THE EFFECTS OF ST. JOHN'S WORT ON THE PHARMACOKINETICS OF

CORTICOSTEROID AND NON-STEROIDAL DRUG PREPARATIONS

Except where reference is made to the work of others, the work described in this dissertation is my own or was done in collaboration with my advisory committee

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THE EFFECTS OF ST. JOHN'S WORT ON THE PHARMACOKINETICS OF CORTICOSTEROID AND NON-STEROIDAL DRUG PREPARATIONS

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VITA

Edward C. Bell was born on January 12, 1976 in Flowood, Mississippi. The son of E.C. and Hilda Bell, he was raised in Jackson, Mississippi. He attended Tougaloo College, Tougaloo, MS, where he earned his Bachelor's of Science degree in Chemistry in May 1998. During his tenure at Tougaloo College, he also participated in an internship as a chemist at Eli Lilly and Company, Indianapolis, Indiana. Bell enrolled in the graduate program in the department of Pharmacal Sciences at Auburn University in 1999. He joined the doctoral program in Pharmaceutics with an emphasis in Pharmacokinetics, and worked under the supervision of Dr. William Ravis. Bell participated in both veterinary and clinical pharmacokinetic investigations during his years at Auburn. Bell was funded by a doctoral fellowship from the Southern Regional Education Board, and was later funded as a graduate research and teaching assistant at Auburn University.

DISSERTATION ABSTRACT

THE EFFECTS OF ST. JOHN'S WORT ON THE PHARMACOKINETICS OF CORTICOSTEROID AND NON-STEROIDAL DRUG PREPARATIONS

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St. John's Wort is a flowering plant whose extracts have gained increasing popularity in the treatment of mild to moderate depression. This investigation examined the impact of long-term St. John's Wort administration upon the pharmacokinetic profiles of the glucocorticoid drugs prednisone and prednisolone, and upon the pharmacokinetic profile of enantiomeric ibuprofen. The pharmacokinetics of prednisone and prednisolone were not significantly changed by St. John's Wort co-administration. Mean prednisone parameters (for control samples and St. John's Wort treatment samples, respectively) were 3.85 +/- 1.49 hrs and 5.10 +/- 2.77 hrs for drug elimination half-life, 115.89 +/- 39.52 µg*hr/L and 128.76 +/- 32.71µg*hr/L for area under the curve (AUC), 15.42 +/- 4.19 µg/L and 15.11 +/- 3.41 µg/L for maximum plasma concentration (Cmax), and 6.44 +/- 0.92 hrs and 8.56 +/- 3.88 hrs for mean residence time (MRT).

Mean prednisolone parameters (for control samples and St. John's Wort treatment samples, respectively) were 2.97 +/- 0.59 hrs and 3.09 +/- 0.83 hrs for drug elimination half-life, 714.19 +/- 153.29 µg*hr/L and 700.74 +/- 89.68 µg*hr/L for AUC, 128.10 +/- 25.33 µg/L and 128.58 +/- 23.65 µg/L for Cmax, and 5.03 +/- 0.88 hrs and 5.23 +/- 1.23 hrs for MRT. Average ratios of prednisolone to prednisone AUC were 6.40 +/- 0.970 and 5.64 +/- 1.15 before and after St. John's Wort co-administration, respectively. Minimal significant changes in ibuprofen pharmacokinetics resulted from long-term St. John's Wort therapy. Mean S(+)-ibuprofen parameters (for control samples and St. John's Wort treatment samples, respectively) were 3.24 +/- 1.61 hrs and 2.17 +/- 0.477 hrs for drug elimination half-life, 131.6 +/- 26.8 µg*hr/mL and 122.4 +/- 32.9 µg*hr/mL for AUC, 31.8 +/- 7.33 µg/mL and 33.6 +/- 7.83 µg/mL for Cmax, and 5.20 +/- 1.95 hrs and 3.60 +/- 0.322 hrs for MRT. A clinically significant decrease in S-enantiomer mean residence time was seen following St. John's Wort treatment. Mean R(-)-ibuprofen parameters (for control samples and St. John's Wort treatment samples, respectively) were 2.28 +/- 1.34 hrs and 1.55 +/- 0.641 for drug elimination half-life, 85.1 +/- 26.6 µg*hr/mL and 87.7 +/- 30.1 µg*hr/mL for AUC, and 28.4 +/- 8.72 µg/mL and 30.0 +/- 8.97 µg/mL for Cmax, and 3.76 +/- 1.71 hrs and 2.78 +/- 0.631 hrs for MRT. Average ratios for S- to R-enantiomer AUC were 1.69 +/- 0.654 before St. John's Wort dosing and 1.49 +/- 0.496 after St. John's Wort co-administration, respectively.

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INTRODUCTION

The proliferation of herbal products has perhaps permanently altered the landscape of modern therapeutics. Many herbal remedies have achieved mainstream popularity in recent years, in some cases becoming relied-upon alternatives to conventional drug treatments. St. John's Wort, for instance, is a popular alternative to synthetic agents. Clinical trials have found the herb to be comparably effective to tricyclic anti-depressants in the treatment of mild to moderate depression. The preparation is easily obtained as an over-the-counter remedy, and therefore is self-prescribed by many without physician advisement. St. John's Wort is an herbal dietary supplement that is not regulated by the United States Food and Drug Administration. St. John's Wort can vary in terms of product purity and active ingredients from batch to batch, and from brand to brand. Furthermore, the product has not undergone the intensive level of safety and efficacy testing as have synthetically derived products; thus, knowledge is yet to be gained concerning its compatibility with many well-established medications.

In recent years, St. John's Wort has received attention due to its apparent capability to alter human hepatic and intestinal cytochrome P450 enzymatic activity. The product consists of multiple xenobiotic components that are individually capable of affecting cytochrome function. Previous case reports and pharmacokinetic studies have shown this supplement to reduce the plasma

concentrations of a wide range of medications metabolized by several cytochrome moieties. Drastic alterations have been observed in various pharmacokinetic parameters of drugs such as warfarin, phenprocoumon, theophylline, cyclosporine, amitryptyline, indinavir, and various oral contraceptives (Henderson et al., 2002).

Ibuprofen is a stereo-isomeric, non-steroidal anti-inflammatory drug that is used in the treatment of body aches most commonly associated with various types of arthritis, dysmenorrhea, and headache. Ibuprofen is also used for body temperature regulation in instances of fever. Developed in the 1960s as an anti-rheumatic agent, the drug has been available without a prescription in the United States since 1984.

A proprionic acid derivative, ibuprofen potently inhibits prostaglandin biosynthesis to elicit its effects. The S(+)-enantiomer displays the majority of pharmacological activity. Ibuprofen is usually administered orally in either conventional or sustained release preparations. Its standard dosing ranges from 200-400 mg, taken every four hours (Davies, 1998). The agent is rapidly absorbed, and exhibits peak plasma concentrations within three hours of administration. Ibuprofen displays nonlinear kinetics at high doses due to its extensive binding to plasma proteins. Ibuprofen is metabolized through enantiomeric inversion of R(-)-ibuprofen to its stereoisomer. In addition, ibuprofen is extensively oxidized to its hydroxy and carboxy metabolites via cytochrome P450 2C9 catalysis (Davies, 1998).

Prednisone and prednisolone are well-known glucocorticoid agents that have been widely used during the past three decades. The corticosteroids act as immunosuppressant and anti-inflammatory agents largely by their inhibition of cytokine secretion. Antibody production is therefore suppressed, and the inflammatory process is slowed and weakened to reduce heat, swelling, redness, and pain in affected areas. The agent also stabilizes the immune system in instances of over-activity (Schimmer and Parker, 2001).

Either prednisone or prednisolone can be administered orally in tablet form in daily doses ranging from 2.5 mg to 100 mg (Frey and Frey, 1990). Oral absorption is rapid and complete. Both compounds display significant plasma protein binding, and prednsiolone in particular binds extensively to transcortin (Rose et al., 1981). The agents are metabolized in the liver via cytochrome P450 3A4 catalysis, resulting in the formation of several hydroxy and ketosteroid metabolites (Frey and Frey, 1990). Prednisone and prednisolone are especially intriguing due to their reversible systemic conversion following the administration of either drug form. However, prednisolone has considerably stronger pharmacological activity than its reversible counterpart. The two drugs are also noted for their dose-dependent pharmacokinetic profiles (Rose et al., 1981).

The effects of treatment with St. John's Wort on the pharmacokinetics and plasma concentrations of prednisone, prednisolone or ibuprofen have not been studied. St. John's Wort has been noted to affect the metabolism and clearance of several drugs that are substrates of cytochrome P450 3A4 and 2C9 isoforms— cytochrome pathways that are largely responsible for the metabolism

of prednisone and prednisolone, and ibuprofen, respectively. Due to their enormous popularity and easy accessibility, prednisone, prednisolone and ibuprofen are worthwhile candidates for investigation of drug interactions with St. John's Wort.

The primary objective of this investigation was to compare pharmacokinetic parameter profiles of prednisone, prednisolone and ibuprofen when the agents were taken with and without the dietary supplement St. John's Wort. Ibuprofen was given to 8 volunteers as a single oral dose followed by blood sampling, and prednisone was given as a single oral dose one week later. The volunteers self-administered St. John's Wort 300 mg TID (900 mg daily) for 4 weeks. After 3 weeks of St. John's Wort administration, a single dose of ibuprofen was again given, followed by blood sampling. Prednisone was administered by single dose 1 week later following a fourth week of herbal supplementation. All plasma samples obtained during the study were placed into frozen storage before extraction of drug residues. Drug concentrations were analyzed by high performance liquid chromatography, and drug pharmacokinetic parameters were determined using model independent methods.

REVIEW OF LITERATURE:

HUMAN METABOLISM AND THE CYTOCHROME P450 SYSTEM

The concept of biological transformation of chemical substrates has been researched extensively for over 50 years. During this time it has been determined that drug metabolism (in its most useful sense) refers to the body's chemical reaction-mediated formation of increasingly polar and water-soluble derivatives of foreign compounds, ultimately resulting in the pharmacological nullification and rapid expulsion of those compounds (Mandel, 1971). Though often understated, the importance of the body's metabolic processes is enormous. It was once boldly estimated, for instance, that the pharmacological effects of the anticonvulsant pentobarbital would linger for a century in the absence of drug metabolism, simply due to its lipophilic nature (Brodie, 1964)!

Water solubility and xenobiotic expulsion

The mechanisms by which drugs are transformed and expelled are as numerous as they are complex. Enhanced water solubility of metabolites is usually accomplished by the addition of a variety of hydrophilic functional groups, or by the addition of adequately lipophobic conjugates to a parent compound. There are some instances in which enhanced water solubility is not achieved through drug biotransformation; however, such scenarios are usually avoided when drug is excreted before metabolites of lower water solubility can be formed. The excretion of some metabolites is enhanced due to their relative ease of

ionization at physiological pH compared to their parent structures, thus favoring the formation of water-soluble salts. In addition, many acidic metabolites are advantageously secreted by the renal tubules of the kidney, leading to even faster drug expulsion (Williams, 1959).

Metabolic systems are not always predictable processes and do not always produce desirable outcomes. Despite the vast majority of drug metabolites possessing reduced pharmacological activity compared to parent compounds, there exist many exceptions to the rule in which metabolites display increased pharmacological activity. The phenomenon can at times be advantageous, as in the case of prodrug catabolism to yield a therapeutically active product. The metabolic conversion of prednisone to its therapeutically superior metabolite prednisolone serves as an example (M. Frey and F. Frey, 1990). However, the phenomenon can also be quite problematic, as in the case of toxic metabolite formation. A notorious example of this occurs in sulfonamide metabolism, which features the production of very water insoluble metabolites. The subsequent crystallization of these metabolities in the renal tubules is capable of causing considerable kidney damage (Ylitalo et al., 1985). The determination of a metabolite's pharmacologic activity is ultimately dependent upon its solubility and interaction with individual tissues and organs (Mandel, 1971).

The most common pathways of drug metabolism feature the structurally-altering processes of oxidation, reduction, hydrolysis, and conjugation. Drug catabolism may often involve several of these reactions in sequence. Drug

catabolism can also occur by simultaneous transformation by several reactions, as the extent of metabolite formation is greatly impacted by relative reaction rates. This versatility of action makes the prediction of a compound's metabolic fate an arduous task. Fortunately, research has provided much understanding regarding the details of drug metabolism. Central to this understanding is an appreciation of the liver and its role in drug metabolism.

The liver's role in drug metabolism

The human liver serves as the body's chief detoxification and metabolic processing center. The organ is highly perfused with blood and therefore is exposed to a large array of hormonal and exogenous substrates. The liver's anatomy is well suited for this rapid transit and processing of blood. The functional units of the liver are the cylindrically-shaped lobules, which number anywhere between 50,000 and 100,000 in the average person. Within each lobule exists a number of hepatic cells that are located amongst an intricate vascular network of central veins, septal portal venules, and hepatic arterioles. Cumulatively, these hepatocytes form a chemically reactive conglomerate capable of a high rate of metabolism. This conglomerate is responsible for the conversion and synthesis of various substrates for further circulation throughout and/or excretion from the body (Guyton and Hall, 1996).

Oxidative metabolism accounts for a large portion of the liver's contribution to drug detoxification. Within each liver hepatocyte exists enzymes called cytochrome P450 (CYP) monooxygenases. Cytochrome monooxygenases are found within the inner membranes of cellular mitochrondria

and endoplasmic reticulum (Shen, 1995). The human cytochrome P450 monooxygenase system is a superfamily of enzymes that act in the oxidative metabolism of a wide range of drugs. In general, oxidative reactions such as various forms of dealkylation and hydroxylation are characteristic processes by which CYPs facilitate the degradation of a long list of lipophilic substrates (Guengerich, 1990).

Phase reactions and cytochrome P450

The metabolic mechanisms involved in cytochrome action are classified as phase I and phase II reactions. Phase I reactions convert parent substrates to more polar forms via the introduction or cleavage of specific functional groups, including -OH, -SH, -CO_2H, and -NH_2. Functional group manipulation can occur by a variety of specific reactions, including deamination, dehalogenation, epoxidation, O-, N- and S- dealkylation, N- and S-oxidation, N-hydroxylation, hydroxylation of alkyl and aryl hydrocarbons, and alkylol derivative formation (Gillette, 1966). Phase I metabolites can be further altered by phase II conjugation reactions such as glucuronidation, sulfation, acetylation, and methylation (Testa and Jenner, 1976). Phase II reactions can also precede phase I conversion of parent compounds (Ioannides, 1996).

An interesting feature of the cytochrome P450 system is its versatility. Singular CYP isoforms are capable of metabolizing multiple xenobiotic substrates; by the same token, some CYPs have substrate activity spectrums that overlap to impose cumulative metabolic effects upon single substrates (Gonzalez, 1992). It comes as no surprise that several isoforms within the

superfamily have been implicated in a number of drug interactions that have been either documented and/or investigated in recent years. In general, drug interactions involving cytochrome metabolism have been associated with either inhibition or induction of drug catalysis (Lewis and Pratt, 1998).

Cytochrome P450 is distinguished from other enzyme classes by virtue of structural and spectroscopic characteristics. Originally discovered in the membrane fractions of rat liver microsomes, the enzymes in this class could not be adequately observed by standard spectroscopy due to the turbidity of microsomal suspensions and their light scattering tendencies. By utilizing difference spectroscopy (ie., subtracting out light scattering through the use of two beams during spectrum measurement), the absorption of cytochrome residues was later determined. A cytochrome isoform displays its trademark absorbance at a wavelength of 450 nm, due in part to the presence of a single protophorphyrin IX prosthetic heme iron at the cytochrome's center (Schenkman, 1993).

The heme group of cytochrome P450 consists of four nitrogens and a thiolate anion. A sixth ligand binding site provides the enzyme the important dimension of binding and activating molecular oxygen. CYP P450 incorporates one of the two oxygen atoms of an oxygen molecule into a given substrate with adjoining reduction of the second oxygen by two electrons to molecular water (Ortiz de Montellano and Correia, 1995). The term "monoxygenase" refers to the process by which cytochrome enzymes incorporate single oxygen atoms into bonded substrates. The arduous task of breaking the oxygen-oxygen bond is

made feasible by the addition of two electrons to the heme iron of P450 complex. The electrons are donated by another protein that binds briefly to the P450 and passes an electron from a prosthetic group. Movement of these electrons between proteins occurs by the electron transfer chain. NADPH or NADH provides the electron source that flows from ferredoxin reductase to ferridoxin and then to the P450 heme iron (Cashman, 2000). The redox reaction adheres to the scheme:

$$RH + O_2 \text{ ----------> } ROH + H_2O$$

where RH is the drug substrate to be oxidized, and ROH is the hydroxylated metabolite that results from enzyme catalysis. The ROH metabolite is more water soluble and better-suited for elimination from the body (Lewis and Pratt, 1998).

The specific steps involved in the cytochrome activation process include:

1. The binding of the substrate species to the enzyme complex.
2. The reduction of the ferric CYP species of the complex to the ferrous CYP state.
3. The binding of molecular oxygen to yield a ferrous CYP – dioxygen complex.
4. The transfer of a second electron to the CYP- dioxygen complex to yield a peroxoiron (III) complex.
5. The protonation and cleavage of the oxygen-oxygen bond with the incorporation of an oxygen into a water molecule and the reaction iron-oxo species.

6. The oxygen atom transfer from the oxo complex to the bound substrate.
7. The dissociation of the product (White et al., 1980).

Each CYP isoform's interaction with a substrate is dependent upon the entry and orientation of the substrate with amino acids in the CYP active site. This interaction is generally non-specific from a configuration standpoint such that several substrate orientations are possible at the active site, resulting in the production of multiple metabolites with regio- and stereospecificity characteristic of each individual CYP (Guengerich, 1995).

The cytochrome P450 superfamily is made up of gene families and gene subfamilies that are classified and grouped according to their amino acid sequences and sequence similarities. Sequences that are greater than 40% identical at the amino acid level belong to the same family, while sequences greater than 55% identical belong to the same subfamily. Some 481 CYP genes and 22 pseudogenes are known to exist across all species (Nelson et al., 1996). Thirty-five of these genes have been identified in humans, occupying 14 families. Human cytochrome families can be separated into two basic classifications by virtue of function. Some families aid in the synthesis and/or metabolism of endogenous compounds such as bile acids, biogenic amines, eicosanoids, fatty acids, phytolexins, retinoids and steroids. The remaining families are responsible for drug metabolism. In fact, 18 gene forms contained within 3 families-- CYP1, CYP2 and CYP3-- impact the metabolism of drug and xenobiotics in humans and largely dominate liver metabolism. These families are further divided into related

subfamilies (eg., CYP1A, CYP2C, and CYP2E) that differ only by their protein structures around their identical heme centers (Shimada et al., 1994).

Cytochromes 1A2, 2A6, 2B6, 2C, 2D6, 2E1 and 3A make up about 70% of all CYPs in the human liver. CYPs 3A and 2C are particularly prevalent, accounting for 30% and 20% of all CYP, respectively (Guengerich and Turvy, 1991). The human genome is known to consist of at least seven genes within the family CYP2C, with 2C8 and 2C9 accounting for 35% and 60% of that total. Similarly, CYP3A4 is the most abundant 3A isoform in human adult liver, accounting for approximately 25% of that family's representation (Romkes et al., 1991).

Various factors can affect CYP isoform activity. Regulation of gene expression often differs from one isoform to another. In addition, an individual's isoform phenotype can influence the metabolic clearances of drugs undergoing CYP biotransformation. Environmental, genetic, and physiological influences can lead to interindividual variability in drug pharmacokinetics and pharmacodynamics (Wrighton and Stevens, 1992).

The contents of individual cytochrome isoforms can vary greatly amongst individuals and amongst populations. For example, a study of human liver microsomes in Japanese and Caucasian volunteers revealed a 6-fold difference in CYP3A4, and 10 to 50-fold differences in CYP2D6 across racial groups (Shimada et al., 1994). Genetic polymorphisms play an important role in this variability as a result of mutations in genes that control drug metabolism. The increased, decreased, or absence of enzyme activity that is a result of

polymorphism may have significant clinical impact (Meyer et al., 1992). In particular, polymorphisms in CYP2D6 and CYP2C19 have a profound impact upon the incidences of rapid and poor metabolizers in certain segments of the population. For example, it is estimated that 5% to 10% of Caucasians are poor metabolizers due to CYP2D6 polymorphism, while 1% to 2% of Asians share similar status. CYP2C19 polymorphism has an opposing effect. In its case, 2% to 6% of Caucasians reveal the poor metabolizer phenotype, while 18% to 22% of Asians exhibit the phenotype. Cases of rapid metabolism have also been noted in small segments of Ethiopian and Swiss populations as a result of CYP2D6 polymorphism (Johansson et al., 1993; Aklillu et al., 1995).

Inhibition of cytochrome-mediated drug metabolism

Cytochrome inhibition is the phenomenon that occurs when one of the seven-steps in the process of catalytic activation is impaired. Of these seven steps, three steps are most vulnerable to the interference that most commonly causes enzyme inhibition:

Step 1-- The binding of substrate to the enzyme.

Step 3-- The binding of molecular oxygen to the cytochrome species.

Step 6-- The oxidation of the substrate through oxygen transfer from the oxo-complex. (Ortiz de Montellano and Correia, 1995)

Cytochrome inhibition can be classified into three categories: inhibition through reversible mechanisms, inhibition through quasi-irreversible mechanisms, and inhibition through irreversible mechanisms.

Reversible enzyme inhibition, the most common form of inhibition, results from the competitive binding of substrates at an enzyme's active site and impacts the first step of catalytic activation. Substances that successfully occupy the cytochrome's hydrophobic domain (via competitive or noncompetitive inhibition) interact with the cytochrome's prosthetic heme iron, or interact with miscellaneous active site regions through hydrogen bonding or ionic interaction. Although this manner of inhibition is temporary in duration, it is very capable of rendering significant metabolic effects (Correia and Ortiz de Montellano, 1993).

The resting ferric state of the heme iron in cytochrome P450 is often bound by strong iron ligands (particularly those of ionic character) that effectively change the redox potential of the enzyme. This redox shift makes the reduction of the enzyme by cytochrome P450 reductase substantially more difficult. Altered reduction potential is therefore as integral a part in cytochrome inhibition as the physical coordination of the heme iron during strong ligand interaction (Guengerich, 1983). Inhibition by substrates binding to the reduced ferrous state of heme also may occur. Carbon monoxide inhibition is a well-known example of this phenomenon. This form of inhibition is quite uncommon, however, due to the fact that many reactions catalyzed by cytochrome P450 are resistant to carbon monoxide interference (Gibbons et al., 1979).

Substrates that bind to both the prosthetic heme iron and to lipophilic portions of the protein structure are more effective in cytochrome inhibition than substrates that can only accomplish one of these feats. An agent that is capable of this optimally balances the properties of hydrophobic character with the ability

to form a strong bond between its heteroatomic lone pair and heme at the CYP active site. Amongst the most potent reversible inhibitors are nitrogen containing substrates, particularly agents possessing pyridine, quinoline, and imidizole moieties. In general, inhibitor potency is largely dependent upon substrate lipophilicity and heme bond strength (Rogerson et al., 1977). Quinoline derivatives such as quinidine are strong reversible inhibitors of debrisoquine 4-hydroxylation, which is a reaction catalyzed by CYP2D in humans. It's interesting to note that although quindine inhibits CYP2D, the drug is metabolized by CYP3A4 (Boobis et al., 1990).

Quasi-irreversible enzyme inhibition is characterized by the oxidation of substrates to metabolite intermediates that form strong, stable complexes with the prosthetic heme of cytochrome P450. This complex renders the enzyme functionally inactive such that it can only be displaced by specific in vitro methods. Under in vivo circumstances, enzyme inhibition in this manner can only be overcome by synthesis of new enzymes (Dickins et al., 1979). The macrolide antibiotic troleandomycin is perhaps the best known agent capable of quasi-irreversible inhibition. The tertiary amine contained within the compound is transformed by a series of reactions to produce a nitroso metabolite intermediate that binds closely to ferrous cytochrome isoforms. Many agents like troleandomycin act as both cytochrome inhibitors and inducers after repeated dosing. Due to rapid inactivation of the enzymes affected by these agents, detection of enzyme induction is often masked by inhibition of metabolic function (Pessarye et al., 1982).

Irreversible inhibition of drug substrates differs in mechanism from quasi-irreversible inhibition by virtue of the step in which inhibition occurs. A substrate that causes this form of inhibition is oxidized upon interaction with a cytochrome isoform, leading to inactivation of the enzyme (Silverman, 1988). During the inhibition process, the heme iron and/or the protein structure of the enzyme is permanently altered following substrate binding. Loss of catalytic activity is dependent upon the modification of essential amino acids, while alteration of the heme iron results in unconditional cytochrome inactivation (Ortiz de Montellano, 1988).

Enzyme inhibition through protein alteration usually involves disruption of oxygen activation or electron transfer processes at the activation site. Some sulfur-containing compounds inhibit enzymes in this manner. These compounds alter cytochrome isoforms by facilitating the creation of reactive sulfur metabolites, which form covalent bonds with the host protein (Lopez-Garcia et al., 1993). For example, chloramphenicol can disrupt electron transfer. Upon cytochrome interaction, chloramphenicol is oxidized to an intermediate that acylates lysine in the enzyme's active center. This acylation prevents the transfer of electrons from CYP reductase and halts enzyme catalysis (Halpert et al., 1985). Other compounds such as spironolactone can generate reactive intermediates capable of covalently modifying both protein and prosthetic heme. Cytochrome-catalyzed hydrolysis of spironolactone transforms its thioester moiety into a free thiol group before further oxidation by the CYP enzyme.

Oxidation of this group creates a highly electrophilic species that covalently bonds and structurally alters both protein and heme (Decker et al., 1986).

Induction of cytochrome-mediated drug metabolism

An intriguing quality of the cytochrome P450 system is that of inducibilty. During this process, exposure to a chemical stimulus causes enzyme activity to increase over time, at times by large orders of magnitude (Whitlock and Denison, 1995). The induction of these enzymes may cause a change in the pharmacological response of the body to a chemical substrate, often resulting in shortened action and lessened effectiveness of the substrate (Remmer and Merker, 1963). Cytochrome isoforms that have been confirmed as inducible in humans include CYPs 1A2, 2C9, 219, 2E1, and 3A4 (Lin and Lu, 2001). Cytochrome induction is believed to serve dual functions at the cellular level. The phenomenon provides a means by which an interacting substrate can facilitate its own metabolism. Additionally, enzyme induction provides an efficient means of cell detoxification, the most striking example of which is the role cytochrome P450 plays in lowering the incidence of neoplasia after animals are exposed to potent chemical carcinogens (Conney, 1982).

Although induction is inherently a mechanism by which cells protect themselves, it can be troublesome phenomenon when it interferes with drug efficacy. Many enzymes are capable of metabolizing multiple drug substrates. Metabolic induction of these enzymes by one drug may affect the metabolism of other substrates that share these enzymes (Park and Breckinridge, 1981). This phenomenon is capable of causing a drastic reduction in the efficacy of

therapeutic agents through increased drug clearance, shortened half-life, and reduced area under the concentration versus time curve. In addition, enzyme induction may be particularly troublesome in the case of enhanced chemical toxicity. For instance, the induction of CYP1A isoforms has been proven to lessen the carcinogenic properties of some compounds. By the same token, its induction has also been found to enhance the carcinogenic qualities of another compound, benzopyrene, by activating the substrate to produce carcinogenic metabolites (Gelboin, 1980). Excessive production of reactive metabolites produced during enzyme induction can eventually lead to systemic toxicity (Lin and Lu, 1998). Acetaminophen toxicity is a notorious example of reactive metabolite production. High concentrations of the analgesic not only saturate detoxification pathways, but also cause an accumulation of metabolites that lead to hepatic necrosis (Thomas, 1993).

Sometimes metabolites formed during enzyme induction possess equal pharmacological activity as the parent drug, a situation that deviates from the expected inductive effect. Collste and colleagues (1979) demonstrated this phenomenon during an investigation of the inductive effects of pentobarbital upon alprenolol metabolism. Pentobarbital administration caused a decrease in alprenolol bioavailability, along with a 45% reduction in AUC. Despite these pharmacokinetic changes, only a slight reduction in pharmacological response was observed, as indicated by inhibition of tachycardia. The comparable pharmacological activity was attributed to the equipotent actions of metabolic 4-hydroxyalprenolol upon beta-adrenergic receptor function.

Dose-adjustment, though a commonly-used approach by many physicians to combat the effects of enzyme induction, can become problematic in cases of severe induction. The metabolically-induced medication can hazardously accumulate when the enzyme inducer is withdrawn. For instance, the discontinued use of phenobarbital by heart patients who were also taking anticoagulant medications caused severe bleeding once anticoagulant induction was no longer apparent (Mutschler and Derendort, 1995).

Cytochrome induction occurs in a time-dependent manner. Whereas drug inhibition is virtually instantaneous, drug induction has a slower onset and varies with inducer potency. For example, rifampicin induces CYPs 1A2, 2C, and 3A4 within 24 hours of administration, whereas phenobarbital requires one week of consistent administration before induction is seen. St. John's Wort generally requires dosing for two weeks or more before its effects become apparent (Lin and Lu, 2001).

The molecular mechanisms involved in CYP induction are varied, but many studies suggest that major classes of CYP genes are selectively regulated by certain ligand-activated nuclear receptors. Two types of nuclear receptors commonly implicated in enzyme induction are the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR). These receptors code for transcription factors that transform extracellular and intracellular signals into cellular responses by initiating nuclear receptor target gene transcription (Honkakoski and Negishi, 2000). Nuclear receptors consist of a DNA binding domain and a ligand binding domain, not unlike the makeup of classical steroid

receptors. These DNA binding domains are tightly wrapped within histone proteins to form chromatin structural units. When a ligand binds to a nuclear receptor, structural changes in the receptor's ligand binding domain cause the formation of a sufficiently hydrophobic region that can be accessed by co-activators. Such co-activators may possess histone acetyltransferase activity capable of altering chromatin structure to expose DNA molecules for activation of gene transcription (Mangelsdorf et al., 1995). In general, most transcriptional processes consist of:

1. Activation of nuclear receptors, usually by ligand-dependent receptor dimerization. In some cases, protein phosphorylation and dephosphorylation facilitate the transcriptional activation of nuclear receptors.
2. Interaction between transcription factors and drug-responsive enhancers.
3. Induction signaling from the enhancer to a transcription promoter.
4. Alteration of chromatin structure to facilitate transcription (Yan and Caldwell, 2001).

Various cytochrome genes are regulated by various transcriptional factors. The constitutive androstane receptor, for example, regulates the induction of cytochrome 3A and can be activated by a broad range of substrates, including antibiotics, barbiturates, and glucocorticoids (Wei et al., 2000).

The majority of cases of enzyme induction involve increases in gene transcription; however, there also exist examples of enzyme induction by non-transcriptional processes. The CYP3A4 inhibitor troleandomycin is also capable

of non-transcriptional induction by simply preventing the enzyme's degradation (Park et al., 1995). Isoniazid's induction of CYP2E1 can similarly be attributed to protein stabilization (Park et al., 1993).

Inducible cytochrome P450s fall into five catagories:

- Aromatic hydrocarbon inducible, whose drug substrates are chiefly metabolized by CYP1A1 and are known to increase the rate of carcinogen metabolism in rodents (Conney, 1982). CYP1A1 provides one of only a few well-understood examples of how transcriptional induction can take place (Okey, 1992).
- Peroxisome inducible, whose drug substrates tend to be structurally dissimilar. They induce hepatic peroxisome proliferation, cause an increase in hepatic smooth endoplasmic reticulum, and are metabolized by CYP4A1 (Lock et al., 1989).
- Phenobarbital inducible, which include a wide range of isoforms such as CYP2A1, CYP2B2, and CYP3A2. They induce hepatic ER proliferation and increase expression of phase I and II enzymes (Waxman and Azaroff, 1992).
- Ethanol inducible, namely CYP2E1, whose induction produces an increase in the amount of enzyme protein while causing a stabilization of CYP mRNA. This induction action is implicated with ethanol's infamous toxicity with increased dosing (Song et al., 1987).
- Steroid inducible, characterized by the famously abundant CYP3A isoform, whose induction results in the proliferation of hepatic endoplasmic reticulum

and an increase in hepatic CYP3A enzyme content (Elshourbagy and Guzelian, 1980).

The CYP2 enzyme family and CYP2C9

The cytochrome 2 family is the largest cytochrome family in humans. Many are strongly involved in steroid hydroxylation and serve an important purpose in assisting in the detoxification of food products. Members of this family include CYPs 2A, 2B, 2C, 2D, 2E, and 2F (Hamman et al., 1997).

Specifically, members of the CYP2C subfamily were among the first to be purified from human liver and used in animal models (Wang et al., 1980). The subfamily is quite extensive, consisting of at least seven different genes and operating under a variety of different modes of regulation, including inducibility and polymorphism. The CYP2C subfamily initially drew interest because of its involvement in human S-mephenytoin polymorphism. This phenomenon is observed in 2% to 5% of Caucasian populations, and is noted in 23% of Japanese individuals who altogether lack S-mephenytoin 4'-hydroxylation activity (Wilkinson et al., 1989). Interest in the subfamily inevitably led to thorough examination of CYP2C9, by far the most abundant hepatic member of the 2C subfamily (Hamman et al., 1997).

CYP2C9 is one of four known members of subfamily 2C, sharing an 82% amino acid identity with isoforms 2C8, 2C18, and 2C19 (Goldstein and de Morais, 1994). CYP2C9 displays a high catalytic activity in the metabolism of widely-used drugs such as warfarin, phenytoin, tolbutamide, and several non-steroidal agents. For instance: CYP2C9 is the chief facilitator of (S)-warfarin 7-

hydroxylation, as evidenced by the emergence and predominance of warfarin's 7-hydroxy metabolite in humans (Rettie et al., 1992). It has also been suggested that the troublesome interpatient variability displayed in humans after warfarin administration may have a direct relation to the interindividual variation of CYP2C9 expression in humans. Phenytoin metabolism by 2C9 occurs by phenytoin 4'-hydroxylation to its primary metabolite 5-(4 p-hydroxyphenyl)-5-phenylhydantoin, accounting for about 80% of phenytoin elimination (Veronese et al., 1991). Tolbutamide is similarly catalyzed by tolbutamide methyl hydroxylation (Brian et al., 1989). In addition, the oxidation of tolbutamide has been shown to be favored by barbiturates and rifampicin, thus indicating the inducibility of 2C9 (Zilly et al., 1977). CYP2C9 has proven important to the oxidative metabolism of acetic acid, propionic acid, fenemate and oxicam NSAIDs. Inhibition and kinetic studies have indicated that 2C9 is responsible for the 4'-hydroxylation of enantiomeric flurbiprofen (Tracy et al., 1995) and the O-demethylation of S-naproxen (Miners et al., 1996).

The subject of polymorphism as it relates to CYP2C9 has garnered much attention in recent years. As early as the 1970s, polymorphism was reported in 2C9 substrates phenytoin and tolbutamide, but it wasn't until years later that the responsible allele, CYP2C9*3, was identified. The CYP2C9*3 allele has been detected in approximately 6% of Caucasian and Asian populations, with a homozygous poor metabolizer frequency of about 0.3% (Sullivan-Close et al., 1996). CYP2C9*3 carries a Leu 359 mutation that can manifest itself in poor metabolizers of 2C9 substrates like losartan and tolbutamide. For example, a

homozygous carrier of the CYP2C9*3 allele exhibited decreased clearance of S-warfarin and associated untoward effects, not unlike previously observed instances of warfarin toxicity seen in CYP2C9 poor metabolizers (Steward et al., 1997). In addition, recombinant CYP2C9*3 has been linked with lowered *in vitro* Vmax values for the hydroxylations of piroxcam, tenoxicam, and mefenamic acid, in turn resulting in decreased intrinsic clearance. These results have been attributed (in part) to changes in substrate -- enzyme affinity. Slower metabolism of non-steroidal agents in CYP2C9*3 homozygous individuals has also been implied (Takanashi et al., 2000).

Another mutant CYP2C9 allele, CYP2C9*2, has also been observed in humans, and has exhibited decreased catalytic activity upon some 2C9 substrates *in vitro*. Effects upon warfarin metabolism have been particularly intriguing. Higher incidences of the variant CYP2C9*2 allele have been observed in individuals requiring lower does of warfarin (Furuya et al., 1995), as has been the case in individuals with higher incidences of combination (2C9*2 and 2C9*3) variant alleles (Aithal et al., 1999). The CYP2C9*2 allele has essentially no expression in Asian populations, but is found in about 8% of Caucasian populations (Sullivan-Close et al., 1996).

Inhibition of CYP2C9 enzymes has been noted upon administration of several drugs hailing from distinctly different classes. In fact, reports of enzyme inhibition have been more common than that of enzyme induction both *in vitro* and *in vivo*.

- Fluconazole potently inhibited CYP2C9 during in vitro investigations. Studies have indicated fluconazole inhibition of the metabolic hydroxylations of tolbuamide (Back et al., 1998), diclofenac (Hargreaves et al., 1994) and warfarin (Kunze et al., 1996). In *in vivo* investigations, fluconazole pretreatment increased AUC values of several medications, including S-warfarin by 184% (Black et al., 1996), tolbutamide by 109% (Lazar and Wilner, 1990), phenytoin by 75% (Blum et al., 1991), and losartan by 153% (Kazeriad et al., 1997). Other azole antifungals have inhibited CYP isoforms in *in vitro* and *in vivo* studies. O'Reilly et al. reported that miconazole reduced plasma clearance of S-warfarin by 80% (O'Reilly et al., 1992). The potent CYP3A4 inhibitor ketoconazole has shown little effect upon clearances of commonly sensitive CYP2C9 substrates, however (McCrea et al., 1996; Touchette et al., 1992).
- Another potent CYP2C9 substrate, phenylbutone, competitively inhibited human liver microsomal activity *in vitro*, and affected the metabolism of warfarin and tolbutamide. Interestingly, phenybutazone pretreatment caused stereoselective reduction of S-warfarin clearance, while it simultaneously elevated clearance of R-warfarin (Lewis et al., 1974). Moderately long (1 week) treatment of phenylbutazone also significantly increased the elimination half-life of tolbutamide (Pond et al., 1977). The pyrazolone congener sulphinpyrazone similarly decreased S-warfarin plasma clearance while it increased R-warfarin clearance, and also decreased the plasma clearance of tolbutamide by 40% (Miners et al., 1982).

- Amiodarone administration has been linked with potentiation warfarin effects in humans, as evidenced by the O'Reilly et al. study (1987) in which R- and S- warfarin plasma clearances were reduced by 33% and 47% following amiodarone treatment for 3 days. It was later determined that amiodarone inhibited all oxidation pathways of warfarin in human microsomal studies (Heimark et al., 1992). Amiodarone has also been implicated with elevation of phenytoin AUC by 40% and resistance of metabolite formation (Skovsted et al., 1976).
- Cimetidine has provided low-level inhibition of CYP2C9 *in vitro* (Miners et al., 1988). *In vivo*, cimetidine administration in high doses decreased tolbutamide by 40% (Back et al., 1988), while cimetidine caused clinically significant increases in phenytoin after long-term anti-convulsant treatment (Nation et al., 1990). In addition, cimetidine pretreatment reduced warfarin plasma clearance by 25% in normal subjects (Serlin et al., 1979), and produced stereoselective reduction in the plasma clearance of R-warfarin due to inhibition of hydroxy metabolite formations (Toon et al., 1987; Niopas et al., 1991).

Enzyme induction of CYP2C9 has been observed in both *in vitro* and *in vivo* investigations. Evidence of *in vitro* induction has been indicated in pregnane X receptor-mediated interactions of rifampicin, phenobarbital and hyperforin with CYP2C9 (Chen et al., 2004). Barbiturate medications have shown inconsistent enzyme induction during *in vivo* studies, exemplified by phenobarbitone's impact on warfarin pharmacokinetics. Phenobarbitone administration to patients on

long-term warfarin therapy resulted in decreased steady-state plasma concentrations of the anticoagulant, as well as decreased effectiveness of the agent (Serlin and Breckinridge, 1983). In addition, long-term rifampicin treatment has been shown to approximately double the clearances of drugs such as phenytoin (Kay et al., 1985), tolbutamide (Zilly et al., 1975), S-warfarin and losartan (Heimark et al., 1987).

The CYP3A enzyme subfamily

The CYP3A subfamily of enzymes is the single most abundant of the human P450 subfamilies, representing about 30% of total CYP protein content in the human liver (de Wildt et al., 1999) and 70% of the total CYPs in the intestines (Kolars et al., 1992). Members of the subfamily have also been found in locations as diverse as the lungs and kidneys (Haehner et al., 1996). Remarkably, CYP3A is responsible for CYP-mediated phase-I metabolism of over 50% of all drugs administered in humans. The subfamily's most important and well-recognized isoform is 3A4, the major CYP3A protein found in the liver, small intestine, colon and pancreas (Zhang and Benet, 2001). CYP3A4 has a remarkably wide spectrum of substrate activity and therefore has a profound impact on the metabolism of a large number of drugs and xenobiotics. 3A4's presence in the small intestine has been contraindicated in the metabolism of orally administered drugs during drug transport across the intestinal wall (Kolars et la., 1991).

The major metabolic pathways catalyzed by CYP3A4 include dehalogenation, dehydration, nitroreduction, C-dealkylation, N-dealkylation, and

C-hydroxylation (Li et al., 1995). This broad catalytic activity of 3A4 exists despite drastic conformational differences amongst its substrates. It has been observed that with each interacting substrate there is amazing regio- and stereoselectivity that directs substrate oxidation. For example, the substrate testosterone is oxidized exclusively at the 6-beta position on CYP3A4's active site implying that the 3A4 active site features a complex structure, in spite of its ability to accommodate substrates of varied sizes (Waxman et al., 1988). It is noteworthy that catalysis of many CYP3A4 substrates has an added dimension of intestinal metabolism. The fates of some CYP3A4 substrates are largely decided by a second factor, the efflux pump P-glycoprotein, which acts to enhance the effects of enzymatic induction and inhibition (Wacher et al., 2001).

CYP3A4 inhibition

CYP3A4 inhibition has been involved in several cases of clinically significant drug interactions. Such interactions have occurred via metabolic interference of structurally unique substrates with differing potencies of action. Among the most potent agents are various macrolide antibiotics, protease inhibitors, azol antifungals and even xenobiotic components found in grapefruit juice (Dresser et al., 2000). Particularly important pharmacokinetic and pharmacodynamic interactions are listed below:

- Sedative medications metabolized by CYP3A4 such as the benzodiazepine midazolam and its congeners, and non-benzodiazepines like buspirone have been implicated in drug inhibition capable of causing excessive sedation in humans. For example, midazolam AUC increased 800% above baseline

values when the drug was administered alongside itraconazole (Backman et al., 1998). In another study, co-administration of midazolam with itraconazole and its congener ketoconazole resulted in increased midazolam AUCs 10- to 15-fold in both scenarios (Olkkola et al., 1994). Macrolide antibiotics erythromycin and clarithromycin have also significantly altered midazolam pharmacokinetics, as the agents caused 4-fold and 3.5-fold increases in midazolam AUC after oral dosing (Olkkola et al., 1993; Yeates et al., 1996). In addition, midazolam AUC, Cmax and Tmax values increased 52%, 56%, and 79%, respectively, when combined with grapefruit juice (Kupferschmidt et al., 1995).

- Significant interactions have been reported for dihydropyridine antihypertensive medications. Felodipine exhibited 8-fold, 6-fold, and 2-fold increases in Cmax, AUC, and elimination half-life, respectively, when administered with itraconazole (Jalava et al., 1997). Itraconazole similarly increased the average trough concentration of another antihypertensive, nifedipine, by 4.4-fold (Tailor et al., 1996). Nifedipine metabolism was also inhibited when the drug was given with grapefruit juice, as evidenced by significantly increased AUC and bioavailability (1.6-fold) (Rashid et al., 1995).
- CYP3A4 is responsible for the metabolism of HMG-CoA reductase inhibitors, namely lovastatin, simvastatin, atorvastatin, and cervastatin. When administered with itraconazole, simvastatin and lovastatin exhibited significant increases in Cmax (17-fold and 13-fold, respectively) and AUC (19-fold and 20-fold, respectively) after oral dosing (Neuvonen et al., 1998, Neuvonen and

Jalava, 1996). Elevation of plasma concentrations of drugs in this class have been linked to severe rhabdomyolysis and eventual renal failure (Corpier et al., 1988).

- The antiepileptic agent carbamazepine is substrate of CYP3A4 that induces its own metabolism, among other agents. It is also prone to enzyme inhibition by other 3A4 substrates, including clarithromycin, erythromycin, diltiazem, fluoxetine, and fluvoxamine. For example, carbamazepine coadministration with fluoxetine caused an increase in AUC of carbamazepine by 127% (Barzaghi et al., 1987). In addition, administration of carbamazepine with erythromycin doubled serum carbamazepine concentrations (Miles and Tennison, 1989).
- Co-administration of ketoconazole and the antihistamine terfenadine was reported to cause excessive increases in terfenadine plasma concentrations and fatal ventricular arrhythmia in several patients. This outcome occurred despite terfenadine's extensive first-pass metabolism that generally maintains the drug at undetectable plasma levels (Honig et al., 1993). Similar alterations upon terfenadine's plasma profile were observed following itraconanzole (Crane and Shih, 1993) and erythromycin (Honig et al., 1992) co-administration. The antihistamine was eventually either withdrawn or greatly restricted in several countries due to its susceptibility to CYP3A4 inhibition (Lin and Lu, 1998).

The enzymatic induction of CYP3A4 is an issue that impacts the efficacy of administered drugs moreso than the safety of those agents. In comparison to

CYP3A4 inhibition, the number of xenobiotics and drugs that induce CYP3A4 is limited. In most cases, enzyme induction by a capable drug substrate requires a sufficiently high dose and/or sustained drug/receptor interaction to render significant changes to drug action and body profile. The influence of the intestinal component P-glycoprotein is more prominent in 3A4 induction by virtue of this lower occurrence (Smith, 2000). Interestingly, there exists a large overlap of substrate coverage and tissue distribution between CYP3A and P-glycoprotein (Wacher et al., 1995).

P-glycoprotein as a facilitator of drug induction

P-glycoprotein is a large plasma membrane-bound protein classified as one of a large family of ATP-binding cassette transporters (Higgins, 1992). The glycoprotein functions as a drug efflux pump that lowers intracellular drug concentrations, and was first recognized as a transport protein that facilitated multi-drug resistance in cancer cells. P-glycoprotein is expressed in tumor cells and normal tissues, acts upon a wide range of drug substrates, and can play a very important role in both enzyme inhibition and enzyme induction (Cordon-Cardo, et al., 1990). P-glycoprotein can be found on the apical surfaces of epithelial cells in the liver, kidney, and pancreas, and both intestines (Thiebaut et al., 1987). The protein is even expressed in the capillary endothelia of the brain and the testes (Schinkel et al., 1994). P-glycoprotein has a relatively homogenous distribution in the liver and other regions, whereas its expression is quite uneven along the intestine. In fact, the protein is most abundantly

expressed in the small intestine, found only in mature enterocytes lining the villi (Fojo et al., 1987).

The magnitude of P-glycoprotein's effect on metabolism is in part dependent upon its proximity to CYP3A enzymes. P-glycoprotein in the liver and kidney is localized along exit sites of hepatocytes and renal epithelial cells; therefore, it only interacts with drug molecules after those molecules undergo cellular uptake, cellular distribution, and metabolism. This situation is in stark contrast with that of intestinal P-glycoprotein, which is localized at the entrance site of epithelial cells of the intestines. Intestinal P-glycoprotein is exposed to drug molecules before cellular distribution and metabolism (Lin, 2003). Intestinal P-glycoprotein extrudes drug molecules from within epithelial cells into the intestinal lumen. By the same token, a portion of the drug molecules extruded by P-glycoprotein can be reabsorbed into epithelial cells. The processes of extrusion and reabsorption occur continuously to increase the availability of drug species to catalytic enzymes via an extension of the intercellular residence times of drug molecules. Thus, intestinal P-glycoprotein plays a greater role in intestinal metabolism than in other organs where the protein is significantly expressed (Thummel et al., 1997). Evidence of P-glycoprotein's influence upon orally administered drugs metabolized by CYP3A4 has been substantial, as the protein has been observed to significantly contribute to the first-pass metabolism of various agents. P-glycoprotein has also been implicated in the poor bioavailability observed in many CYP3A4 joint substrates (Wacher et al., 1995).

CYP3A4 induction

CYP3A4 induction has been most commonly observed during the long-term treatment of potent inducers such as rifampicin, and the anti-convulsants phenytoin, phenobarbital and pentobarbital. Several examples of the impact of enzyme induction are described below:

- Rifampicin has been involved in several cases of CYP3A4 enzyme induction. For instance, concomitant administration of the drug with fexofenadine significantly increased fexofenadine average clearance by 2-fold and significantly reduced fexofenadine average peak serum concentrations by 1.8-fold (Mitchell et al., 2001). Rifampicin decreased the total AUCs of simvastatin and its active metabolite by 87% and 93%, respectively (Kyrkland et al., 2000), and decreased the total AUC and peak plasma concentration of the anti-diabetic medication repaglinide by 57% and 41%, respectively (Niemi et al., 2000). Benzodiazepines are sensitive to induction as well, as pretreatment with rifampicin decreased AUCs of midazolam and triazolam to 3% and 5% of control values, respectively (Backman et al., 1996, Villikka et al., 1997). Diazepam total body clearance increased 300% after rifampicin interaction (Ohnhaus et al., 1987). In addition, anti-retroviral agents such as indinavir, amprenavir and saquinavir caused reductions in AUC of 82%, 82% and 70%, respectively, after rifampicin co-administration (McCrea et al., 1997, Polk et al., 2001, Grub et al., 2001).
- The anti-convulsant medications phenobarbital and phenytoin are strong inducers of CYP activity, both *in vitro* and *in vivo*. Phenobarbital increased

the level of CYP3A4 mRNA transcripts by 536% and 205% in human hepatocytes in separate *in vitro* investigations (Raucy, 2003, Raucy et al., 2002). In addition, phenobarbital significantly increased the oral clearance and decreased the AUC and half-life of metronidazole *in vivo* (Eradiri et al., 1988). Similarly, phenytoin induced the metabolism of itraconazole and its metabolite by lowering AUCs by 93% and 95%, respectively, and by lowering half-lives by 83% and 75%, respectively, after co-administration of the two drugs (Ducharme et al., 1995).

- The glucocorticoid agent dexamethasone caused dehydrogenation of the calcium channel blocker nifedipine (5-fold) when both drugs were applied to human hepatocytes (Meunier et al, 2000). Dexamethasone treatment also caused a 2-fold to 7-fold increase in CYP3A4 activity in human hepatocytes, as monitored by the erythromycin breath test (McCune et al., 2000).

CYP3A4's expression is sex-dependent. The enzyme is expressed twice as extensively in the livers of women than in men. In a study conducted by Wolbold et al. (2003), surgically excised female human liver samples not only yielded higher levels of CYP3A4 in female volunteers, but also exhibited a fifty percent increase in CYP3A-dependent dealkylation of the substrate verapamil. It was noted that this sex-based difference was not the result of preferential induction in women, but rather linked to a higher prevalence of CYP3A4 messenger RNA transcripts. This sex difference may well be worth considering alongside the results of the investigation described in this document, as only male volunteers were used.

REVIEW OF LITERATURE:

ST. JOHN'S WORT AND HERB- DRUG INTERACTION

The herbal explosion and herb-drug interactions

Human beings have long desired control of their health and well-being. Consistent with that desire, herbal medications have become widely-used alternatives to synthetic drugs for the treatment of common ailments. Natural products are generally viewed as benign and easily accessible to the general public, thus tremendously augmenting their mass appeal (Henderson et al., 2002). The economic impact of herbal medicines has been staggering in recent years, as evidenced by the proliferation of various exotic, now household-name products featured in drug, discount, and grocery stores across the United States. It is estimated that between 1988 and 1997, U.S. herbal product sales surged from $200 million to $3.3 billion (Mahady, 2001). Herbal product use has steadily increased since then, with about 50% of all Americans relying upon them, and recent sales estimates exceeding $10 billion (Stys et al., 2004).

The results of a recent survey revealed an estimated 16% of prescription drug users also used some type of herbal remedy concurrently (Kaufman et al., 2002). Despite the widespread use of herbal products, overall preclinical and clinical information (ie., case reports and interaction studies) regarding herb-drug

interactions are limited in number. Herb-drug interactions may be underreported or underestimated for various reasons, including:

- Lack of herbal usage disclosure by patients to their care providers, estimated as common as in 70% of all cases (Eisenberg et al., 1993).
- Lack of requirement of rigorous preclinical and clinical testing of herbal supplements by regulatory authorities due to their natural product profile.
- Limited value of clinical investigations, due to poor study designs, small sample sizes, and usage of herbal products with uncertain active ingredient compositions and batch consistency (Goldman, 2001).
- Lack of case reporting of herb-drug interactions due to underestimation of herbal potency.
- Presence of multiple active ingredients in a given herbal product, each with distinct pharmacological actions (Zhou et al., 2003).

Herbal medicines are characterized by their pharmacological makeup. These medicines are most commonly mixtures of multiple active ingredients; therefore, ambiguity regarding which and how many herbal constituents possess pharmacological activity is not uncommon. The situation is complicated by the fact that herbal medicines generally lack uniformity in terms of ingredient compositions among brands and batches, thus making these medications difficult to analyze and to evaluate (Izzo and Ernst, 2001).

It has been estimated that most herbal drug users additionally use prescription medications for chronic ailments, thereby increasing the likelihood of pharmacokinetic and/or pharmacodynamic interaction (Eisenberg et al., 1998).

Both pharmacodynamic and pharmacokinetic interactions involving herbal products have been identified in case reports and controlled investigations. Intriguing instances of herb-drug interaction have involved several popular herbal supplements, including ginkgo biloba, ginseng, garlic, kava, echinacea, and St. John's Wort.

Depression as a disease state

Depression is one of the most common disorders treated clinically, with about 75% of depressive illnesses of mild to moderate severity treated in primary care settings (Muller and Volz, 1996). According to World Health Organization estimates, severe depression will become the number two cause of death within the next ten years. It is also estimated that approximately 10% to 17% of the U.S. population will experience a major episode of depression at least once (Keith and Matthews, 1993). The disorder is often difficult to accurately diagnose due to its ambiguous symptoms, and is often difficult to treat. Specifically, both patients and physicians are at times reluctant to utilize standard antidepressant therapies due to side effects and the high cost of such medications (Gaster and J. Holroyd, 2000). It is not uncommon for patients prescribed synthetic antidepressants to discontinue treatment prematurely. In general, 65% to 75% of patients receiving conventional drug therapy in the treatment of depression achieve successful regression of symptoms, while only 40% to 50% achieve complete recoveries (Muller and Volz, 1996). These conditions have contributed to the gradual rise in popularity of St. John's Wort as a treatment in depression,

despite conflicting research claims and views regarding the supplement's effectiveness.

The duplicitous nature St. John's Wort

Saint John's Wort (hypericum perforatum) is a flowering perennial plant found in various regions of Europe, Africa, and Asia, and in parts of the western United States. The plant derives its name from its yellow flowers, traditionally gathered for the feast of St. John the Baptist. In ancient times, the plant was revered for its medicinal properties. St. John's Wort was used for a wide range of conditions, including neuralgic ailments, sciatica, and even poisonous reptile bites. In recent times, the herb has been used for the treatment of psychiatric disorders ranging from anxiety, neurosis, depression and even hysteria (Rasmussen, 1998), and has even enjoyed use as an external treatment for wounds, sprains, and bruises (Snow, 1996). The leaves and flowers of the plant consist of over two dozen constituents owing to its pharmacological properties, with 0.1% of the herb consisting of the naphthodianthrones hypericin (a key active ingredient) and pseudohypericin. Also contained within the plants are the flavonoids amentoflavone, luteolin, kaempferol, and quercetin, the glycosides hyperoside and rutinoside, and the phloroglutinol derivative hyperforin (Barnes et al., 2001).

St. John's Wort is marketed in Europe as a mood and sleep enhancer. The supplement generated $6 billion (U.S.) in European sales in 1998 alone (Harrison, 1998). Similarly, St. John's Wort has seen an unprecedented rise in popularity in the United States since the FDA's Dietary Supplement Health and

Education Act of 1994, with its sales increasing from $20 million in 1995 to $200 million in 1997 (Brevoort, 1998).

Crude or purified Hypericum perforatum L. extracts are obtained using mixtures of methanol/water or ethanol. Important components such hypericin are then standardized by UV spectroscopy. The United States Pharmacopoeia requires that standardized St. John's Wort extracts contain no less than 0.2% hypericin and pseudohypericin and no less than 3% hyperforin as assayed by HPLC (U.S.P., 1999). Complicating the situation is the fact that St. John's Wort constituents are very light- and temperature-sensitive, resulting in unreliable label claims by some manufacturers due to ingredient decay (Bilia et al., 2001). For example, when ten separate St. John's Wort products were tested for active ingredient content in a 1998 survey, it was found that half the products surveyed contained less than 80% stated hypericin, while a third of the products contained less than 50% of stated hypericin (Monmaney, 1998).

St. John's Wort's mechanism of action in the treatment of depression and anxiety has been difficult to ascertain. Hypericin, hyperforin, pseudohypericin, and quercetin are constituents believed to be responsible for St. John's Wort antidepressant effects. Research has suggested that St. John's Wort's effectiveness is dependent upon the supplement's impact on several neurotransmitters in the body. Although the manipulation of an individual neurotransmitter may not be potent enough to account for trademark xenobiotic effects, the manipulation of several may combine to achieve these results (Wills et al., 2000).

St. John's Wort extracts have exhibited the ability to prevent serotonin uptake in animal models. Specifically, it has been demonstrated that hydromethanolic hypericin extracts rapidly deplete 5-HT storage vesicles, dramatically raising 5-HT concentrations in the cytoplasm and inhibiting its cellular uptake. The herbal supplement inhibits serotonin uptake through the elevation of free intracellular sodium ion concentrations via increased Na^+/H^+ exchange. Prolongation of neurotransmitter availability augments antidepressant effects. However, these seritonergic effects are less potent than those of the SSRI class of antidepressants (Gobbi et al., 1999). St. John's Wort similarly increases dopaminergic effects through apparent dopamine-beta-hydroxylase inhibition. This inhibition is thought to increase synaptic dopamine concentrations (Kleber et al., 1999). Furthermore, St. John's Wort hydroethanolic extracts weakly inhibit monoamine oxidase types A and B (Suzuki et al., 1984), and also inhibit catechol-O-methyltransferase. It has been concluded, however, that the antidepressant action of St. John's Wort relies neither upon MAO inhibition alone nor COMT inhibition alone (Thiede and Walper, 1994).

Specific components of St. John's Wort have been implicated in alterations of neurotransmitter action in animal studies. Hyperforin, for instance, induces increased levels of extracellular dopamine, norepinephrine, and glutamate through uptake inhibition (Muller et al., 1997). Hyperforin also inhibits the uptake of 5-HT, GABA, and L-Glutamate (Singer et al., 1999; Kaehler et al., 1999). The component quercetin inhibits mono-amine oxidase levels in rat brain;

however, St. John's Wort does not significantly affect MAO in humans at clinical dosages (Demisch et al., 1989). The key component hypericin is believed to impact cellular ion channels, acting to antagonize NMDA receptors by inhibiting AMPA- and GABA-mediated responses. Hypericin has shown inhibitory activity upon dopamine- beta-hydroxylase (Kleber et al., 1999), and has also shown strong inhibitive affinity for muscarinic cholinergic and sigma receptors (Raffa, 1998). In addition, biflavone components in St. John's Wort have displayed partial agonist effects on brain benzodiazepine receptors *in vitro*, although the fact that hypericum does not readily cross the blood brain barrier lowers the probability of a clinical effect (Nielsen et al., 1988).

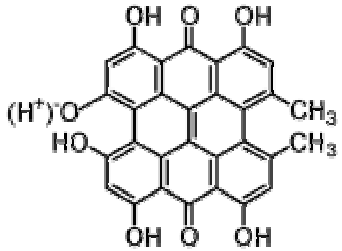


Figure 2.1: Structure of hypericin.

St. John's Wort and mainstream therapy

Studies undertaken to examine St. John's Wort antidepressant effects have yielded mixed results. Investigations comparing St. John's Wort to synthetic antidepressants produced promising results, including several studies involving imipramine. In one such study, no significant difference was found between imipramine and St. John's Wort extract treatments in terms of both von Zerssen depression scale and clinical global impression scale scores (Woelk, 2000). Similarly, St. John's Wort extract was deemed therapeutically equivalent

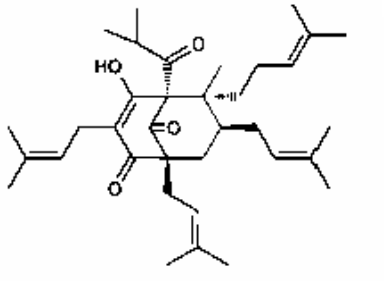
to various doses (100 mg, 150 mg daily) of imipramine by virtue of Hamilton anxiety score, global impression score, and Zung self rating depression score evaluations (Vorbach et al., 1994; Philipp et al., 1999; Vorbach et al., 1997). When compared to amitriptyline, fluoxetine, and maprotiline, St. John's Wort displayed no significant differences compared to the tricyclic antidepressants in overall anti-depressive outcomes (Harrer et al., 1994; Wheatley, 1997; Schader, 2000). In large, multicenter studies, St. John's Wort was consistently effective in the treatment of mild to moderate depression. For instance, in a study involving 1060 patients, a 66% improvement rate and a 27% "symptom free" rate was seen in patients who received the supplement for four weeks (Smet and Nolen, 1996). St. John's Wort was found to be 77% and 76% effective by patient assessment in separate studies involving 2404 and 1606 volunteers, respectively (Bilia et al., 2002). Success was even observed when St. John's Wort extract was given to post-menopausal women in a multicenter study. Post-menopausal climacteric symptoms decreased or disappeared in 79% of women based upon physician clinical global impression scale assessment (Grube et al., 1999). St. John's Wort, however, has produced less impressive results in severe depression. For example, in a study conducted by Shelton et al. (2001), St. John's Wort failed to distinguish itself from placebo action in the treatment of individuals diagnosed with severe depression.

St. John's Wort's popularity is greatly strengthened by its high tolerability and low incidence of adverse effects, particularly when compared to the side effect profiles of commonly-used synthetic anti-depressants. The supplement

has been associated with mild, self-limiting adversities such as fatigue, anxiety, confusion, dizziness, headache, dry mouth, and upset stomach. Several studies have deemed St. John's Wort adverse effects as comparable to those of placebo treatments (Linde et al., 1996). In a large, multicenter study of 3250 adult patients in primary care settings, adverse effects included gastrointestinal irritation or dyspepsia (0.6%), allergic reactions (0.5%), lethargy and drowsiness (0.4%), and descriptions of anxiety or restlessness (0.3%). Only 1.5% of patients who participated in the study required discontinuation of therapy due to adverse effects (Woelk et al., 1994). Other studies have reported supplement adverse effects to occur at a rate of 1% to 3% (Schulz, 2001). In addition, a review of several clinical trials (totaling 1757 patients) reported adverse reactions in 19.8% of patients who received St. John's Wort treatment. The review reported adverse reactions in 52.8% of patients who received conventional anti-depressants (Wheatley, 1998).

Photosensitization is a rare but intriguing side effect associated with St. John's Wort use. Photosensitivity reactions cause serious sunburn and often result in blisters or lesions of the skin. The side effect has long been observed in grazing animals following ingestion of the St. John's Wort plant (Araya and Ford, 1981). Studies have yielded mixed results concerning photosensitivity in humans, although most results suggest a correlation between increased dose and increased sensitivity (Brockmoller et al., 1997). Investigations have also indicated a higher prevalence of photosensitivity in HIV and hepatitis C infected patients (Gulick et al., 1999; Jacobson et al., 2001).

St. John's Wort has gained recent notoriety for the role it plays in various herb-drug metabolic interactions, with most attention directed toward the supplement's interaction with cytochrome P450 monooygenase system.



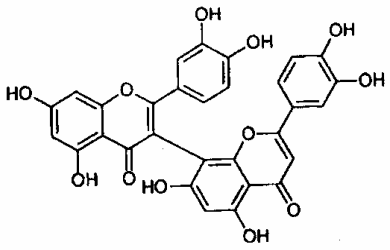


Figure 2.2: Structure of hyperforin. Figure 2.3: Structure of I3, II8 biapigenin.

St. John's Wort: *in vitro* and *in vivo* interface with cytochrome P450

Components of St. John's Wort extract inhibit the activity of various CYP subfamilies. For instance, Moore and colleagues (2000) reported a marked increase in CYP3A4 mRNA levels following hyperforin exposure to human hepatocytes. An *in vitro* study found the flavonoid compound biapigenin to be a potent, competitive inhibitor of CYP3A4 (Ki= 0.038 μM), CYP2C9 (Ki= 0.32 μM), and CYP1A2 (Ki= 0.95 μM), and a 50% inhibitor of CYPs 2D6, 3A4, and 2C9 at concentrations below 10 μM. Hyperforin was a potent noncompetitive inhibitor of CYP2D6 activity (Ki =1.5 μM) and was a competitive inhibitor of CYP2C9 and CYP3A4 (1.8 μM and 0.48 μM, respectively). At concentrations less than 10 μM, the constituent exhibited 50% inhibition of CYPs 2D6, 3A4, and 2C9. Hypericin similarly inhibited CYP2C9 competitively with an inhibition constant of 1.4 μM. Like hyperforin, hypericin displayed 50% inhibition of CYPs 2D6, 3A4, and 2C9. Quercetin, a fourth constituent of St. John's Wort, displayed 50% inhibition

(concentration below 10 μM) in only one cytochrome subfamily, CYP1A2 (Obach, 2000). In another *in vitro* investigation, the herbal constituent hyperforin was found to inhibit CYPs 2C9, 2C19, 2D6, 3A4 and 1A2, while quercetin inhibited CYPs 2C9, 2D6, 3A4, and 1A2. Notably, hyperforin produced very potent inhibition of cytochrome subfamilies 2C9 and 2C19, resulting in IC50 values of 0.01 μM and 0.02 μM, respectively (Zou et al., 2000). Hypericin potently inhibited CYP3A4 *in vitro* in both tincture and ethanolic extract forms, with IC_{50} values of 0.04% (of full strength preparation) and 0.33 μM, respectively (Budzinski et al., 2000).

Despite substantial in vitro evidence of St. John's Wort and its extracts inhibiting cytochrome isoforms, in vivo reports have indicated enzyme induction. A number of in vivo drug interactions have been observed involving St. John's Wort and widely used medications. The interactions have been largely pharmacokinetic in nature and have indicated alterations of normal drug metabolism through several cytochrome pathways, including CYPs 2C9, 3A4, 1A2, 2C19 and 2D6.

Several case reports have indicated St. John's Wort to impact the metabolism of CYP2C9 substrates warfarin and phenprocoumon. For example, between 1998 and 2000 there were 22 case reports of decreased international normalized ratio (INR) in patients taking both warfarin and the herbal supplement (Ernst, 1999). An altered profile of the anticoagulant's pharmacologically active S-enantiomer suggests enzyme induction in cases of decreased INR (Yue et al, 2000); however, neither *in vitro* nor *in vivo* testing has been performed to confirm

this induction. It has even been hypothesized that such interactions involving warfarin may be the result of P-glycoprotein induction, possibly in concert with induction of adjacent cytochrome pathways CYP1A2 and CYP3A4 (Cott, 2001; Kaminsky and Zhang, 1997). In addition, a clinical study examining the effect of St. John's Wort dosing upon phenprocoumon revealed a significant decrease in anticoagulant AUC after 11 days of supplementation (Donath et al., 1999). A randomized *in vivo* study involving St. John's Wort co-administration with a drug cocktail that included the CYP2C9 substrate tolbutamide yielded no significant pharmacokinetic effects on drug metabolism in 12 volunteers (Wang et al., 2001).

St. John's Wort has been linked to CYP2C19 induction in polymorphic volunteers. In a study conducted by Wang et al, the pharmacokinetic profile of the proton pump inhibitor omeprazole and its hydroxylated metabolites were affected by coadministered St. John's Wort after 14 days of supplement treatment. Specifically, .Cmax and AUC-extrapolated values in CYP2C19 mutant-allele individuals were significantly decreased (37.5 % and 37.9%, respectively) after St. John's Wort treatment. Likewise, significant differences in Cmax and AUC-extrapolated were observed in wild-type allele carriers as well (49.6% and 43.9%, respectively). Additionally, St. John's Wort produced significant increases in Cmax and AUC_{extrap} values in omeprazole's hydroxylated metabolite (Wang et al., 2004).

CYP1A2, too, is believed to be susceptible to enzyme induction by St. John's Wort. In a well-documented case report, a patient experienced lowered theophylline levels with co-administration of St. John's Wort. Theophylline

dosing was increased substantially to overcome St. John's Wort's inductive effect, and discontinuation of the supplement's administration resulted in a doubling of the patient's plasma theophylline concentrations (Nebel et al., 1999).

Perhaps the most infamous cases of drug interaction involving St. John's Wort have involved the CYP3A4 isoform. CYP3A induction by St. John's Wort occurs through the pregnane-X receptor, a member of the steroid/thyroid receptor family that mediates CYP3A expression (Moore et al., 2000). The pregnane-X receptor is strongly favored by St. John's Wort constituent hyperforin, implicating this component as a chief inducer of CYP3A4 (Wentworth et al., 2000). This fact is also consistent with the *in vitro* findings of Budzinsky et al (2000), in which hyperforin was identified as a potent CYP3A4 inhibitor. Administration of St. John's Wort with a broad range of CYP3A substrates has resulted in dramatic examples of drug induction:

- The interaction between St. John's Wort and cyclosporin is well-documented as a potentially fatal herb-drug interaction. The popular immunosuppressant cyclosporine is often used following instances of organ transplantation and has a very small therapeutic concentration range. Drug plasma levels that fall below the minimum effective concentration can lead to tissue or organ rejection and an increased risk of kidney and/or liver toxicity. Several instances of organ rejection in the presence of St. John's Wort have been reported in recent years (Izzo, 2004). For instance, Ruschitzka and associates (2000) observed acute cellular transplant rejection in heart transplant patients who were

stabilized on cyclosporine therapy for 11 months and 20 months, respectively. Significantly lowered cyclosporine plasma trough concentrations and transplant rejection were also associated with St. John's Wort co-administration in a patient 25 years after kidney transplantation (Mai et al, 2000). Furthermore, St. John's Wort significantly lowered cyclosporin plasma concentrations in 30 kidney transplant patients with a mean decrease of 47%. After discontinuation of St. John's Wort therapy, cyclosporin levels exhibited a mean increase of 187% (Breidenbach et al., 2000). Cessation of St. John's Wort treatment generally resulted in spontaneous recovery in patients who experienced kidney, heart, and pancreas transplants. Some recoveries were augmented by additional immunosuppressive therapy (Rey and Walter, 1998).

- Analogous results were seen in the pharmacokinetics of another immunosuppressant, tacrolimous. Fourteen days of St. John's Wort extract administration decreased its AUC_{0-12} by greater than 50%. Reductions in tacrolimus peak and trough plasma concentrations were observed as well. Again, dose adjustment was necessary to achieve pharmacological effects. However, the immunosuppressant mycophenolic acid displayed no pharmacokinetic alterations under identical circumstances, (Mai et al., 2003).
- Long-term (2 or more weeks) administration of St. John's Wort caused a significant increase in midazolam clearance (121.8 L/hr to 254.5 L/hr)

after oral midalzolam dosing. Likewise, oral bioavailability of midazolam significantly decreased (0.28 +/- 0.15 to 0.17 +/- 0.06), matched with a greater than 50 percent decrease in AUC during the same time span. Although a non-significant change, midazolam AUC after intravenous dosing was decreased by 21% following two weeks of St. John's Wort administration (Wang et al., 2001). St. John's Wort also affected the pharmacokinetics of the congener alprazolam, resulting in a 2-fold decrease in the drug's AUC, a 2-fold increase in clearance, and a 2-fold decrease in half-life after two weeks of St. Johns' Wort administration (Markowitz et al., 2003).

- Volunteers administered racemic verapamil exhibited 80% and 78% decreases in plasma AUC values for the S- and R- enantiomers, respectively, following 14-day treatments of St. John's Wort. In addition, 78% and 76% decreases in S- and R- verapamil Cmax values were observed. Metabolic norverapamil S- and R- AUCs declined by 63% and 51%, respectively (Tannergren et al., 2004).
- St. John's Wort has been shown to interact with HIV protease inhibitors. Fourteen days of administration of the herbal supplement caused a decrease in indinavir mean AUC by 57% in a study consisting of healthy volunteers. Similarly, mean C_{max} values for indinavir decreased by 28% following St. John's Wort treatment (Piscitelli et al., 2000).
- The tricyclic antidepressant amitriptyline is metabolized by both CYP3A4 and CYP2C19, while its active metabolite nortriptyline is metabolized by

both CYP3A4 and CYP2D6 (Venkatakrishnan et al., 1998). Co-administration of St. John's Wort with amitriptyline resulted in 20% and 40% decreases in AUC parameters for amitriptyline and nortriptyline, respectively. It was hypothesized that St. John's Wort caused an up-regulation of CYP3A4 to yield these results (Roots et al., 2000).

- Fourteen days of St. John's Wort treatment lowered plasma AUC and C_{max} parameters of the HMG CoA reductase inhibitor simvastatin after single dosing, although the reductions were not statistically significant. However, the AUC value for simvastatin's hydroxyl acid metabolite was significantly reduced following supplementation. St. John's Wort did not affect the pharmacokinetics of the pravastatin, though. The difference in induction results was speculated to be due to differences in P-glycoprotein influence between the two congeners (Sugimoto et al., 2001).
- Several reports of breakthrough bleeding have been observed in cases involving the co-administration of oral contraceptives and St. John's Wort. In all reports, women reported no breakthrough bleeding for at least seven months prior to supplement treatment. Unplanned pregnancies, too, have been reported in some women following simultaneous use of birth control and St. John's Wort (Izzo, 2004). For example, one pregnancy occurred when synthetic antidepressant therapy was substituted with herbal supplementation by a woman who also used ethinyl estradiol/ dienogesterol contraceptive (Schwartz et al., 2003). In

clinical investigations, the supplement caused a clinically significant increase in the oral clearance of the contraceptive agent norethindrone (Gorski et al., 2002), a significant reduction in ethinyl estradiol half-life, and a 3.5-fold increase in breakthrough bleeding in human volunteers (Hall et al., 2003).

P-glycoprotein and St. John's Wort

Induction of P-glycoprotein, too, may be a result of long-term St. John's Wort therapy and thus may dramatically affect the metabolism of co-administered drugs. Intestinal P-glycoprotein is responsible for the efflux of drugs from the intestinal lumen. Increased efflux resulting from pump induction may reduce the bioavailability of P-glycoprotein substrate drugs, therefore antagonizing the absorptive transport and/or diffusion of the drugs (Durr et al., 2000). Although St. John's Wort induces hepatic CYP3A expression and activity, there has been little evidence of increased drug clearance following intravenous administration of the supplement. The majority of reports regarding cytochrome induction occur following oral administration of drugs (Perloff et al., 2001), perhaps emphasizing the importance of intestinal induction and P-glycoprotein's possible mediation of the process.

A study by Hennessy et al. (2002) revealed that St. John's Wort increases the expression and activity of P-glycoprotein, as indicated by peripheral blood lymphocyte measurements. Several constituents of St. John's Wort, too, may contribute to P-glycoprotein induction, as the xenobiotic contains flavonoid compounds proven to interact with the transporter (Conseil et al., 1998).

Furthermore, evidence suggests that hypericin may be a significant facilitator of this protein's induction. Hypericin caused potent low-concentration induction of P-glycoprotein in an *in vitro* investigation (Perloff et al., 2001).

Vast overlap amongst substrates of intestinal CYP3A4 and P-glycoprotein has been observed in various xenobiotics, and it even appears that the two intestinal structures are coordinately regulated in some instances. For example, CYP3A4 and P-glycoprotein have been located in close quarters on the same chromosome, and studies have indicated a tendency for tumors that express P-glycoprotein to also express CYP3A4 (Wacher et al., 1995). It has been postulated that P-glycoprotein and CYP3A4 act synergistically to control drug metabolism in spite of differing mechanisms of action (Katoh et al., 2001). Three popular scenarios by which this synergism may occur include:

1. P-glycoprotein acts to divergently limit proximal bowel absorption. Reduction in the rate of drug absorption by P-glycoprotein may facilitate a shift in the site of absorption from the proximal bowel to distal segments that possess lower concentrations of CYP3A4. Such regions of the intestine are catalytically less efficient; hence, the net result of this process may be decreased drug metabolism and increased bioavailability (Homsy et al., 1995).
2. P-glycoprotein acts to prolong the duration of exposure to intestinal CYP3A4. In this popular scenario, a drug substrate of P-glycoprotein circulates between luminal and epithelial cells in the intestine. The process repeats in such a fashion that the drug receives maximal

exposure to CYP3A4 for metabolic oxidation, in turn increasing the rate of drug metabolism while reducing oral bioavailability. This process has been noted in the metabolism of cyclosporin A in the small intestine (Gan et al., 1996).

3. P-glycoprotein acts to remove primary drug metabolites from enterocyte structures. This process prevents further metabolism of these by-products by CYP3A4 and thus accelerates the rate of parent drug metabolism. Hence, the process is contingent upon the affinity of primary metabolites for P-glycoprotein efflux being greater than that of the parent drug. Cyclosporin A has been observed to be metabolized in this manner, particularly in individuals with high P-glycoprotein expression (Christians and Sewing, 1993).

St. John's Wort has indicated significant inductive effects upon intestinal P-glycoprotein and CYP3A4 in vivo; therefore, the supplement is likely to interact with a wide range of medications whose metabolism is affected by these intestinal proteins. Several case reports and studies conducted in both animals and humans have implicated St. John's Wort in P-glycoprotein mediated interactions. For instance, studies in rats indicated a 3.8-fold increase in P-glycoprotein expression. Male rats given St. John's Wort for 14 days exhibited an 18% decrease in digoxin plasma concentrations, and exhibited 1.4-fold increase in duodenal P-glycoprotein and 1.5-fold increase in duodenal CYP3A4 (Durr et al., 2000). CYP3A influence on digoxin metabolism has not been detected in humans, as evidenced in *in vitro* investigations involving human

hepatocytes and human liver microsomes (Lacarelle et al., 1991). In a human investigation by Johne and associates (1999), digoxin AUC values were reduced by 25% in 25 healthy subjects after ten days of St. John's Wort treatment, although no clinically significant alterations in digoxin pharmacokinetics were seen. In a similar investigation by Dresser et al. (2003), fexofenadine AUC decreased by 49% in human volunteers after 12 days of herbal therapy, an effect also believed to be mediated by P-glycoprotein.

Other case reports and clinical investigations have suggested a combined influence of P-glycoprotein and CYP3A4 in altered drug metabolism. For example, de Maat reported a 35% reduction in nevirapine plasma levels in 5 human volunteers after St. John's Wort administration (de Maat et al., 2001). Nifedipine plasma values were decreased by 53% after 18 days of St. John's Wort treatment in 22 volunteers (Henderson et al., 2002). As alluded before, the immunosuppressant cyclosporine has been involved in several incidences of P-glycoprotein/CYP3A4 metabolic induction. Reports range from singular incidences of organ transplant rejections resulting from cyclosporine plasma reductions (Henderson et al., 2002; Mai et al., 2000), to controlled clinical trials confirming reduced cyclosporine efficacy after long-term St. John's Wort therapy (Breidenbach et al., 2000).

There even exist situations in which P-glycoprotein and CYP3A-mediated metabolism may be further complicated by the production of potent metabolites that may also alter enzyme function. Studies have indicated that CYP3A4-mediated metabolism may have inhibitory effects upon P-glycoprotein, as in the

work performed by Katoh and colleagues (2001). Transcellular transport of tritiated daunorubicin and digoxin were strongly inhibited by CYP3A4 substrates azelastine and their corresponding desethylamiodarone and desmethylazelastine metabolites following parent drug treatment upon intestinal epithelia. These findings present a cautionary reminder of the unpredictable nature of drug interactions, particularly when active metabolite scenarios are taken into account.

In recent years, St. John's Wort has enjoyed an unprecedented rise in popularity for the treatment of mild depression in Europe and the United States, due in part to its seemingly benign adversity profile. The supplement's liberal use, lenient regulation, and dispersed effects on metabolism via the cytochrome P450 monoxygenase system has generated much attention and legitimate concern regarding its interaction with other agents that may be metabolized by common pathways. St. John's Wort and several of its individual components have shown considerable activity upon multiple cytochrome P450 isoforms. This xenobiotic has also been implicated in an alarming number of herb-drug interactions citing pharmacokinetic and pharmacodynamic influences. St. John's Wort's broad usage, combined with its strong safety profile, make it a relevant and convenient candidate for use in *in vivo* studies.

REVIEW OF LITERATURE:

OVERVIEW OF PREDNISONE AND PREDNISOLONE

Corticosteroid drugs: history, mechanism of action and pharmacology

The corticosteroid era of drug therapy began in the 1930s, when adrenal steroids such as cortisol, cortisone and corticosterone were purified and identified. By the late 1940s, corticosteroids suitable for human use were developed and subsequently introduced to the public for the treatment of immune system-exacerbated conditions such as rheumatoid arthritis and respiratory disorders such as asthma. Although therapeutically effective, these compounds were hampered by troublesome mineralocorticoid side effects that threatened to limit their therapeutic value. Considerable research was devoted to the development of cortisone analogs with equivalent-to-superior therapeutic profiles and diminished side-effects. Researchers soon learned that modifications to corticosteroid structure could be performed to greatly manipulate either glucocorticoid or mineralocorticoid potency. This triggered a rapid proliferation of adrenal steroid compounds such as prednisolone and dexamethasone that have since become widely-utilized, front-line medications across the globe (Barnes and Mueller, 1995).

Corticosteroids elicit their effects through either positive or negative control of gene expression. Glucocorticoid action, for instance, is initiated when the drug enters target tissue cells by diffusion across plasma membranes. Drug

molecules bind tightly with human glucocorticoid receptors found both on the surface of the cell membrane and within the cellular cytoplasm (Schimmer and Parker, 2001). The binding of drug to a glucocorticoid receptor causes a conformational change of the receptor in route to the formation of a receptor steroid-receptor complex. The structural change also enhances the DNA binding affinity of the receptor complex. The newly-modified receptor complex is then transported to the cell nucleus for chromatin binding and activation of glucocorticoid response elements. Located near promoter regions of glucocorticoid-responsive genes, the response elements become conduits through which the steroid receptor complex regulates gene promoter activity and messenger RNA transcription. Increased or decreased accumulation of mRNA transcripts within the target cell causes changes in the rate of protein synthesis. The proteins that arise from this process code for the production of enzymes that influence either anabolic or catabolic processes in affected tissues to render glucocorticoid effects (Wahli and Martinez, 1991).

The effects of corticosteroid treatment on the body's homeostatic processes are well-understood. In general, corticosteroid agents either exhibit predominate glucocorticoid properties, predominate mineralocorticoid properties, or some combination of the two. Early functional assays prompted the classification of corticosteroids into these categories by virtue of their ability to either promote sodium retention by the kidney or to promote liver glycogen deposition (Szpilfogel, 1984). Mineralocorticoids, for example, possess the ability to enhance sodium reabsorption in the kidney. Potassium and hydrogen

excretion is increased as well, thus affecting electrolyte and water balance within the body. Some glucocorticoids also affect electrolyte and water balance by affecting sodium ion retention (Schimmer and Parker, 2001).

Energy storage and utilization are glucocorticoid functions executed through the manipulation of endogenous insulin, glucagon, and catecholamines. Glucocorticoids promote energy storage in the liver through the formation of glycogen, whereas glucocorticoid peripheral actions include decreased utilization of glucose and mobilization of amino acid residues. Transposed amino acids reach the liver as building-block substrates for gluconeogenesis. Endogenous cortisol, for instance, stimulates new enzyme synthesis within the liver through interaction with the glucocortoid receptor. The rise of such enzymes—phosphoenolpyruvate carboxykinase and glucose-6-phosphatase among the most important— augment the ability of glucagon and catecholamines to trigger gluconeogenesis. Like cortisol, synthetic glucocorticoid analogues powerfully alter carbohydrate, protein, and lipid metabolism such that glycogen stores are conserved and enhanced. The agents inhibit glucose uptake and utilization from peripheral tissues, and support gluconeogenic processes by promoting protein and triglyceride catalysis (Orth et al., 1992; Schimmer and Parker, 2001).

The cardiovascular, nervous, and skeletal systems may be significantly impacted by corticosteroid therapy. For instance, a mineralocorticoid-induced increase in sodium ion retention can lead to hypertension and a cascade of adverse effects on the cardiovascular system. Glucocorticoid-induced hypertension can also occur through increased expression and affinity of

adrenergic receptors in the heart and vascular lining. Corticosteroid insufficiency or excess may impair muscle function and work capacity. Furthermore, corticosteroid effects upon the CNS include variable changes in mood, behavior, and brain excitability during conditions of glucocorticoid insufficiency or excess (Schimmer and Parker, 2001).

The anti-inflammatory profile of glucocorticoid compounds is well-documented. The agents inhibit either the production or action of leukocytes that mediate inflammation. Glucocorticoids inhibit the release of vasoactive factors, reduce the secretion of proteolytic enzymes, decrease leukocyte migration to areas of injury, and decrease fibrosis. Glucocorticoids also induce lipocortin production to inhibit phospholipase A_2 for prevention of arachidonic acid release. The net result of glucocorticoid action is the prevention or suppression of inflammatory response to radiant, mechanical, chemical, and immunological stimuli (Schimmer and Parker, 2001).

Side effects of long-term steroid dosing include prolonged suppression of ACTH and cortisol via negative feedback upon the pituitary gland. Musculoskeletal pain, fever, and lethargy are adverse symptoms associated with steroid withdrawal. More serious adversities associated with cortisol suppression include hypertensive episodes, osteoporosis, capillary fragility, fluid retention, increased appetite with weight gain, hyperglycemia and glycosuria, peptic ulceration, cataracts, and even arrested growth in children. Single dose and short-term, low dose corticosteroid treatment are generally regarded as safe alternatives, however (Barnes and Mueller, 1995).

Prednisone and prednisolone: pharmacokinetics and dose-dependency

The glucocorticoid compounds prednisone and prednisolone are synthetic analogues of endogenous cortisol, and are used in the treatment and management of a broad range of ailments including severe asthma and various rheumatic, gastrointestinal, and hematological disorders. Although the actions of these agents are largely palliative rather than curative, they remain first-line options for many physicians in the treatment of disease (Schimmer and Parker, 2001).

Prednisone and prednisolone are generally administered as prodrugs in oral and intravenous dosage forms. Prednisolone is rapidly absorbed in humans and features very little interindividual variability. The agent is metabolized in the liver and is characterized by a plasma half-life of about 2.5 hours and a biologic half-life of 24 hours (Barnes and Mueller, 1995). Prednisolone is highly bound to plasma proteins. Percent bound values for the drug ranged from 50% to 90% in previous investigations (B.M. Frey and F.J. Frey, 1990).

Prednisone is also metabolized in the liver and is characterized by a half-life of about 3.5 hours with an average plasma protein binding of 75% (B.M. Frey and F.J. Frey, 1990). The two glucocorticoids undergo reversible metabolism upon introduction of either agent into the body. Prednisolone has slightly greater pharmacological activity than prednisone (Schimmer and Parker, 2001).

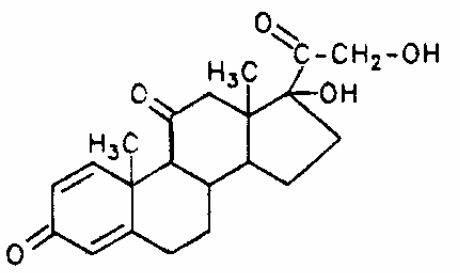


Figure 3.1: Structure of prednisone.

Prednisone and prednisolone undergo oxidative metabolism in the liver and kidneys to yield several inactive metabolites, including the major metabolites 6-beta- , 20-beta- and 20-alpha hydroxyprednisolone, along with 20-beta hydroxyprednisone. 20-alpha hydroxyprednisone is produced to a lesser extent (Garg and Jusko, 1991). 6-beta hydroxyprednisolone production depends upon the enzymatic activity of 6-beta hydroxylase as dictated by cytochrome P4503A4 (Waxman et al., 1988), while 20-alpha- and 20-beta metabolite production is facilitated by the 11-beta hydroxysteroid dehydrogenase enzyme system. The 11-beta hydroxysteroid system is also responsible for the interconversion of prednisolone and prednisone (Escher et al., 1998). Urinary excretion of prednisone and prednisolone are 3% and 15%, respectively, after intravenous dosing, regardless of which drug is administered (Schimmer and Parker, 2001).

Prednisone and prednisolone bioavailability assessments are commonly made with reference to prednisolone concentration. Prednisone and prednisolone administered to humans have complete absorption in low-dose formulations. Their bioavailabilities are unaffected by the presence of food (Henderson et al., 1979). When equivalent does of prednisone and prednisolone are given to the same individuals, the resulting prednisolone

plasma concentrations are nearly identical (Gambertoglio et al., 1980). Lower prednisolone bioavailability has been observed upon ingestion of high-dose prednisone, however, with some values as low as 60% (F.J. Frey et al., 1986). An investigation by Garg and Jusko (1994) largely attributed this discrepancy to less efficient prednisone absorption compared to hydrophilic prednisolone, possibly due to inferior dissolution of the former. Interconversion also plays a role in steroid bioavailability, as it allows for both removal and conservation of drug action via production of active and inactive drug forms. In addition, a larger fraction of the steroid dose may undergo first pass metabolism in the liver after high dose administration (Legler et al., 1982).

Prednisolone is notorious for its dose-dependent pharmacokinetic profile in several species. This characteristic is not shared by its reversible metabolite (Boudinot and Jusko, 1986; F.J. Frey et al., 1980). Volume of distribution and plasma clearance values for prednisolone increase with increased dose amounts in humans over dose ranges commonly used for maintenance therapy. In a study conducted by Uribe et al. (1978), normal volunteers exhibited average apparent volume of distribution (V_d/F) values of 0.69 L/kg, 1.43 L/kg, and 2.44 L/kg at 10, 20, and 30 mg doses of prednisone, respectively. Similarly, Pickup et al. (1977) reported mean volumes of distribution of 0.22 L/kg, 0.41 L/kg, and 0.64 L/kg, along with respective plasma clearances of 1.02 ml/min/kg, 1.44 ml/min/kg, and 2.0 ml/min/kg following tracer, 0.15 mg/kg and 0.3 mg/kg doses of prednisolone. This trend could also be seen at higher dose levels. Volume of distribution increased from 49 L to 132 L, and clearance increased from 155

mL/min to 382 mL/min as prednisolone dose rose from 10 mg to 100 mg, respectively (Tanner et al., 1979).

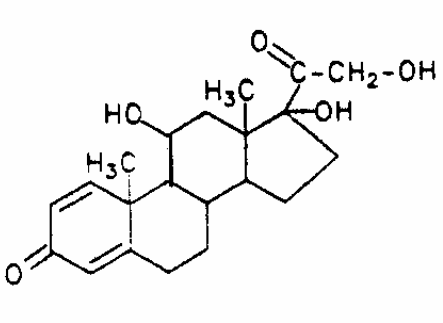


Figure 3.2: Structure of prednisolone.

Prednisolone's dose dependency is due in part to the compound's concentration-dependent plasma protein binding. Prednisolone has the ability to bind and eventually saturate plasma transcortin (Rose et al., 1981; Legler et al., 1982). Transcortin binds prednisolone with a high affinity yet low capacity (Chen et al., 1961; Rocci et al., 1980). High-dose prednisolone, for instance, is associated with an increased drug fraction-free in plasma and increased apparent volume of distribution and clearance values (F.J. Frey et al., 1981). Prednisolone's dose-dependency can also be attributed to its concentration-dependent clearance in the unbound form. Legler and colleagues (1982) reported that prednisolone's unbound clearance was 30% greater following a high infusion rate compared to a low infusion rate. Prednisolone has no effect upon glomerular filtration, as was determined by proportional drug/creatinine clearance ratios with higher dosing (F.J. Frey et al., 1986). It should be noted that prednisone binds to plasma transcortin with less resiliency than does its metabolic counterpart. Prednisolone, in fact, competitively inhibits prednisone

binding by transcortin (Boudinot and Jusko, 1984). Plasma albumin equally binds prednisone and prednisolone, though the interactions are relatively weak (Pugeat et al., 1981).

The interconversion of prednisone and prednisolone also appears to be concentration-dependent, as evidenced by increased prednisolone to prednisone plasma concentration ratio vs. time at higher dose levels. The cause of this ratio shift has yet to be fully understood. Prednisone concentrations plateau at about 50 to 60 μg/mL after high doses of intravenous prednisolone or oral prednisone. Researchers have ruled out the possibility of saturation of 11-beta-hydroxydehydrogenase by pointing out that an expected decrease of unbound prednisolone clearance does not occur at high doses (B.M. Frey and F.J. Frey, 1990). In addition, prednisone's low plasma protein binding (Boudinot and Jusko, 1984), combined with its dose-independent urinary excretion, weakens the argument that this phenomenon results from a dose-dependent clearance of prednisone (B.M. Frey and F.J. Frey, 1990).

Reversible metabolism

The phenomenon of reversible metabolism is a complex but increasingly important issue in modern biopharmaceutics. Reversible metabolism has been observed in drugs such as sulindac, chlorpromazine and haloperidol, in addition to various sulfonamide and steroid compounds. The phenomenon has long been overlooked due to a lack of pharmacokinetic studies involving metabolite dosing (Jann et al., 1994). Elucidation of reversible metabolism between prednisone and prednisolone has been especially difficult, due in part to the absence of a

federally-approved intravenous formulation of prednisone for use in humans (Garg and Jusko, 1994).

A problematic feature of reversible metabolism is the notion that measurement of key pharmacokinetic parameters may not provide an accurate representation of traditional parameters. Characterization of prednisone/ prednisolone pharmacokinetics was the subject of continuous debate during the 1970s and 1980s, highlighted by the creation and use of various models. Compartmental, non-compartmental, and even what can be described as hybrid methods were utilized by various investigators in hopes of appropriately profiling the compounds (B.M. Frey and F.J. Frey, 1990).

There are three general models used to describe reversible metabolism following prednisone or prednisolone administration, as shown in figure 3.3 below.

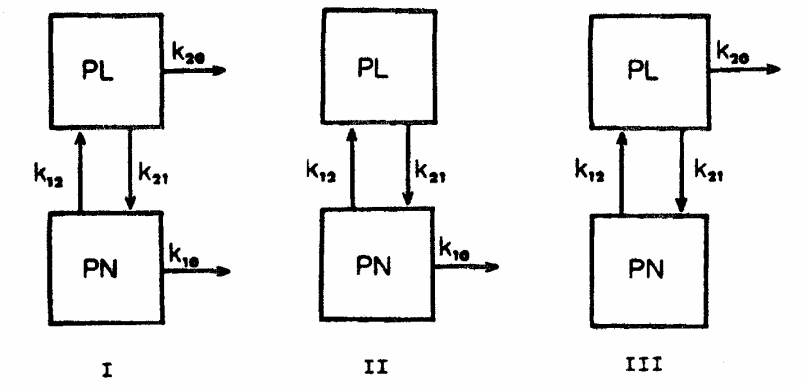


Figure 3.3: Models of prednisone/prednisolone interconversion. (Ferry and Wagner, 1986).

Boxes PN and PL represent drug and interconvertible metabolite in the central compartment. The parameters k_{10} and k_{20} represent the rate constants for the

irreversible elimination pathways for prednisone and prednisolone, respectively, while k_{12} and k_{21} represent the reversible pathways for each compound. The models assume no saturation of elimination or interconversion pathways, and also assume that reversible metabolism is linear and occurs systemically.

Figures 3.3 (II) and 3.3 (III) illustrate linear metabolism scenarios in which elimination pathways may saturate, evidenced by the lack of an elimination rate constant for either of the corticosteroid analogs. Wagner et al. (1981) suggested this scenario as feasible after low dose administration of prednisone and prednisolone to humans. Complicating the situation are the scenarios involving saturation of interconversion pathways, and this has been noted in investigations involving corticosteroid administration in dogs and in humans (F.J. Frey et al., 1980; Rose et al., 1981). It has since been suggested that nonlinear reversible metabolism is associated with higher levels (50 mg or greater oral administration) of prednisone or prednisolone dosing (Huang and Jusko, 1990).

Prednisone/ prednisolone enzymatic inhibition and induction

The effects of enzymatic inhibition upon prednisone/prednisolone metabolism have been studied by several investigators. Known cytochrome 3A4 inhibitors such as ketoconazole, cimetidine, and cyclosporine have been frequently used in these investigations, yielding variable results.

- A study conducted by Ludwig et al. reported no significant differences in mean residence time, terminal slope, volume of distribution, and clearance of prednisolone or prednisone after six days of ketoconazole treatment (Lugwig et al., 1989). Only four subjects were used in this

investigation, however, bringing sample size into immediate question (B.M. Frey and F.J. Frey, 1990).

- Zurcher et al. (1989) reported that repeated administration of ketoconazole (200 mg) was found to inhibit 6- beta hydroxylase activity, which was measured by the fractional urinary excretion of 6-beta hydroxyprednisolone. This reduced activity was associated with decreased prednisolone clearance in spite of unaltered oral prednisone bioavailability and unaltered prednisone/prednisolone AUC ratio. However, the mean AUC of unbound prednisolone rose by 50% during the investigation.
- Itraconazole, an antifungal observed to be 10-fold less potent in CYP3A4 inhibition than ketoconazole, was administered in 200 mg doses to ten volunteers for 4 days. Itraconazole significantly increased prednisolone AUC (24%) and elimination half-life (29%) compared to placebo (Varis et al., 2000).
- Four days of cimetidine (300 mg every 6 hours) and ranitidine (150 mg twice a day) treatment resulted in no significant effects upon prednisolone plasma concentration, AUC, half-life, or volume of distribution after a single prednisone dose (Sirgo et al., 1985). This outcome reinforced a previous investigation in which cimetidine yielded no significant effects upon the plasma profile of enteric-coated prednisolone (Morrison et al., 1980).

- While co-administration of the macrolide antibiotic troleandomycin caused a significant alteration of methylprednisolone clearance and elimination, no significant effects upon prednisolone metabolism were observed (Szefler et al., 1982).
- Cyclosporin displayed no significant effects upon prednisolone volume of distribution, bioavailability, and metabolic or renal clearance in renal transplant patients (F.J. Frey et al., 1987).
- Significant reductions in unbound prednisolone clearance were observed in women taking oral contraceptives containing ethinyl estrogens (B.M. Frey et al., 1984). A 50% increase in mean AUC values was also noted for unbound prednisolone following prednisone or prednisolone co-administration (Boekenhogen et al., 1983; Gustavson et al., 1986).

Enzyme induction has also been associated with prednisolone metabolism. Prednisolone induced liver enzymes *in vivo*, as illustrated by significantly reduced half-lives of both antipyrine and chloramphenicol in rabbits (Shukla et al., 1984). Several investigators have suggested prednisolone auto-induction following long-term treatment of the agent in patients with liver disease. These patients displayed shortened prednisolone elimination half-lives compared to patients with liver disease who had not received previous prednisolone therapy, although the differences were not significant (Schalm et al., 1977). In another investigation, prednisolone clearance rate increased by 23% after 6 weeks of prednisone dosing, in comparison to clearances recorded before daily administration (Kozowzer et al., 1974). Several studies involving common

medications have been conducted to assess prednisolone induction, and are listed below.

- In a study by F. Frey and B. Frey (1983), prednisolone unbound drug clearance was significantly increased and 6-beta-hydroxyprednisolone urinary excretion was increased following 8 days of (300 mg) phenytoin administration. In addition, the prednisolone/prednisone ratio uncharacteristically increased as prednisolone plasma concentration decreased, indicating a possible phenytoin induction effect upon corticosteroid interconversion, or a phenytoin-induced alteration of prednisone volume of distribution.
- Anticonvulsant medications phenobarbital, phenytoin, and carbamazepine significantly increased the plasma clearance of prednisolone in pediatric patients by 41%, 79%, and 77%, respectively, compared to control subjects (Bartoszek et al., 1987). Similarly, Petereit and Meikle (1977) reported a 45% decrease in prednisolone mean elimination half-life and a 77% increase in prednisolone mean metabolic clearance in patients on phenytoin therapy for 3 weeks.
- Three weeks of rifampicin therapy resulted in reduced prednisolone mean elimination half-life (44.8%) and reduced mean AUC (47.5%) for total drug. In addition, unbound prednisolone AUC decreased 56.5%, and prednisolone total body clearance increased by 91% (Bergrem and Refvem, 1983).

Drug induction and prednisone/ prednisolone pharmacodynamics

Enzyme induction has proven an important issue for prednisone/prednisolone dosing from an efficacy standpoint as well as from a pharmacokinetic standpoint. Various *in vitro* and *in vivo* findings highlight the hazards of prednisolone induction.

- B. Frey and F. Frey (1984) reported lowered inhibition of lymphocyte cultures in the plasma of humans treated with phenytoin and prednisone. Reduced lymphocyte inhibition was not seen in plasma of individuals treated with prednisone alone.
- Another investigation involving rats that received kidney and heart transplants revealed that rats treated with prednisolone in combination with phenytoin displayed shorter organ survival times in comparison to rats that received prednisolone alone (Kort et al., 1979).
- Phenobarbital and phenytoin co-administration with prednisone resulted in an increased incidence of cadaver allograft rejection in renal transplant patients. Survival rates for 20 index allografts were significantly lower than rates observed in control allografts (Wassner et al., 1976). In further studies, phenytoin-induced allograft adversities were combated by either discontinuing anticonvulsant therapy or increasing prednisone dosage. Discontinued anticonvulsant treatment resulted in allograft stabilization, while elevation of prednisone dosage resulted in 50% allograft failure (Wassner et al., 1977).

- Increased allograft rejection rates were observed in renal transplant patients following coadministration of phenytoin or phenobarbital with prednisolone. Decreased total and unbound prednisolone half-life, combined with increased prednisolone clearance, were associated with the rejection results (Gambertoglio et al., 1984).
- Rifampicin and prednisolone treatment in patients with tuberculosis resulted in decreased AUC and increased clearance of prednisolone to produce adverse outcomes. Patient improvement was seen when rifampicin was discontinued (McAllister et al., 1983). A previous investigation observed a progressive loss of allograft function in renal allograft recipients who also received rifampicin as tuberculosis therapy (Buffington et al., 1976).
- Asthmatic patients treated with rifampicin displayed significant decreases in prednisolone bioavailability and half-life in spite of a 93% increase in dose. In addition, inferior asthma control in rifampicin-treated patients was observed compared to patients who received prednisolone alone (Powell-Jackson, 1983).
- Patients with nephrotic syndrome on corticosteroid therapy displayed treatment failures after corticosteroid/rifampicin coadministration due to inductive processes as well (Hendrickse et al., 1979).

Prednisone and prednisolone are widely used corticosteroids. The agents present a pharmacokinetic challenge due to their reversible metabolism-- a process complicated by prednisolone's infamous dose-dependency. The short

half-lives of prednisone and prednisolone, combined with their relative safety at low doses, make the agents attractive candidates for single dose pharmacokinetic drug interaction studies.

REVIEW OF LITERATURE:

OVERVIEW OF IBUPROFEN

Non-steroidal agents: mechanism of action and pharmacology

Ibuprofen is a widely-used, non-steroidal anti-inflammatory drug (NSAID). Like its prototypical ancestor aspirin, ibuprofen possesses useful anti-inflammatory, analgesic, and antipyretic capabilities. NSAID medications combine the anti-inflammatory properties associated with corticosteroid agents with the analgesic properties associated with opiate agents, although achieving their effects through alternate mechanisms of action. Despite their tremendous structural diversity, members of the NSAID class are remarkably similar in terms of overall pharmacological profile and side effects. The advantage of NSAID diversity is that it allows specialization of agents in the treatment of disease. The analgesic potency of a given NSAID, for instance, may not match the potency of one of its analogs. By the same token, the analog may be less effective in the management of inflammation (Roberts and Morrow, 2001).

The events of the inflammatory process are characterized by local vasodilation and capillary permeability, infiltration of leukocytes and phagocytes, and tissue degeneration and fibrosis. The inflammatory process is exacerbated by the appearance of chemical mediators such as histamine, prostaglandins, and the enzymatic contents of ruptured lysosomes. NSAID agents are generally effective in inhibiting prostaglandin synthesis and promote a reversal of

vasodilation and edematous conditions associated with the inflammatory response (Roberts and Morrow, 2001).

The generation and maintenance of pain is dependent in part upon the release of prostaglandins. Prostaglandins are important sensitizers of pain receptors in the human body. Although NSAIDs generally do not affect the pain response caused by prostaglandin action, they are quite effective in the management of pain through the prevention of prostaglandin production (Roberts and Morrow, 2001).

Fever is the result of loss of thermostat control in the hypothalamus. The phenomenon can be triggered by various stimuli, including tissue damage, malignancy, inflammation, or other ailments. Prostaglandins contribute to the elevation of body temperature by affecting hypothalamic function. NSAIDs alleviate fever through the inhibition of pyrogen synthesis rather than through direct action upon prostaglandin synthesis (Roberts and Morrow, 2001).

Inhibition of prostaglandin synthesis is greatly dependent upon NSAID interaction with the cyclooxygenase enzyme. NSAID interface with cyclooxygenase interrupts the conversion of arachidonic acid to the prostaglandin intermediate endoperoxide. The precise manner in which cyclooxygenase is inhibited varies from compound to compound. For example, while most NSAIDs impact cyclooxygenase in an irreversible fashion, some compounds (such as ibuprofen) produce similar effects through reversible means (Roberts and Morrow, 2001).

The phenylpropionic acid ibuprofen: pharmacokinetics and disposition

Phenylpropionic acid derivatives are a structurally diverse group of NSAIDs noted for their effectiveness in anti-inflammation and analgesia. They are particularly useful in the management of inflammatory disorders such as osteoarthritis and ankylosing spondylitis, and are useful in the management of dysmenorrheal and post-surgical pain. Included in this class are widely used agents like ibuprofen, naproxen, ketoprofen, fenoprofen, and flurbiprofen. The number of members of this class expanded rapidly following the introduction of ibuprofen in the 1960s. Despite the large number of compounds, propionic acid derivatives have remarkably similar therapeutic actions and side effects across the board (McNamara and Mayeux, 1995)

Upon their introduction, propionic acid derivatives rapidly rose in popularity due to their superior tolerability compared to aspirin and other older medications. Similar to many NSAIDs, propionic acid derivatives can cause erosions of the stomach and intestines, but to a lesser extent than earlier NSAID medications like aspirin and indomethacin. All members of the class exhibit characteristic NSAID anti-platelet activities, and their adverse effects are most commonly associated with alterations in platelet function upon administration with agents with similar anti-platelet activity (Roberts and Morrow, 2001).

Ibuprofen was the first propionic acid derivative to achieve general use in the United States. The medication was marketed for over-the-counter use in 1984 for low dose formulations (200 mg). Low dose ibuprofen is more effective in the management of pain than in the management of inflammation, but the drug

has better potency against inflammation than aspirin at analogous doses (McNamara and Mayeux, 1995).

Ibuprofen is generally marketed as a racemate mixture with R- and S-enantiomers. Ibuprofen is quickly and completely absorbed following oral administration in man, with peak drug concentrations appearing 1 to 2 hours after dosing. While past investigations assert that food has no significant affect upon ibuprofen absorption (Giesslinger and Dietzel, 1989; Levine et al., 1992), more recent findings suggest that food administered prior to dosing can affect ibuprofen pharmacokinetics in terms of S- and R- enantiomer initial concentrations. Food may also affect S-/R- enantiomer ratio through increased peak plasma concentration (C_{max}) and area under the curve (AUC) values (Siemon et al., 1997). The drug is 99% bound to plasma proteins and interacts strongly with plasma albumin. Average volume of distribution values in man are 0.15 L/kg, with clearance values averaging 0.75 ml/min/kg. Ibuprofen has an average elimination half-life of 2 hours. Drug bioavailability is usually better than 80%, with 90% of ingested doses excreted in the urine in the form of metabolites. Ibuprofen metabolism is largely mediated by cytochrome P4502C9 to form major oxidative metabolites such as 2-hydroxyibuprofen and carboxyibuprofen. Minor products such as 1-hydroxy- and 3-hydroxyibuprofen are also produced by this pathway (Leeman et al., 1993). The hydroxylated and carboxylated metabolites possess no known pharmacological activity (Adams et al., 1970). Ibuprofen and its metabolites also undergo glucuronide conjugation to yield acylglucuronide

products. These substances are suggested by some to contribute to ibuprofen toxicity and rare anaphylactic reactions in humans (Castillo et al., 1995).

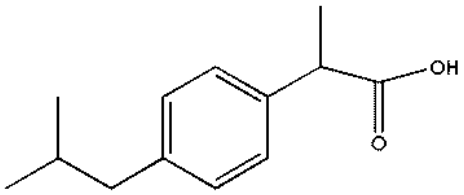


Figure 4.1: Structure of ibuprofen.

Ibuprofen has stereoselective pharmacokinetic and pharmacodynamic activity, and its characteristics are dominated by the action of its S-enantiomer. S(+)-ibuprofen possesses the bulk of the drug's pharmacological activity and therefore receives more attention than its R-counterpart. S(+)-ibuprofen has been estimated to be 160 times more potent than R(-)-ibuprofen in the inhibition of prostaglandin synthesis by *in vitro* measurements (Geisslinger et al., 1989; Evans, 1992). Moreover, S(+)-ibuprofen has twice the potency of racemate drug and about 4 times the potency of R(+)-ibuprofen in the inhibition of platelet aggregation and thromboxane formation *in vitro* (Villanueva et al., 1993). In addition, virtually all of ibuprofen's anti-inflammatory effectiveness has been associated with the S-enantiomer. Enantioselectivity not only characterizes ibuprofen's pharmacological activity, but also ibuprofen's disposition profile (Evans, 1992).

Studies indicate ibuprofen enantiomers bind to plasma proteins in a competitive and stereoselective manner. An investigation by Hage and associates identified R(-)- and S(+)-ibuprofen to have one common binding site

on human serum albumin (Hage et al., 1995). In addition, a second site on the molecule was found to bind the S-enantiomer exclusively. An investigation by Hansen et al. (1985) strengthened the notion of competition and stereoselectivity when equilibrium dialysis revealed an S/R free concentration ratio of 1.7 over a broad concentration range. Stereoselective protein binding by ibuprofen was also noted in several other studies that featured larger unbound fractions of S(+)-ibuprofen in comparison to its enantiomer (Davies, 1998).

Ibuprofen exhibits saturable plasma protein binding at high plasma concentrations, which results in nonlinear pharmacokinetics. This was illustrated by decreased AUC/dose ratio upon plasma protein saturation in several studies (Lockwood et al., 1983; Albert and Gernaat, 1984). Both R(-)- and S(+)-ibuprofen exhibited saturable binding as drug concentrations were increased from 2 mg/L to 100 mg/L (Hansen et al., 1985). As ibuprofen dose increased from 50 mg to 1200 mg, there was a gradual increase in the clearances of S- and R-enantiomers with no change in S/R AUC ratio (Jamali et al., 1992). In another investigation, a nonlinear increase in clearance of R(-)- ibuprofen was observed after increased oral administration from 200 mg to 800 mg, although this nonlinear behavior was not displayed by the S-enantiomer (Hall et al., 1993). A binding study by Cheruvallath and associates (1997) provided stereospecific information regarding ibuprofen and human albumin interactions, with S(+)-ibuprofen alone displaying a greater apparent binding constant (K_{app}= 8.0 +/- 0.11 * 10^5 L/mol) than R(-)-ibuprofen (K_{app}= 4.8 +/- 0.07 * 10^5 L/mol) and racemate drug (K_{app}= 1.4 +/- 0.04 * 10^5 L/mol).

Ibuprofen's disposition is stereoselective. While its characteristic volume of distribution in humans indicates extensive binding to plasma proteins (Davies, 1998), ibuprofen also distributes into synovial fluid, cerebrospinal fluid, and sparingly into tissues. Ibuprofen tissue distribution is in a stereoselective fashion (Fears, 1985). Cerebrospinal fluid concentrations of both ibuprofen enantiomers were lower and reached their maximum values later than the enantiomers in plasma. Additionally, the elimination half-lives of R(-)- ibuprofen and S(-)-ibuprofen were 3.9 hours and 7.9 hours in cerebrospinal fluid, respectively, in contrast to their respective half-lives (1.7 hours, 2.5 hours) in plasma (Bannwarth et al., 1995).

Infiltration of ibuprofen into the synovial fluid is believed to be an important event in ibuprofen's anti-inflammatory course of action. Ibuprofen's effectiveness in rheumatoid arthritis is, in part, a function of its success in reaching the synovial membrane. When utilized in the treatment of rheumatoid and osteoarthritis, ibuprofen was noted to have a longer t_{max} and elimination half life in synovial fluid compared to plasma. In addition, ibuprofen concentration was observed to be higher in plasma during the first 2 ½ hours after administration, while the drug was predominately distributed in synovial fluid thereafter (Glass and Swannell, 1978). Similar results in distribution characteristics were observed in arthritic children treated with ibuprofen (Janssen and Venema, 1985). Although differences in the total concentrations of ibuprofen could be seen between plasma and synovial fluid, free drug concentrations were not significantly different in the two locales (Whitlam et al., 1981). Thus, plasma albumin binding plays an

important role in synovial fluid distribution. Ibuprofen S-/R- enantiomer concentration ratios were the same in synovial fluid as in plasma, though lower C_{max} values were reported for both enantiomers in synovial fluid when compared to plasma (Cox et al., 1991).

Studies in rats revealed deposition of R(-)- ibuprofen in adipose tissue due to formation of glycerolipids, or “hybrid” triglycerides. These triglycerides have been observed to act as drug reservoirs allowing delayed release of ibuprofen (Williams et al., 1986), and it has been speculated that their action may have a disruptive effect upon lipid metabolism and may indirectly contribute to CNS toxicity (Fears, 1985). The triglyceride phenomenon has not been seen following administration of S(+)-ibuprofen alone, although R-enantiomer distribution is believed to occur during R to S enantiomeric conversion and thioester formation (Williams et al., 1986).

Ibuprofen stereoselectivity and enantiomeric metabolism

Ibuprofen metabolism includes the conversion of R(-)-ibuprofen to S(+)-ibuprofen. The mechanism by which this occurs begins with the stereoselective enzymatic activation of R(-)-ibuprofen to R(-)-ibuprofenyl-adenylate. An acyl coenzyme A synthetase acts upon this derivative to form an acyl CoA thoiester, R-ibuprofen CoA. Epimerization of R-ibuprofen CoA leads to the formation of R(-)- and S(+)-ibuprofen enantiomers. Since S(+)-ibuprofen is activated to the adenylate intermediate, reversible metabolism does not occur between the enantiomers (Nakamura et al., 1981; Menzel et al., 1994). Data from several investigations suggest R(-)-ibuprofen is converted to its S(+)-enantiomer to an

extent as low as 52% to as high as 69% in humans (Rudy et al., 1995). Extent of inversion is often determined by administration of equal amounts of individual enantiomer, during which the AUC of the S-enantiomer (conversion product) is compared to the AUC of the R-enantiomer (Davies, 1998).

The process of ibuprofen's enantiomeric inversion is believed by some to be influenced by drug absorption rate. It has been suggested that pre-systemic inversion takes place within the gastrointestinal tract. Accordingly, drug residence time within the G.I. tract has been observed to affect the extent of enantiomer inversion, with longer residence times translating into an increase in the conversion activity (Jamali et al., 1988). In support of this idea, Cox et al. (1988) demonstrated that increased dose did not affect S-/R-ibuprofen AUC ratio after ibuprofen tablet administration in humans, whereas this ratio was significantly decreased after administration of liquid dosage forms. Opposing viewpoints regarding pre-systemic inversion exist as well, strengthened by observations of Hall et al. (1993), in which no differences in AUC ratio were seen between oral and intravenous treatments. However, systemic inversion has been confirmed in humans following intravenous administration of R(-)-ibuprofen to form the S-enantiomer (Cheng et al., 1994). In addition, gradual increases in S-/R-ibuprofen ratio over an 8-hour infusion period support the notion that systemic inversion strongly impacts ibuprofen metabolism and pharmacokinetics (Hall et al., 1993). It should be noted, however, that S-/R- ratio is not necessarily the ideal measure of drug inversion because it assumes pharmacokinetic linearity within the system, and because it assumes that there is no

pharmacokinetic interaction between the enantiomers (Davies, 1998). It does not take into account, for example, mitigating factors such as plasma protein binding, particularly in the case of saturation of protein binding (Mehvar et al., 1998).

Concurrent administration of equivalent quantities of enantiomeric and racemate ibuprofen has indicated inversion-mediated kinetic differences in multiple investigations. Romero et al. (1991) reported that the AUC of a 400 mg dose of S(-)- ibuprofen (93.1mg/L*hr) was notably less than the AUC value of the S-enantiomer (128 mg/L*hr) after 800 mg of racemate administration. Analogously, the AUC value of a 400 mg dose of R(-)- ibuprofen (101 mg/L*hr) was greater than that of the R-enantiomer after administration of 800 mg of racemate drug (82.3 mg/L*hr). In another investigation featuring concurrent administration of 300 mg of S- and R-enantiomeric ibuprofen and 600 mg of racemate drug, greater peak plasma concentrations of S(+)- ibuprofen were observed after administration of the individual enantiomer than were observed after racemate mixture dosing, with mean values of 19.1 mg/L and 16.4 mg/L, respectively. This difference was not statistically significant, however. Non-significant differences in T_{max} were also observed after administration of S-enantiomer individually (1.44 hr), and after racemate administration (1.8 hr). In addition, S(+)-ibuprofen generated from conversion of the R-stereoisomer in racemate drug accounted for one-third of the AUC obtained from single enantiomer administration (Geisslinger et al., 1990).

The usefulness of racemate ibuprofen has been the subject of much debate in recent years. Although R(-)-ibuprofen has very little pharmacological

activity, it is considered valuable by many due to its considerable conversion to its active S- enantiomer. Recent data have suggested that administration of individual S(+)-ibuprofen produces a more desirable pharmacological profile than racemate administration. For example, a 200 mg dose of S(+)- ibuprofen was more effective in the treatment of pain than 400 mg of racemate ibuprofen, the latter of which was theoretically equivalent to a 300 mg dose of S-enantiomer (Dionne and McCullagh, 1998).

Ibuprofen and drug interactions

Ibuprofen possesses a unique drug interaction profile. Pharmacokinetic and pharmacodynamic interactions have been observed between ibuprofen and a broad array of agents by a range of mechanisms:

- Ibuprofen has a mixed interaction profile with H_2 –antagonists. Cimetidine yielded no significant effects upon the disposition and pharmacokinetics of ibuprofen as reported by Conrad et al (1984). Another study reported non-significant increases in ibuprofen peak concentrations following cimetidine co-administration as opposed to control values (64 mg/L to 56 mg/L). Cimetidine also impaired ibuprofen clearance to a small degree. However, ranitidine co-administration resulted in no change in ibuprofen pharmacokinetics (Jenkinson et al., 1998). Li et al. (1989) revealed that cimetidine caused a 23% increase in S(+)-ibuprofen C_{max} and a 28% increase in R(-)-ibuprofen C_{max}. Both were statistically significant alterations. In addition, cimetidine caused a 37% increase in R(-)-ibuprofen AUC and a 19% increase in S(+)-ibuprofen AUC. Stephenson et al. (1988) reported no significant effects upon

ibuprofen steady-state pharmacokinetics by cimetidine or ranitidine. Ibuprofen and its metabolites showed no pharmacokinetic differences due to race during a stereospecific investigation (Evans et al., 1989).

- Ibuprofen co-administration with aspirin significantly lowered ibuprofen plasma concentrations in patients with rheumatoid arthritis. The interaction occurred during high-dose therapy of ibuprofen (1600 mg) and was believed to be caused by ibuprofen displacement from plasma proteins by salicylate, resulting in a doubling in ibuprofen unbound fraction (Grennan et al., 1979). An interaction with aspirin was also detected in rheumatoid patients when the total ibuprofen synovial fluid/plasma ratio was decreased following co-administration of the two drugs (Whitlam et al., 1981).
- Co-administration of magnesium hydroxide with ibuprofen caused a 65% increase in ibuprofen's AUC and a 31% increase in C_{max} during the first hour of therapy. It was believed that magnesium hydroxide increased the solubility of ibuprofen in the G.I. tract to increase the NSAID's absorption rate (Neuvonen, 1991).
- Ibuprofen AUC and C_{max} were decreased by 11.8% and 6.5%, respectively, when the drug was administered with sucralfate in early studies (Pugh et al., 1984). Later investigations revealed significant alterations in ibuprofen absorption rate and pharmacokinetics after co-administration of sucralfate (Gambaro et al., 1985), in addition to significant reductions in peak concentrations of both ibuprofen enantiomers in contrast with controls.

Ibuprofen pharmacokinetic parameters were not significantly affected by sucralfate in another study (Levine et al., 1992).

- The hypolipidemic agent, clofibrate, significantly increased the clearances of R-ibuprofen (239%) and radio-labeled S-ibuprofen (61%) after patients were treated with pseudo-racemate drug. It was further determined that R(-)-ibuprofen clearance was also significantly increased following clofibrate administration. In addition, maximum plasma concentrations of R-ibuprofen were decreased by 44%, and significant decreases in AUC were observed for each enantiomer as well (Scheuerer et al., 1998).
- Cholestyramine co-administration resulted in a significant reduction in ibuprofen AUC (26%) and C_{max} (34%). Peak concentration time was prolonged by 80%. Significant changes in ibuprofen bioavailability were also observed following cholestyramine in other studies (Al-Meshal et al., 1994).

Ibuprofen administration also affects the pharmacokinetics of other drugs. Co-administration of paracetamol and ibuprofen, for instance, resulted in alterations of paracetamol plasma concentrations, although significant changes were not observed in paracetamol bioavailability and pharmacokinetics (Wright et al., 1983). In addition, daily dosing of ibuprofen significantly increased digoxin concentrations (Quattrocchi et al., 1983). Ibuprofen similarly increased lithium plasma concentrations by an average of 34% in nine patients, although interindividual variation was highly evident (Ragheb, 1987).

Cytochrome 2C9 and ibuprofen catabolism

Cytochrome P4502C is responsible for the metabolism and (at least in part) the pharmacological and pharmacokinetic profiles of ibuprofen. CYP2C9 has been shown to be the chief oxidative pathway for the metabolism of non-steroidal agents, as illustrated by *in vitro* investigations. Microsomal studies by Hamman and associates (1997) noted high cDNA expression of CYP2C9 with reference to ibuprofen hydroxylation as measured by K_m values. The finding was supported by the observation that sulfaphenazole potently and competitively inhibited ibuprofen hydroxylation, with K_i values for sulfaphenazole inhibition of ibuprofen hydroxylation being comparable to that of S-warfarin hydroxylation, another well-known CYP2C9 mediated oxidation. In addition, microsomal studies revealed high correlations between all ibuprofen hydroxylations and the oxidative processes of prototypical 2C9 transformations phenytoin 4'-hydroxylation and tolbutamide methylhydroxylation.

Investigations have identified a second CYP isoform, 2C8, in the oxidative metabolism of ibuprofen. CYP2C9 substrates ibuprofen and tolbutamide were found to be additionally affected by CYP2C8. This result was in spite of microsomal studies which indicated a decreased inhibition of ibuprofen hydroxylation by sulfaphenazole at CYP2C8 as compared to CYP2C9. Studies also revealed differences in the sulfaphenazole inhibition of 3-hydroxylation (0.06 μM) compared to inhibition of 2-hydroxylation (0.12 μM). It has since been suggested that ibuprofen metabolism by 3-hydroxylation is exclusively mediated by CYP2C9, whereas metabolism by 2-hydroxylation is in part dependent upon

other cytochrome isoforms, CYP2C8 in particular (Veronese et al., 1993; Rettie et al., 1994). These findings imply that relative expression of CYPs 2C9 and 2C8 could greatly contribute to the large individual and interethnic variability often observed in ibuprofen pharmacokinetics, especially from an enantiomeric standpoint. Effects on CYP2C8 in an individual, for example, could theoretically affect the stereoselective clearance of ibuprofen, perhaps favoring the conservation of the S-enantiomer while having no discernible effect upon the R-enantiomer (Hamman et al., 1997). Arachidonic acid, whose stereoselective epoxidization during NSAID therapy may strongly impact regulation of organ function (Daihk et al., 1994), was affected by variations in CYP2C8 expression, suggesting possible alterations in NSAID pharmacological actions (Hamman et al., 1997).

The impact of polymorphism on CYP2C metabolism

Cytochrome P450 2C9 is also characterized by genetic polymorphism. CYP2C9 consists of three known polymorphic variations, each differing only by the nucleic acid identity at codon sites 144 and/or 359. CYP2C9 variants are identified as 2C9*1 (wild-type), 2C9*2, and 2C9*3. *In vitro* and human pharmacokinetic investigations have indicated the enzymatic activity of CYP2C9*2 to be slightly less than that of the wild type, while the enzymatic activity of CYP2C9*3 generally varies between 10% and 30% of the wild type. The activity of 2C9*3 is also substrate-dependent (Sullivan-Klose et al., 1996).

Genetic polymorphism of CYP2C9 has been found to impact NSAID pharmacokinetics and effectiveness. An investigation by Kirchheiner and

colleagues (2002) revealed statistically relevant differences in pharmacokinetic parameters among polymorphs. In general, C_{max} and AUC parameters were significantly higher for racemate and S(+)-ibuprofen in humans who were carriers of the CYP2C9*3 genotype as opposed to individuals with wild-type genotypes. Specifically, AUC values for racemate and S(+)- ibuprofen in 2C9*3 homozygous carriers were approximately double that of wild-type carriers. Elimination half-life of racemate ibuprofen was significantly different in CYP2C9*3 homozygous individuals compared to wild-type individuals, due almost exclusively to differences in S(+)-ibuprofen values. Stereospecific metabolism was also apparent in the case of ibuprofen clearance. Clearance values corresponding to racemate drug differed by 50% in 2C9*3 homozygotes compared to wild types, while clearance values for the S-enantiomer differed by 100% in homozygotes compared to wild-type carriers. In addition, it was reported that the median C_{max} values for metabolic 2-hydroxyibuprofen in 2C9*3 homozygotes were lower (0.85 mg/L) compared to corresponding values for 2C9*1 carriers (1.34 mg/L). Likewise, median plasma concentrations of the metabolite were lower in 2C9*3 homozygous carriers, although not in a significant fashion.

Pharmacodynamic differences have also been noted among CYP2C9 polymorphs. Measurement of thromboxane B_2 and prostaglandin E_2 activity was used to evaluate the impact of CYP2C9 polymorphism on ibuprofen efficacy. Individuals who were homozygous or heterozygous carriers of the 2C9*3 genotype exhibited prolonged inhibition of thromboxane formation and delayed prostaglandin recovery, effects assumed to result from prolonged drug half-life.

In general, 50% inhibition values for cyclooxygenase 1 were longer in individuals who carried variant 2C9 alleles than in wild-type carriers, with inhibition reaching significance in the cases of 2C9 *2/*3 heterozygotes and 2C9*3 homozygotes. Not surprisingly, ibuprofen pharmacokinetic parameters correlated with prostaglandin E_2 data revealed that the S-enantiomer displayed long elimination half-lives and stronger inhibitory effects in 2C9*3 homozygotes compared to wild types (Kirchheiner et al., 2002).

Inconsistent metabolism due to genetic variations in CYP2C9 expression in humans has been offered as a possible explanation for high NSAID tissue concentrations and adverse effects in some subjects. Adversities such as gastric bleeding, deterioration of kidney function, or sodium retention to antagonize heart function are believed by some researchers to have increased prevalence in individuals who carry CYP2C9 variants (Patrono et al., 2001).

Ibuprofen is an intriguing compound for use in drug interaction investigations because of its widespread use and stereospecific pharmacokinetic and pharmacodynamic drug profile. Ibuprofen's metabolism can be altered by the co-administration of a diverse group of agents through various mechanisms of interaction. Changes in the drug's stereoselective clearance and/or conversion impact its effectiveness in humans. The NSAID agent also possesses a short half-life and mild side effects profile, making it a safe and manageable compound for small-scale pharmacokinetic investigations.

PHARMACOKINETICS OF PREDNISONE AND PREDNISOLONE: IMPACT OF ST. JOHN'S WORT SUPPLEMENTATION

Abstract

Purpose. The purpose of this study was to examine the effect of long-term St. John's Wort administration upon the pharmacokinetics of prednisone and its reversible metabolite, prednisolone. **Methods.** Plasma corticosteroid concentrations were determined using a normal phase HPLC assay. Model independent methods were used to evaluate corticosteroid pharmacokinetics.

Results. Twenty-eight days of St. John's Wort treatment resulted in no significant alterations in the pharmacokinetic parameter means for prednisone or prednisolone. Oral administration of prednisone resulted in prednisone mean elimination half-lives of 3.85 +/- 1.49 hrs (mean +/- S.D.) in control samples and 5.10 +/- 2.77 hrs after 28 days of St. John's Wort treatment. Mean prednisone AUCs were determined to be 115.89 +/- 39.52 μg*hr/L in control samples and 128.76 +/- 32.71 μg*hr/L after 28 days of treatment with St. John's Wort. Prednisolone elimination half-lives were 2.97 +/- 0.59 hrs in control samples and 3.09 +/- 0.83 hrs in St. John's Wort treatment samples, while mean prednisolone AUCs were 714.19 +/- 153.29 μg*hr/L and 700.74 +/- 89.68 μg*hr/L, respectively. The ratio of AUCs for prednisolone/ prednisone was 6.40 +/- 0.970 before St. John's Wort treatment and 5.64 +/- 1.15 after St. John's Wort treatment.

Conclusions. St. John's Wort appears to have no significant treatment effects upon the pharmacokinetic parameters of prednisone and prednisolone. Factors such as diet and ethnicity may require closer examination in future interaction studies that involve these agents.

Introduction

The herbal supplement St. John's Wort (SJW) has achieved widespread appeal for its comparable effectiveness and superior tolerability profile to tricyclic antidepressants in the treatment of mild to moderate depression (Harrer et al., 1994; Wheatley, 1997; Schader, 2000). Recent case reports and animal and clinical studies have implicated St. John's Wort in a number of herb-drug interactions, raising many questions regarding the safety of the supplement (Henderson et al., 2002; Izzo, 2004).

Evidence suggests St. John's Wort can potently impact cytochrome 3A4-mediated drug metabolism. CYP3A4 is involved in the metabolism of a broad spectrum of drugs and xenobiotics; therefore, the possibility of interaction with individual 3A4 substrates becomes a relevant issue for further study.

Prednisolone is a well-known glucocorticoid that has been long-relied upon in the treatment of inflammation and immune-exacerbated adversities. Prednisolone is often administered orally in its prodrug form, prednisone, which has weaker pharmacological activity. Prednisone and prednisolone undergo interconversion following oral administration, and the pharmacokinetic profiles of each agent are different due to prednisolone's saturable plasma transcortin binding and nonlinear elimination at high corticosteroid doses. Prednisolone and

prednisone are substrates of CYP3A4, the pathway by which their irreversible metabolism occurs (Waxman et al., 1988).

The relative popularities of prednisolone and St. John's Wort, combined with their shared CYP3A4 linkages, present this herb-drug combination as an intriguing candidate for an interaction study. This investigation compared the single-dose pharmacokinetic profiles of prednisone and its reversible metabolite prednisolone before and after 28-day administration of St. John's Wort in humans.

Materials and Methods

Materials:

St. John's Wort (standardized with 0.3% hypericin) was obtained in capsule form from HBC Protocols®, Santa Monica, CA. Uncoated prednisone tablets were obtained via prescription by the herb-drug investigation's supervising physician from East Alabama Medical Center, Opelika, AL. Reagent-grade prednisone, prednisolone, and methylprednisolone corticosteroids were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO. The analytical column used in this investigation was purchased from Phenomenex ®, Torrance, CA. Plasma storage tubes were purchased from Starstedt ®, Newton, N.C. All other clinical and analytical supplies, including HPLC solvents, methylene chloride, heptane, and ethanol were purchased from Fisher Scientific, Pittsburg, PA.

Experimental Protocol:

All study procedures were disclosed in a study protocol, which was later reviewed and approved by the Auburn University Institutional Review Board, Human Subjects Office, Auburn University, AL.

Eight male volunteers between the ages of 19 and 36 were recruited for participation in the drug interaction study. A sample size of n=8 volunteers was chosen in an effort to balance investigational costs with statistical power. Only males were utilized in the investigation due to previously published concerns regarding St. John's Wort's effects upon oral contraception (Gorski et al., 2002; Hall et al., 2003). Mandatory informed consent was obtained from all volunteers.

Volunteers were directed to East Alabama Medical Center, Opelika, AL for pre-investigational health screenings and physical examinations. All physical examinations were performed within 21 days of the study's start date for determination of study eligibility. Physical examinations included tests of visual acuity, neurological condition, and peripheral pulses. Vital signs, weight measurements, and clinical laboratory tests (hematology, biochemistry, and urinalysis) were also conducted, along with drug screens and hepatitis B and C surface antigen tests. Volunteers were subject to exclusion from the study if:

1. They were deemed unfit to participate in the study as a result of physical examination or blood screens.
2. They were hypersensitive to any study medications or materials.

3. They used unauthorized medications during the study's duration. Volunteers were only permitted to take acetaminophen or ibuprofen within study duration.
4. They drank alcohol during study duration.
5. They donated blood within 30 days of the start of the study.

The duration of the herb-drug investigation was 29 days. On Day 1, each volunteer received a single 20 mg dose of an uncoated prednisone tablet 1 hour following a light breakfast. Blood samples were obtained by either nurse-assisted venipuncture or intravenous catheterization at -0.25 hours pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 16 hours post-dose. Catheters were filled with sterile dilute heparin and saline solutions after each blood draw. The saline/heparin solutions were used to rinse the catheters and to prevent blood clotting.

Each blood sample, approximately 7 mL, was placed into heparinized Vacutainer™ blood tubes. Collected blood was immediately placed in a refrigerated centrifuge and spun for 10 minutes at 2000 rpm for separation of plasma. Plasma samples were transferred by disposable pipet into Starstedt® plasma vials and then stored at -70˚ C until drug analysis.

Volunteers were allowed to drink water freely, and they received lunch and dinner at 4.5 hours and 12 hours post-dose, respectively. During days 2-29, volunteers were instructed by handout to self-administer 300 mg of St. John's Wort capsules 3 times a day with meals. The St. John's Wort dose used in the study has been regarded as the standard dosing regimen recommended by St.

John's Wort manufacturers (Monmaney, 1998), and has been used in similar drug interaction investigations involving St. John's Wort (Wang et al., 2001; Markowitz et al., 2003; Piscitelli et al., 2000). Volunteers were instructed to take the supplement with meals to lessen the possibility of upset stomach. Volunteers were contacted once a week to assess study compliance and side effects.

After four weeks of St. John's Wort administration, volunteers returned on Day 29 to again receive 20 mg of uncoated prednisone after a light breakfast. Blood samples were collected at -0.25 hours pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 16 hours post-dose. Saint John's Wort dosing was continued throughout the final blood-collection day, with the first SJW dose taken with the light breakfast at least 1 hour before prednisone administration.

Time Schedule	Treatment	Description
Pre-dose period	Screening/Physicals	
Day 1	Prednisone – 20 mg	Blood sampling over 16 hours
Day 2 – 29	St. John's Wort	300 mg three times daily
Day 29	Prednisone – 20 mg & St. John's Wort	Blood sampling over 16 hours

Table 5.1: Schedule of prednisone single dosing and St. John's Wort treatment.

HPLC Assay:

Corticosteroid assays were conducted using an HPLC system featuring a Waters Associates Model 519 pump and Waters Associates WISP 717

autosampler linked to a Jasko Plus UV-2075 UV/VIS detector. Plasma samples were processed and analyzed largely in accordance with a normal phase chromatography method recommended by Jusko and associates (1994). Adjustments were made in terms of solvent ratio, flow rate, and internal standard utilized. A de-gassed mobile phase consisting of 60:34.5:4.5:1 methylene chloride: heptane: ethanol: glacial acetic acid was pumped at a flow rate of 1.6 mL/min during analysis. The compounds of interest (prednisone and prednisolone) and the internal standard (methylprednisolone) were monitored at a wavelength of 253 nm and were separated on an Agilent Technologies® Zorbax SIL column (4.6 x 250 mm, 5 µm). Drug standard curves were constructed for prednisone and prednisolone between the concentrations 5 ng/mL and 200 ng/mL. The lower limit of quantitation was 5 ng/mL, and the limit of detection was below 2.5 ng/mL. Inter-day variation ranged between 5%-9% for prednisone and prednisolone. Drug retention times were 10.0, 19.4, and 22.1 minutes for prednisone, methylprednisolone, and prednisolone, respectively.

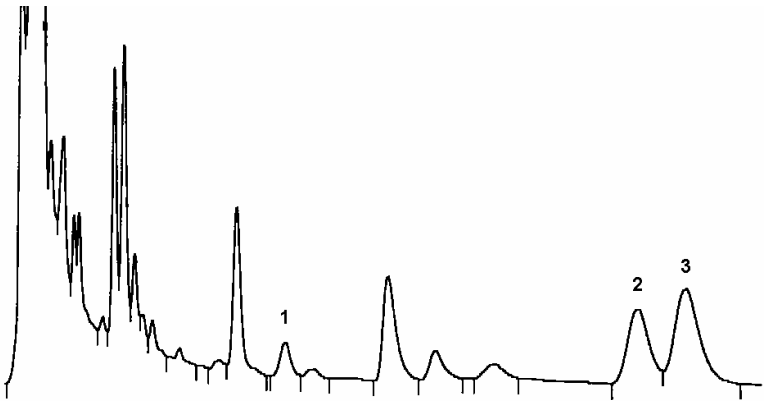


Figure 5.1: Representative chromatogram for the corticosteroid assay. Identified by number are (1) prednisone, (2) methylprednisolone and (3) prednisolone.

Extraction:

Frozen plasma samples were allowed to thaw for 30 minutes at room temperature. To each 750 µL aliquot of plasma was added 40 µL of 10 µg/mL methylprednisolone followed by 200 µL of 0.6 M H_2SO_4 acid solution for plasma protein denaturation. Each sample was briefly shaken before 7 mL of methylene chloride was added for drug extraction. Samples were then vortex-mixed for 30 seconds and centrifuged at 3500 x g for 10 minutes. After centrifugation, aqueous phases consisting of separated plasma residues were discarded to isolate organic phases. The organic phase was then washed using 1 mL of distilled water followed by brief manual mixing and centrifugation at 3500 rpm for 3 minutes. The aqueous phase was again discarded, and the remaining organic phase was placed under nitrogen gas for evaporation. Dry drug residues were reconstituted in 100 µL of mobile phase, transferred to HPLC injection vials and injected into the HPLC system at a volume of 90 µL.

Data Analysis:

Model independent methods were used to estimate pharmacokinetic parameters for both prednisolone and prednisone. Log-linear regression was performed upon the terminal portion of the concentration-time curve to determine the terminal rate constant. Plasma concentration versus time (AUC) and area under the moment curve (AUMC) were determined by the log trapezoidal rule with areas to infinity calculated using both the terminal rate constant and concentrations corresponding with the last sampling time. The mean residence time (MRT) of each agent was calculated using the ratio of AUMC to AUC. Drug

half-lives and prednisone apparent clearance (Cl_T/F) were calculated using $0.693/ K_E$ and the ratio of dose and AUC, respectively. Data generation was accomplished using Microsoft Excel® spreadsheet software, and tests of data significance were executed using Statistical Applications Software® and WINNONLIN® software. Log transformed data was evaluated by 2-way ANOVA and confidence interval testing. Confidence intervals were constructed based on a two one-sided test procedure by Schuirmann (1987).

Results and Discussion

Plasma samples collected prior to St. John's Wort administration were classified as control samples, whereas plasma samples drawn after St. John's Wort administration were classified as treatment samples. In an effort to maintain uniformity, only data obtained through 12 hours of blood sampling was utilized for pharmacokinetic profiling. Pharmacokinetic parameters and confidence interval values corresponding to prednisone and prednisolone are given in tables 5.2-5.8. The plasma concentration curves for individual volunteers and treatment means are displayed in figures 5.3-5.20. Graphical comparisons of treatment effects on selected parameters are presented in figures 5.21-5.26.

Plasma concentration profiles revealed that mean half-lives for prednisone control samples and St. John's Wort treatment samples were 3.85 +/- 1.49 hrs and 5.10 +/- 2.77 hrs, respectively. Prednisone control samples and treatment samples revealed mean AUCs of 116 +/- 39.5 µg*hr/L and 129 +/- 32.7 µg*hr/L, respectively. Furthermore, prednisone control sample and treatment sample

apparent clearances were 2.48 +/- 0.532 L/hr/kg and 2.21 +/- 0.499 L/hr/kg, respectively. Mean Vd_{area}/F were 185 +/- 70.6 mL/kg and 211 +/- 111 mL/kg for control samples and treatment samples, respectively. Prednisone Cmax values were 15.4 +/- 4.19 µg/L and 15.1+/- 3.41 µg/L for respective control samples and treatment samples. Average mean residence times for prednisone were 6.44 +/- 0.920 hrs and 8.56 +/- 3.88 hrs for control samples and treatment samples, respectively.

For prednisolone, mean terminal half-lives of 2.97 +/- 0.590 hrs and 3.09 +/- 0.830 hrs were observed for control samples and St. John's Wort treatment samples, respectively. Mean drug AUC values were 714 +/- 153 µg*hr/L and 701 +/- 89.7 µg*hr/L, respectively, for prednisolone control samples and treatment samples. Prednisolone Cmax values were 128 +/- 25.3 µg/L and 129 +/- 23.7 µg/L for corresponding control samples and St. John's Wort treatment samples, while the average mean residence times were 5.03 +/- 0.880 hrs and 5.23 +/- 1.23 hrs for respective prednisolone control samples and treatment samples. Mean total drug clearances and Vd_{area}/F values were not obtained for prednisolone. The average ratios of prednisolone to prednisone were 6.40 +/- 0.970 and 5.64 +/- 1.15 before and after St. John's Wort co-administration, respectively.

Prednisolone and prednisone log-parameter data evaluations revealed no significant ($p > 0.05$) differences in prednisone or prednisolone pharmacokinetic parameters after 4 weeks of St. John's Wort dosing. No significant difference in prednisolone to prednisone AUC ratio was seen between treatments. However,

a within-volunteer effect in prednisone Ln (AUC_{0-12}) was statistically significant (p= 0.04). The term "within-volunteer" refers to a random effect which may be observed in a longitudinal study in which the subjects act as their own control subjects (Rao, 1998). This random effect is in contrast to the fixed treatment effect of St. John's Wort usage. A trend towards within-volunteer effects in Ln (Cmax) for both prednisone (p= 0.06) and prednisolone (p= 0.07) were also observed, although these differences failed to reach statistical significance.

Confidence interval testing of pharmacokinetic parameters for prednisone and prednisolone also confirmed a lack of herbal effect upon prednisone and prednisolone pharmacokinetics following oral prednisone administration. Overlap of 90% confidence intervals for the ratio of geometric means upon the null hypothesis mean (ratio of 1) warrants acceptance of the null hypothesis, which states that St. John's Wort does not alter prednisone or prednisolone pharmacokinetics by more than 20%. The 90% confidence intervals of all log-transformed pharmacokinetic data from this study lie within the acceptance range of 80% -125% as proposed by Schuirmann (1987). All confidence interval test results are shown in tables 5.7 and 5.8.

Reversible metabolism of prednisone and prednisolone was considered during the analysis of data. Prednisone parameter calculations were conducted under the assumption of low dose therapy. Thus, the 20 mg prednisone dose was considered to cause limited saturation of prednisolone protein binding and nonlinear pharmacokinetics as compared to higher dose (> 50 mg) administration

(B. Frey and F. Frey, 1990). A popular interconversion scheme for prednisone and prednisolone was presented by Ferry and Wagner (1986).

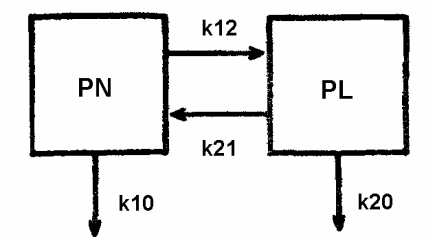


Figure 5.2: Ferry-Wagner Interconversion Scheme

Due to the difficulty involved in fully defining reversible processes without separate administration of prednisone and prednisolone, prednisolone clearance and volume of distribution values cannot be considered as traditional pharmacokinetic parameters. These parameters are best characterized with reference to prednisone dose, as was expressed in a corticosteroid investigation by Rose and colleagues (1981). Prednisolone clearance and volume of distribution are not presented in tables 5.4 and 5.5 because they are particularly complex parameters.

This investigation revealed a lack of significant change in mean pharmacokinetic parameters for both prednisone and prednisolone after long-term administration of St. John's Wort. Although St. John's Wort components have been implicated in both inductive and inhibitive metabolic processes, it was expected that this supplement would induce the metabolism of either (or both) corticosteroids considered in this investigation. The lack of inductive effect was noted in spite of the relatively long (4-week) duration of St. John's Wort treatment. This St. John's Wort dosing regimen has been shown to effectively

induce CYP3A metabolism in previous studies (Tannergren et al., 2004; Piscitelli et al., 2000; Sugimoto et al., 2001).

Volunteer compliance in the study was monitored by the retrieval of St. John's Wort tablets at the study's conclusion. Retrieved tablets represented missed doses during self administration periods. Table 6.9 details each individual's compliance. In addition, statistical power values for AUC_{0-12} for prednisone (87.2%) and prednisolone (89.8%) indicate suitable sample size. Along with the analysis of variance of log transformed parameters, the combination of power value data with confidence interval tests provides further evidence of St. John's Wort's lack of pharmacokinetic effects upon the single dose profiles of these corticosteroids.

Furthermore, pharmacokinetic parameter values such as elimination half-life and Cmax for both prednisone and prednisolone during both treatment periods are similar to parameter values encountered in previous studies (Bergrem et al., 1983; Pickup, 1979). Previously reported elimination half-lives range from 2.1 hrs to 3.5 hrs for prednisolone and range from 3.3 hrs to 3.8 hrs for prednisone. Following a 20 mg dose of prednisone, Cmax values for prednisolone have been reported between 119 µg/L and 500 µg/L, while prednisone Cmax values have been reported between 22 µg/L and 81 µg/L (Pickup, 1979). In addition, the prednisolone/ prednisone AUC ratios found during this study fit comfortably around the ratio of 6.1 reported in a previous investigation at an identical dose level (Rose et al., 1981).

In regards to intersubject variability, AUC and AUC_{0-12} values appeared more variable during pretreatment periods as opposed to results obtained after St. John's Wort treatment. The percent coefficient of variation of AUC_{0-12} for prednisone was 24.2% during control sampling period and 11.3% during St. John's Wort treatment sampling period. For prednisolone, the percent coefficient for AUC_{0-12} was 18.5% and 10.5% for control and treatment periods, respectively. The degree of intersubject variation is also visible in figures 5.21-5.24. The small number of subjects and study design does not allow this variability to be statistically evaluated. The reason for potential differences in intersubject variability between treatments is unknown. Graphical illustrations (figures 5.21-5.26) showing canceling trends further support the lack of herbal effects upon corticosteroid pharmacokinetics.

The irreversible metabolism of prednisone and prednisolone is mediated by the cytochrome P450 monooxygenase system (Waxman et al., 1988). The reversible metabolism of these agents is mediated by the 11-beta hydroxysteroid system (Escher et al., 1998). In theory, prednisone/ prednisolone reversible conversion could mask changes in individual metabolite clearances during an interaction event. An observation of no alteration in prednisone and/or prednisolone pharmacokinetics could result from such a scenario. The effect of St. John's Wort upon 11-betahydroxysteroid system function has not been studied in past investigations; therefore, it is unknown whether prednisone/prednisolone reversible metabolism can be affected by supplement use. However, it was anticipated that long-term administration of St. John's Wort

might alter the irreversible metabolism of either or both corticosteroids in this investigation via CYP 3A4.

One explanation for the apparent lack of herbal effects in this study may be related to P-glycoprotein. P-glycoprotein is a membrane-associated multidrug efflux pump found in the intestines and within peripheral blood lymphocytes (Hennessy et al., 2002). Intestinal P-glycoprotein and CYP 3A4 are believed to be closely localized and coordinately regulated in some instances (Wacher et al., 1995). The mechanism by which St. John's Wort increases P-glycoprotein expression in route to drug induction has not been determined. Neither prednisone nor prednisolone are known substrates of P-glycoprotein, in contrast with various dual CYP3A/ P-glycoprotein substrates noted for their altered metabolism during St. John's Wort administration. It is possible that this factor's lack of known influence upon prednisone or prednisolone absorption impacts the results of this investigation.

Volunteer demographics and dietary habits are also worth examination. Six of the eight volunteers who participated in this study were of Asian Indian decent. Genetic variations in cytochrome systems have been noted amongst racial groups (Shimada et al., 1994), and the underrepresentation of Asian Indians in many drug investigations may be a relevant issue. Previous reports have indicated, for example, that various ingredients of Indian food preparations may impact metabolic processes. The curry component curcumin, for example, has been studied for its chemopreventive effects and its interaction with various cellular entities, including cytochrome P450 (Leu and Maa, 2002). Another

Indian food additive, garam masala, has been found to affect cytochrome P450 along with several other hepatic enzyme systems (Singh and Rao, 1992). It was noted that higher AUC values (though minor in most instances) in both prednisone and prednisolone occurred in 5 of 6 Asian Indian volunteers (A, D, F, G, and H) after St. John's Wort treatment, whereas 2 American volunteers each displayed decreased AUC for both drugs.

Past St. John's Wort-drug interaction studies have given little attention to subject variables such as gender, race, age, and diet as they relate to herb-drug metabolism. For instance, investigations have generally involved single-gender demographics. Many investigations have featured all-male study enrollments, as seen in St. John's Wort interaction studies involving simvastatin (Sugimoto et al., 2001), omeprazole (Wang et al., 2004), and verapamil (Tannergren et al., 2004). An all-female study enrollment was used in an investigation conducted by Hall et al. (2003) involving St. John's Wort co-administration with oral contraceptive agents. Gender differences in alprazolam drug metabolism were reported in a St. John's Wort interaction study consisting of 6 men and 6 women (Markowitz et al., 2003). In addition, gender was reported to be a major determinant in human expression of cytochrome P450 3A4. CYP3A4 expression has been reported to be 2-fold greater in women compared to men (Wolbold et al., 2003). No race, age, or diet-specific pharmacokinetic data has been reported in previous St. John's Wort interaction studies.

This investigation showed no significant change in prednisone or prednisolone single dose pharmacokinetics as a result of long-term St. John's

Wort administration. Lack of parameter changes was judged by both ANOVA testing and confidence interval testing. Aside from one volunteer report of upset stomach that was attributed to taking St. John's Wort without food, no adverse effects were reported as a result of St. John's Wort administration. The impact of genetics, race, and diet upon St. John's Wort-drug interactions warrants further study.

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Table 5.2: Model independent pharmacokinetic parameters for prednisone following a 20 mg oral dose of prednisone.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
PRED (CONTROL)											
K_E (hr^{-1})	0.290	0.338	0.102	0.199	0.145	0.213	0.185	0.161	0.204	0.0771	37.8
$T_{1/2}$ (hr)	2.39	2.05	6.78	3.48	4.79	3.25	3.75	4.31	3.85	1.49	38.7
AUC_{0-12} (µg*hr/L)	102	104	127	67.5	122	107	82.4	64.1	97.0	23.5	24.2
AUC (µg*hr/L)	108	107	193	74.7	152	119	96.7	76.8	116	39.5	34.1
MRT (hr)	5.68	5.18	7.46	5.65	7.39	5.96	7.01	7.19	6.44	0.916	14.2
CL/F (L/hr/kg)	2.60	2.22	1.45	3.04	2.41	2.39	2.57	3.18	2.48	0.532	21.4
Vd area/F (mL/kg)	127	77.9	219	173	306	159	173	242	185	70.6	38.3
r^2	0.986	0.941	0.874	0.978	0.980	0.975	0.992	0.975	0.963	0.0390	4.05
Cmax (µg/L)	18.1	16.3	14.8	12.6	22.9	17.3	11.1	10.1	15.4	4.19	27.2
Tmax (hr)	3.0	2.5	2.5	1.0	1.0	2.5	4.0	1.5	2.25	1.04	46.0

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-12} = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; CL/F = apparent total body clearance after an oral dose; Vd area/F = apparent volume of distribution after an oral dose; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 5.3: Model independent pharmacokinetic parameters for prednisolone following a 20 mg oral dose of prednisone.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
PRL (CONTROL)											
K_E (hr^{-1})	0.231	0.257	0.158	0.255	0.245	0.251	0.278	0.238	0.239	0.0358	15.0
$T_{1/2}$ (hr)	3.00	2.70	4.39	2.72	2.83	2.76	2.49	2.92	2.97	0.593	19.9
AUC_{0-12} (µg*hr/L)	632	787	824	495	746	639	646	503	659	122	18.5
AUC (µg*hr/L)	686	837	982	522	790	682	676	538	714	153	21.5
MRT (hr)	5.27	4.98	7.04	4.33	4.39	4.99	4.44	4.77	5.03	0.880	17.5
r^2	0.957	0.998	0.976	0.996	0.998	0.994	0.994	0.993	0.988	0.0143	1.45
AUC L/P	6.33	7.80	5.10	6.99	5.20	5.75	6.99	7.00	6.40	0.970	15.2
Cmax (µg/L)	119	153	117	110	180	116	124	105	128	25.3	19.8
Tmax (hr)	2.5	2.0	1.5	1.0	1.0	2.5	1.0	1.5	1.63	0.641	39.4

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-12} = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; AUC L/P = ratio of the AUCs of prednisolone to prednisone; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 5.4: Model independent pharmacokinetic parameters for prednisone following a 20 mg oral dose of prednisone, combined with long-term St. John's Wort administration.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
PRED (SJW)											
K_E (hr^{-1})	0.143	0.279	0.300	0.119	0.102	0.193	0.064	0.167	0.171	0.0833	48.8
$T_{1/2}$ (hr)	4.86	2.49	2.31	5.83	6.82	3.59	10.8	4.14	5.11	2.78	54.4
AUC_{0-12} (µg*hr/L)	97.3	101	109	86.6	95.9	112	97.6	78.7	97.3	11.0	11.3
AUC (µg*hr/L)	124	107	115	118	143	131	200	92.4	129	32.7	25.4
MRT (hr)	7.50	5.05	5.21	9.39	10.6	6.86	16.9	6.93	8.56	3.88	45.3
CL/F (L/hr/kg)	2.28	2.23	2.70	1.92	2.56	2.17	1.16	2.65	2.21	0.499	22.6
Vd area/F (mL/kg)	226	95.0	140	183	462	159	225	193	211	111	52.6
r^2	0.492	0.998	0.973	0.961	0.890	0.997	0.749	0.931	0.874	0.174	19.9
Cmax (µg/L)	19.0	14.8	20.0	12.0	13.8	17.6	13.4	10.4	15.1	3.41	22.5
Tmax (hr)	3.0	1.5	2.0	2.5	3.0	4.0	3.0	1.5	2.56	0.863	33.7

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-12} = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; CL/F = total body clearance after an oral dose; Vd area = apparent volume of distribution after an oral dose; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 5.5: Model independent pharmacokinetic parameters for prednisolone following a 20 mg oral dose of prednisone, combined with long-term St. John's Wort administration.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
PRL (SJW)											
K_E (hr^{-1})	0.136	0.289	0.238	0.225	0.245	0.239	0.261	0.244	0.235	0.0442	18.8
$T_{1/2}$ (hr)	5.09	2.40	2.91	3.08	2.83	2.90	2.65	2.85	3.09	0.833	27.0
AUC_{0-12} (µg*hr/L)	625	603	724	547	606	629	751	611	637	67.2	10.5
AUC (µg*hr/L)	838	625	777	596	644	684	793	648	701	89.7	12.8
MRT (hr)	7.99	3.95	5.03	5.37	4.53	5.63	4.66	4.67	5.23	1.23	23.5
r^2	0.361	0.993	0.993	0.996	0.999	0.994	0.991	0.990	0.914	0.224	24.5
AUC L/P	6.79	5.85	6.77	5.05	4.49	5.23	3.96	7.02	5.64	1.15	20.3
Cmax (µg/L)	138	164	116	96.3	131	108	157	117	129	23.7	18.4
Tmax (hr)	2.0	0.5	1.0	2.0	1.0	3.0	1.0	1.0	1.44	0.821	57.1

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-12} = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; AUC L/P = ratio of the AUCs of prednisolone to prednisone; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 5.6: Summary of mean pharmacokinetic parameters corresponding to prednisone and prednisolone. Control treatments and St. John's Wort treatments are featured.

Parameters	PRD (CONTROL)			PRD (SJW)		
	Mean	**SD**	**% C. V.**	**Mean**	**SD**	**% C. V.**
K_E (hr^{-1})	0.204	0.0771	37.8	0.171	0.0833	48.8
$T_{1/2}$ (hr)	3.85	1.49	38.7	5.11	2.78	54.4
$AUC_{0\text{-}12}$ (µg*hr/L)	97.0	23.5	24.2	97.3	11.0	11.3
AUC (µg*hr/L)	116	39.5	34.1	129	32.7	25.4
MRT (hr)	6.44	0.916	14.2	8.56	3.88	45.3
CL/F (L/hr/kg)	2.48	0.532	21.4	2.21	0.499	22.6
Vd area/F (mL/kg)	185	70.6	38.3	211	111	52.6
r^2	0.963	0.0390	4.05	0.874	0.174	19.9
Cmax (µg/L)	15.4	4.19	27.2	15.1	3.41	22.5
Tmax (hr)	2.25	1.04	46.0	2.56	0.863	33.7

Parameters	PRL (CONTROL)			PRL (SJW)		
	Mean	**SD**	**% C. V.**	**Mean**	**SD**	**% C. V.**
K_E (hr^{-1})	0.239	0.0358	15.0	0.235	0.0442	18.8
$T_{1/2}$ (hr)	2.98	0.593	19.9	3.09	0.833	27.0
$AUC_{0\text{-}12}$ (µg*hr/L)	659	122	18.5	637	67.2	10.5
AUC (µg*hr/L)	714	153	21.5	701	89.7	12.8
MRT (hr)	5.03	0.880	17.5	5.23	1.23	23.5
r^2	0.988	0.0143	1.45	0.914	0.224	24.5
AUC L/P	6.40	0.970	15.2	5.64	1.15	20.3
Cmax (µg/L)	128	25.3	19.8	129	23.7	18.4
Tmax (hr)	1.63	0.641	39.4	1.44	0.821	57.1

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; $AUC_{0\text{-}12}$ = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; CL/F = apparent total body clearance after an oral dose; Vd area/F = apparent volume of distribution after an oral dose; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; AUC L/P = ratio of the AUCs of prednisolone to prednisone; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 5.7: Ratios and 90% confidence intervals for prednisone pharmacokinetic parameters for control and
St. John's Wort treatments.

Prednisone Parameters	***Control***		***SJW***				
	N	**LSM**	**N**	**LSM**	**Ratio**	**90% C.I.**	**P Value**
AUC_{0-12} (µg*hr/L)	8	97.0	8	97.3	1.03	0.912, 1.15	0.698
AUC (µg*hr/L)	8	116	8	129	1.14	0.888, 1.45	0.359
Cmax (µg/L)	8	15.4	8	15.1	0.990	0.845, 1.16	0.907
K_E (hr^{-1})	8	0.204	8	0.171	0.797	0.521, 1.22	0.346
MRT (hr)	8	6.44	8	8.56	1.24	0.964, 1.60	0.148

Abbreviations: AUC_{0-12} = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; Cmax = maximum plasma concentration; K_E = elimination rate constant; MRT = mean residence time

Table 5.8: Ratios and 90% confidence intervals for prednisolone pharmacokinetic parameters for control and
St. John's Wort treatments.

Prednisolone Parameters	***Control***		***SJW***				
	N	**LSM**	**N**	**LSM**	**Ratio**	**90% C.I.**	**P Value**
AUC_{0-12} (µg*hr/L)	8	659	8	637	0.977	0.874, 1.01	0.712
AUC (µg*hr/L)	8	714	8	701	0.994	0.865, 1.14	0.937
Cmax (µg/L)	8	128	8	129	1.00	0.892, 1.13	0.948
K_E (hr^{-1})	8	0.239	8	0.235	0.974	0.817, 1.16	0.357
MRT (hr)	8	5.03	8	5.23	1.03	0.879, 1.21	0.730

Abbreviations: AUC_{0-12} = area under the plasma concentration versus time curve to the 12-hour sampling point; AUC = area under the plasma concentration versus time curve; Cmax = maximum plasma concentration; K_E = elimination rate constant; MRT = mean residence time

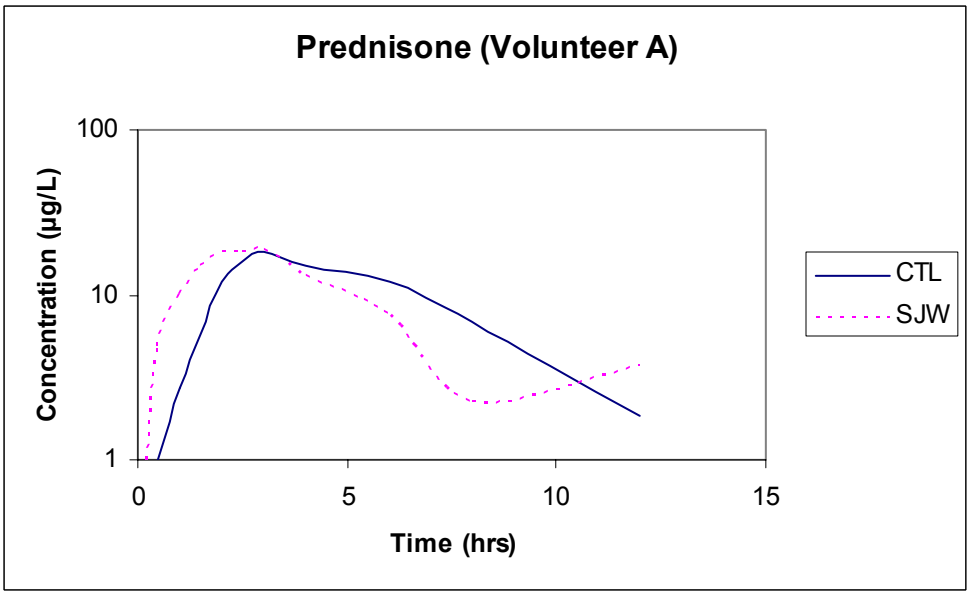

Figure 5.3: Prednisone log plasma concentrations versus time for volunteer A. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

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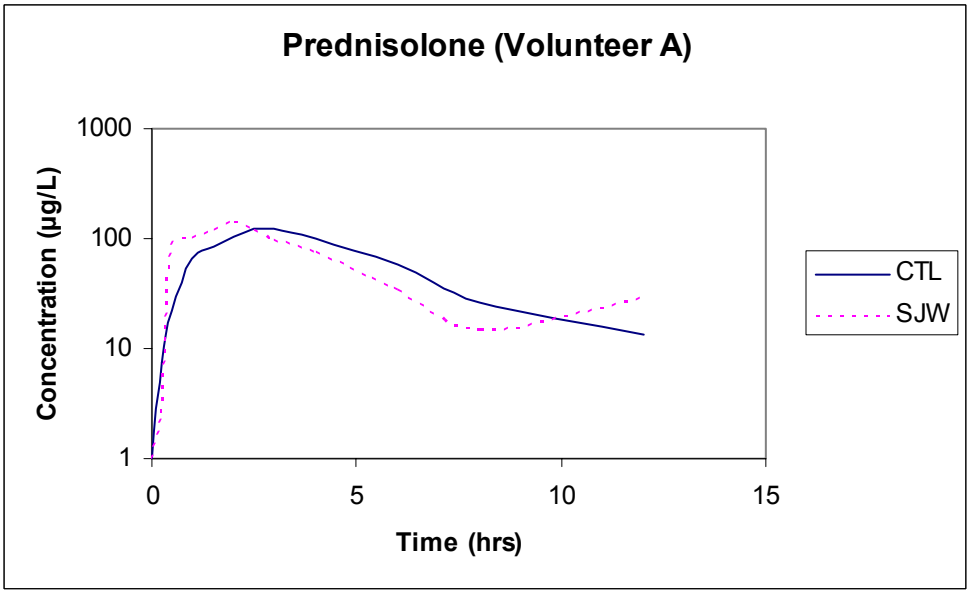

Figure 5.4: Prednisolone log plasma concentrations versus time for volunteer A. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

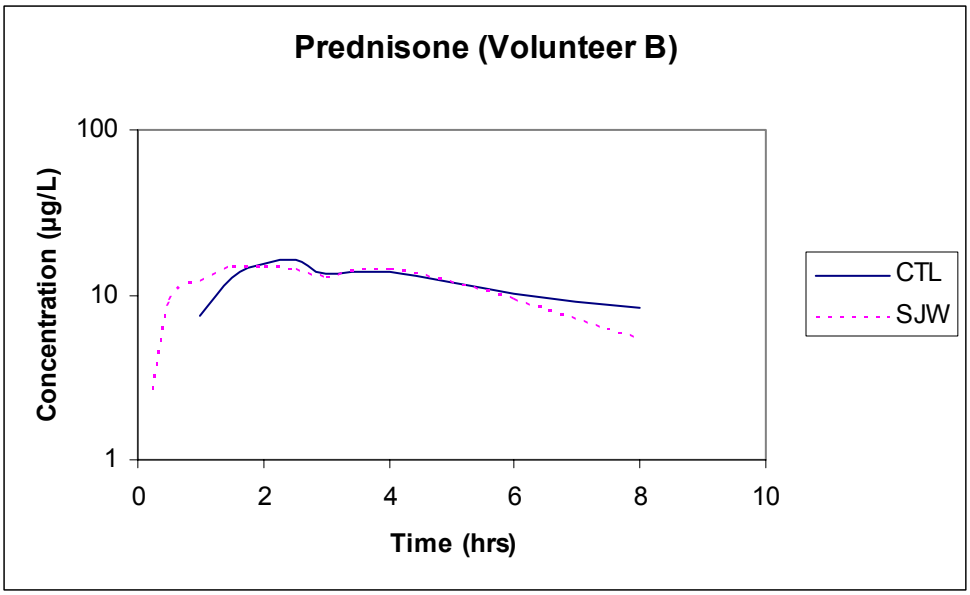

Figure 5.5: Prednisone log plasma concentrations versus time for volunteer B. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

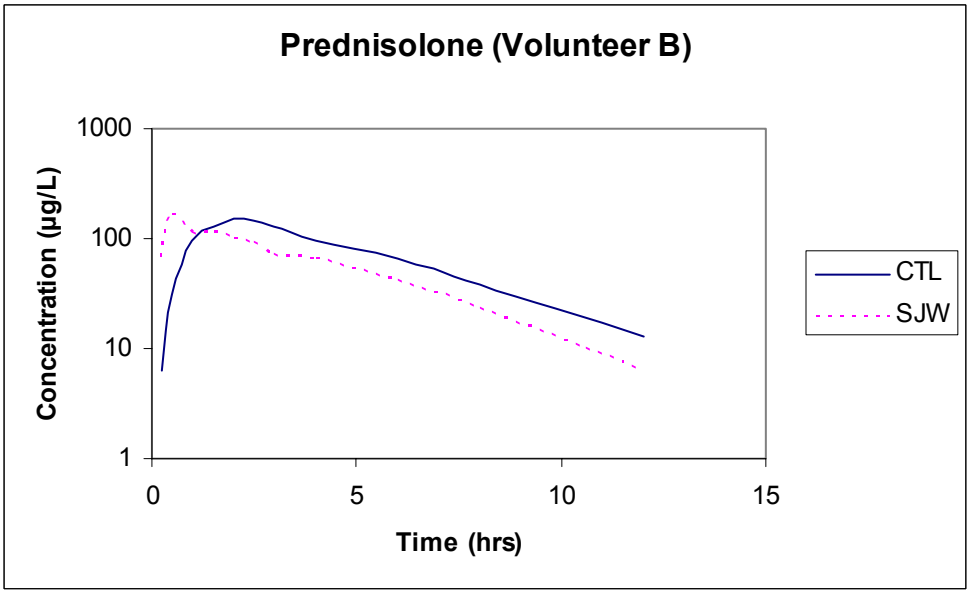

Figure 5.6: Prednisolone log plasma concentrations versus time for volunteer B. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

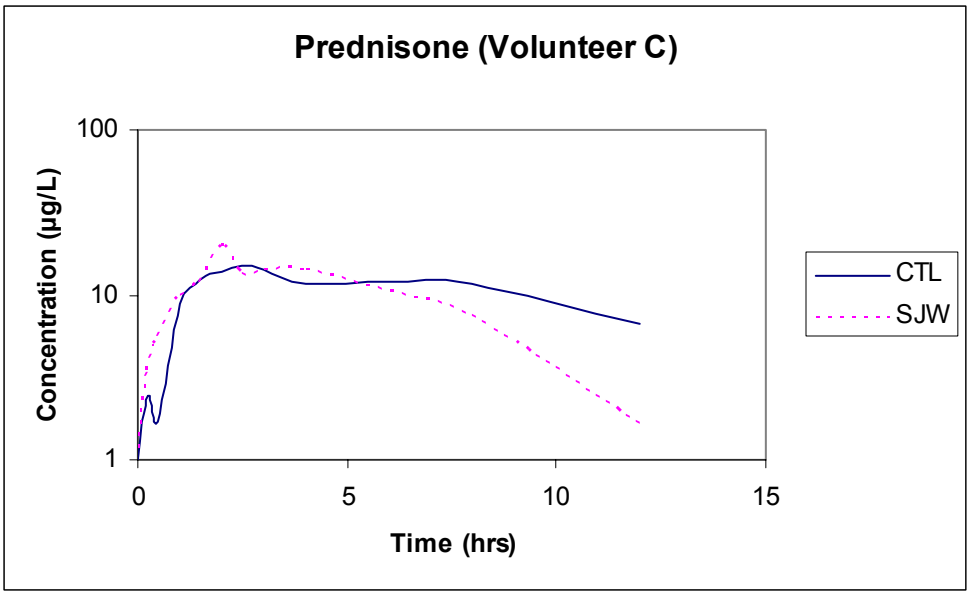

Figure 5.7: Prednisone log plasma concentrations versus time for volunteer C. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

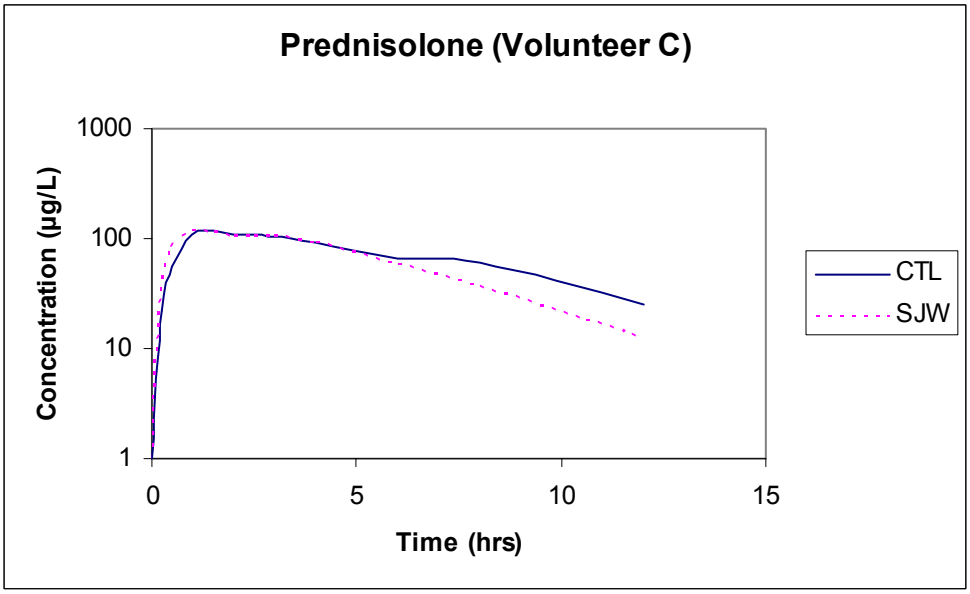

Figure 5.8: Prednisolone log plasma concentrations versus time for volunteer C. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

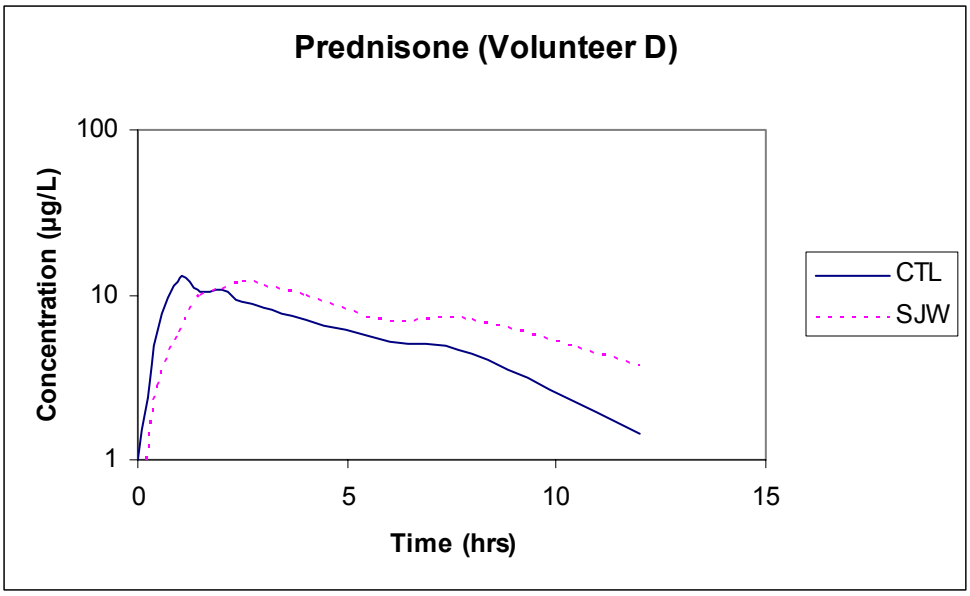

Figure 5.9: Prednisone log plasma concentrations versus time for volunteer D. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

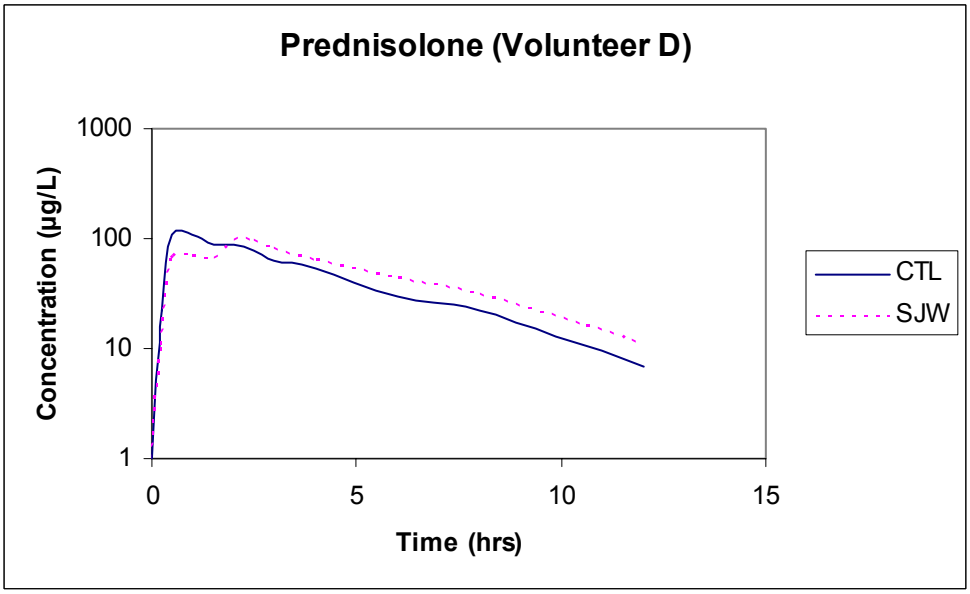

Figure 5.10: Prednisolone log plasma concentrations versus time for volunteer D. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

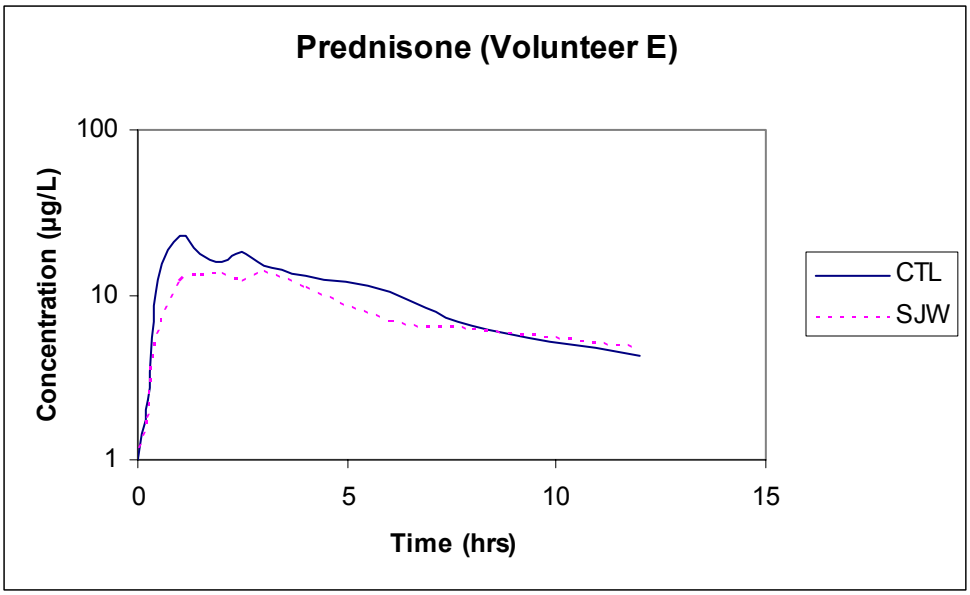

Figure 5.11: Prednisone log plasma concentrations versus time for volunteer E. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

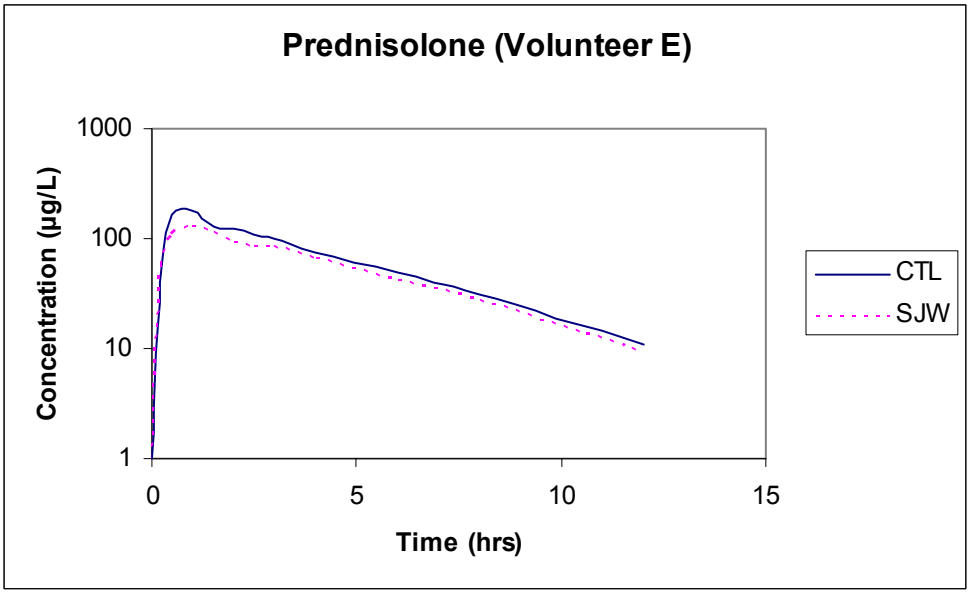

Figure 5.12: Prednisolone log plasma concentrations versus time for volunteer E. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

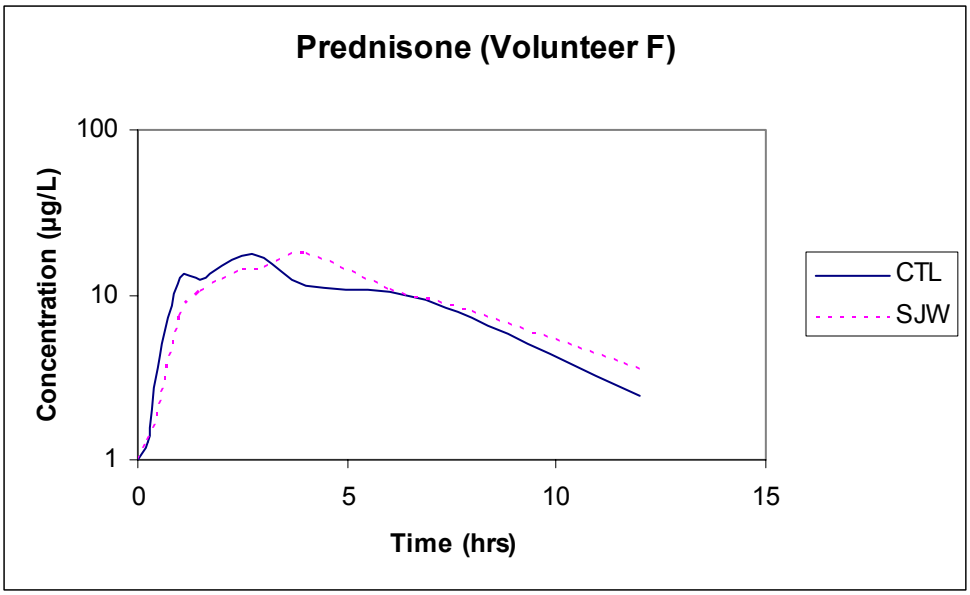

Figure 5.13: Prednisone log plasma concentrations versus time for volunteer F. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

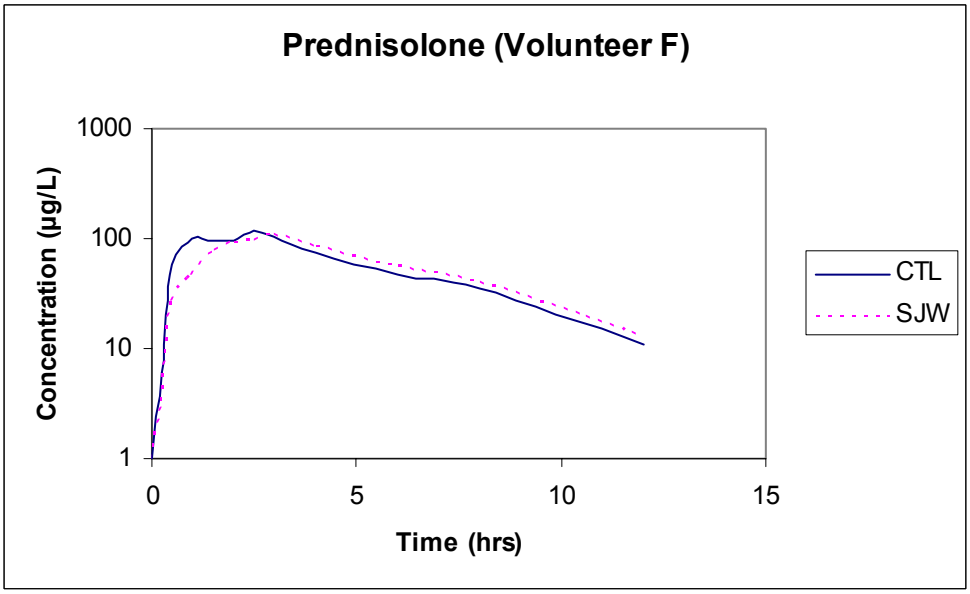

Figure 5.14: Prednisolone log plasma concentrations versus time for volunteer F. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

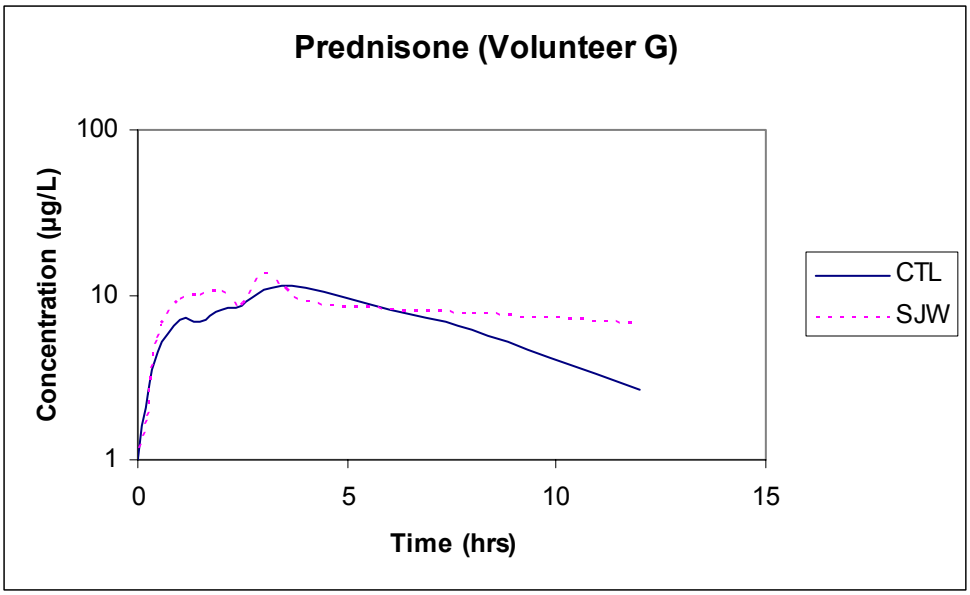

Figure 5.15: Prednisone log plasma concentrations versus time for volunteer G. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

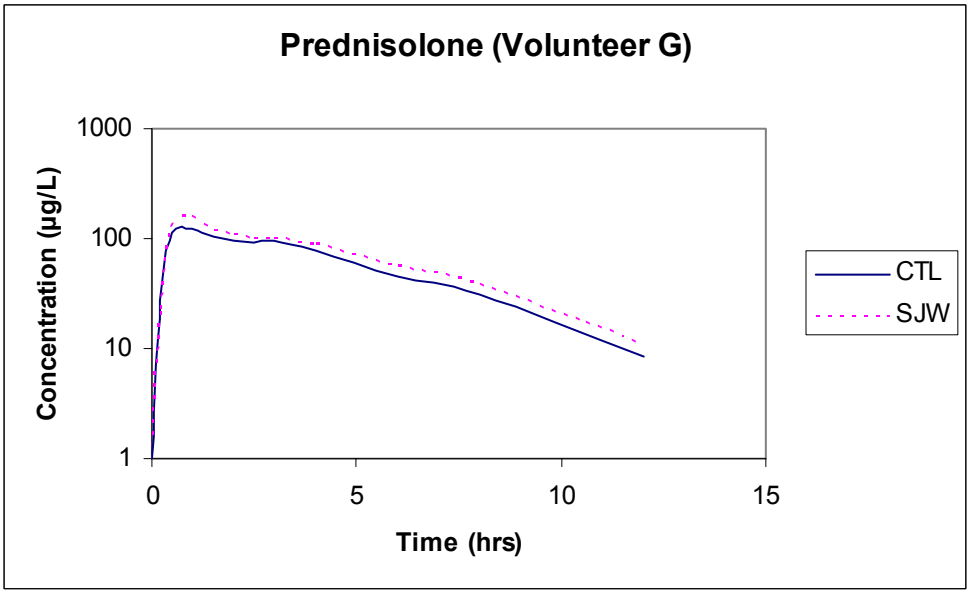

Figure 5.16: Prednisolone log plasma concentrations versus time for volunteer G. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

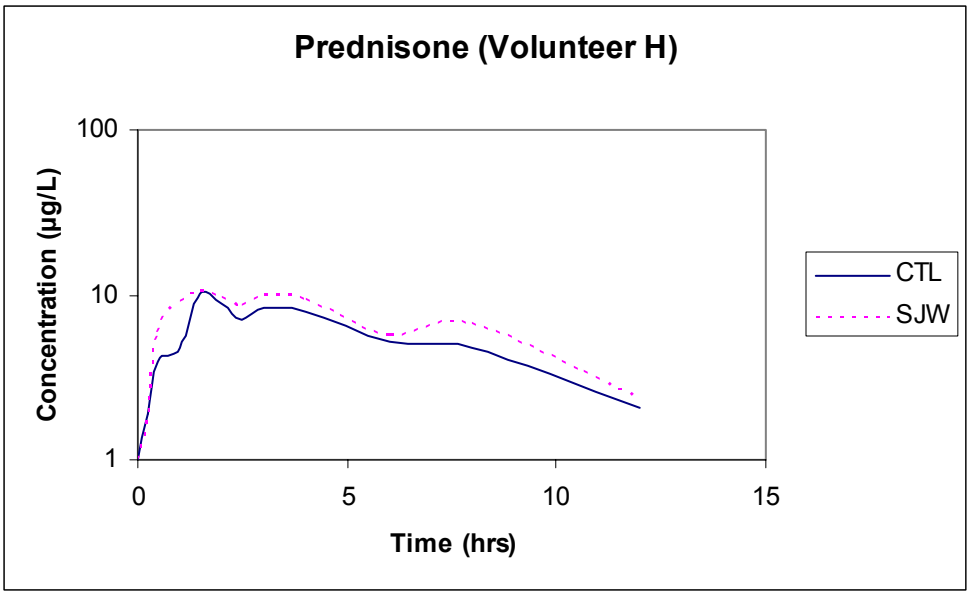

Figure 5.17: Prednisone log plasma concentrations versus time for volunteer H. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

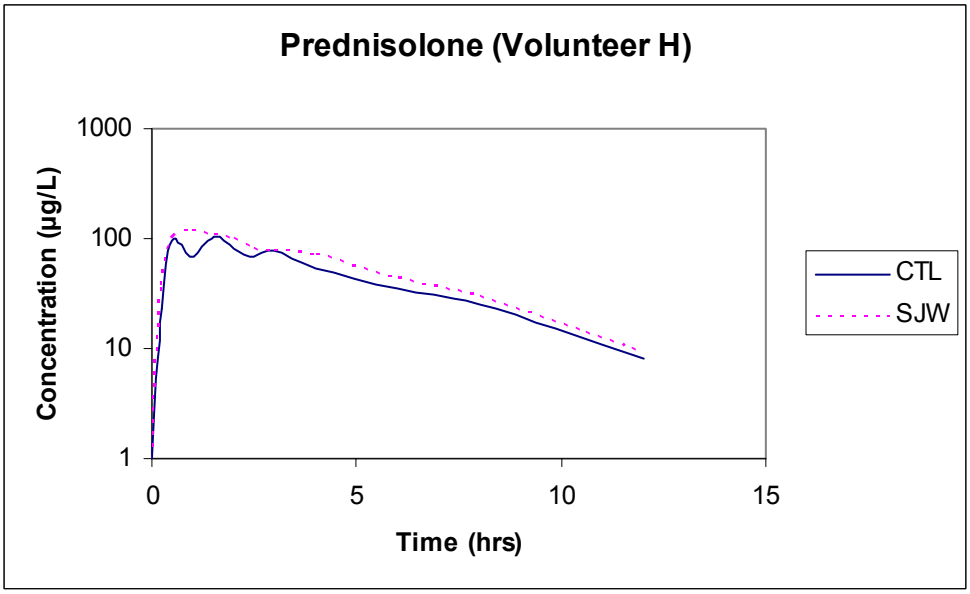

Figure 5.18: Prednisolone log plasma concentrations versus time for volunteer H. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

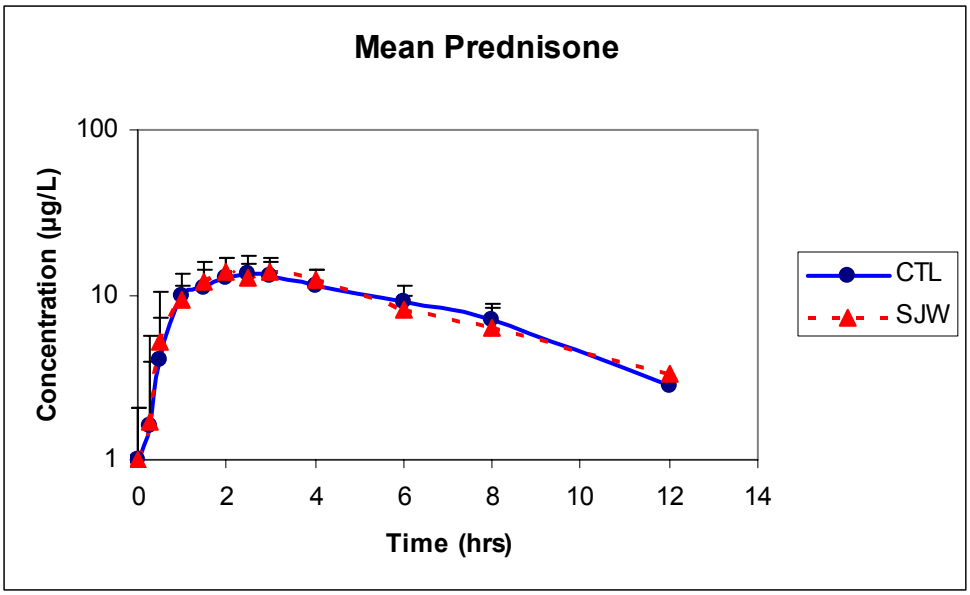

Figure 5.19: Average prednisone log plasma concentrations versus time. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

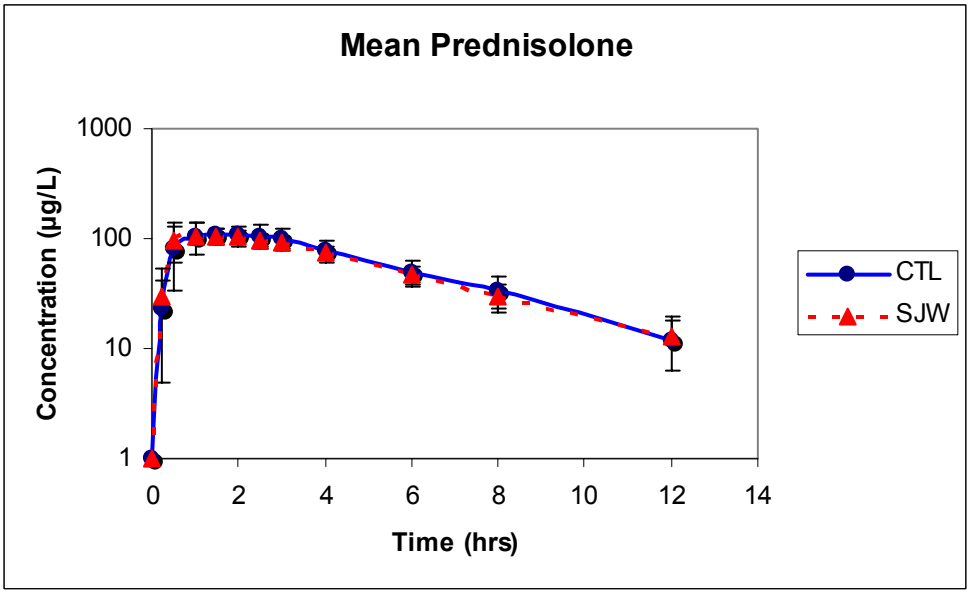

Figure 5.20: Average prednisolone log plasma concentrations versus time. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 28 days of St. John's Wort (SJW) treatment.

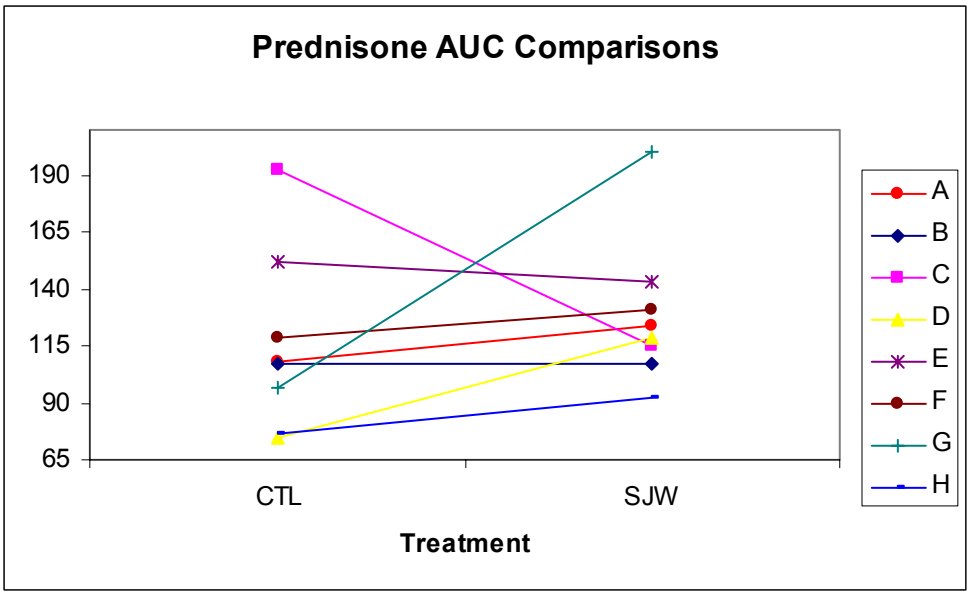

Figure 5.21: Graphical comparisons of individual prednisone AUC values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

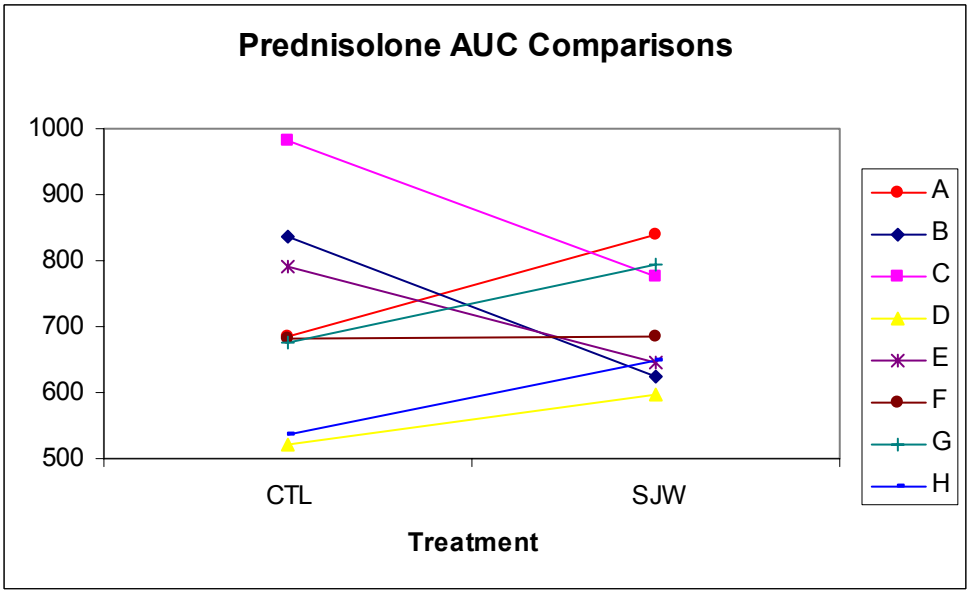

Figure 5.22: Graphical comparisons of individual prednisolone AUC values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

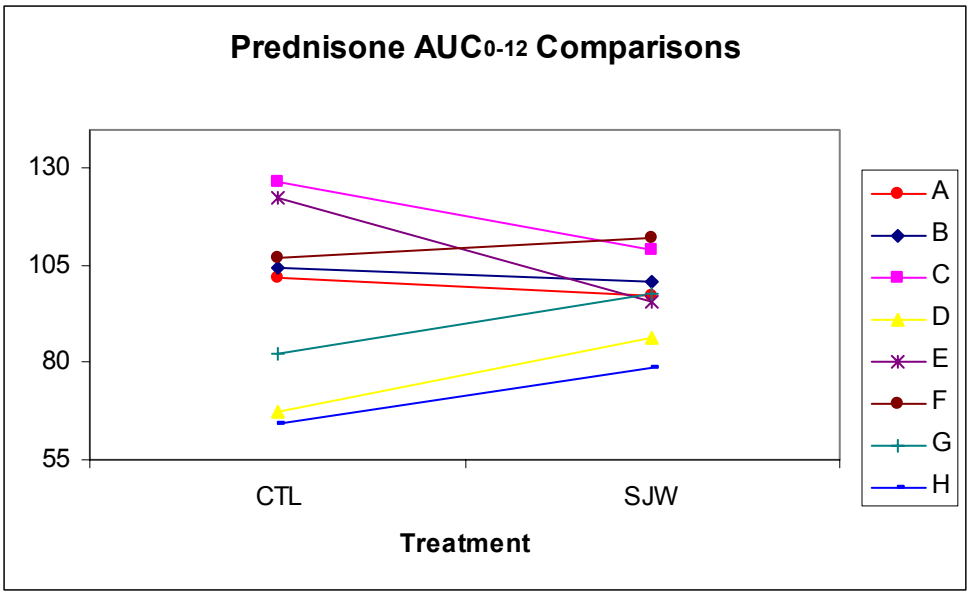

Figure 5.23: Graphical comparisons of individual prednisone AUC_{0-12} values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

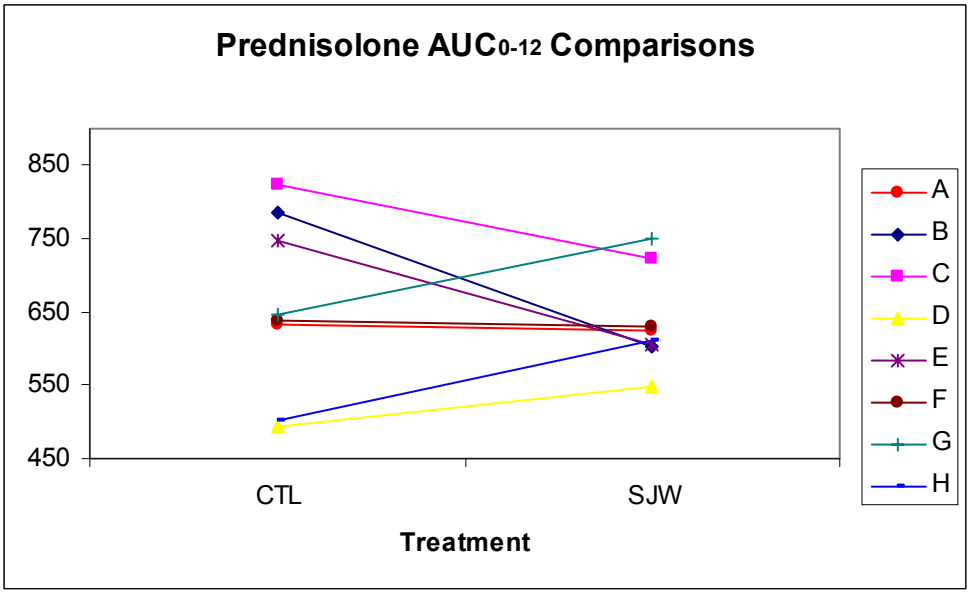

Figure 5.24: Graphical comparisons of individual prednisolone AUC_{0-12} values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

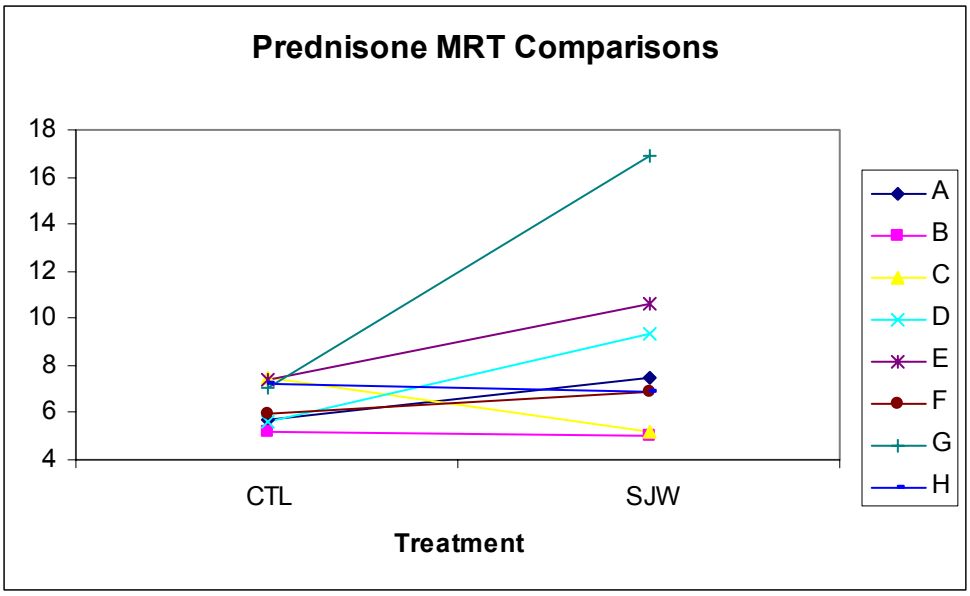

Figure 5.25: Graphical comparisons of individual prednisone MRT values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

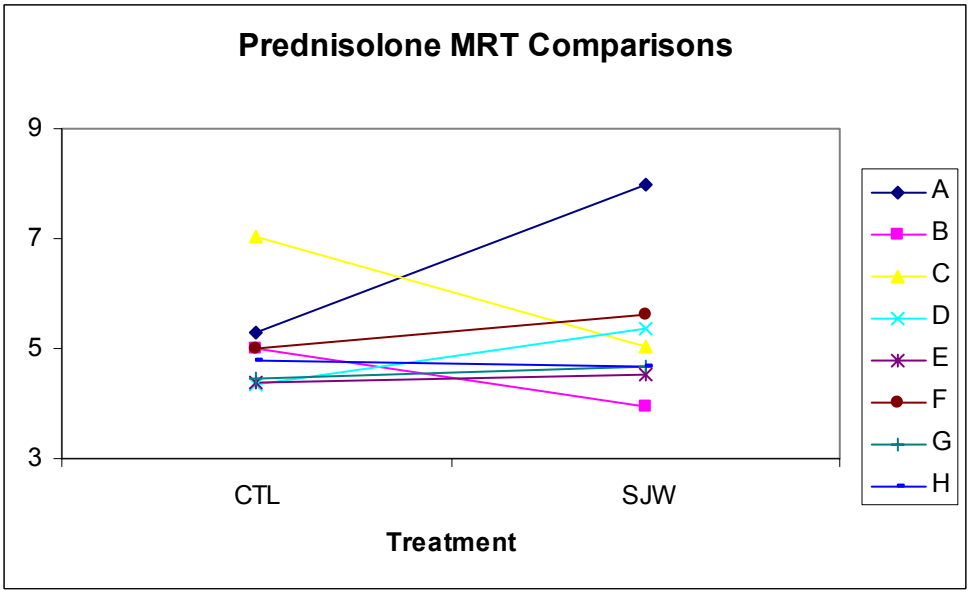

Figure 5.26: Graphical comparisons of individual prednisolone MRT values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

IBUPROFEN PHARMACOKINETICS AND ST. JOHN'S WORT SUPPLEMENTATION: A COMPARATIVE STUDY

Abstract

Purpose. The purpose of this study was to examine the effect of long-term St. John's Wort administration upon the stereoselective pharmacokinetics of the NSAID drug ibuprofen. **Methods.** Plasma ibuprofen concentrations were determined using a stereoselective, reversed phase HPLC assay. Model independent methods were used to evaluate the pharmacokinetics of each ibuprofen enantiomer. **Results.** Administration of St. John's Wort for 21 days resulted in one significant parameter difference, that of S(+)-ibuprofen mean residence time ($p= 0.02$). A near-significant difference was detected in S(+)-ibuprofen's log elimination rate constant ($p= 0.07$) and in R(-)-ibuprofen mean residence time ($p= 0.08$). S(+)-ibuprofen elimination half-lives were 3.24 +/- 1.61 hrs (mean +/- S.D.) in control samples and 2.17 +/- 0.477 hrs following St. John's Wort (SJW) treatment, while mean AUC values were 131.6 +/- 26.8 µg*hr/mL for control samples and 122.4 +/- 32.9 µg*hr/mL for SJW treatment samples. R(-)-ibuprofen elimination half-lives were 2.28 +/- 1.34 hrs and 1.55 +/- 0.641 hrs for control samples and treatment samples, respectively. R(-)-ibuprofen mean AUC values were 85.1 +/- 26.6 µg*hr/mL and 87.7 +/- 30.1 µg*hr/mL for respective control samples and SJW treatment samples. **Conclusions.** St. John's Wort administration had a significant effect upon S(+)- ibuprofen mean residence time,

but had no apparent impact upon key S(+)- and R(-)-ibuprofen pharmacokinetic parameters such as AUC and Cmax.

Introduction

The herbal supplement St. John's Wort has achieved widespread appeal for its comparable effectiveness and superior tolerability profile to tricyclic antidepressants in the treatment of mild to moderate depression. Recent case reports, animal and clinical studies have implicated St. John's Wort in a number of herb-drug interactions, raising many questions regarding the safety of the supplement.

In vitro and *in vivo* findings indicate that St. John's Wort may influence cytochrome 2C9-mediated drug metabolism (Obach, 2000; Back et al., 1998; Hargreaves et al., 1994). CYP2C9 is involved in the metabolism of several well known and widely used drug substrates, including phenytoin, tolbutamide, and warfarin (Kay et al., 1985; Zilly et al., 1975; O'Reilly et al., 1987). St. John's Wort's possible interaction with other prime CYP2C9 substrates is relevant for further study.

Ibuprofen is a stereoisomeric non-steroidal anti-inflammatory drug (NSAID) used in the management of pain, inflammation and fever. Ibuprofen is most often orally administered as a racemic mixture. Although both of ibuprofen's enantiomers possess pharmacological activity, the S-enantiomer is most responsible for drug effectiveness. The drug's R-enantiomer undergoes partial conversion to its S-enantiomer systemically, contributing to distinct

differences in each enantiomer's pharmacokinetic profile. Ibuprofen's irreversible metabolism is largely mediated by CYP2C9.

The wide spread usage of both ibuprofen and St. John's Wort, combined with their common CYP2C9 linkages, present this herb-drug combination as an intriguing candidate for study. This investigation compared the single-dose pharmacokinetic profiles of enantiomeric ibuprofen with and without the co-administration of St. John's Wort in humans.

Materials and Methods

Materials:

St. John's Wort (standardized with 0.3% hypericin) was obtained in capsule form from HBC Protocols ®, Santa Monica, CA. Advil ™ brand ibuprofen tablets were purchased over-the-counter for volunteer administration. Reagent-grade racemic ibuprofen and racemic fenoprofen were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO. The analytical column (C18) used in this study was obtained from Waters ®, Milford, MA. Plasma storage tubes were purchased from Starstedt ®, Newton, N.C. Other clinical and analytical supplies, including HPLC solvents acetonitrile and isopropanol, were purchased from Fisher Scientific, Pittsburg, PA.

Experimental Protocol:

All study procedures were disclosed in a study protocol later reviewed and approved by the Auburn University Institutional Review Board, Human Subjects Office, Auburn, AL.

Eight male volunteers were recruited for the drug interaction study. A sample size of n=8 volunteers was chosen in an effort to balance investigational costs with statistical power. All volunteers were between the ages of 19 and 36. Mandatory informed consent was obtained from all volunteers, and pre-investigational health screenings and physical examinations were conducted at East Alabama Medical Center, Opelika, AL. Physical examinations were performed within 21 days of the study's start date for determination of study eligibility. Physical examinations included tests of visual acuity, neurological condition, and peripheral pulses. In addition, vital signs, weight measurements, and clinical laboratory tests (hematology, biochemistry, and urinalysis) were conducted, along with drug screens and hepatitis B and C surface antigen tests. Volunteers were subject to exclusion from the study if:

1. They were deemed unfit to participate in the study as a result of physical examination or blood screens.
2. They were hypersensitive to any study medications or materials.
3. They used unauthorized medications during the study's duration. Volunteers were only permitted to take acetaminophen or ibuprofen within study duration.
4. They drank alcohol during study duration.
5. They donated blood within 30 days of the start of the study.

The duration of this herb-drug investigation was 22 days. On Day 1, each volunteer received a single 400 mg dose of ibuprofen following a light breakfast. Blood samples were obtained by either nurse-assisted intravenous

catheterization or venipuncture at -0.25 hours pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 16 hours post-dose. Catheters were filled with sterile, dilute heparin and saline solutions after each blood draw to rinse the catheters and to prevent blood clotting.

Approximately 7 mL of blood was collected and placed in heparinized Vacutainer™ blood tubes during each sampling period. Blood was immediately placed in a refrigerated centrifuge for 10 minutes at 2000 rpm for plasma separation. Plasma was transferred into Starstedt® plasma vials and then stored at -70˚ C until drug analysis.

Volunteers were allowed to drink water freely, and they received lunch and dinner at 4.5 hours and 12 hours post-dose, respectively. During outpatient days (2-22), volunteers were given a handout that instructed them to self-administer 300 mg of Saint John's Wort capsules 3 times a day with meals. The St. John's Wort dose used in the study has been regarded as the standard dosing regimen recommended by St. John's Wort manufacturers (Monmaney, 1998), and has been used in similar drug interaction investigations involving St. John's Wort (Wang et al., 2001; Markowitz et al., 2003; Piscitelli et al., 2000). Volunteers were instructed to take the supplement with meals to lessen the possibility of upset stomach. Volunteers were contacted weekly for assessment of study compliance and side effects.

Volunteers returned after three weeks of St. John's Wort administration to receive a 400 mg dose of ibuprofen following a light breakfast. Blood samples were again collected at -0.25 hours pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4,

6, 8, 12, and 16 hours post dose. Saint John's Wort dosing was continued during the final day of blood-collection. The first herbal dose was taken with the light breakfast at least 1 hour before ibuprofen dosing.

Time Schedule	Treatment	Description
Pre-dose period	Screening/Physicals	
Day 1	Ibuprofen – 400 mg	Blood sampling over 16 hours
Day 2 – 22	St. John's Wort	300 mg three times daily
Day 22	Ibuprofen – 400 mg & St. John's Wort	Blood sampling over 16 hours

Table 6.1: Schedule of ibuprofen single dosing and St. John's Wort treatment.

HPLC Assay:

Ibuprofen was characterized using an HPLC system featuring a Waters Associates Model 519 pump and Waters Associates WISP 717 autosampler linked to a Thermo Separations Products Spectrasystem FL3000 fluorescence detector. Plasma samples were processed and analyzed in accordance to the reversed phase chromatography method recommended by Canaparo and associates (2000). Solvent ratio, flow rate, and internal standard conditions were altered during assay development. A de-gassed mobile phase consisting of 52:48:0.1:0.02 acetonitrile: water: glacial acetic acid: triethylamine was pumped at a flow rate of 1.1 mL/min during analysis. S(+)- ibuprofen, R(-)-ibuprofen, and the internal standard (S-fenoprofen) were monitored at 280 nm excitation and

320 nm emission wavelengths. The compounds were separated on a Waters® Symmetry C18 column (4.6 x 75 mm, 3.5 µm). Drug standard curves were constructed for S(+) and R(-)-ibuprofen between the concentrations of 1 µg/mL and 30 µg/mL. The lower limit of quantitation was 1 ng/mL and the limit of detection was below 0.25 ng/mL. Inter-day variation ranged between 6%-13% for S(+)-ibuprofen and R(-)-ibuprofen. Drug retention times were 12.8 and 14.4 minutes for S(+)- and R(-)-ibuprofen, respectively.

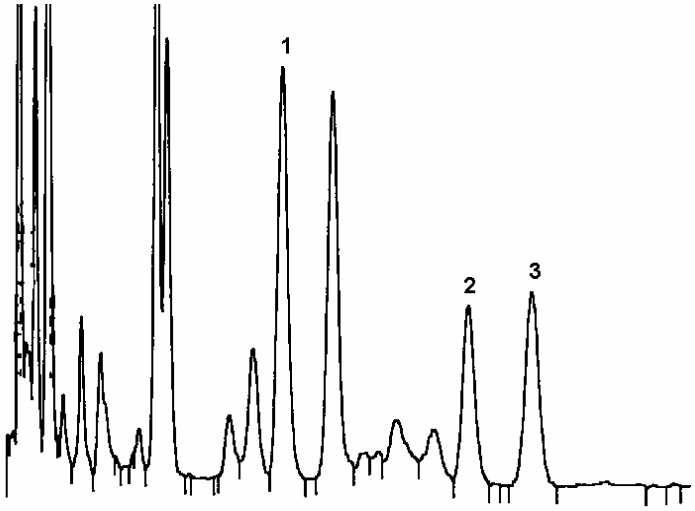

Figure 6.1: Representative chromatogram for the ibuprofen assay. Identified by number are (1) S-fenoprofen, (2) S(+)-ibuprofen and (3) R(-)-ibuprofen.

Extraction:

Frozen plasma samples were allowed to thaw for 30 minutes at room temperature. To each 500 µL aliquot of plasma was added 25 µL of 20 µg/mL fenoprofen followed by 200 µL of 0.6 M H_2SO_4 solution for plasma protein denaturation. Each sample was briefly vortexed before 3.5 mL of (30:70) ethyl

actetate: hexane was added. Samples were then vortex-mixed for 30 seconds and centrifuged at 4000 rpm for 5 minutes. After centrifugation, the isolated organic phase was transferred into a clean test tube, and remaining aqueous residues were discarded. The ethyl acetate/hexane drug solution was then placed under nitrogen gas for evaporation.

Derivatization:

Dry drug residues were reconstituted in 125 µL of 50 µM triethylamine solution in acetonitrile, followed by 50 µL of 6mM ethylchloroformate solution, also in acetonitrile. The solution was briefly vortexed and allowed to stand for 1 minute. After the minute passed, 25 µL of 0.5 M S-(alpha) phenylethylamine solution (in acetonitrile) was added. After 4 minutes, 50 µL of HPLC grade water was added to halt the reaction. The resultant solution was transferred to HPLC injection vials and injected into the HPLC system at a volume of 60 µL.

Data Analysis:

Pharmacokinetic parameters for both S- and R- enantiomers of ibuprofen were estimated using model independent (noncompartmental) methods. The terminal rate constant (K_E) was determined using log-linear regression upon the terminal portion of the ln concentration-time curve. Area of the plasma concentration versus time curve (AUC) and area under the moment curve (AUMC) were determined by the log trapezoidal rule with areas to infinity calculated using both the terminal rate constant and concentrations corresponding with the last sampling time. The mean residence time (MRT) of each agent was calculated using the ratio of AUMC to AUC. Drug half-lives and

clearances were calculated as 0.693/ K_E and the dose/ AUC ratio, respectively. Microsoft Excel® spreadsheet software, WINNONLIN® software, and Statistical Applications Software® were utilized for data generation and assessment. Log transformed values were evaluated by 2-way ANOVA as well as confidence interval testing. Ninety percent confidence intervals were constructed based on the two one-sided test method by Schuirmann (1987).

Results and Discussion

Plasma samples collected prior to St. John's Wort administration were classified as control samples, whereas plasma samples drawn after St. John's Wort administration were classified as treatment samples. S(+)-ibuprofen and R(-)-ibuprofen pharmacokinetic parameters and confidence interval data are given in tables 6.2 through 6.8. Plasma concentration profiles for individual volunteers and treatment means are given in figures 6.2 through 6.19. Comparisons of treatment effects on selected parameters are presented in figures 6.20-6.25.

Plasma concentration profiles revealed mean AUCs for R(-)-ibuprofen control samples and St. John's Wort treatment samples were 85.1 +/- 26.6 μg*hr/mL and 87.7 +/- 30.1 μg*hr/mL, respectively. R(-)-ibuprofen average elimination half-lives were 2.28 +/- 1.34 hrs and 1.55 +/- 0.641 hrs for respective control samples and St. John's Wort treatment samples. R(-)-ibuprofen Cmax values were 28.4 +/- 8.72 μg/mL and 30.0 +/- 8.97 μg/mL, respectively, for control samples and treatment samples. R(-)-ibuprofen control samples and St. John's Wort treatment samples revealed average mean residence times of 3.76

+/- 1.71 hrs and 2.78 +/- 0.631 hrs, respectively. Apparent total drug clearances (Dose/AUC) were 35.0 +/- 12.8 mL/hr/kg for control samples and 33.5 +/- 7.94 mL/hr/kg for treatment samples for R(-)-ibuprofen. Furthermore, mean Vd_{area}/F values were 1.54 +/- 0.943 mL/kg and 0.993 +/- 0.417 mL/kg for respective R(-)-ibuprofen control samples and treatment samples.

Mean AUCs of 132 +/- 26.8 µg*hr/mL and 122 +/- 32.9 µg*hr/mL were observed for S(+)-ibuprofen control samples and St. John's Wort treatment samples, respectively. Mean elimination half-lives were 3.24 +/- 1.61 hrs for S(+)-ibuprofen control samples and 2.17 +/- 0.477 hrs for St. John's Wort treatment samples. S(+)-ibuprofen Cmax values were 31.8 +/- 7.33 µg/mL and 33.6 +/- 7.83 µg/mL for corresponding control samples and treatment samples. Average mean residence time values were 5.20 +/- 1.95 hrs and 3.60 +/- 0.322 hrs, respectively, for S(+)-ibuprofen control samples and St. John's Wort treatment samples. S(+)-ibuprofen clearance and volume of distribution values were not calculated due to R(-)-ibuprofen's extensive (52% to 69%) conversion to its S-enantiomer (Rudy et al., 1995). Full characterization of S(+)-ibuprofen would have required administration of the single enantiomer rather than the racemate mixture. The average ratio of S(+)-ibuprofen to R(-)-ibuprofen was 1.69 +/- 0.654 without St. John's Wort treatment, and was 1.49 +/- 0.496 with St. John's Wort administration.

Comparison of treatment effects by ANOVA of log transformed parameters showed no significant ($p > 0.05$) effect from St. John's Wort treatment on AUCs, Cmax, or Tmax for either S(+)- or R(-)-ibuprofen. Evaluation

of log-parameter data did reveal a statistically-significant treatment effect in log mean residence time for S(+)-ibuprofen following 3 weeks of St. John's Wort administration (p= 0.02). A notable treatment effect was also seen in the S-enantiomer's log elimination rate constant, but this difference failed to reach statistical significance (p= 0.07) by ANOVA or confidence interval testing. Log MRT of R(-)-ibuprofen exhibited a noteworthy treatment effect following long-term St. John's Wort administration (p=0.08). No significant change in the ratio of S(+)- to R(-)-ibuprofen AUC was detected after St. John's Wort treatment.

The term "within-volunteer" refers to a random effect which may be observed in a longitudinal study in which the subjects act as their own control subjects (Rao, 1998). This random effect is in contrast with the fixed treatment effect of St. John's Wort usage. Notable within-volunteer effects were observed for log AUC and log K_E for S(+)-ibuprofen. However, these differences were not significant (p= 0.08 and p= 0.09, respectively).

The application of 90% confidence interval testing of log transformed pharmacokinetic parameters provides evidence of significant changes. In this study, the mean MRT for S(+)-ibuprofen showed a statistically significant treatment effect. There was no confidence interval (0.599, 0.891) overlap upon the null hypothesis parameter mean ($\theta = 1$), as illustrated in table 6.7. This supports acceptance of the research hypothesis that St. John's Wort administration significantly alters S-enantiomer mean residence time. No overlap of confidence intervals upon null parameter means ($\theta = 1$) could be seen in S(+)-ibuprofen K_E (1.03, 1.79) and R(-)-ibuprofen MRT (0.610, 0.982) (Tables 6.7 and

6.8). However, these outcomes do not provide convincing support for null hypothesis rejection, in light of discrepant p-value data corresponding to these parameters (p= 0.08; p= 0.09).

Evaluation of ibuprofen pharmacokinetics revealed no significant treatment effects upon major pharmacokinetic parameters of S- and R-ibuprofen after St. John's Wort treatment. Both ANOVA evaluations and confidence interval testing showed that 3 weeks of St. John's Wort administration did not change the AUC or Cmax values of either ibuprofen stereoisomer. However, the average mean residence time of S(+)-ibuprofen significantly decreased from 5.20 hrs (control) to 3.60 hrs (treatment). The statistically significant lowering of S-enantiomer mean residence time after herbal supplementation is possibly indicative of cytochrome 2C9 induction by St. John's Wort. Near-significant changes in S-enantiomer half-life and R-enantiomer MRT (weakly) support the idea of drug induction. In spite of this result, this study primarily showed an apparent lack of herbal treatment effects upon ibuprofen disposition.

A decrease in mean residence time of S(+)-ibuprofen following St. John's Wort treatment could be due to an increased clearance of this enantiomer, a faster absorption of either S- or R-enantiomer, a decreased volume of distribution of either enantiomer, a faster conversion of R- to S-enantiomer, and/or a decrease in the mean residence time of the R-enantiomer. R-Ibuprofen mean residence time did show a trend (p=0.08) towards decreased times following treatment (3.76 +/- 1.71 hr vs. 2.78 +/- 0.631 hr). Offsetting alterations in the clearance, absorption, or conversion of either enantiomer could appear to cancel

any observable effects of St. John's Wort administration upon the AUC of ibuprofen enantiomers and their ratio.

As seen in mean plasma concentration versus time graphs (figure 6.18 and 6.19), plasma concentrations of both ibuprofen enantiomers appeared superimposible up to 12 hours after drug administration. Key ibuprofen pharmacokinetic parameters for both enantiomers were largely unchanged following long-term St. John's Wort treatment. Lack of a consistent change or trend in ibuprofen AUC and Cmax values following treatment is supported by the observation that ibuprofen pharmacokinetic parameters changed in opposing directions among various individuals. For instance, S(+)-ibuprofen AUC values for volunteer B decreased from 179 μg*hr/mL to 118 μg*hr/mL after three weeks of St. John's Wort supplementation, while S(+)-ibuprofen AUC values corresponding to volunteer C increased from 121 μg*hr/mL to 182 μg*hr/mL after supplementation. This canceling effect can also be seen in other parameters across volunteers, including (but not limited to) R-enantiomer values for AUC_{0-16} and mean residence time (Figures 6.23 and 6.25).

Statistical power values corresponding to AUC_{0-16} were low for S- and R-ibuprofen (40.3% and 22.0%, respectively). The low power of this study may be a reflection of the small sample size used. Closer examination of the 90% confidence interval values for the two near-significant parameters, S(+)-ibuprofen K_E and R(-)-ibuprofen MRT, suggests these parameters may have reached clinical significance provided a larger sampling pool was utilized. The confidence interval measurements for both parameters do not include null hypothesis

parameter means. In theory, expansion of sample size would be accompanied by contraction of interval width at an identical significance level (10%). The confidence interval boundaries would shift farther from the null means and in turn strengthen the argument of a significant herbal effect (Rao, 1998). However, based on results of ANOVA, confidence interval testing, and trends, it is unlikely that an increase in sample size would show significant effects of St. John Wort treatment upon AUC and Cmax of S- and R-ibuprofen.

These results are in contrast to other *in vitro* and *in vivo* investigations. For instance, St. John's Wort components biapigenin, hypericin, and hyperforin altered cytochrome 2C9 function (Obach 2000), and St. John's Wort intact supplement altered the metabolism of warfarin (Ernst, 1999). Ibuprofen's irreversible metabolism is largely mediated by CYP2C9 (Leeman et al., 1993). In addition, R(-)-ibuprofen metabolism has been reported to be strongly influenced by CYP2C8 and genetic polymorphism (Kirchheiner et al., 2002). Lack of supplementation effect is not likely to be associated with duration of treatment. Volunteers were instructed to self-administer St. John's Wort for 21 days, and volunteer compliance was monitored throughout the study period. Failure of St. John's Wort to induce or inhibit cytochrome 2C9 is a plausible explanation for the observed lack of effect. Evidence of CYP2C9 alteration is sparse compared to the more abundant, broader spectrum CYP3A4 subfamily (Guengerich and Turvy, 1991; Chen et al., 2004).

The values for key pharmacokinetic parameters such as elimination half-life, Cmax, Tmax, and volume of distribution were all within the range of

parameter values encountered during past studies involving enantiomeric ibuprofen. Half-lives of S(+)- and R(-)-ibuprofen have been reported to range from 1.77 – 3.40 hours and 1.84 - 4.30 hours, respectively (Davies, 1998). Ratios of AUCs for S/R have been cited as ranging from 1.34 – 2.10 and are dependent upon absorption rate and saturation of ibuprofen's protein binding. Some interindividual variation was observed amongst volunteer data, although the possibility of a variation-concealed alteration of ibuprofen pharmacokinetics is unlikely for most of the parameters measured in this study. Similar variation has been reported for ibuprofen in past studies (Ragheb, 1987), confirming that the phenomenon is not uncommon in ibuprofen pharmacokinetics studies.

Despite the small sample size, there appears to be minimal significant change in the single dose pharmacokinetics of S(+)- or R(-)-ibuprofen following 3 weeks of St. John's Wort treatment. Although St. John's Wort treatment may affect the mean residence time of one or both stereoisomers, no recommendation of ibuprofen dose changes when given with St. John's Wort can be made based on the lack of treatment effects seen in the AUC and Cmax values of both enantiomers.

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Table 6.2: Model independent pharmacokinetic parameters for S(+)-ibuprofen following a 400 mg oral dose of racemate drug.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
S-IBU (CONTROL)											
K_E (hr^{-1})	0.203	0.237	0.107	0.385	0.216	0.165	0.634	0.246	0.274	0.166	60.4
T ½ (hr)	3.41	2.92	6.45	1.80	3.21	4.21	1.09	2.82	3.24	1.61	49.8
AUC_{0-16} (µg*hr/mL)	132	174	100	87.4	119	130	112	139	124	26.4	21.3
AUC (µg*hr/mL)	140	179	121	87.6	127	138	112	147	132	26.8	20.4
MRT (hr)	7.06	5.39	8.92	3.17	4.10	5.24	3.51	4.20	5.20	1.95	37.5
r^2	0.971	0.979	0.712	0.965	0.949	0.670	0.924	0.982	0.894	0.127	14.2
AUC S/R	1.19	2.87	2.46	1.56	1.13	1.56	1.05	1.69	1.69	0.654	38.7
Cmax (µg/mL)	22.4	32.7	22.9	27.3	38.2	43.5	33.8	33.9	31.9	7.33	23.0
Tmax (hr)	3.0	2.5	2.5	1.0	1.5	2.0	1.5	1.5	1.94	0.678	35.0

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-16} = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; AUC S/R = ratio of the AUCs of S(+)- and R(-)- ibuprofen; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 6.3: Model independent pharmacokinetic parameters for R(+)-ibuprofen following a 400 mg oral dose of racemate drug.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
R-IBU (CONTROL)											
K_E (hr^{-1})	0.155	0.450	0.290	0.523	0.601	0.320	0.670	0.167	0.397	0.194	48.9
T ½ (hr)	4.47	1.54	2.39	1.32	1.15	2.17	1.04	4.15	2.28	1.34	58.9
AUC_{0-16} (µg*hr/mL)	106	61.7	49.1	55.3	110	85.9	107	85.1	82.6	24.6	29.8
AUC (µg*hr/mL)	118	62.3	49.3	56.1	112	88.7	107	86.9	85.1	26.6	31.3
MRT (hr)	7.69	3.73	3.58	2.12	2.63	4.08	3.08	3.15	3.76	1.71	45.4
CL/F (mL/hr/kg)	23.9	38.2	62.8	40.4	32.8	32.0	23.1	28.1	35.2	12.8	36.3
Vd area/F (mL/kg)	2.17	1.01	3.36	0.876	1.00	1.42	0.429	2.06	1.54	0.943	61.2
r^2	0.993	0.999	0.953	1.00	0.981	0.0280	0.993	0.453	0.800	0.364	45.5
Cmax (µg/mL)	22.7	16.9	20.3	27.1	42.2	38.1	32.5	27.7	28.4	8.72	30.7
Tmax (hr)	2.5	3.0	2.5	1.0	1.5	2.0	1.5	1.5	1.94	0.678	35.0

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-16} = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; CL/F = total body clearance after an oral dose; Vd area/F = apparent volume of distribution after an oral dose; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 6.4: Model independent pharmacokinetic parameters for S(+)-ibuprofen following a 400 mg oral dose of racemate drug, combined with long-term St. John's Wort administration.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
S-IBU (SJW)											
K_E (hr^{-1})	0.361	0.366	0.297	0.429	0.287	0.231	0.418	0.279	0.334	0.071	21.3
T ½ (hr)	1.92	1.89	2.33	1.61	2.41	3.00	1.66	2.49	2.17	0.477	22.0
AUC_{0-16} (µg*hr/mL)	125	116	179	63.9	99.6	130	122	126	120	32.0	26.7
AUC (µg*hr/mL)	126	118	182	64.2	103	134	122	130	122	32.9	26.9
MRT (hr)	3.75	3.21	4.15	3.24	3.46	3.90	3.58	3.54	3.60	0.322	8.94
r^2	0.975	0.984	0.999	0.985	0.983	0.966	0.999	0.953	0.980	0.0160	1.59
AUC S/R	1.20	2.25	1.36	1.11	0.880	1.67	1.29	2.17	1.49	0.496	33.3
Cmax (µg/mL)	33.9	37.5	45.1	17.8	36.6	35.0	29.3	33.4	33.6	7.83	23.3
Tmax (hr)	1.5	1.0	2.0	2.0	0.50	1.5	1.5	1.0	1.38	0.518	37.6

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-16} = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; AUC S/R = ratio of the AUCs of S(+)- and R(-)- ibuprofen; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 6.5: Model independent pharmacokinetic parameters for R(+)-ibuprofen following a 400 mg oral dose of racemate drug, combined with long-term St. John's Wort administration.

	Volunteer										
Parameters	A	B	C	D	E	F	G	H	**Mean**	**SD**	**% C.V.**
R-IBU (SJW)											
K_E (hr^{-1})	0.340	0.835	0.463	0.248	0.413	0.561	0.472	0.778	0.514	0.204	39.7
T ½ (hr)	2.04	0.830	1.50	2.80	1.68	1.23	1.47	0.891	1.55	0.641	41.2
AUC_{0-16} (µg*hr/mL)	103	52.2	134	57.3	116	80.2	91.3	59.5	86.6	29.7	34.3
AUC (µg*hr/mL)	105	52.3	134	57.9	117	80.2	95.2	60.1	87.7	30.1	34.3
MRT (hr)	3.73	1.72	3.31	2.50	2.59	2.63	3.26	2.48	2.78	0.631	22.7
CL/F (mL/hr/kg)	26.8	45.4	23.1	39.2	31.5	35.4	26.1	40.7	33.5	7.94	23.7
Vd area/F (mL/kg)	1.11	0.647	0.773	1.80	1.40	0.894	0.687	0.640	0.993	0.417	42.0
r^2	0.998	0.996	0.999	0.480	0.993	0.952	0.999	0.959	0.922	0.180	19.5
Cmax (µg/mL)	29.5	30.7	38.3	19.2	47.1	27.1	25.8	22.5	30.0	8.97	29.9
Tmax (hr)	1.5	0.50	2.0	1.0	0.5	1.5	2.0	1.0	1.25	0.598	47.8

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; AUC_{0-16} = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; CL/F = total body clearance after an oral dose; Vd area/F = apparent volume of distribution after an oral dose; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 6.6: Summary of mean pharmacokinetic parameters corresponding to S(+)-ibuprofen and R(-)-ibuprofen. Control treatments and St. John's Wort treatments are featured.

Parameters	S-IB (CONTROL)			S-IB (SJW)		
	Mean	**SD**	**% C. V.**	**Mean**	**SD**	**% C. V.**
K_E (hr^{-1})	0.274	0.166	60.4	0.334	0.0710	21.3
T½ (hr)	3.24	1.61	49.8	2.17	0.477	22.0
$AUC_{0\text{-}16}$ (µg*hr/mL)	124	26.4	21.3	120	32.0	26.7
AUC (µg*hr/mL)	132	26.8	20.4	122	32.9	26.9
MRT (hr)	5.20	1.95	37.5	3.60	0.322	8.94
r^2	0.894	0.127	14.2	0.980	0.0160	1.59
AUC S/R	1.69	0.654	38.7	1.49	0.496	33.3
Cmax (µg/mL)	31.8	7.33	23.0	33.6	7.83	23.3
Tmax (hr)	1.94	0.678	35.0	1.38	0.518	37.6

Parameters	R-IB (CONTROL)			R-IB (SJW)		
	Mean	**SD**	**% C. V.**	**Mean**	**SD**	**% C. V.**
K_E (hr^{-1})	0.397	0.194	48.9	0.514	0.204	39.7
T½ (hr)	2.28	1.342	58.9	1.55	0.641	41.2
$AUC_{0\text{-}16}$ (µg*hr/mL)	82.6	24.6	29.8	86.6	29.7	34.3
AUC (µg*hr/mL)	85.1	26.6	31.3	87.7	30.1	34.3
MRT (hr)	3.76	1.71	45.4	2.78	0.631	22.7
CL/F (mL/hr/kg)	35.2	12.8	36.3	33.5	7.94	23.7
Vd area/ F (mL/kg)	1.54	0.943	61.2	0.993	0.417	42.0
r^2	0.800	0.364	45.5	0.922	0.180	19.5
Cmax (µg/mL)	28.4	8.72	30.7	30.0	8.97	29.9
Tmax (hr)	1.94	0.678	35.0	1.25	0.598	47.8

Abbreviations: K_E = elimination rate constant; $T_{1/2}$ = half-life; $AUC_{0\text{-}16}$ = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; MRT = mean residence time; CL/F = total body clearance after an oral dose; Vd area = apparent volume of distribution after an oral dose; r^2 = correlation coefficient of log plasma concentration vs. time in terminal phase; AUC S/R = ratio of the AUCs of S(+)- and R(-)- ibuprofen; Cmax = maximum plasma concentration; Tmax = time of peak plasma concentration

Table 6.7: Ratios and 90% confidence intervals for S(+)-ibuprofen pharmacokinetic parameters for control and St. John's Wort treatments.

S-ibuprofen Parameters	***Control***		***SJW***				
	N	**LSM**	**N**	**LSM**	**Ratio**	**90% C.I.**	**P Value**
AUC_{0-16} (µg*hr/mL)	8	124	8	120	0.952	0.778, 1.16	0.654
AUC (µg*hr/mL)	8	132	8	122	0.915	0.772, 1.09	0.358
Cmax (µg/mL)	8	31.8	8	33.6	1.05	0.827, 1.33	0.713
K_E (hr^{-1})	8	0.274	8	0.334	1.36	1.03, 1.79	0.0720
MRT (hr)	8	5.20	8	3.60	0.730	0.599, 0.891	0.0200

Abbreviations: AUC_{0-16} = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; Cmax = maximum plasma concentration; K_E = elimination rate constant; MRT = mean residence time

Table 6.8: Ratios and 90% confidence intervals for R(-)-ibuprofen pharmacokinetic parameters for control and St. John's Wort treatments.

R-Ibuprofen Parameters	***Control***		***SJW***				
	N	**LSM**	**N**	**LSM**	**Ratio**	**90% C.I.**	**P Value**
AUC_{0-16} (µg*hr/mL)	8	82.6	8	86.6	1.04	0.788, 1.37	0.804
AUC (µg*hr/mL)	8	85.1	8	87.7	1.03	0.776, 1.35	0.873
Cmax (µg/mL)	8	28.4	8	30.0	1.06	0.810, 1.39	0.687
K_E (hr^{-1})	8	0.397	8	0.514	1.69	0.852, 3.34	0.190
MRT (hr)	8	3.76	8	2.78	0.774	0.610, 0.982	0.081

Abbreviations: AUC_{0-16} = area under the plasma concentration versus time curve through the 16-hour sampling point; AUC = area under the plasma concentration versus time curve; Cmax = maximum plasma concentration; K_E = elimination rate constant; MRT = mean residence time

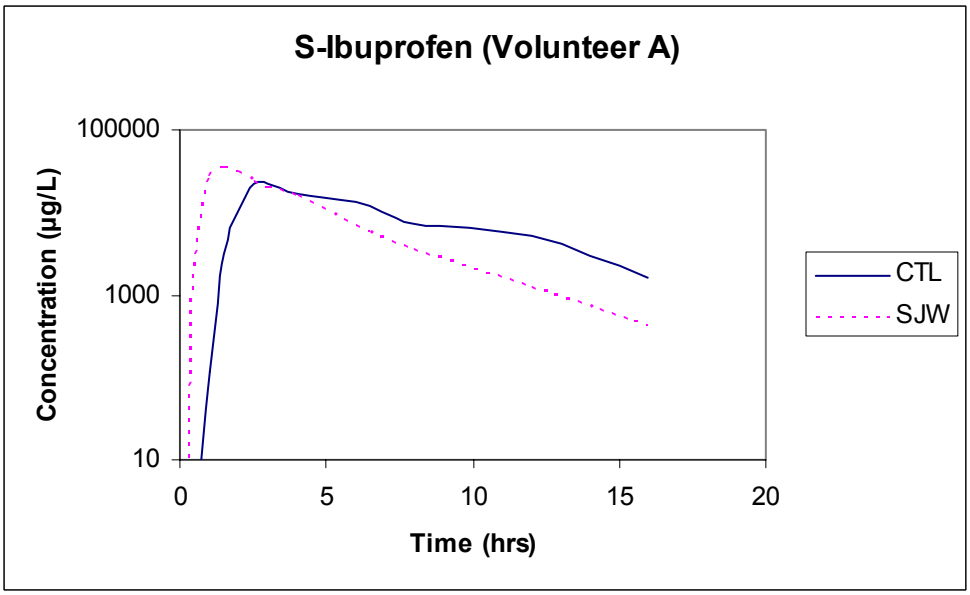

Figure 6.2: S-ibuprofen log plasma concentrations versus time for volunteer A. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

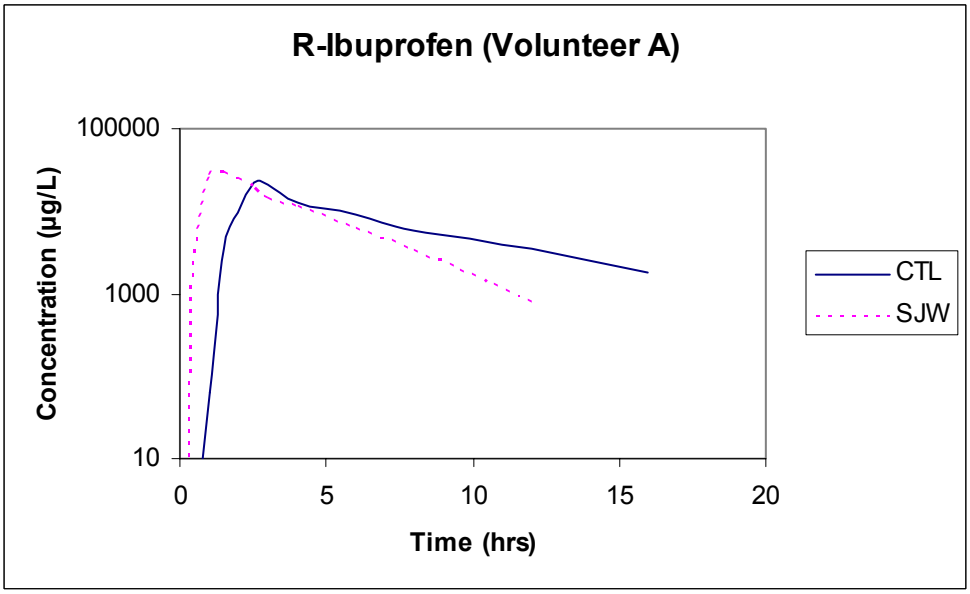

Figure 6.3: R-ibuprofen log plasma concentrations versus time for volunteer A. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

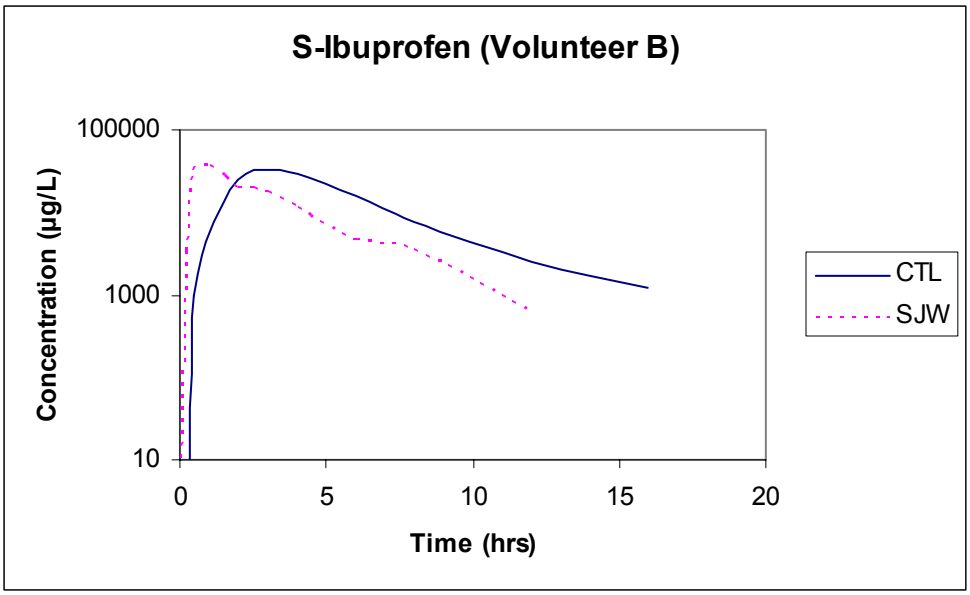

Figure 6.4: S-ibuprofen log plasma concentrations versus time for volunteer B. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

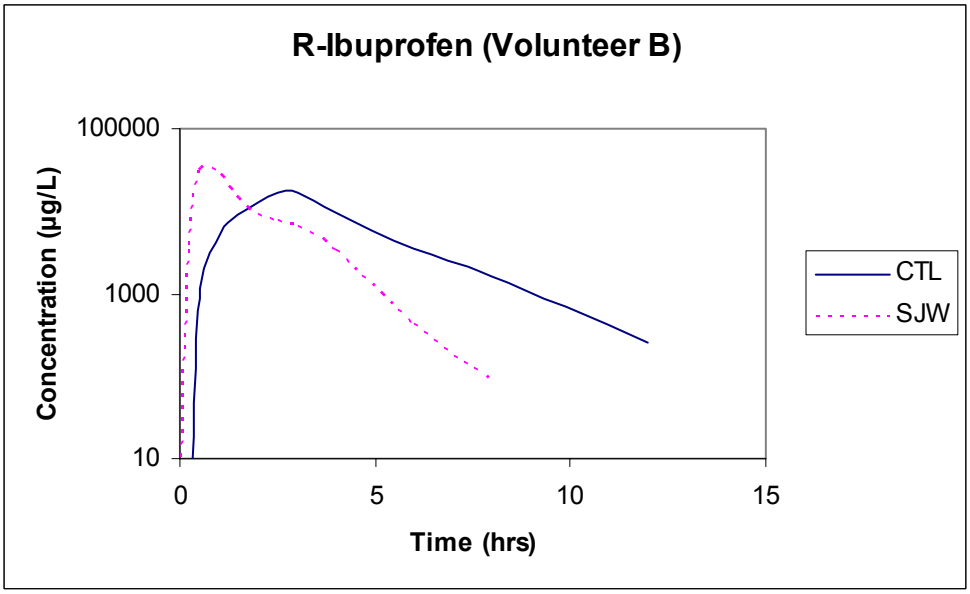

Figure 6.5: R-ibuprofen log plasma concentrations versus time for volunteer B. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

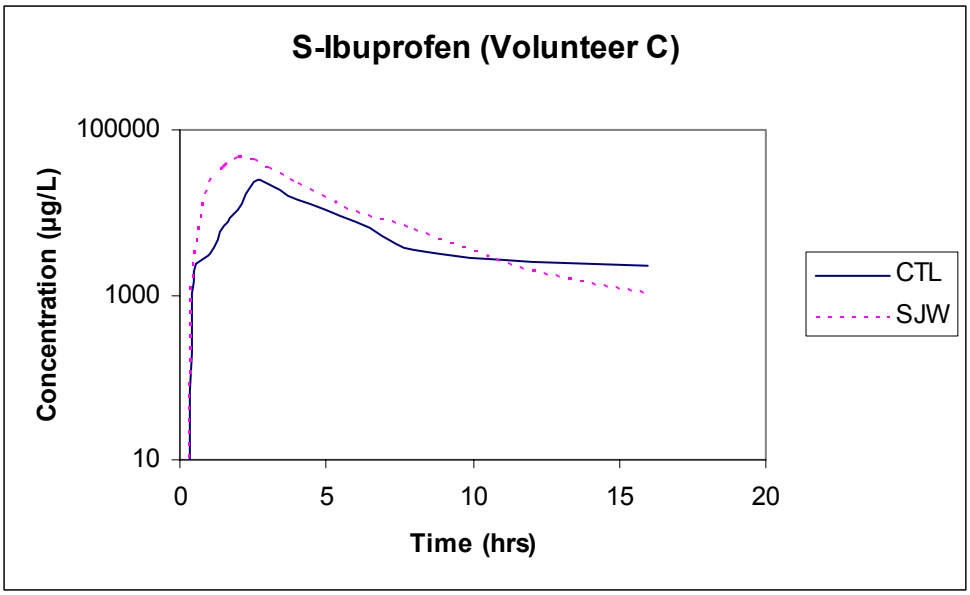

Figure 6.6: S-ibuprofen log plasma concentrations versus time for volunteer C. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

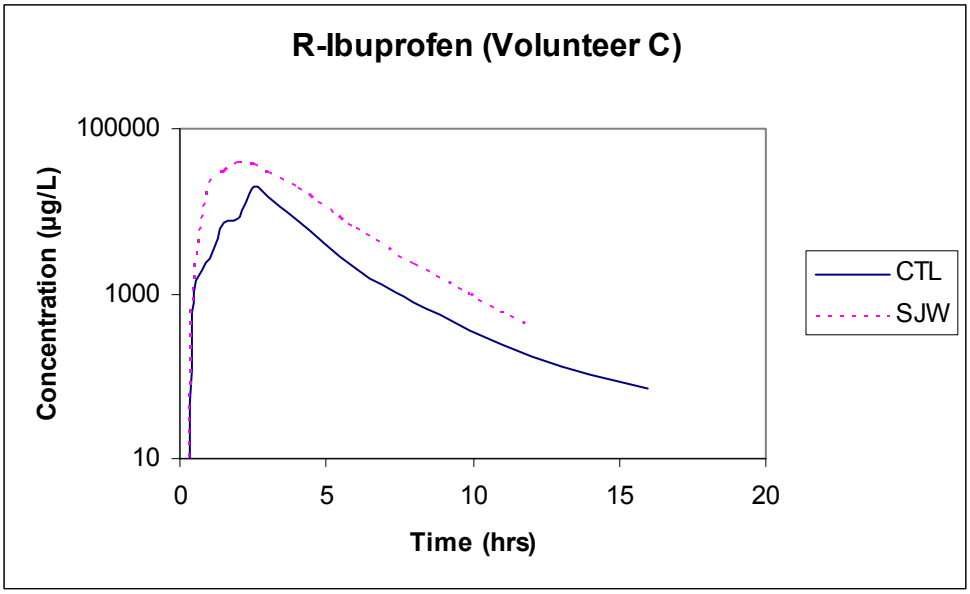

Figure 6.7: R-ibuprofen log plasma concentrations versus time for volunteer C. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

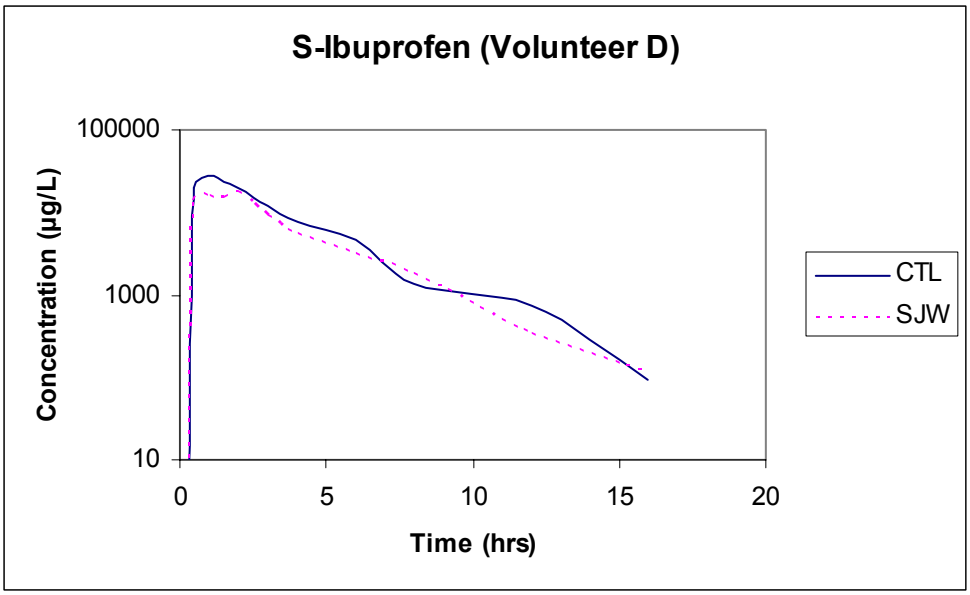

Figure 6.8: S-ibuprofen log plasma concentrations versus time for volunteer D. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

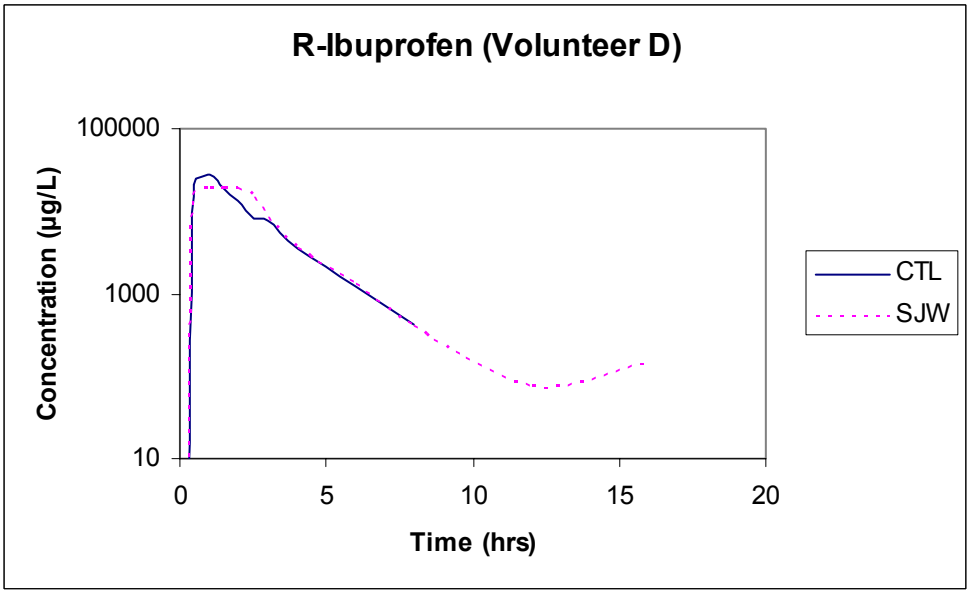

Figure 6.9: R-ibuprofen log plasma concentrations versus time for volunteer D. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

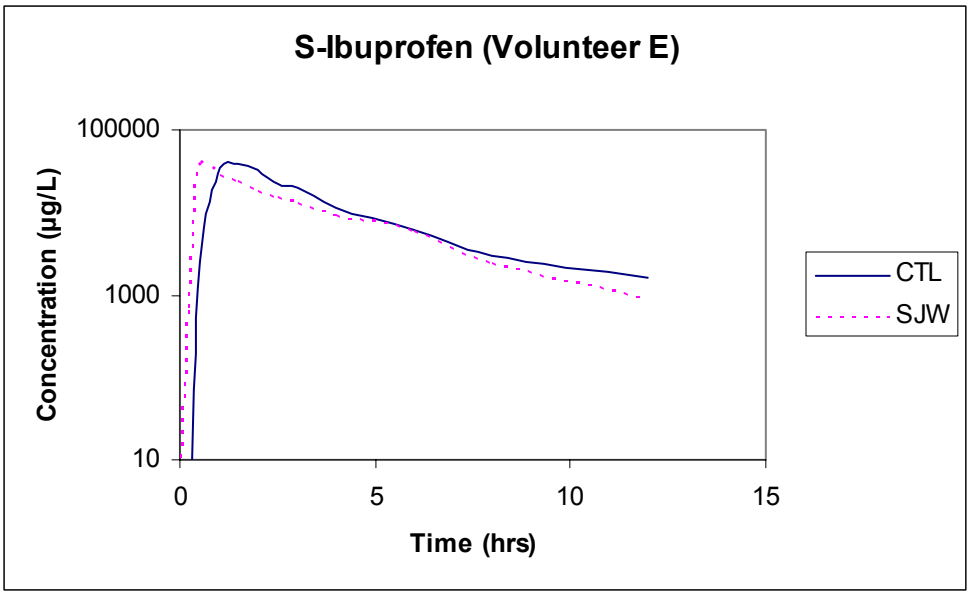

Figure 6.10: S-ibuprofen log plasma concentrations versus time for volunteer E. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

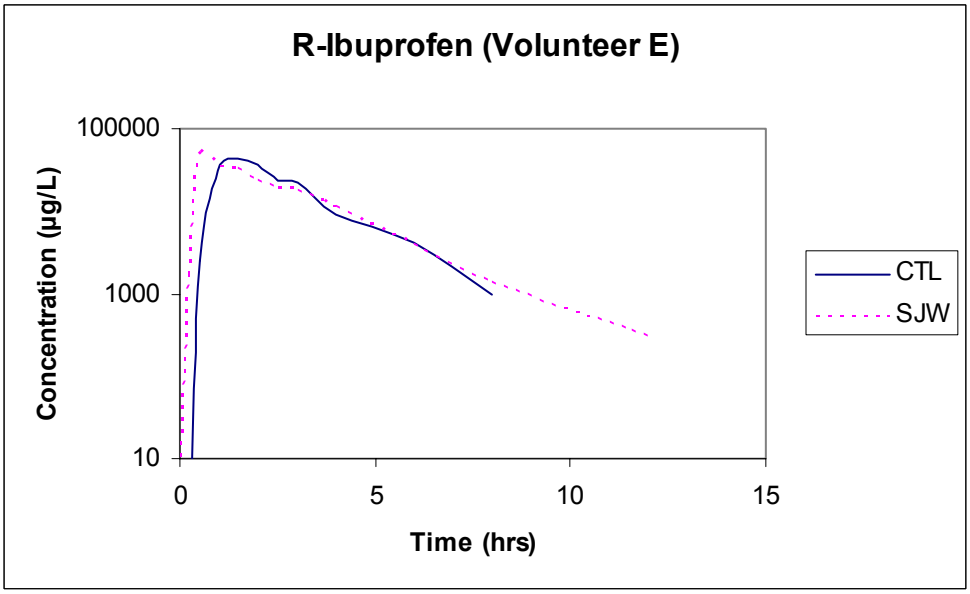

Figure 6.11: R-ibuprofen log plasma concentrations versus time for volunteer E. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

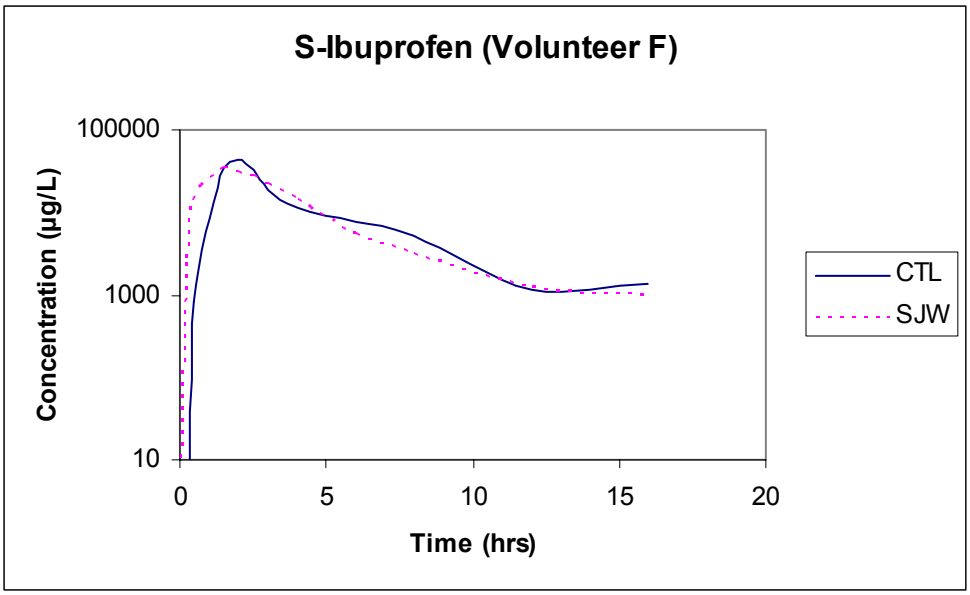

Figure 6.12: S-ibuprofen log plasma concentrations versus time for volunteer F. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

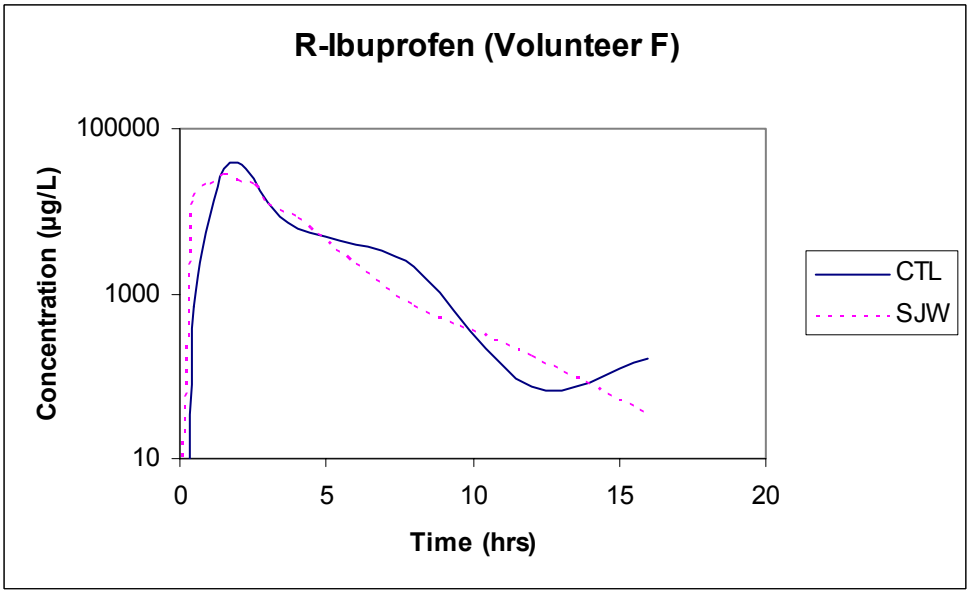

Figure 6.13: R-ibuprofen log plasma concentrations versus time for volunteer F. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

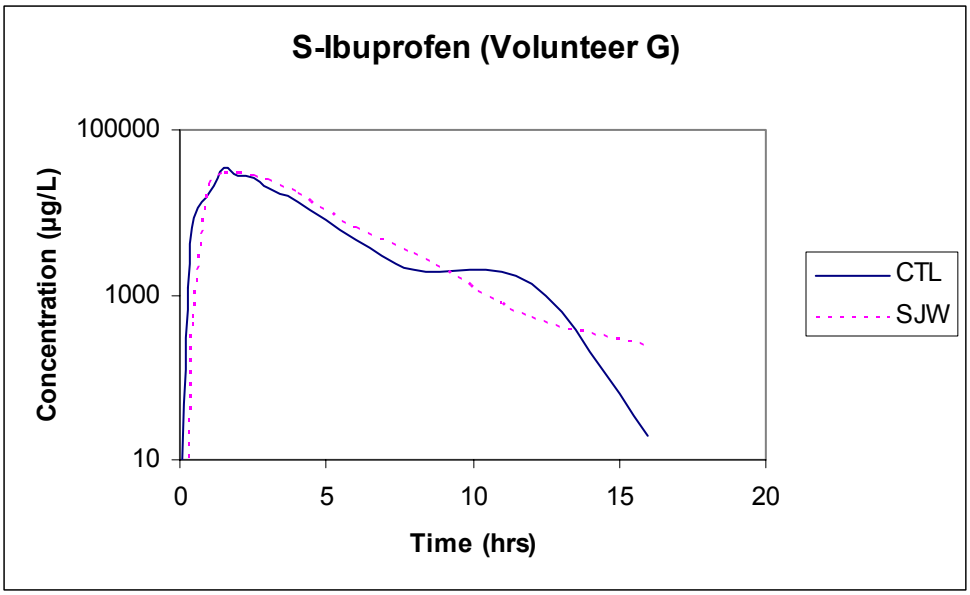

Figure 6.14: S-ibuprofen log plasma concentrations versus time for volunteer G. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

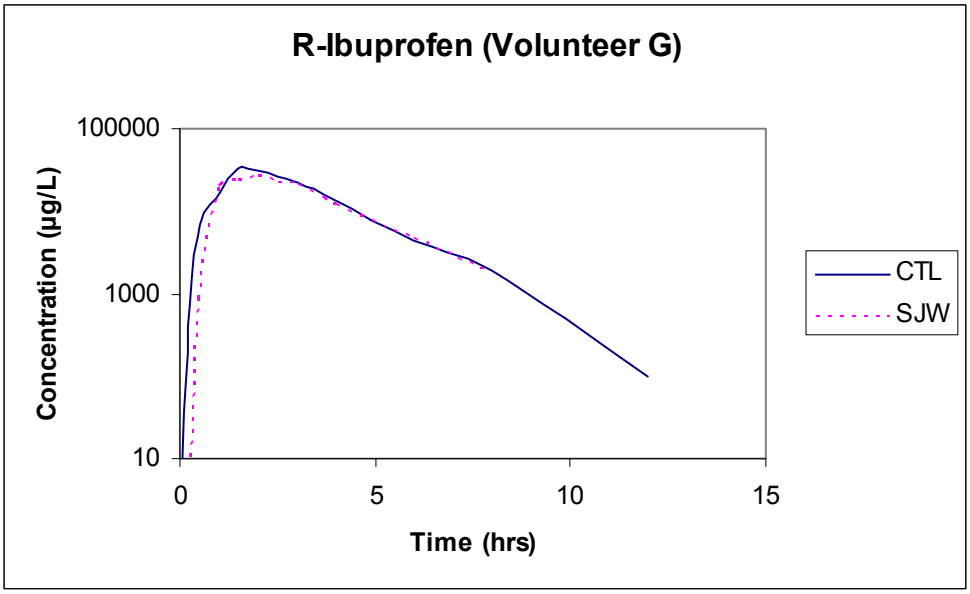

Figure 6.15: R-ibuprofen log plasma concentrations versus time for volunteer G. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

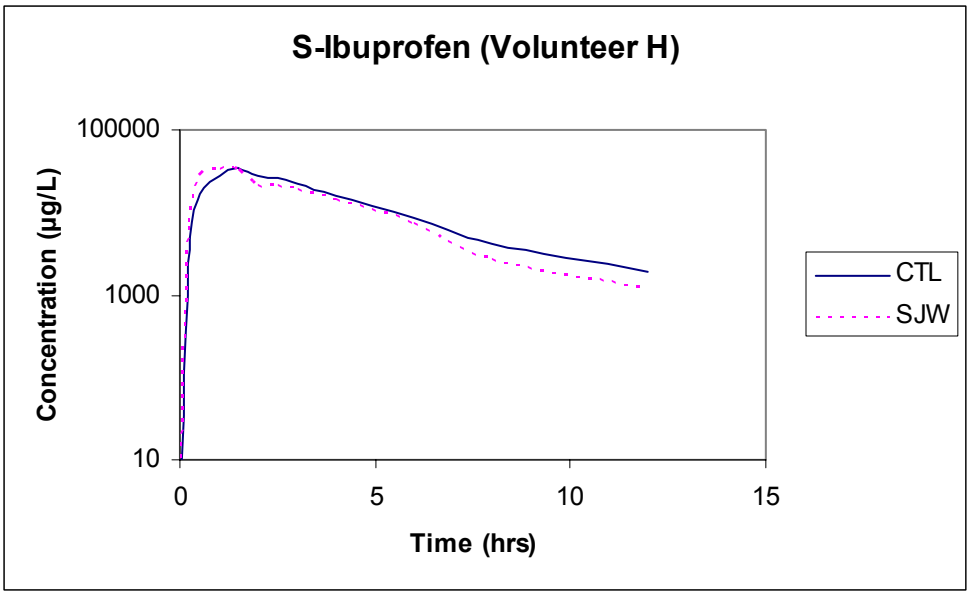

Figure 6.16: S-ibuprofen log plasma concentrations versus time for volunteer H. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

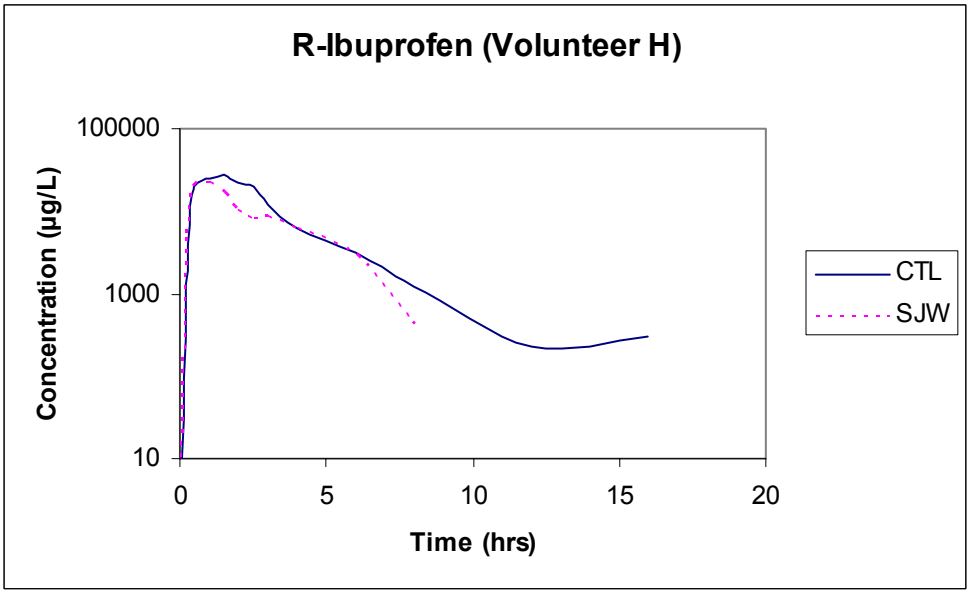

Figure 6.17: R-ibuprofen log plasma concentrations versus time for volunteer H. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

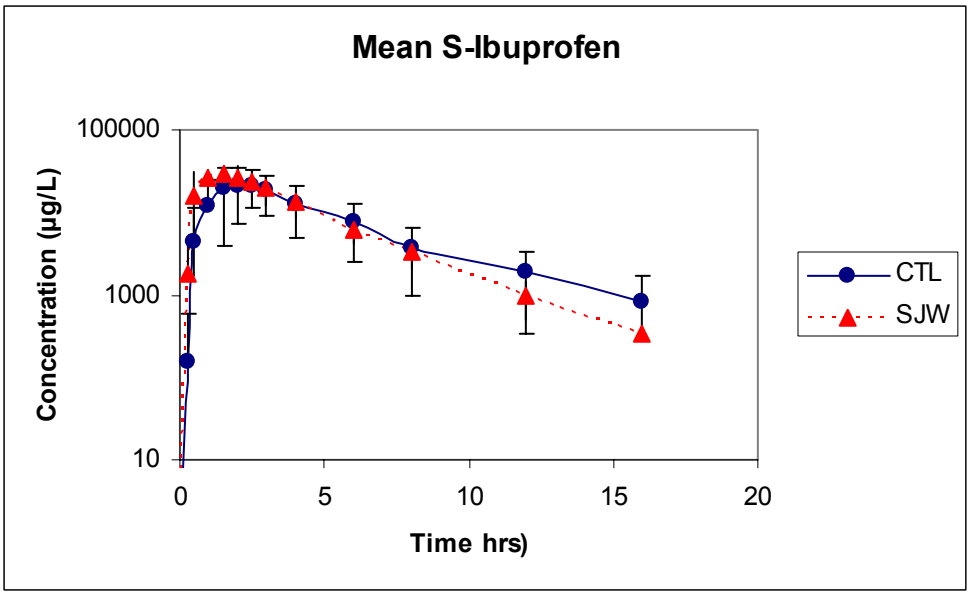

Figure 6.18: Average S-ibuprofen log plasma concentrations versus time. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

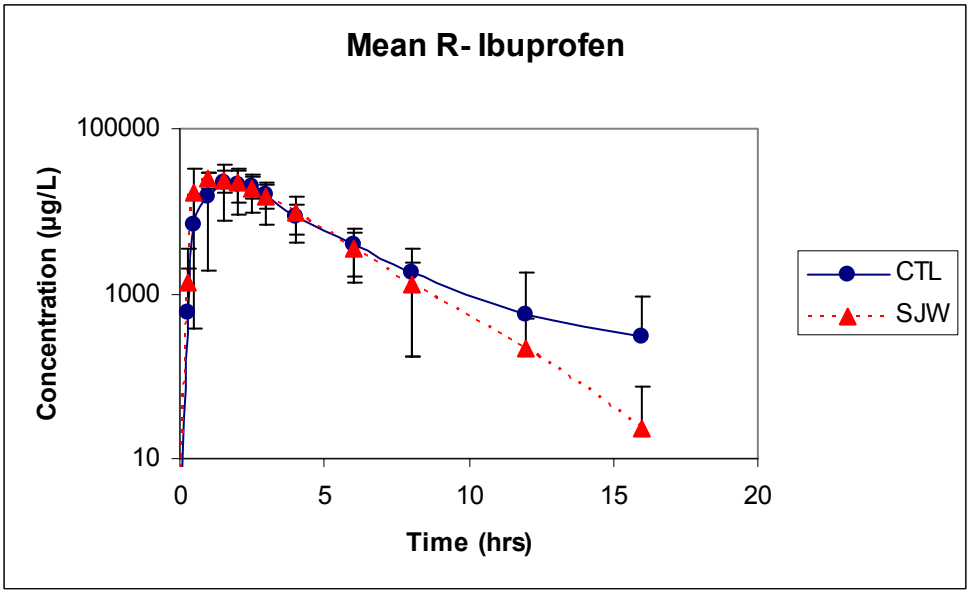

Figure 6.19: Average R-ibuprofen log plasma concentrations versus time. Respective solid and dotted curves represent plasma samples taken before (CTL) and after 21 days of St. John's Wort (SJW) treatment.

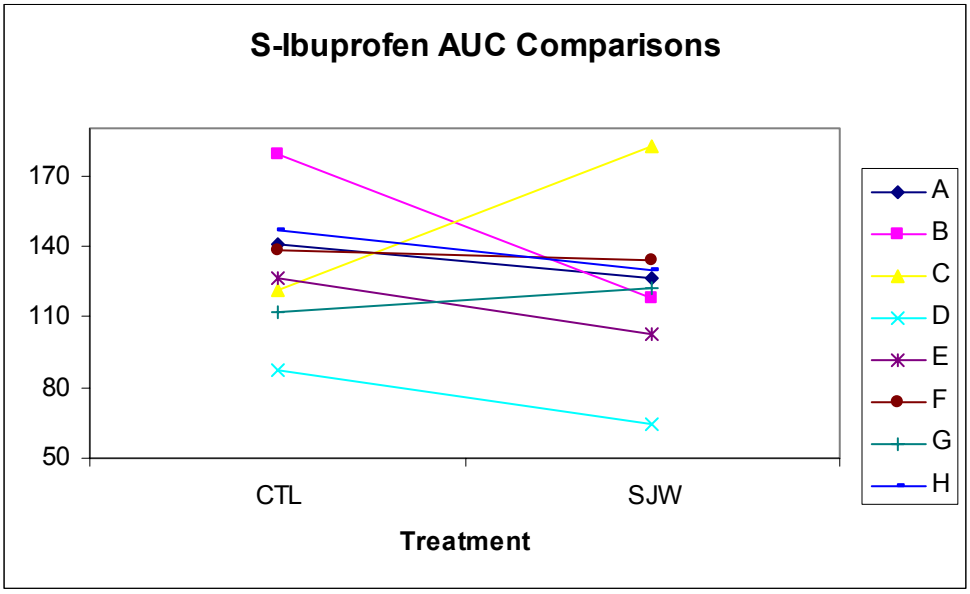

Figure 6.20: Graphical comparisons of individual S-ibuprofen AUC values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

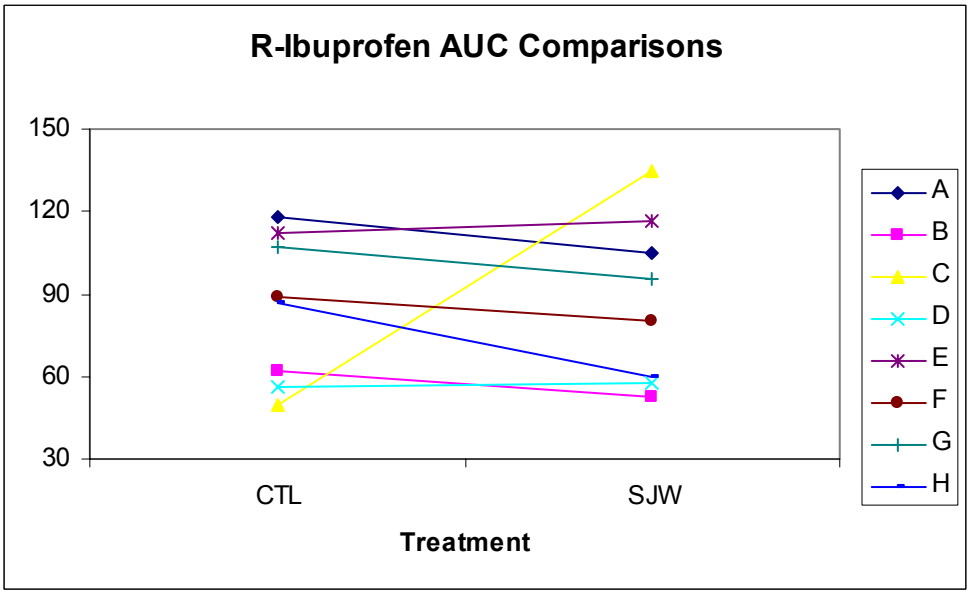

Figure 6.21: Graphical comparisons of individual R-ibuprofen AUC values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

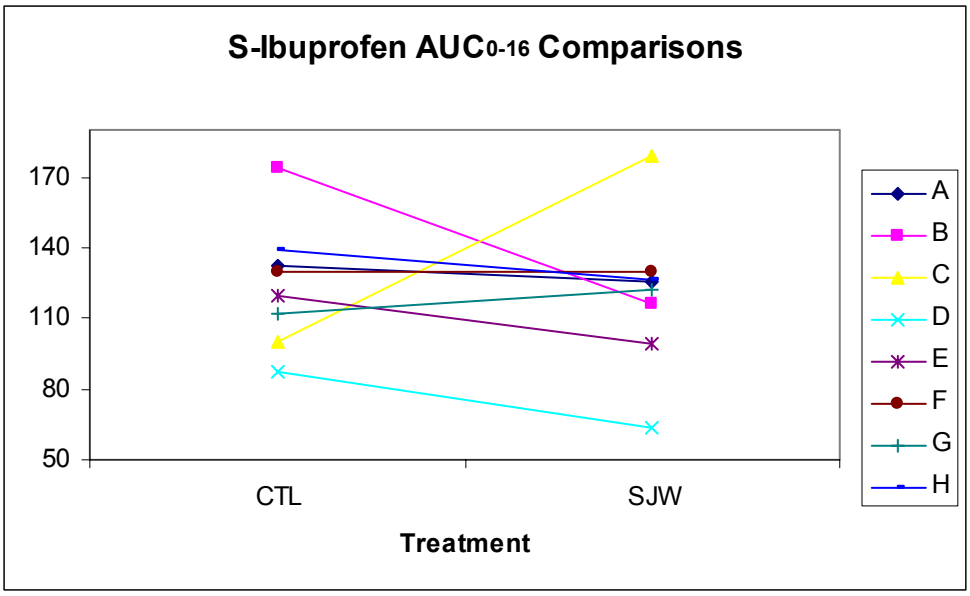

Figure 6.22: Graphical comparisons of individual S-ibuprofen AUC_{0-16} values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

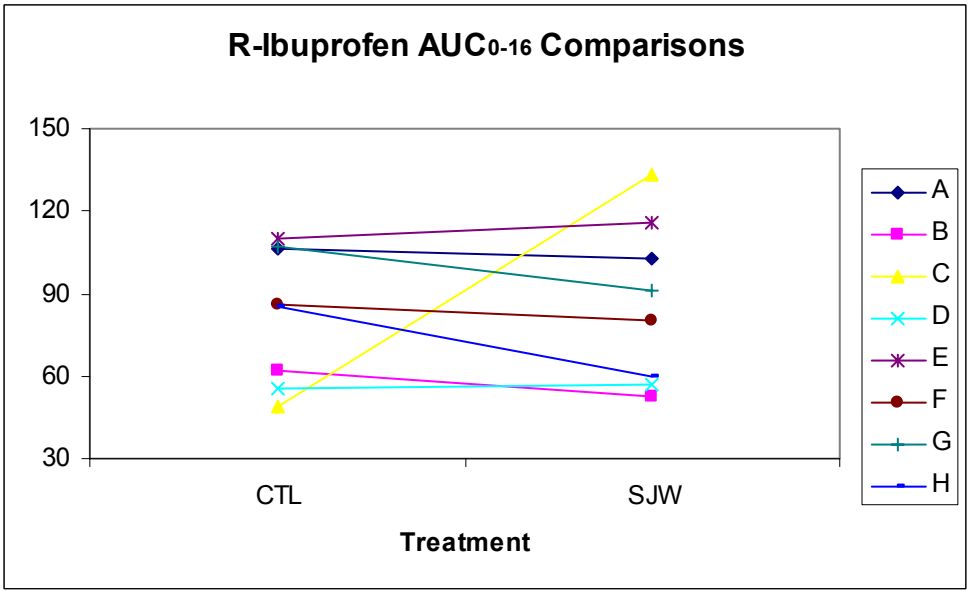

Figure 6.23: Graphical comparisons of individual R-ibuprofen AUC_{0-16} values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

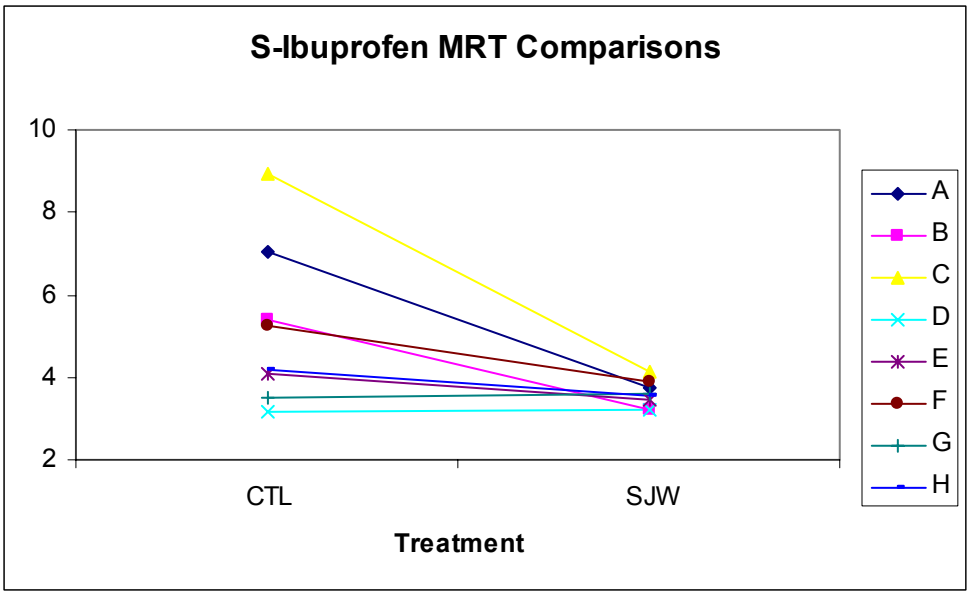

Figure 6.24: Graphical comparisons of individual S-ibuprofen MRT values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

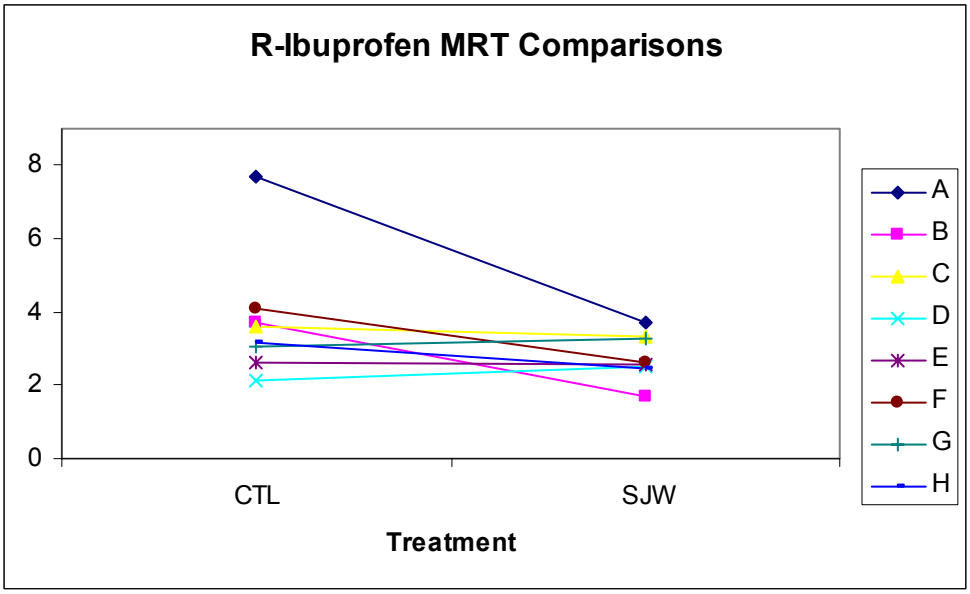

Figure 6.25: Graphical comparisons of individual R-ibuprofen MRT values corresponding to Control (CTL) and St. John's Wort (SJW) treatment groups.

Table 6.9: Record of volunteer compliance during self-administration of St. John's Wort. Record of compliance encompasses both prednisone/prednisolone and ibuprofen investigations. Percent compliance is the ratio of herb tablets taken and tablets distributed to volunteers during the investigation period.

Volunteer	**Missed Doses**	**Percent Compliance**
A	3	96.6
B	4	95.4
C	0	100
D	9	89.7
E	13	85.1
F	0	100
G	12	86.2
H	5	94.3
	Total	**Cumulative Percentage**
	46	93.4

SUMMARY AND CONCLUSIONS

St. John's Wort is a well-known, promising herbal medication that has enjoyed great success in the treatment of mild to moderate depression (Linde et al., 1996; Bilia et al., 2002). Its relatively benign adversity profile is undermined, however, by its propensity to alter the metabolism of synthetically derived agents that have become relied upon in the treatment of various disease states (Wang et al., 2001; Breidenbach et al., 2000). The metabolic pathway by which St. John's Wort affects drug metabolism is cytochrome P450, the diffuse enzyme family largely responsible for the body's chemical detoxification efforts.

Cytochrome P450 is divided into several enzyme subfamilies that mediate metabolism through the addition or removal of specific functional groups on drug and xenobiotic substrates, including -OH, -SH, $-CO_2H$, and $-NH_2$. The ultimate goal of the enzyme system is the creation of more polar forms of chemical substrates that can be more easily expelled from the body (Gillette, 1966). The two cytochrome subfamily isoforms of interest in this study were CYPs 3A4 and 2C9. CYPs 3A4 and 2C9 account for the vast majority of human liver cytochromes. Cytochrome 3A4 is the most common CYP 3A isoform in the human liver and accounts for the vast majority (> 50%) of all phase I drug degradation. The cumulative impact of CYP 3A4 and the P-glycoprotein drug transporter has proven a formidable combination in human drug metabolism. Cytochrome 2C9 is the most abundant member of the large CYP 2 family, and is

responsible for the metabolism of very important drugs, including phenytoin and warfarin. St. John's Wort has been implicated in the functional alteration of CYPs 3A4 and 2C9 in both *in vitro* and *in vivo* investigations.

The NSAID drug ibuprofen, along with the corticosteroid agents prednisone and prednisolone, are long-trusted for their effectiveness in the treatment of inflammation-mediated disorders. Prednisone and prednisolone are early generation glucocorticoids that are cytochrome 3A4 substrates. The compounds are characterized by their reversible metabolism and, particularly in the case of prednisolone, its dose-dependent pharmacokinetic profile. Ibuprofen is one of the most famous substrates of cytochrome 2C9, and is characterized by its racemate administration and enantioselective pharmacology and pharmacokinetics. The drug has been an over-the-counter favorite for more than two decades.

Eight human volunteers between ages 19 and 36 participated in a drug interaction study to examine whether long-term St. John's Wort administration could significantly affect the pharmacokinetics of prednisone, prednisolone or ibuprofen. Volunteers were given 20 mg and 400 mg single doses of prednisone and ibuprofen, respectively, followed by blood sampling for drug assessment. During an outpatient period, the volunteers were instructed to self-administer 900 mg per day of St. John's Wort. At the end of the self administration period, the volunteers were again given 20 mg and 400 mg single doses of prednisone and ibuprofen, respectively, followed by blood sampling at identical time points as used prior to St. John's Wort treatment.

The St. John's Wort investigation was conducted in a staggered fashion. The studies were longitudinal such that each volunteer served as his own control subject. All volunteers received a single 400 mg oral dose of ibuprofen on Day 1 of the investigation, followed by blood sampling at -0.25 hours pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 16 hours post-dose. On Day 8, the volunteers received a single 20 mg oral dose of prednisone, again followed by blood sampling at -0.25 hours pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 16 hours post-dose. Volunteers were instructed to self-administer 300 mg of St. John's Wort 3 times per day for 28 days thereafter. On Day 29 (day 21 of St. John's Wort administration), volunteers again received a final 400 mg oral dose of ibuprofen, followed by blood sampling at the identical time points used on Day 1. On Day 36 (day 28 of St. John's Wort administration), the volunteers received a final 20 mg oral dose of prednisone. Subsequent blood sampling was performed at the identical time points used on Day 8 of the investigation.

The half-lives of ibuprofen, prednisone, and prednisolone are generally short (< 3 hours), and washout times of one week or more were used following single dose administration of ibuprofen and prednisone. Although glucocorticoid agents have been observed to induce cytochrome systems in past investigations (You, 2004; Pascussi et al., 2000), no interaction has ever been documented between ibuprofen and prednisone or prednisolone. In addition, prednisone was administered one week after ibuprofen during single dose portions of the study, thus eliminating any possibility of glucocorticoid induction effects upon ibuprofen.

The staggered study design that was selected should have had no impact upon study results.

The results of both drug interaction inquiries presented in this manuscript suggest that long-term administration of St. John's Wort elicits little to no effect upon important pharmacokinetic indices for prednisone, prednisolone and ibuprofen. The mean pharmacokinetic parameters of prednisone and prednisolone were largely unchanged following herbal administration. ANOVA evaluation of log parameters for prednisone indicated no significant treatment effects in $AUC_{0\text{-}12}$ ($p= 0.70$), AUC ($p= 0.36$), Cmax ($p= 0.91$), Tmax ($p= 0.41$), K_E ($p= 0.35$), and MRT ($p= 0.15$). Similarly, no significant treatment effects were observed in prednisolone log parameters: $AUC_{0\text{-}12}$ ($p= 0.71$), AUC ($p= 0.94$), Cmax ($p= 0. 95$), Tmax ($p= 0.39$), K_E ($p= 0.78$), and MRT ($p= 0.73$). A clinically significant volunteer effect was observed in Ln $AUC_{0\text{-}12}$ for prednisone ($p= 0.04$). Near-significant volunteer effects on Ln Cmax for both prednisone ($p= 0.06$) and prednisolone ($p= 0.07$) were also seen. St. John's Wort's apparent lack of effect upon prednisone and prednisolone pharmacokinetic parameters after long-term administration was additionally supported by confidence interval evaluation. The 90% confidence intervals of log-transformed pharmacokinetic data fell within 80% -125% acceptance range, indicating that St. John's Wort failed to change prednisone or prednisolone pharmacokinetic parameters by more than 20%.

Ibuprofen mean pharmacokinetics parameters, too, displayed little change after herbal administration. ANOVA evaluation of log parameters for S(+)-ibuprofen indicated a significant treatment effect for MRT ($p= 0.02$), but did not

indicate significant treatment effects for AUC (p= 0.36), Cmax (p =0.71), and K_E (p= 0.07). Log parameters for R(-)- ibuprofen revealed no significant treatment effects for MRT (p= 0.08), AUC (p= 0.87), Cmax (p= 0.69), and K_E (p= 0.19). As was the case in the corticosteroid study, near significant volunteer effects were observed for Ln AUC (p= 0.08) and Ln K_E (p= 0.09) in S(+)- ibuprofen. Confidence interval testing for the ratio of geometric means based on log transformed pharmacokinetic parameters revealed a clinically significant change in mean MRT for S(+)- ibuprofen. The 90% confidence intervals for S(+)- ibuprofen K_E and R(-)- ibuprofen MRT did not fall completely within the 80% - 125% acceptance range. However, non-significant ANOVA results corresponding to these parameters were indicative of a lack of an herbal effect, and thus supercede confidence interval information.

In a literature review presented by Mills et al. (2004), 19 documented drug interaction investigations involving St. John's Wort were examined. The results of these studies varied from no effect upon conventional drug pharmacokinetics, as in the case of carbamazepine (Burstein et al., 2000), to dramatic pharmacokinetic changes, as in the case of indinavir (Piscitelli et al., 2000). The Mills et al. (2004) review also revealed that 16 of 19 St. John's Wort drug interaction studies (84%) reported significant effects upon conventional drug AUC following St. John's wort administration in healthy volunteers. The remaining 3 studies (16%) reported a lack of effect after St. John's Wort administration. Mills et al. (2004) measured significant effects (or lack thereof) by 95% confidence interval testing of reported drug AUC values. Lack of effect was

assumed when confidence interval assessment revealed less than a 20% reduction in drug AUC. Similarly, AUC value differences corresponding to prednisone, prednisolone, and ibuprofen treatments indicated 'no effect' following 90% confidence interval assessments.

The apparent failure of St. John's Wort to significantly induce or inhibit the metabolism of drugs in this investigation may be explained by:

1. Insufficient St. John's Wort potency upon cytochrome systems or inability of drug substrates to be induced or inhibited. The use of a standardized St. John's Wort preparation, combined with its long duration of use, lessens the possibility of inadequate potency. It is more plausible that the selected drugs are not easily susceptible to alterations in their metabolism by herbal co-administration. This notion supports the safe usage of these drug combinations. The fact that P-glycoprotein has no known contribution to the metabolism of any of the drugs considered in this work may be relevant as well.
2. Low statistical power values, combined with discrepant 90% confidence interval/ p-value data for S(+)- ibuprofen elimination rate constant and R(-)-ibuprofen MRT indicate that sample size may have influenced study results. It is possible that a sample size increase might have yielded significant results for these parameters at the same significance level (10%). In contrast, high statistical power values encountered in the prednisone study provided solid evidence of St. John's Wort's non-effect upon prednisone and prednisolone pharmacokinetics. Furthermore,

volunteer compliance for St. John's Wort usage (85.1% to 100%) was sufficient to allow confident assessment of St. John's Wort effects.

3. Volunteer demographics and diet may have been relevant to this study. Six of eight study volunteers were of Asian Indian decent, which raises questions about the influence of genetic differences in metabolic drug interaction studies. In the case of ibuprofen, genetic CYP2C9 polymorphism may be relevant, since allelic 2C9*2 has virtually no expression in Asian populations. It has also been noted that certain ingredients of common Indian cuisines may strongly impact cytochrome P450-mediated metabolic processes. Future studies involving long-term herbal dosing may require more careful consideration of these factors to avoid data bias.

CUMULATIVE BIBLIOGRAPHY

Adams, S.S., Bough, R.G., Cliffe, E.E. et al., 1970. Some aspects of the pharmacology, metabolism and toxicology of ibuprofen. Rheumatol. Phys. Med. 9, 9-26.

Aithal, G.P., Day, C., Kesteven, P.J., 1999. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet. 353, 717-719.

Aklillu, E., Persson, I., Bertilsson, L. et al., 1995. Frequent distribution of ultrarapid metabolizers of debrisoquine in an Ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. Journal of Pharmacology and Experimental Therapeutics. 278, 441-446.

Albert, K.S., Gernaat, C.M., 1984. Pharmacokinetics of ibuprofen. Am. J. Med. 23, 40-6.

Al-Meshal, M.A., El-Sayed, Y.M., Al-Balla, S.R. et al., 1994. The effect of colestipol and cholestyramine on ibuprofen bioavailability in man. Biopharm. Drug Disp. 15, 463-471.

Araya, O.S., Ford, E.J., 1981. An investigation of the type of photosensitization caused by the ingestion of St. John's Wort in calves. Journal of Comparative Pathology. 91, 135-141.

Back, D.J., Tija, J.F., Karbwang, J. et al, 1998. In vitro inhibition studies of tolbutamide hydroxylase activity of human liver microsomes by azoles, sulphonamides and quinolones. British Journal of Clinical Pharmacology. 25, 23-29.

Back, D.J., Tija, J., Ohnhaus, E.E. et al, 1998. Selective inhibition of drug oxidation after simultaneous administration of two probe drugs, antipyrine and tolbutamide. European Journal of Clinical Pharmacology. 34, 157-163.

Backman, J.T., Olkkola, K.T., Neuvonen, P.J., 1996. Rifampin drastically reduces plasma concerntrations and effects of oral midazolam. Clinical Pharmacology and Therapeutics. 59, 7-13.

Backman, J.T., Kivisto, K.T., Olkkola, K.T. et al., 1998 The area under the plasma concentration-time curve for oral midazolam is 400-fold larger during treatment with itraconazole than with rifampicin. European Journal of Clinical Pharmacology. 54, 53-58 (1998).

Bannwarth, B., Lapique, F., Pehourq, F. et al., 1995. Stereoselective disposition of ibuprofen enantiomers in cerebrospinal fluid. British Journal of Clinical Pharmacology. 40, 266-269.

Barnes, J., Anderson, L.A., Phillipson, J.D., 2001. St. John's Wort (Hypericum perforatum L.): a review of its chemistry, pharmacology, and clinical properties. Journal of Pharmacy and Pharmacology. 53, 583-600.

Barnes, P.J., Mueller, R.A., 1995. Anti-inflammatory management of bronchospastic disease. In P.L. Munson, (ed.), Principals of Pharmacology. Chapman and Hall, New York. 599- 603.

Bartoszek, M., Brenner, A.M., Szefler, S.J., 1987. Prednisolone and methylprednisolone kinetics in children receiving anticonvulsant therapy. Clinical Pharmacology and Therapeutics. 42(4), 424-432.

Barzaghi, N., Gatti, G., Crema, F. et al, 1987. Inhibition by erythromycin of the conversion of carbamezepine to its active 10,11-expoxide metabolite. British Journal of Clinical Pharmacology. 24, 836-838.

Bergrem, H., Refvem, O.K., 1983. Altered prednisolone pharmacokinetics in patients treated with rifampicin. Acta Medica Scandinavica. 213 (5), 339-343.

Bilia, A.R., Bergonzi, M.C., Morgenni, F. et al., 2001. Evaluation of stability of St. John's wort commercial extract and some preparations. International Journal of Pharmaceutics. 213, 199-208.

Bilia, A.R., Gallori, S., Vincieri, F., 2002. St. John's wort and depression. Efficacy, safety and tolerability-- an update. Life Sciences. 70, 3077-3096.

Black, D.J., Kunze, K.L., Weinkers, L.C. et al., 1996. Warfarin-fluconazole II: A metabolically-based drug interaction. Drug Metabolism and Disposition. 24, 422-428.

Blum, R.A., Wilton, J.H., Hilligoss, D.M. et al., 1991. Effect of fluconazole on the disposition of phenytoin. Clinical Pharmacology and Therapeutics. 49, 420-425.

Boekenhogen, S.J., Szefler, S.J., Jusko, W.J., 1983. Prednisolone disposition and protein binding in oral contraceptive users. Journal of Clinical Endocrinology and Metabolism. 56, 702-709.

Boobis, A.R., Sesardic, D., Murray, B.P. et al., 1990. Species variation in the response of cytochrome P450-dependent monooxygenase system to inducers and inhibitors. Xenobiotics. 20, 1139-61.

Boudinot, F.D., Jusko, W.J., 1984. Plasma protein binding interaction of prednisone and prednisolone. Journal of Steroid Biochemistry. 21(3), 337-339.

Boudinot, F.D., Jusko, W.J., 1986. Dose-dependent pharmacokinetics of predniolosone in normal and adrenalectomized rats. Journal of Pharmacokinetics and Biopharmaceutics. 14, 453-467.

Breidenbach, T.H., Kliem, V., Burg, M. et al., 2000. Profound drop in cyclosporine A whole blood trough levels caused by St. John's Wort (Hypericum perforatum). Transplantation. 69, 2229-2230.

Brevoort, P., 1998. The booming U.S. botanical market. Herbal Gram. 44, 33-48.

Brian, W.R., Srivastava, P.K., Umbenhauer, D.R. et al., 1989. Expression of a human liver cytochrome P-450 protein with tolbutamide hydroxylase activity in Saccharomyces cerevisiae. Biochemistry. 28, 4993-4999.

Brockmoller, J., Reum, T., Bauer, S. et al., 1997. Hypericin and pseudohypericin: pharmacokinetics and effects on photosensitivity in humans. Pharmacopsychiatry. 30 (Suppl. 2), 94-101.

Brodie, B.B., 1964. Distribution and fate of drugs; therapeutic implications. In T.B. Binns, (ed.), Absorption and distribution of drugs. Williams and Wilkins Company, Baltimore. 199-255.

Budzinski, J.W., Foster, B.C., Vandenhoek, S. et al., 2000. An in vitro evaluation of human cytochrome P450 3A4 inhibition by selected commercial herbal extracts and tinctures. Phytomedicine. 7(4), 273-282.

Buffington, G.A., Dominguez, J.H., Piering, W.F. et al, 1976. Interaction of rifampin and glucocorticoids. Adverse effect on renal allograft function. JAMA. 236(17), 1958-1960.

Canaparo, R., Muntoni, E., Zara, G.P. et al., 2000. Determination of ibuprofen in human plasma by high-performance liquid chromatography: validation and application in pharmacokinetic study. Biochemical Chromatography. 12, 219-226.

Cashman, J.R., 2000. Human flavin-containing monooxygenase: substrate specificity and role in drug metabolism. Current Drug Metab. 1(2), 181-191.

Castillo, M., Lam, F., Dooley, M.A. et al., 1995. Disposition and covalent binding of ibuprofen and its acylglucuronide in the elderly. Clinical Pharmacology Therapeutics. 57, 636-644.

Chen, P.S., Mills, I.H. Bartter, F.C., 1961. Ultrafiltration studies of steroid-protein binding. Journal of Endocrinology. 23, 129-137.

Chen, Y., Ferguson, S., Negishi, M. et al., 2004. Induction of Human CYP2C9 by rifampicin, hyperforin, and phenobarbital is mediated by the pregnane X receptor. Journal of Pharmacology and Experimental Therapeutics. 308, 495-501.

Cheng, H., Rogers, J.D. Demetriades, J.L. et al., 1994. Pharmacokinetics and bioinversion of ibuprofen enantiomers in humans. Pharmaceutical Research. 11, 824-830.

Cheruvallath, V.K., Riley, C.M., Narayanan, S.R. et al, 1997. A quantitative circular dichroic investigation of the binding of the enantiomers of ibuprofen and diclofenac to human serum albumin. J. Pharm. Biomed. Anal. 15, 1719-1724.

Christians, U., Sewing, K.F., 1993. Cyclosporin metabolism in transplant patients. Pharmacology and Therapeutics. 57, 291-345.

Collste, P., Seideman, P., Borg, K.O. et al., 1979. Influence of pentobarbital on effect and plasma levels of alprenolol and 4-hydroxyalprenolol. Clin. Pharmacol. Ther. 25, 423-427.

Conney, A.H., 1982. Induction of microsomal enzymes by foreign compounds and carcinogenesis by polycyclic aromatic hydrocarbons. Cancer Research. 42, 4875-4917.

Conrad, K.A., Mayershohn, M., Bliss, M., 1984. Cimetidine does not alter ibuprofen kinetics after a single dose. British Journal of Clinical Pharmacology. 18, 624-626.

Conseil, G., Baubichon-Cortay, H., Dayan, G. et al., 1998. Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroid-binding sites on mouse P-glycoprotein. Proceedings of the National Academy of Sciences of the USA. 95, 9831-9836.

Cordon-Cardo, C., O'Brien, J.P., Boccia, J. et al., 1990. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tisses. Journal of Histochemistry and Cytochemistry. 38, 1277-1287.

Corpier, C.L., Jones, P.H., Suki, W.M. et al., 1988. Rhabdomyolysis and renal injury with lovastatin use: report of two cases in cardiac transplant recipients. JAMA. 260, 239-241.

Correia, M.A., Ortiz de Montellano, P.R., 1993. Inhibition of cytochrome P450 and possibilities for their therapeutic application. In K. Ruckpauland and H. Rein, (eds.), Medicinal implications in cytochrome P450 catalyzed biotransformations. Akademie Verlag, Berlin. 74-146.

Cott, J.M., 2001. Herb-drug interactions. CNS Spectrums. 6, 827-832.

Cox, S.R., Gall, E.P., Forbes, K.K. et al., 1991. Pharmacoikinetocs of the R(-) and S(+) enantiomers of ibuprofen in the serum and synovial fluid of arthritic patients. Journal of Clinical Pharmacology. 31, 88-94.

Cox, S.R. Brown, M.A., Squires, D.J. et al., 1988. Comparative human study of ibuprofen enantiomer plasma concentrations produced by two commercially available ibuprofen tablets. Biopharm. Drug Disp. 9, 539-549.

Crane, J.K. and Shih, H-T., 1993. Syncope and cardiac arrhythmia due to an interaction between itraconazole and terfenadine. Am. J. Med. 95, 445-446.

Daihk, B.E., Lasker, J.M., Raucy, J.L. et al., 1994. Regio- and stereoselective epoxidation of arachdonic acid by human cytochrome P450 2C8 and 2C9. J. Pharmacol. Exp. Ther. 271, 1427-1433.

Davies, N.M., 1998. Clinical pharmacokinetics of ibuprofen. The first 30 years. Clinical Pharmacokinetics. 34(2), 101-154.

Decker, C., Sugiyama, K., Underwood, M. et al., 1986. Inactivation of rat hepatic cytochrome P-450 by spironolactone. Biochem. Biophys. Res. Commun. 136(3), 1162-1169.

de Maat, M.M., Hoetelmans, R.M., Matht, R.A. et al., 2001. Drug interaction between St. John's Wort and nevirapine, AIDS. 15, 420-421.

Demisch, L., Holzl, J., Gollnik, B. et al., 1989. Identification of selective MAO-type-A inhibitors in Hypericum perforatum L. (Hyperforat). Pharmacopsychiatry. 22, 194.

De Smet, D P.A., Nolen, W.A., 1996. St. John's Wort as an antidepressant. British Medical Journal. 313(7052), 241-242 (1996).

de Wildt, S.N., Kearns, G.L., Leeder, J.S. et al., 1999. Cytochrome P450 3A: ontogeny and drug disposition. Clinical Pharmacokinetics, 37, 485-505.

Dickins, M., Elcombe, C.R., Moloney, S.J. et al., 1979. Further studies on the dissociation of the isosafrole metabolite-cytochrome P450 complex. Biochemical Pharmacology. 28, 231-238 (1979).

Dionne, R.A., McCullagh, L., 1998. Enhanced analgesia and suppression of plasma beta-endorphin by the S(+) isomer of ibuprofen. Clinical Pharmacology and Therapeutics. 63, 694-701.

Donath, F., Roots, I., Langerheinrich, M. et al., 1999. Interaction of St. John's wort extract with phenprocoumon. European Journal of Clinical Pharmacology. 55, A22.

Dresser, G.K., Spence, J.D., Bailey, D.G., 2000. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clinical Pharmacokinetics. 38(1), 41-57.

Dresser, G.K., Schwarz, U.I., Wilkinson, G.R. et al., 2003. Coordinate induction of both cytochrome P4503A and MDR1 by St. John's Wort in healthy subjects. Clinical Pharmacology and Therapeutics. 73(1), 41-50.

Ducharme, M.P., Slaughter, R.L., Warbasse, L.H. et al., 1995 Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin. Clinical Pharmacology and Therapeutics. 58(6), 617-24.

Durr, D. Steiger, B., Kullak-Ublick, G. et al., 2000. St. John's Wort induces intestinal P-glycoprotein /MDR1 and intestinal and hepatic CYP3A4. Clinical Pharmacology and Therapeutics. 68, 598-604.

Eisenberg, D.M., Kessler, R.C., Foster, C. et al., 1993. Unconventional medicine in the United States: prevalence, costs, and patterns of use. New England Journal of Medicine. 328, 246-252.

Eisenberg, D., David, R.B., Ettner, S.L. et al., 1998. Trends in alternative medicine use in the United States, 1990-1997. JAMA. 280, 1569-1575.

Elshourbagy, N.A., Guzelian, P.S., 1980. Separation, purification, and characterization of a novel form of hepatic cytochrome P450 from rats treated with pregnenolone- 16 alpha – carbonitrile. Journal Biol. Chem. 255, 1279-1285.

Eradiri, O., Jamali, F., Thomson, A.B., 1988. Interaction of metronidazole with phenobarbital, cimetidine, prednisone, and sulfasalazine in Crohn's disease. Biopharmaceutics and Drug Disposition. 9(2), 219-227.

Ernst, E., 1999. Second thoughts about safety of SJW. Lancet. 354, 2014-2016.

Escher, G., Vogt, B., Beck, T. et al., 1998. Reduced 11 beta-hydroxysteroid dehydrogenase activity in the remaining kidney following nephrectomy. Endocrinology. 139 (4), 1533-1539.

Evans, A.M., 1992. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs. European Journal of Clinical Pharmacology. 42, 237-256.

Evans, A.M., Nation, R.L., Sansom, L.N., 1989. Lack of effect of cimetidine on the pharmacokinetics of R(-)- and S(+)- ibuprofen. British Journal of Clinical Pharmacology. 28, 143-149.

Fears, S., 1985. Lipophilic xenobiotic conjugates: the pharmacological and toxicological consequences of the participation of drugs and other foreign compounds as substrates in lipid biosynthesis. Prog. Lipid Res. 24, 177-195.

Ferry, J.J. Jr., Wagner, J.G., 1986. The non-linear pharmacokinetics of prednisone and prednisolone. I. Theoretical. Biopharmaceutics & Drug Disposition. 7(1), 91-101.

Fojo, A.T., Ueda, K., Slamon, D.J. et al., 1987. Expression of a multidrug resistance gene in human tumors and tissues. Procedures of the National Academy of Sciences of the U.S.A. 84, 265-269.

Frey, B.M., Frey, F.J., 1984. Phenytoin modulates the pharmacokinetics of prednisolone and the pharmacodynamics of prednisolone assessed by the inhibition of the mixed lymphocyte reaction in humans. European Journal of Clinical Investigation. 14, 1-6.

Frey, B.M., Frey, F., 1990. Clinical pharmacokinetics of prednisone and prednisolone. Clinical Pharmacokinetics. 19(2), 126-146.

Frey, B.M., Schaad, H.J., Frey, F.J., 1984. Pharmacokinetic interaction of contraceptive steroids with prednisone and prednisolone. European Journal of Clinical Pharmacology. 26, 505-511.

Frey, F.J., Frey, B.M., 1983. Urinary 6-beta-hydroxyprednisolone excretion indicates enhanced prednisolone catabolism. Journal of Laboratory and Clincial Medicine. 101, 593-604.

Frey, F.J., Frey, B.M., Greither, A. et al., 1980. Prednisolone clearance at steady state in dogs. Journal of Pharmacology and Experimental Therapeutics. 215, 287-291.

Frey, F.J., Amend, J.W., Lozada, F. et al., 1981. Pharmacokinetics of prednisolone and endogenous hydrocortisone levels in cushinoid and non-cushinoid patients. European Journal of Clinical Pharmacology. 21, 235-243.

Frey, F.J., Ruegsegger, M.K., Frey, B.M., 1986. The dose-dependent systemic availability of prednisone: one reason for the reduced biological effect of alternate-day prednisone. British Journal of Clinical Pharmacology. 21, 183-189.

Frey, F.J., Frey, B.M., Schnetzer, A. et al., 1987. Evidence that cyclosporine A does not affect the metabolism of prednisolone after renal transplantation. Transplantation. 43, 494-498.

Furuya, H., Fernandez-Salguero, P., Gregory, W. et al., 1995. Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. Pharmacogenetics. 5, 389-392 (1995).

Gambaro, V., Caligara, M., Benvenuti, C. et al., 1985. Pharmacokinetics of ibuprofen microencapsulated granules. Il Farmaco. 40, 407-415.

Gambertoglio, J.G., Amend Jr., W.J.C., Benet, L.Z., 1980. Pharmacokinetics and bioavailability of prednisone and prednisolone in healthy volunteers and patients: a review. Journal of Pharmacokinetics and Biopharmaceutics. 8, 1-52.

Gambertoglio, J.G., Holford, N.H., Kapusnik, J.E. et al., 1984. Disposition of total and unbound prednisolone in renal patients receiving anticonvulsants. Kidney International. 25(1), 119-123.

Gan, L-S.L., Mosley, M.A., Khosla, B. et al., 1996. CYP3A-Like cytochrome P450-mediated metabolism and polarized efflux of cyclosporine A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport. Drug Metabolism and Disposition. 24, 344-349.

Garg, V., Jusko, W.J., 1991. Simultaneous analysis of prednisone, prednisolone and their major hydroxylated metabolites in urine by high-perfomance liquid chromatography. Journal of Chromatography. A. 567(1), 38-47.

Garg, V., Jusko, W.J., 1994. Bioavailability and reversible metabolism of prednisone and prednisolone in man. Biopharmaceutics and Drug Disposition. 15, 163-172.

Gaster, B., Holroyd, J., 2000. St. John's Wort for depression: A systematic review. Archives of Internal Medicine. 160(2), 152-156.

Geisslinger, G., Dietzel, K., 1989. High-performance liquid chromatographic determination of ibuprofen, its metabolites and enantiomers in biological fluids. Journal of Chromatography. 491, 139-149.

Geisslinger, G., Stock, K.P., Bach, G.L. et al., 1989. Pharmacological differences between R(-)- and S(+)- ibuprofen. Agents Actions. 27, 455-457.

Geisslinger, G., Schuster, O., Stock, K.P. et al., 1990. Pharmacokinetics of S (+)- and R (-)- ibuprofen in volunteers and first clinical experience of S(+)- ibuprofen in rheumatoid arthritis. European Journal of Clinical Pharmacology. 38, 493-497.

Gelboin, H.V., 1980. Benzopyrene metabolism, activation, and carcinogenesis: Role and regulation of mixed function oxidases and related enzymes. Physio. Rev. 60, 1107-1166.

Gibbons, G.F., Pullinger, C.R., Mitropoulos, K., 1979. Studies on the mechanism of lanosterol 14-alpha demethylation: A requirement for two distinict types of mixed function oxidase systems. Biochem. J. 183, 309-315.

Gillette, J.R., 1966. Biochemistry of drug oxidation and reduction by enzymes in hepatic endoplasmic reticulum. Advan. Pharmacol. 4, 219-261.

Glass, R.C., Swannell, A.J., 1978. Concentrations of ibuprofen in serum and synovial fluid from patients with arthritis. Br. J. Clin. Pract. Sym. Suppl. 6, 453P-454P.

Gobbi, M., Dalla Valle, S.M., Ciapparelli, C. et al., 1999. Hypericum perforatum L. extract does not inhibit 5-HT transporter in rat brain cortex. Naunyn-Schmiedebergs-Achives of Pharmacology. 360, 262-269.

Goldman, P., 2001. Herbal medicines and the roots of modern pharmacology. Annals of Internal Medicine. 135, 594-600.

Goldstein, J.A. and de Morais, S.M.F., 1994. Biochemistry and molecular biology of the human CYP2C subfamily. Pharmacogenetics. 4, 285-299.

Gonzalez., F.J., 1992. Human cytochrome P450: problem and prospects. Trends in Pharmacol. Sci. 13, 346-352.

Gorski, J.C., Hamman, M.A., Wang, Z. et al., 2002. The effect of St. John's Wort on the efficacy of oral contraception (abstract). Clinical Pharmacology and Therapeutics. 71(2), 25.

Grennan, D.W, Ferry, D.G., Ashworth, M.E. et al., 1979. The aspirin-ibuprofen interaction in rheumatoid arthritis. British Journal of Clinical Pharmacology. 8, 497-503.

Grub, S., Bryson, H., Goggin, T. et al., 2001. The interaction of saquinavir (soft gelatin capsule) with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. European Journal of Clinical Pharmacology. 57, 115-121.

Grube, B., Walper, A., Wheatley, D., 1999. St. John's Wort extract: efficacy for menopausal symptoms of psychological origin. Advances in Therapy. 16, 177-186.

Guengerich, F.P., 1983. Oxidation-reduction properties of rat liver cytochromes P450 and NADPH- cytochrome P450 reductase related to catalysis in reconstituted systems. Biochemistry. 22, 2811-2820.

Guengerich, F.P., 1990. Enzymatic oxidation of xenobiotic chemicals. Critical Reviews in Biochemistry and Molecular Biology. 25, 97-103.

Guengerich, F.P., 1995. Human cytochrome P450 enzymes. In P.R. Ortiz de Montellano, (ed.), Cytochrome P450: Structure, Mechanism and Biochemistry, 2nd ed. Plenium Press, New York. 473-535.

Guengerich, F.P., Turvy, C.G., 1991. Comparision of levels of human microsomal cytochrome P450 enzymes and epoxide hydrolase in normal and disease states using immunochemical analysis of surgical samples. Journal of Pharmacology and Experimental Therapeutics. 256, 1189-1194.

Gulick, R.M., McAuliffe, V., Holden-Wiltse, J. et al., 1999. Phase I studies of hypericin, the active compound in St. John's Wort, as an antiretroviral agent in HIV-infected adults: AIDS Clinical Trials Group Protocls 150 and 258. Annals of Internal Medicine. 130, 510-514.

Gustavson, L.E., Legler, U.F. Benet, L.Z., 1986. Impairment of prednisolone disposition in women taking oral contraceptives or conjugated estrogens. Journal of Clinical Endocrinology and Metabolism. 62, 234-237.

Guyton, A.C., Hall, J. E., 1996. Textbook of Medical Physiology, 9th Edition. W.B Saunders Company, Philadelphia. 883-884.

Haehner, B.D., Gorski, J.C., Vandenbranden, M. et al., 1996. Bimodal distribution of renal cytochrome P450 3A activity in humans. Molecular Pharmacology. 50, 52-59.

Hage, D.S., Noctor, T.A.G., Wainer, I.W., 1995. Characterization of the protein binding of chiral drugs by high-performance affinity chromatography interactions of R- and S- ibuprofen and human serum albumin. Journal of Chromatography. 693, 23-32.

Hall, S.D., Rudy, A.C., Knight, P.M. et al., 1993. Lack of presystemic inversion of (R)- to (S)- ibuprofen in humans. Clinical Pharmacology and Therapeutics. 53, 393-400.

Hall, S.D., Wang, Z., Huang, S.-M., 2003. The interaction between St. John's Wort and an oral contraceptive. Clinical Pharmacology and Therapeutics. 74, 525-535.

Halpert, J.R., Miller, N.E., Gorsky, L.D., 1985. On the mechanism of the inactivationof the major phenobarbital-inducible isozymes of rat liver cytochrome P450 by chloramphenicol. Journal Biol. Chem. 260, 8397-8403.

Hamman, M.A., Thompson, G., Hall, S., 1997. Regioselective and stereoselective metabolism of ibuprofen by human cytochrome P450 2C. Biochemical Pharmacology. 54, 33-41.

Hansen, T., Day, R., Williams, K. et al., 1985. The assay and in vitro binding of the enantiomers of ibuprofen. The assay and in vitro binding of the enantiomers of ibuprofen. Clin. Exp. Pharmacol. Physiol. 9, 82-83.

Hargreaves, J.A., Jezequel, S., Houston, J.B., 1994. Effect of azole antifungals on human microsomal metabolism of diclofenac and midazolam. British Journal of Clinical Pharmacology. 38, 175.

Harrer, G., Hobner, W.D., Podzuweit, H., 1994. Effectiveness and tolerance of the hypericum extract LI160 compared to maprotiline: a multicenter double-blind study. Journal of Geriatric Psychiatry and Neurology 7, S24-S28.

Harrison, P., 1998. Herbal medicines take root in Germany. Canadian Medical Association Journal. 158, 637-639.

Heimark, L.D. Gibaldi, M., Trager, W.F. et al., 1987. The mechanism of warfarin-rifampin drug interaction in humans. Clinical Pharmacology and Therapeutics. 42, 388-394.

Heimark, L.D., Weinkers, L., Kunze, K. et al., 1992. The mechanism of the interaction between amiodarone and warfarin in humans. Clinical Pharmacology and Therapeutics. 51, 398-407.

Henderson, L., Yue, Q.Y., Bergquist, C. et al., 2002. St. John's Wort (Hypericum perforatum): drug interaction and clinical outcomes. Br. J. Clin. Pharmacol. 54, 349-356.

Henderson, R.G., Wheatley, T., English, J. et al., 1979. Variation in plasma prednisolone concentrations in renal transplant recipients given enteric-coated prednisolone. British Medical Journal. 1, 1534-1536.

Hendrickse, W. Mickierman, J., Pickup, M. et al., 1979. Rifampicin-induced non-responsiveness to corticosteroid treatment in nephrotic syndrome. British Medical Journal. 1, 306.

Hennessy, M., Kelleher, D., Spiers, J.P. et al., 2002. St. John's Wort increases expression of P-glycoprotein: Implications for drug interactions. British Journal of Clinical Pharmacology. 53, 75-82.

Higgins, C.F., 1992. ABC transporters: from microorganisms to man. Annual Rev. Cell Biol. 8, 67-113.

Higgs, G.A., Flower, R.J., 1981. Anti-inflammatory drugs and the inhibition of arachidonate lipoxygenase. In P.J. Piper (ed), SRS-A and Leukotrienes, John Wiley and Sons, Ltd., London. 197-207.

Homsy, W., Caille, G., duSouich, P., 1995. The site of absorption in the small intestine determines diltiazem bioavailability in the rabbit. Pharmaceutical Research. 12, 1722-1726.

Honig, P.K. Wortham, D.C., Zamani, K. et al., 1993. Terfenadine- ketoconazole interaction: pharmacokinetic and electrocardiographic consequences. JAMA. 269, 1535-1539.

Honig, P.K., Woosley, R.L., Zamani, K. et al., 1992. Changes in pharmacokinetics and electrocardiographic pharmacodynamics of terfenadine with concomitant administration of erythromycin. Clin. Pharmacol. Ther. 52, 231-238.

Honkakoski, P., Negishi, M., 2000. Regulation of cytochrome P450 (CYP) genes by nuclear receptors. Biochem. Journal. 347(2), 321-337.

Huang, M.L., Jusko, W.J., 1990. Nonlinear pharmacokinetics and interconversion of prednisolone and prednisone in rats. Journal of Pharmacokinetics and Biopharmaceutics. 18(5), 410-421.

Ioannides, C., 1996. Cytochromes P450 Metabolic and Toxicological Aspects. CRC Press Inc, New York.

Izzo, A.A., 2004. Drug interactions with St. John's Wort (Hypericum perforatum): a review of the clinical evidence. International Journal of Clinical Pharmacology and Therapeutics. 42(3), 139-148.

Izzo, A.A., Ernst, E., 2001. Interactions between herbal medicines and prescribed drugs. Drugs. 61(15), 2163-2175.

Jacobson, J.M. Feinman, L. Liebes, L. et al., 2001. Pharmacokinetics, safety, and antiviral effects of hypericin, a derivative of St. John's Wort plant, in patients with chronic hepatitis C virus infection. Antimicrobial Agents and Chemotherapy. 45, 517-524.

Jalava, K.M., Olkkola, K.T., Neuvonen, P.J., 1997. Itraconazole greatly increases plasma concentrations and effects of felodipine. Clinical Pharmacology and Therapeutics. 61, 410-415.

Jamali, F., Singh, N.N., Pasutto, F.M. et al., 1988. Pharmacokinetics of ibuprofen enantiomers in man following oral administration of tablets with different absorption rates. Pharmaceutical Research. 5, 40-43.

Jamali, F., Mehvar, R., Russell, A.S. et al., 1992. Human pharmacokinetics of ibuprofen enantiomers following different doses and formulations: intestinal chiral inversion. Journal of Pharmaceutical Sciences. 81, 221-225 (1992).

Jann, M.W., Francis, Y.W., Gray, E.C. et al., 1994. Reversible metabolism of drugs. Drug Metabolism and Drug Interactions. 11(1), 1-24.

Janssen, G.M.E. and Venema, J.F., 1985. Ibuprofen: plasma concentrations in man. J. Int. Med. Res. 13, 68-73.

Jenkinson, M.L. Fitzpatrick, R., Streete, P.J. et al., 1998. The relationship between plasma ibuprofen concentrations and toxicity in acute ibuprofen overdose. Human Toxicol. 7, 319-324.

Johansson, I., Lundqvist, E., Bertilsson, L. et al., 1993. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. Procedures of the National Academy of Sciences of the U.S.A. 90, 11825-11829.

Johne, A., Brockmoller, J., Bauer, S. et al., 1999. Pharmacokinetic interaction of digoxin with an herbal extract from St. John's Wort. Pharmacokinetics and Drug Disposition. 66(4), 338-345.

Jusko, W.J., Pyszczynski, N.A., Bushway, M.S. et al., 1994. Fifteen years of operation of a high-performance liquid chromatographic assay for prednisolone, cortisol and prednisone in plasma. Journal of Chromatography B. 658, 47-54.

Kaehler, S.T., Sinner, C., Chatterjee, S.S. et al., 1999. Hyperforin enhances the extracellular concentrations of catecholamines, serotonin and glutamate in the rat locus coeruleus. Neuroscience Letters. 262(3), 199-202.

Kaminsky, L.C., Zhang, Z.Y., 1997. Human P450 metabolism of warfarin. Pharmacology and Therapeutics. 73, 67-74.

Katoh, M., Nakajima, M., Hiroshi, Y. et al., 2001. Inhibitory effects of CYP3A substrates and their metabolites on P-glycoprotein-mediated transport. European Journal of Pharmaceutical Sciences. 12, 505-513.

Kay, L., Kampmann, J.P., Svendsen, T.L. et al., 1985. Influence of rifampin and isoniazid on the kinetics of phenytoin. British Journal of Clinical Pharmacology. 20, 323-326.

Kaufman, D.W., Kelly, J.P., Rosenberg, L. et al., 2002. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. JAMA. 287, 337-344.

Kazeriad, D.J., Martin, D.E., Tenero, D. et al., 1997. Fluconazole significantly alters the pharmacokinetics of losartan but not eprosartan. Clinical Pharmacology and Therapeutics. 61, 203.

Keith, S.J., Matthews, S.M., 1993. The value of psychiatric treatment: its efficacy in severe mental disorders. Psychopharmacology Bulletin. 29, 427-430.

Kirchheiner, J., Meineke, I., Freytag, G. et al., 2002. Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2. Clinical Pharmacology and Therapeutics. 72(1), 62-75.

Kleber, E., Obry, T., Hippeli, S. et al., 1999. Biochemical activity of extracts from Hypericum perforatum L. 1st communication: inhibition of dopamine-beta-hydroxylase. Drug Research. 49, 106-109.

Kolars, J.C., Awni, W.M., Werion, R.M. et al., 1991. First-pass metabolism of cyclosporine in the gut. Lancet. 338, 1488-1490.

Kolars, J.C., Schmiedlin-Ren, P., Schuetz, J.D. et al., 1992. Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. Journal of Clinical Investigation. 90, 1871-1878.

Kort, W.J., Weijma, I.M., Westbroek, D.L., 1979. Reductive effect of phenobarbital on graft survival in prednisolone-treated rats. European Surgical Research. 11, 317-324.

Kozowzer, M., Veatch, L., Kaplan, M.M., 1974. Decreased clearance of prednisolone, a factor in the development of corticosteroid side effect. Journal of Clinical Endocrinology and Metabolism. 38, 407-412.

Kunze, K.L., Wienkers, L.C., Thummel, K.L. et al, 1996. Warfarin-fluconazole I. Inhibition of human cytochrome P450 dependent metabolism of warfarin by fluconazole: in vitro studies. Drug metabolism and Disposition. 24, 414-421.

Kupferschmidt, H.H., Ha, H.R., Ziegler, W.H. et al., 1995. Interaction between grapefruit juice and midazolam in humans. Clinical Pharmacology and Therapeutics. 58, 20-28.

Kyrkland, C., Backman, J.T., Kivisto, K.T. et al., 2000. Rifampin greatly reduces plasma simvastatin and simvastatin acid concentrations. Clinical Pharmacology Therapeutics. 68, 592-597.

Lacarelle, B., Rahmani, R., Desousa, G. et al., 1991. Metabolism of digoxin, digoxigenin digitoxosides and digoxigenin in human hepatocytes and liver-microsomes. Fundamental and Clinical Pharmacology. 5, 567-582.

Lazar, J.D., Wilner, K.D., 1990. Drug interactions with fluconazole. Reviews of Infect. Dis. 12 (Suppl 3), S327-S333.

Leeman, T.D., Tanson, C., Bonnabry, C. et al., 1993. A major role cytochrome $P450_{TB}$ (CYP2C) subfamily in the actions of non-steroidal anti-inflammatory drugs. Drugs Exp. Clin. Res. 19, 189-195.

Legler, U.F., Frey, F.J., Benet, L.Z., 1982. Prednisolone clearance at steady state in man. Journal of Clinical Endocrinology and Metabolism. 55, 762-767.

Levine, M.A.H., Walker, S.E., Paton, T.W., 1992. The effect of food or sucralfate on the bioavailability of S(+) and R(-) enantiomers of ibuprofen. Journal of Clinical Pharmacology. 32, 1110-1114.

Lewis, D.F., Pratt, J.M., 1998. The P450 catalyic cycle and oxygenation mechanism. Drug Metab. Rev. 30(4), 739-786.

Lewis, R.J., Trager, W.F., Chan, K.E. et al., 1974. Warfarin—Stereochemical aspects of its metabolism and the interaction with phenylbutazone. Journal of Clinical Investigation. 53, 1607-1617.

Li, A.P., Kaminski, D., Rasmussen, A., 1995. Substrates of human hepatic cytochrome P450 3A4. Toxicology. 104, 1-8.

Li, G., Treiber, G., Klotz, U., 1989. The ibuprofen-cimetidine interaction: stereochemical considerations. Drug Invest. 1, 11-17.

Lin, J.H., 2003. Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. Advanced Drug Delivery Reviews. 55, 53-81.

Lin, J.H., Lu, A.Y., 1998. Induction and induction and clinical implications. Clinical Pharmacokinetics. 35(5), 361-390.

Lin, J.H., Lu, A.Y.H., 2001. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. Annu. Rev. Pharmacol. Toxicol. 41, 535-567.

Linde, K., Ramirez, G., Mulrow, C.D. et al., 1996. St. John's Wort for depression—an overview and meta-analysis of randomized clinical trials. BMJ. 313 (7052), 253-258.

Lock, E.A., Mitchell, A.M., Elcombe, C.R., 1989. Biochemical mechanisms of induction of hepatic peroxisome proliferation. Ann. Rev. in Pharmacol. and Toxicol. 29, 145-163.

Lockwood, G.F., Albert, K.S., Gillespie, W.R. et al., 1983. Pharmacokinetics of ibuprofen in man. 1: Free and total area/ dose relationships. Clinical Pharmacology and Therapeutics. 31, 97-103.

Lopez-Garcia, M.P., Dansette, P.M., Mansuy, D., 1993. Thiophene derivatives as new mechanism-based inhibitors of cytochromes P450: inactivation of yeast-expressed human liver P450 2C9 by tienilic acid. Biochemistry. 33, 166-175.

Leu, T.H., Maa, M.C., 2002. The molecular mechanisms for the antitumorigenic effect of curcumin. Current Medicinal Chemistry- Anti-Cancer Agents. 2(3), 357-370.

Lugwig, E.A., Slaughter, R., Savliwala, M. et al., 1989. Steroid-specific effects of ketoconazole on corticosteroid disposition: unaltered prednisolone elimination. DICP. 23, 858-861.

Mahady, G.B., 2001. Global harmonization of herbal health claims. Journal of Nutrition, 131(3s), 1120S-3S.

Mai, I., Kruger, H., Buddle, K. et al., 2000. Hazardous pharmacokinetic interaction of Saint John's Wort (Hypericum perforatum) with the immunosuppressant cyclosporine. International Journal of Clinical Pharmacology and Therapeutics. 38, 500-502.

Mai, I., Stormer, E., Bauer, S. et al., 2003. Impact of St. John's Wort treatment on the pharmacokinetics of tacrolimus and mycophenolic acid un renal transplant patients. Nephrol. Dial Transplant. 18, 819-822.

Makman, M.H., Dvorkin, B., White, A., 1971. Evidence for induction by cortisol in vitro of a protein inhibitor of transport and phosphorylation in rat thymocytes. Proc. Natl. Acad. Sci U.S.A. 68, 1269-1273.

Mandel, H.G., 1971. Pathways of drug biotransformation: Biochemical conjugations. In B.N. LaDu et al., (eds.), Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins Company, Baltimore. 149-186.

Mangelsdorf, D.J., Thummel, C., Beato, M. et al., 1995. The nuclear receptor superfamily: the second decade. Cell. 83, 835-839.

Markowitz, J.S., Jennifer, J.L., DeVane, C. et al., 2003. Effect of St. John's Wort on drug metabolism by induction of cytochrome P450 3A4 enzyme. JAMA. 290 (11), 1500-1504.

McAllister, W.A., Thompson, P.J., Al-Habet, S.M. et al., 1983. Rifampicin reduces effectiveness and bioavailability of prednisolone. British Medical Journal Clinical Research Ed. 286 (6369), 923-925.

McCrea, J.B., Lo, M.W., Furtek, C.I. et al., 1996. Ketoconazole does not affect the systemic conversion of losartan to to E-3174. Clinical Pharmacology and Therapeutics. 59, 169.

McCrea, J., Wyss, D., Stone, J. et al., 1997. Pharmacokinetic interaction between indinavir and rifampin. Clinical Pharmacology and Therapeutics. 61, 152.

McCune, J.S., Hawke, R.L., LeCluyse, E.L. et al., 2000. In vivo and in vitro induction of human cytochrome P4503A4 by dexamethasone. Clinical Pharmacology and Therapeutics. 68(4), 356-366.

McNamara, D.B., Mayeux, P.R., 1995. Nonopiate analgesics and anti-inflammatory drugs. Principles of Pharmacology. Chapman & Hall, New York, 1995, pp. 1159-1174.

Mehvar, R., Jamali, F., Pasutto, F.M., 1998. Liquid-chromatographic assay of ibuprofen enantiomers in plasma. Clinical Chemistry. 34, 493-496.

Menzel, S., Waibel, R., Brune, K. et al., 1994. Is the formation of R- ibuprofenyl-adenylate the first stereoselective step of chiral inversion? Biochem. Pharmacol. 48 (5), 1056-1058.

Meunier, V., Bourrie, M., Julian, B. et al., 2000. Expression and induction of CYP1A1/ 1A2, CYP2A6, CYP3A4 in primary cultures of human hepatocytes. Xenobiotica. 30(6), 589-607.

Meyer, U.A., Skoda, R.C., Zanger, U.M. et al., 1992. The genetic polymorphism of of debrisoquine/sparteine metabolism: molecular mechanisms. In W. Kalow, (ed.), Pharmacogenetics of Drug Metabolism. Pergamon Press, New York. 609-623.

Miles, M.V., Tennison, M.B., 1989. Erythromycin effects on multiple-dose carbamazepine kinetics. Therapeutic Drug Monitoring. 11, 47-52.

Mills, E., Montori, V.M., Wu, P., 2004. Interaction of St. John's Wort with conventional drugs: systematic review of clinical trials. BMJ. 329 (7456), 27-30.

Miners, J.O., Foenander, T., Wanwinolruk, S. et al., 1982. The effect of sulfinpyrazone on oxidative drug metabolism in man: Inhibition of tolbutamide elimination. European Journal of Clinical Pharmacology. 22, 31-326.

Miners, J.O., Smith, K.J., Robson, R.A. et al., 1988. Tolbutamide hydroxylation by human liver microsomes: Kinetic characterization and relationship to other cytochrome P450 dependent xenobiotic oxidation. Biochemical Pharmacology. 37, 1137-1144.

Miners, J.O., Coulter, S., Tukey, R.H. et al., 1996. Cytochromes P450 1A2 and 2C9 are responsible for the human hepatic O-demethylation of (R)- and (S)-naproxen. Biochemical Pharmacology. 51, 1003-1008.

Mitchell, B.S., Hamman, A., Melissa, A. et al., 2001. The effect of rifampin administration on the disposition of fexofenadine. Clinical Pharmacology and Therapeutics. 69, 114-121.

Monmaney, T., 1998. Analysis finds claims often inaccurate. Los Angeles Times. A10, August 31.

Moore, L.B., Goodwin, B., Jones, S.A. et al., 2000. St. John's Wort induces drug metabolism through the activation of the pregnane X receptor. Proceedings of the National Academy of Sciences of the USA. 97, 7500-7502.

Morrison, P.J., Rogers, H.J., Bradbrook, I.D. et al., 1980. Concurrent administration of cimetidine and enteric-coated prednisolone: effect on plasma levels of prednisolone. British Journal of Clinical Pharmacology. 10, 87-89.

Muller H.J., Volz, H.P., 1996. Drug treatment of depression in the 1990s. An overview of achievements and future possibilities. Drugs. 52(5), 625-638.

Muller, W.E., Rolli, M., Schafer, C. et al., 1997. Effects of Hypericum extract in in biochemical models of anti-depressant activity. Pharmacopsychiatry. 30(2), 102-107.

Mutschler, E., Derendort, H., 1995. Drug interaction. In E. Mutschler, H. Derendort, (ed.), Drug Actions: Basic and Therapeutic Aspects. CRC Press, Boca Raton. 80-84.

Nakamura, Y., Yamaguchi, T., Takahashi, S. et al., 1981. Optical isomerization mechanism of R(-)- hydratropic acid derivatives. J. Pharmacobiodyn. 4, S-1.

Nation, R.L., Evans, A.M., Milne, R.W., 1990. Pharmacokinetic drug interactions with phenytoin. Clinical Pharmacokinetics. 18, 37-60.

Nebel, A., Schneider, B.J., Baker, R.K. et al., 1999. Potential metabolic interaction between St. John's Wort and theophylline. Annals of Pharmacotherapy. 33, 502.

Nelson, D.R., Koymans, L., Kamataki, T. et al, 1996. P450 superfamily, update on new sequences, gene mapping, accession number and nomenclature. Pharmacogenetics. 6, 1-42.

Neuvonen, P.J., 1991. The effect of magnesium hydroxide on the oral absorption of ibuprofen, ketoprofen and diclofenac. British Journal of Clinical Pharmacology. 31, 263-266.

Neuvonen, P.J., Jalava, M.D., 1996. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. Clinical Pharmacology and Therapeutics. 60, 54-61.

Neuvonen, P.J., Kantola, T., Krivisto, K.T., 1998. Simvastatin, but not pravastatin, very susceptible to interact with the CYP3A inhibitor itraconazole. Clinical Pharmacology and Therapeutics. 63, 332-341.

Nielsen, M., Frokjaer, S., Braestrup, C., 1988. High affinity of the naturally-occurring biflavonoid, amentoflavon, to brain benzodiazepine receptors in vitro. Biochem. Pharmacol. 37, 3285-3287.

Niemi, M., Backman, J.T., Neuvonen, M. et al., 2000. Rifampin decreases the plasma concentrations and effects of repaglinide. Clinical Pharmacology Therapeutics. 69, 495-500.

Niopas, I.,Toon, S., and Rowland, M., 1991. Further insight into the stereoselective interaction between warfarin and cimetidine in man. British Journal of Clinical Pharmacology. 32, 508-511.

Obach, R.S., 2000. Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. The Journal of Pharmacology and Experimental Therapeutics. 294, 88-95.

Ohnhaus, E.E., Brockmeyer, N., Dylewicz, P. et al., 1987. The effect of antipyrine and rifampin on the metabolism of diazepam. Clinical Pharmacology and Therapeutics. 42, 148-156.

Okey, A.B., 1992. Enzyme induction in the cytochrome P450 system. W. Kalow, (ed.), Pharmacogenetics of Drug Metabolism. Pergamon Press, New York. 549-608.

Olkkola, K.T., Aranko, K., Luurila, H. et al., 1993. A potentially hazardous interaction between erythromycin and midazolam. Clinical Pharmacology and Therapeutics. 53, 298-305.

Olkkola, K.T., Backman, J.T., Neuvonen, P.J. et al., 1994. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. Clinical Pharmacology and Therapeutics. 55(5), 481-485.

Ortiz de Montellano, P.R., 1988. Suicide substrate for drug metabolizing enzymes: mechanisms and biological consequences. In G.G. Gibson, (ed.), Progress in Drug Metabolism, Vol. 11. Taylor and Francis, New York. 99-148.

Ortiz de Montellano, P.R., Correia, M.A., 1995. Inhibition of cytochrome P450 enzymes. In P.R. Ortiz de Montellano, (ed.), Cytochrome P450: Structure, Mechanism and Biochemistry, 2nd ed. Plenium Press, New York. 305-364.

O'Reilly, R.A., Wienkers, L., Kunze, K. et al., 1987. Interaction of amiodarone with racemic warfarin and its separated enantiomorphs in humans. Clinical Pharmacology and Therapeutics. 42, 290-294.

O'Reilly, R.A., Goulart, D.A., Kunze, K.L. et al., 1992 Mechanisms of the stereoselective interaction between miconazole and racemic warfarin in human subjects. Clinical Pharmacology and Therapeutics. 51, 656-667.

Orth, D.N., Kovacs, W.J., Debold, C.R., 1992. The adrenal cortex. In J.D. Wilson and D.W. Foster, (eds.), Textbook of Endocrinology, 8th edition. W.B. Saunders Publishing, Philadelphia. 489-619.

Park, B.K., Breckinridge, A.M., 1981. Clinical implications of enzyme induction and enzyme inhibiton. Clinical Pharmacokinetics. 6, 1-24.

Park, K.S., Sohn, D.H., Veech, R. et al., 1993 Translational activation of ethanol-inducible cytochrome P450 (CYP2E1) by isoniazid. European Journal of Pharmacol., Environ. Toxicol. Pharmacol. Sect. 248(1), 7-14.

Park, Y., Kemper, B., Whitlock, J.P. et al., 1995. Induction of cytochrome P450 enzymes that metabolize xenobiotics. In P.R. Ortiz de Montellano, (ed.), Cytochrome P450: structure, mechanism and biochemistry, 2nd ed. Plenium Press, New York. 367-390.

Pascussi, J.M., Drocourt, L., Fabre, J.M. et al., 2000. Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. Molecular Pharmacology. 58(2), 361-372.

Patrono, C., Patrignami, P., Garcia Rodriguez, L.A., 2001. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. J. Clin. Invest. 108, 7-13.

Perloff, M.D., von Moltke, L.L., Stormer, E. et al., 2001. Saint John's Wort: An in vitro analysis of P-glycoprotein induction due to extended exposure. British Journal of Pharmacology. 134, 1601-1608.

Pessarye, D., Descatoire, V., Tinel, M. et al., 1982. Self-induction of oleandomycin of its own transformation into a metabolite forming an inactive complex with reduced cytochrome P450: comparison with troleandomycin. Journal of Pharmacology and Experimental Therapeutics. 221, 215-221.

Petereit, L.B., Meikle, A.W., 1977. Effectiveness of prednisolone during phenytoin therapy. Clinical Pharmacology and Therapeutics. 22(6), 912-916.

Philipp, M., Kohnen, R., Hiller, K.O., 1999. Hypericum extract versus imipramine or placebo in patients with moderate depression: randomized multicenter study of treatment for eight weeks. British Medical Journal. 319, 1534-1539.

Pickup, ME., 1979. Clinical pharmacokinetics of prednisone and prednisolone. Clinical Pharmacokinetics. 4(2), 111-28.

Pickup, M.E., Lowe, J.R., Leatham, P.A. et al., 1977. Dose-dependent pharmacokinetics of prednisolone. European Journal of Clincial Pharmacology. 12, 213-219.

Piscitelli, S.C., Burstein, A.H., Chaitt, D. et al., 2000. Indinavir concentrations and St. John's Wort. The Lancet. 355, 547-548.

Polk, R.E., Brophy, D.F., Israel, D.S. et al., 2001. Pharmacokinetic interaction between amprenavir and rifabutin and rifampin in healthy males. Antimicro. Agents Chemother. 45, 502-508.

Pond, S.M., Birkett, D.J., Wade, D.N., 1977. Mechanisms of inhibition of tolbutamide metabolism: phenylbutazone, oxybutazone, sulphaphenazole. Clinical Pharmacology and Therapeutics. 22, 573-579.

Powell-Jackson, P.R., Gray, B.J., Heaton, R.W. et al., 1983. Adverse effect of rifampicin administration on steroid-dependent asthma. American Review of Respiratory Disease. 128(2), 307-310.

Pugeat, M.M., Dunn, J.F., Nisula, B.C., 1981. Transport of steroid hormones: Interaction of 70 drugs with testosterone-binding and corticosteroid-binding globulin in human plasma. Journal of Clinical Endocrinology and Metabolism. 53, 69-75.

Pugh, M.C., Small, R.E., Garnett, W.R. et al., 1984. Effect of sucralfate on ibuprofen absorption in normal volunteers. Clin. Pharm. 3, 630-633.

Quattrocchi, F.P., Robinson, J.D., Curry, R.W. et al., 1983 The effects of ibuprofen on serum digoxin concentrations. Drug Intell Clin. Pharm. 17, 286-288.

Raffa, R.B., 1998. Screen of receptor and uptake site activity of hypericum component of St. John's Wort reveals sigma receptor binding. Life Sciences. 62, 265-270.

Ragheb, M., 1987. Ibuprofen can increase serum lithium level in lithium-treated patients. Journal of Clinical Psychiatry. 48(4), 161-163.

Rao, P.V., 1998. Statistical Research Methods in the Life Sciences. Brooks/ Cole Publishing Company, Pacific Grove, CA. 106-111.

Rashid, T.J., Martin, U., Clarke, H., et al. 1995. Factors affect the absolute bioavailability of nifedipine. British Journal of Clinical Pharmacology. 40, 51-58.

Rasmussen, P., 1998. St. John's wort— A review of its use in depression. Australian Journal of Medicine and Herbalism. 10(1), 8-13.

Raucy, J.L., 2003. Regulation of CYP3A4 expression in human hepatocytes by pharmaceutics and natural products. Drug Metabolism and Disposition. 31(5), 533-539.

Raucy, J.L., Mueller, L., Duan, K. et al., 2002. Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. Journal of Pharmacology and Experimental Therapeutics. 302(2), 475-482.

Remmer, H. and Merker, H.J., 1963. Drug-induced changes in the liver endoplasmic reticulum: Association with drug-metabolizing enzymes. Science. 142, 1657-1658.

Rettie, A.E., Korzekwa, K.R., Kunze, K.L. et al., 1992. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: A role for P-4502C9 in the etiology of (S)-warfarin drug interactions. Chem. Res. Toxicology. 5, 54-59.

Rettie, A.E., Wienkers, L.C., Gonzalez, F.J. et al., 1994. Impaired (S)-warfarin metabolism catalyzed by the R144C allelic variant of CYP2C9. Pharmacogenetics. 4, 39-42.

Rey, J.M., Walter, G., 1998. Hypericum perforatum (St. John's Wort) in depression: pest or blessing? MJA. 169, 583-586.

Roberts II, L.J., Morrow, J.D., 2001. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In A.G. Gilman, (ed.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition. McGraw-Hill, New York. 687-731.

Rocci, M.L., Johnson, N.F., Jusko, W.L., 1980. Serum protein binding of prednisolone in four species. Journal of Pharmaceutical Sciences. 69, 977-978.

Rogerson, T.D., Wilkinson, C.F., Hetnarski K., 1977. Steric factors in the inhibitory interactions of imidazoles with microsomal enzymes. Biochemical Pharmacology. 26, 1039-1042.

Romero, A.J., Rackley, R.J., Rhodes, C.T., 1991. An evaluation of ibuprofen bioinversion by simulation. Chirality. 3, 418-421.

Romkes, M., Faletto, M.B., Blaisdell, J.A. et al., 1991. Cloning and expression of complementary DNAs for multiple numbers of the human cytochrome P450 II C subfamily. Biochemistry. 30, 3247-3255.

Roots, I., Johne, A., Schmider, J. et al., 2000. Interaction from herbal extract from St. John's Wort with amitriptyline and its metabolites. Clinical Pharmacology and Therapeutics. 67, PIII69.

Rose, J.Q., Yurchak, A.M., Jusko, W.J., 1981. Dose dependent pharmacokinetics of prednisone and prednisolone in man. Journal of Pharmacokinetics and Biopharmaceutics. 9, 389-417.

Roth, G.R., Siok, C.J., 1978. Acetylation of the NH_2-terminal serine of prostaglandin synthetase by aspirin. J. Biol. Chem. 253, 3782-3784.

Rudy, A.C., Knight, P.M., Brater, D.C. et al., 1995. Enantioselective disposition of ibuprofen in elderly persons with and without renal impairment. Journal of Pharmacology and Experimental Therapeutics. 273, 88-93.

Ruschitzka, F., Meier, P.J., Turina, M. et al., 2000. Acute heart transplant rejection due to St. John's Wort. Lancet. 355, 548-549.

Schader, E., 2000. Equivalence of St. John's Wort extract (Ze 117) and fluoxetine: a randomized, controlled study in mild moderate depression. International Clincal Psychopharmacology. 15, 61-88.

Schalm, S.W., Summerskill, W.H., Go, V.L., 1977. Prednisone for chronic active liver disease: pharmacokinetics, including conversion to prednisolone. Gastroenterology. 72(5 Pt 1), 910-913.

Schenkman, J.B., 1993. Historical background and description of the cytochrome P450 monooxygenase system. Handbook of Experimental Pharmacology: Cytochrome P450., Vol 105. Springer-Verlag, Berlin. 3-10.

Scheuerer, S., Hall, S.D., Williams, K.M et al., 1998. Effect of clofibrate on the chiral inversion of ibuprofen in healthy volunteers. Clinical Pharmacology and Therapeutics. 64(2), 168-176.

Schimmer, B.P., Parker, K.L., 2001. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones. In A.G. Gilman (ed.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition. New York, McGraw-Hill. 1649-1677.

Schinkel, A.H., Smit, J.J., van Tellingen, O. et al., 1994. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell. 77, 491-502.

Schuirmann, D.J., 1987. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. Journal of Pharmacokinetics of Biopharmaceutics. 15(6), 657-680.

Shukla, V.V.K., Garg, S.K., Mathur, V.S., 1984. Influence of prednisolone on antipyrine and chloramphenicol disposition in the rabbit. Pharmacology. 29, 117-120.

Schulz, V., 2001. Incidence and clinical relevance of the interactions and side effects of Hypericum preparations. Phytomedicine. 8, 152-160.

Schwartz, U.I., Buschel, B., Kirch, W., 2003. Unwanted pregnancy on self-medication with St. John's Wort despite hormonal contraception. Br. J. Clin. Pharmacol. 55, 112-113.

Serlin, M.J., Mossman, S., Sibeon, R.G. et al., 1979. Cimetidine: Interaction with oral anticoagulants in man. Lancet. ii, 317-319.

Serlin, M.J., Breckinridge, A.M., 1983. Drugs interactions with warfarin. Drugs. 25, 610-620.

Shelton, R.C., Keller, M.B., Gelemberg, A. et al., 2001. Effectiveness of St. John's Wort in major depression: a randomized controlled trial. Journal of the American Medical Association. 285, 1978-1986.

Shen, W.W., 1995. Cytochrome P450 monooxygenases and interactions of psychotrophic drugs: a five year update. International Journal of Psychiatric Medicine. 25, 277-90.

Shimada, T., Yamazaki, H., Mimura, M. et al., 1994. Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. Journal of Pharmacology and Experimental Therapeutics. 270, 414-423.

Siemon, D., de Vries, J.X., Stozer, F. et al., 1997. Fasting and postprandial disposition of R(-) and S(+)- ibuprofen. J. Med. Res. 2, 215-219.

Silverman, R.B., 1988. Criteria for mechanism-based enzyme inactivation. Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology. CRC Press, Boca Raton, FL. 3-27.

Singer, A., Wonneman, M., Mueller, W.E., 1999. Hyperforin, a major antidepressant constituent of St. John's Wort inhibits serotonin uptake by elevating free intracellular Na +1. Journal of Pharmacology and Experimental Therapeutics. 290, 1363-1368.

Singh, A., Rao, A.R., 1992. Evaluation of the modulatory influence of food additive garam masala on hepatic detoxication system. Indian Journal of Experimental Biology. 30(12), 142-1145.

Sirgo, M.A., Rocci, M.L., Ferguson, R.K. et al., 1985. Effects of cimetidine and ranidine on the conversion of prednisone and prednisolone. Clinical Pharmacology and Therapeutics. 37, 534-538.

Skovsted, L., Kristensen, M., Hansen, J.M. et al, 1976. The effect of different oral anticoagulants on diphenylhydantoin and tolbutamide metabolism. Acta Med Scand. 199, 513-515.

Smith, A., 2000. Induction and drug development. European Journal of Pharmaceutical Sciences. 11 (3), 185-189.

Snow, J.M., 1996. Hypericum perforatum L. (Hyperiaceae). Protocol Journal of Botanical Medicine. 2(1), 16-21.

Song, B.J., Matsunaga, T., Hardwick, J.P. et al., 1987 Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat. Molecular Endocrinology. 1, 542-547.

Stephenson, D.W., Small, R.E., Wood, J.H. et al., 1988. Effect of ranitidine and cimetidine on ibuprofen pharmacokinetics. Clin. Pharm. 7, 317-321 (1988).

Steward, D.J., Haining, R.L., Henne, K.R. et al., 1997. Genetic association between sensitivity to warfarin and expression of CYP2C9*3. Pharmacogenetics. 7, 361-367.

Stys, T., Stys, A., Kelly, P. et al., 2004. Trends in use of herbal and nutritional supplements in cardiovascular patients. Clinical Cardiology. 27(2), 87-90.

Sugimoto, K., Ohmori, M., Tsuruoka, S.et al., 2001. Different effects of St. John's Wort on the pharmacokinetics of simvastatin and pravastatin. Clinical Pharmacology and Therapeutics. 70, 518-524.

Sullivan-Close, T.H., Ghanayem, B.I., Bell, D.A. et al., 1996. The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. Pharmacogenetics. 6, 341-349.

Suzuki, O. Katsumata, Y., Oya, M. et al., 1984. Inhibition of monoamine oxidase by hypericin. Planta Medica. 50, 272-274.

Szefler, S.J., Ellis, E.F., Brenner, M. et al., 1982. Steroid-specific and anticonvulsant interaction aspects of troleandomycin-steroid therapy. Journal of Allergy and Clinical Immunology. 69, 455-460.

Szpilfogel, S.A., 1984. Adrenocortical steroids and their synthetic analogs. In M.J. Parnham and J. Bruinvels, (eds.), Discoveries in Pharmacology, Volume 2. Elsevier, Amsterdam. 253-284.

Takanashi, K., Tainaka, H., Kobayashi, K. et al., 2000. CYP2C9 Ile and Leu359 variants: enzyme kinetic study with seven substrates. Pharmacogenetics. 10, 95-104.

Tailor, S.A., Gupta, A.K., Walker, S.E. et al., 1996. Peripheral edema due to nifedipine-itraconazole interaction: a case report. Archives of Dermatology. 132, 350-352, (1996).

Tanner, A., Bochner, F., Caffin, J. et al., 1979. Dose-dependent prednisolone kinetics. Clinical Pharmacology and Therapeutics. 25, 571-578.

Tannergren, C., Engman, H., Knutson, L.et al., 2004. St. John's Wort decreases the bioavailability of R-and S-verapamil through induction of the first-pass metabolism. Clinical Pharmacology and Therapeutics. 75, 298-309.

Testa, B., Jenner, P., 1976. Drug Metabolism— Chemical and Biochemical Aspects. Marcel Dekker Inc., New York.

Thiebaut, F., Tsuruo, T., Hamada, H. et al., 1987. Cellular localization of multidrug-resistance gene product P-glycoprotein in normal human tissues. Procedures of the National Academy of Sciences of the U.S.A. 84, 7735-8.

Thiede, H.M., Walper, A., 1994. Inhibition of MAO and COMT by hypericum extracts and hypericin. Journal of Geriatric Psychiatry and Neurology. 7 (suppl 1), S54-S56.

Thomas, S.H.L, 1993. Paracetamol (acetaminophen) poisoning. Pharmacology Therap. 60, 91-120.

Thummel, K.E., Kunze, K.L., Shen, D.D. et al., 1997. Enzyme-catalyzed process of first-pass hepatic and intestinal drug extraction. Advanced Drug Delivery Reviews. 27, 99-127.

Toon, S., Hopkins, K.J., Garstang, F.M. et al., 1987. Comparative effects of ranitidine and cimetidine on the pharmacokinetics and pharamcodynamics of warfarin in man. European Journal of Clinical Pharmacology. 32, 165-172.

Touchette, M.A., Chandraskar, P.H., Milad, M.A. et al., 1992. Contrasting effects of fluconazole and ketoconazole on phenytoin and testosterone disposition in man. British Journal of Clinical Pharmacology. 34, 75-78.

Tracy, T.S., Rosebluth, B.W., Wrighton, S.A. et al., 1995. Role of cytochrome P4502C9 and an allelic variant in the 4'-hydroxylation of (R)- and (S)-fluribiprofen. Biochemical Pharmacology. 49, 1269-1275.

United States Pharmacopoeia, 1999. Powdered St. John's Wort extract monograph. Pharmacopoeial Forum of the United State Pharmacopoeia. 25(2).

Uribe, M., Schalm, S.W., Summerskill, W.H.J. et al., 1978. Oral prednisone for chronic active liver disease: Dose responses and bioavailability studies. Gut. 19, 1131-1135.

Varis, T., Kivisto, K.T., Neuvonen, P.J., 2000. The effect of itraconazole on the pharmacokinetics and the pharmacodynamics of oral prednisolone. Pharmacokinetics and Disposition. 56, 57-60.

Venkatakrishnan, K., Greenblatt, D.J., Von Moltke, L.L. et al., 1998. Five distinct human cytochromes mediate amitriptyline N-demethylation in vitro: dominance of CYP2C19 and 3A4. Journal of Clinical Pharmacology. 38, 112-121.

Veronese, M.E., Mackenzie, P.I., Doecke, C.J. et al., 1991. Tolbutamide and phenytoin hydroxylations by cDNA-expressed human liver cytochrome P4502C9. Biochem. Biophys. Res. Commun. 175, 1112-1118.

Veronese, M.E., Doecke, C.J., Mackenzie, P.I. et al., 1993. Site-directed mutation studies of human liver cytochrome P-450 isoenzymes in the CYP2C subfamily. Biochem. J. 289, 533-538.

Villanueva, M., Heckenberger, R., Strobach, H. et al., 1993. Equipotent inhibition by R(-)-, S(+)- and racemic ibuprofen of human polymorphonuclear cell function in vitro. British Journal of Clinical Pharmacology. 35, 235-242.

Villikka, K., Kivisto, K.T., Backman, J.T. 1997. Triazolam is ineffective in patients taking rifampin. Clinical Pharmacology and Therapeutics. 61, 8-14.

Vorbach, E.U., Hubner, W.D., Arnoldt, K.H., 1994. Effectiveness and tolerance of the hypericium extract LI 160 in comparison with imipramine: Randomized double-blind study with 135 outpatients. Journal of Geriatric Psychiatry and Neurology. 7, S19-S23.

Vorbach, E.U., Arnoldt, K.H., Hubner, W.D., 1997. Efficacy and tolerability of St. John's Wort extract LI 160 versus imipramine in patients with severe depressive episodes according to ICD-10. Pharmacopsychiatry. 30, 81-85.

Wacher, V.J., Wu, C.Y., Benet, L.Z., 1995. Overlapping substrate specificities and tissue distribution of cytochrome P4503A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Mol. Carcinogenesis. 13, 129-134.

Wacher, V.J., Salphati L., Benet, L.Z., 2001. Active secretion and enterocytic drug metabolism barriers to drug absorption. Advanced Drug Delivery Reviews. 46, 89-102.

Wagner, J.G., Santo, A.R., Gillespie, W.R. et al., 1981. Reversible metabolism and pharmacokinetics: application to prednisone-prednisolone. Research Communications in Chemical Pathology and Pharmacology. 32(3), 387-404.

Wahli, W., Martinez, E., 1991. Superfamily of steroid nuclear receptors: positive and negative regulators of gene expression. FASEB. 5, 3092-3099.

Wang, L., Zhou, G., Zhu, B. et al., 2004. St. John's Wort induces both cytochrome P450 3A4- catalyzed sulfoxidation and 2C19- dependent hydroxylation of omeprazole. Clinical Pharmacology and Therapeutics. 75, 191-197.

Wang, P., Mason, P.S., Guengerich, F.P., 1980. Purification of human liver cytochrome P-450 and comparison to the enzyme isolated from rat liver. Arch. Biochem. Biophys. 199, 206-219.

Wang, Z., Gorski, J.C., Hamman, M.A. et al., 2001. The effects of St. John's Wort (hypericum perforatum) on human cytochrome P450 activity. Clinical Pharmacology and Therapeutics. 70, 317-326.

Wassner, S.J., Pennisi, A.J., Malekzadeh, M.H. et al., 1976. The adverse effect of anticonvulsant therapy on renal allograft survival. A preliminary report. Journal of Pediatrics. 88(1), 134-137.

Wassner, S.J., Malekzadeh, M.H., Pennisi A.J. et al., 1977. Allograft survival in patients receiving anticonvulsant medications. Clinical Nephrology. 8(1), 293-297.

Waxman, D.J., Azaroff, L., 1992. Phenobarbital induction of cytochrome P-450 gene expression. Biochem. J. 281, 577-592.

Waxman, D.J., Attisano, C., Guengerich, F.P. et al., 1988. Human liver microsomal steroid metabolism: identification of the major microsomal steroid hormone 6β-hydroxylase cytochrome P-450 enzyme. Arch. Biochem. Biophys. 263, 424-436.

Wei, P., Zhang, J., Egan-Hafley, M. et al., 2000. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. Nature. 407 (6806), 920-923.

Wentworth, J.M., Agostini, M., Schwabe J.W. et al., 2000. St. John's Wort, a herbal antidepressant, activates the steroid X receptor. Journal of Endocrinology. 166, R11-R16.

Wheatley, D., 1997. LI 60. an extract of St. John's Wort, versus amitriptiline in mildly to moderately depressed outpatients—a controlled 6-week clinical trial. Pharmacopsychiatry. 30, 77-80.

Wheatley, D., 1998. Hypericum extract—potential in the treatment of depression. CNS Drugs. 9, 431-440.

White, R.E., Sligar, S.G., Coon, M.J., 1980. Evidence for a homolytic mechanism of peroxide oxygen-oxygen bond cleavage during substrate hydroxylation by cytochrome P450. Journal of Biol. Chem. 255, 11108-11111.

Whitlam, J.B., Brown, K.F., Crooks, M.J. et al., 1981. Transsynovial distribution of ibuprofen in arthritic patients. Clinical Pharmacology and Therapeutics. 29, 487-492.

Whitlock, J.P., Denison, M.S., 1995. Induction of cytochrome P450 enzymes that metabolize xenobiotics. In P.R. Ortiz de Montellano, (ed.), Cytochrome P450: structure, mechanism, and biochemistry, 2nd ed. Plenium Press, New York. 367-390.

Wilkinson, G.R., Guengerich, F.P., Branch, R.A., 1989. Genetic polymorphism of S-mephenytoin hydroxylation. Pharmacology and Therapeutics. 43, 53-76.

Wills, R.B.H., Bone, K., Morgan, M., 2000. Herbal products: active constituents, modes of action and quality control. Nutr. Res. Rev. 13, 47-77.

Williams, K., Day, R., Knihinicki, R. et al., 1986. The stereoselective uptake of ibuprofen enantiomers into adipose tissue. Biochemical Pharmacology. 35, 3403-3405.

Williams, R.T., 1959. Detoxification mechanisms. The Metabolism and Detoxification of Drugs, Toxic Substances and other Organic Compounds. John Wiley and Sons, New York.

Woelk, H., Burkard, G., Gruenwald, J., 1994. Benefits and risks of the Hypericum extract LI 160: Drug monitoring study with 3250 patients. Journal of Geriatric Psychiatry and Neurology. 7(1), 34-38.

Woelk, H., 2000. Comparison of St. John's Wort and imipramine for treating depression: randomized controlled trial. British Medical Journal. 321, 536-539.

Wolbold, R., Klein, K., Burk, O. et al., 2003. Sex is major determinant of CYP3A4 expression in human liver. Hepatology. 38, 978-988.

Wright, C.E., Antal, E.J., Gillespie, W.R. et al., 1983. Ibuprofen and acetaminophen kinetics when taken concurrently. Clinical Pharmacology and Therapeutics. 34(5), 707-710.

Wrighton, S.A., Stevens, J.C., 1992. The human hepatic cytochromes P450 involved in drug metabolism. Crit. Rev. Toxicol. 22, 1-21.

Yan, Z., Caldwell, G.W., 2001. Metabolism, profiling, and cytochrome P450 inhibition and induction in drug discovery. Current Topics in Medicinal Chemistry. 1, 403-425.

Yeates, R.A., Lauren, H., Zimmerman, T., 1996. Interaction between midazolam and clarithromycin: comparison with azithromycin. International Journal of Clinical Pharmacology and Therapeutics. 34, 400-405.

Ylitalo, P., Janas, M., Wilen, G. et al., 1985. Sulfadiazine versus sulfafurazole excretion in urine and risk of crystallization in children with conventional dosage regimen. Arzneimittel-Forschung. 35(6), 988-990.

You, L., 2004. Steroid hormone biotransformation and xenobiotic induction of hepatic steroid metabolizing enzymes. Chemico-Biological Interactions. 147(3), 233-246.

Yue, Q-Y., Bergquist, C., Gerden, B., 2000. Safety of St. John's Wort (Hypericum perforatum). Lancet. 355, 576-577.

Zhang, Y., Benet, L., 2001. The gut as a barrier to drug absorption. Combined role of cytochrome P450 3A and P-glycoprotein. Clinical Pharmacokinetics. 40 (3), 159-168.

Zhou, S., Gao, Y., Jiang, W. et al., 2003. Interactions of herbs with cytochrome P450. Drug Metabolism Reviews. 35 (1), 35-98.

Zilly, W., Breimer, D.D., Richter, E., 1975. Induction of drug metabolism in man after rifampicin treatment measured by increased hexobarbital and tolbutamide clearance. European Journal of Clinical Pharmacology. 9, 219-227.

Zilly, W., Breimer, D.D., Richter, E., 1977. Stimulation of drug metabolism by rifampicin in patients with cirrhosis or cholestasis measured by increased hexobarbital and tolbutamide clearance. European Journal of Clinical Pharmacology. 11, 287-293.

Zou, L., Harkey, M.R., Henderson, G.L., 2002. Effects of herbal components on cDNA-expressed cytochrome P450 enzyme activity. Life Sciences. 71, 1579-1589.

Zurcher, R.M., Frey, B.M., Frey, F.J., 1989. Impact of ketoconazole on the metabolism of prednisolone. Clinical Pharmacology and Therapeutics. 45, 366-372.