Transdermal Iontophoretic Delivery of Selegiline and Prochlorperazine

by

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Abstract

Transdermal iontophoretic delivery of selegiline hydrochloride (SH) across dermatomed human skin was studied. SH was stable under the influence of electrical field. Increase in drug concentration from 1 to 20 mg/ml, increased the iontophoretic flux by 13 fold. Overall, with 20 mg/ml of SH, pH 5 and 100 mM NaCl concentration and 0.4 mA/ cm^2, a maximum flux of 305.5μg/cm^2/hr was obtained. Based on the reported pharmacokinetic parameters, with a surface area of 40 cm^2, Iontophoretic delivery can provide 6-7 times higher levels of SH than the target delivery rate, which enables lowering of dose and/or patch surface area. Further in vivo studies will prove the efficacy of ionophoresis for enhanced delivery of SH.

Transdermal prochlorperazine edisylate (PE) was studied. Skin was microporated using a Dermaroller™ to study the effect of solid microneedles on the transport of PE with and without the influence of an electric current. Effect of PE concentration (20, 50 and 100 mg/ml), number of passes of the Dermaroller ^{TM} (0, 5, 10 and 20) and the combined effect of microneedle and iontophoresis of PE was determined. Samples were assayed by HPLC. Permeation of PE in iontophoresis was increased the flux by 53 fold as compared to passive delivery. When skin is microporated (5 passes) the total iontophoretic flux was increased by 92 fold as compared to the control (passive delivery). A synergistic increase in the transdermal transport of PE was observed when iontophoresis (0.4 mA/cm^2) was used in conjunction with microporation (5 passes). The combined system can provide a flux of 4 μg/cm^2/h, which is 2 fold higher than the target delivery rate (2 μg/cm^2/h), to provide effective plasma concentrations.

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List of Abbreviations

CTZ	Chemoreceptor Trigger Zone
MDD	Major Depressive Disorder
MN	Microneedles
PE	Prochlorperazine Edisylate
SH	Selegiline Hydrochloride
TDS	Transdermal Delivery System

I. LITERATURE REVIEW

Skin

The skin is the largest human organ of the integumentary system (Hadgraft, 2001), and also an excellent biological barrier. Though it is normally less than 2mm thick, the skin is 10^2-10^4 times less permeable to water than a blood capillary wall. It is composed of three major components: the epidermis, dermis, and subcutaneous fat layer (hypodermis). The outer skin part (epidermis) in humans is typically 50-150μm thick; it contains five histologically distinct layers, which are the stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum from the inside to the outside. The horny skin layer, the stratum corneum, is composed of large, flat, polyhedral, plate-like envelopes filled with keratin, which is made up of dead cells that have migrated up from the stratum granulosum. Although the stratum corneum comprises the outermost 5-20μm of thickness, it is the primary skin barrier to drug and chemical substances' diffusion. The impermeability of skin is a considerable problem in the delivery of drugs both to and through the skin (Hadgraft, 2001; Trommer and Neubert, 2006). The region below the epidermis is called the dermis. It consists of the outer papillary and the inner reticular dermis, which taken together are usually 4-20 times thicker than epidermis and thus normally measure up to 2 mm in thickness. These two inner skin regions contain only a few cells, predominantly fibroblasts and adipocytes, but a lot of collagen (~70% wt). The latter forms fibrous, many micrometers long bundles to shelter, as well as support, sweat glands, some immunologically active cells, nerves, plus dermal lymph and blood capillaries.

Each individual skin layer is mechanically different, because of its unique biochemical and anatomical properties. The stratum corneum is rather stiff. Its effective elastic modulus in the 10^7~10^8 Pa range but decreases with temperature and hydration (static: ~3-22 MPa; dynamic: ~50-240 MPa). The underlying epidermis and papillary dermis are less resistant to mechanical stress (static stiffness: 0.2 kPa).

Lipids Organization in the Stratum Corneum and Skin Pathways

Lipids organization in the stratum corneum has been extensively reviewed in the literature. The extracellular space in primary skin barrier is filled with a quasi-continuous matrix, largely in crystalline estate and in the form of multi-lamellae with repeat distance of ~5.5 nm. Interlamellar regions in the stratum corneum contain less ordered lipids and more flexible hydrophobic chains. Fluid lipids in skin barrier are crucially important for trans-epidermal diffusion of the lipidic and amphiphilic molecules, as they secure the "free volume" needed for such molecules insertion and migration through intercellular lipid layers. The hydrophilic molecules behave conversely that they diffuse predominantly "laterally". Water filled inter-lamellar spaces or through such volumes, polar molecules can also utilize the free space between a lamella and a corneocyte outer membrane to the same end. The exact drug permeation means against its barrier has been investigated over many years. Recent interesting insights into the mechanisms of absorption at a molecular level showed a number of ways in which the drug can cross the stratum corneum (Guy and Hadgraft, 1989; Roberts and Walters, 1998; Hadgraft, 2001). These include intercellular, transcellular, and trans-appendageal route through the pores and hair follicles. Intracellular macromolecular matrix within the stratum corneum does not contribute directly to the skin diffusive barrier but supports mechanical stability and thus intactness of the stratum corneum. The transcellular and intercellular route together is known as transepidermal

route. Although transcellular route is the shortest channel for the molecules, the drug molecules encounter significant resistance to permeation because they have to cross both lipophilic, hydrophilic and the cytoplasm of the dead keratinocytes structures (Hadgraft, 2001), and it is practically unimportant for transdermal drug transport (Cevc and Vierl, 2010). The intercellular route is more common as the molecules are able to overcome the stratum corneum by passing between the corneocytes (Trommer and Neubert, 2006). Skin appendages like hair follicles and the sweat and sebaceous glands also contribute to the permeation as named transappendageal pathway, but the amount is small as they only occupy 0.1% of the total skin surface. Recently, research regarding follicular absorption has shown to be of more significance. This is more for micro and nanoemusions which have the ability to be absorbed into the hair shaft and reside there to provide a significant local effect (Cevc and Vierl, 2010).

Transdermal Drug Delivery Systems

These are classified as two types; 1) adhesive matrix transdermal delivery systems and 2) reservoir type (membrane moderated) transdermal systems. Adhesive system typically consists of an impermeable backing, adhesive polymer matrix loaded with drug and a release liner. This yields a thinner and/or smaller transdermal patch. The release profiles of drug from this type of transdermal delivery system follows matrix diffusion process where skin serves as a rate limiting barrier for drug absorption. This type of patch is best illustrated by the nitroglycerin-releasing transdermal patch, the Minitran^{TM} system and the Nitro-DurII^{TM} system and the isosorbide dinitrate-releasing transdermal delivery system (TDS) (Frandol Tape) for once-a-day medication of angina-pectoris (Chien, 1992).

Reservoir system consist of 1) impermeable backing; 2) reservoir of gel containing a drug, enhancer and anti-irritants, and gelling agents; 3) membrane; 4) adhesive. These patches could

mostly be placed on top of abdomen, upper arm, back and thigh. The rate of drug release from these TDS can be tailored by varying the composition of the drug reservoir formulation and the permeability coefficient and/or thickness of the rate controlling membrane. Several TDS have been successfully developed from this technology and approved by FDA for marketing (e.g., Transderm-Nitro™, Transderm-Scop™, Catapress-TTS™, Estraderm™, Duragesic™) (Chien, 1992; Southam, 1995).

Transdermal Drug Products Overview

The first transdermal patch was approved in 1981 for the relief of the symptoms of motion sickness, nausea and vomiting. Over the last three decades, more than 35 transdermal patch products were approved by US Food and Drug Administration (Table 1).

Table 1: Summary of Marketed Transdermal Products
(Subedi et al., 2010)

Drug	Disease/treatment	Product
Scopolamine	Motion sickness	Transderm-Scop®
Nitroglycerin	Angina pectoris	Transderm-Nitro®, Nitrodisc®, Deponit®, Minitran®, Nitro-dur®, Nicotinell®
Nocotine	Smoking cessation	Nicoderm®, Nicostop®, Habitrol®, Nicotrol®, Prostep®
Estradiol	Postmenstrual syndrome	Estraderm®, Estran®, Climaderm®, Climara®, Alora®, Fematrix®, Fempatch®, Vivelle®
Testosterone	Hypogonadism	TestoDermTTS®, AndroDerm®
Clonidine	Hypertension	Catapress-TTS®
Fentanyl	Analgesia	Duragesic®, Matrifen®
Buprenorphine	Analgesia	BuTrans®
Progestin/estrogen	Cotraceptives	OrthoEvra®

Estradiol/Norethindrone	Hormone replacement therapy (HRT)	CombiPatch®
Estrgen/Progesterone	HRT	Nuvelle TS®
Selegiline	Depression	EmSam®
Rotigotine	Parkinson's	Neupro®
Methylphenidate	ADHD (Attention deficit hyperactivity disorder)	Daytrana®
Lidocaine	Post-herpetic neuralgia	Lidoderm®, Synera® (lidocaine+Tetracaine)
Ketoprofen, Piroxicam, Diclofenac	Inflamation/pain	Ketotop®, Trast®, Rheumastop®, Nupatch®
Rivastigmine	Alzheimer's disease	Exelon®
Oxybutynin	Hyper active bladder	Oxytol® (USA), Kentera® (Europe)
Granusetron	Nausea, vomiting	Sansuco®
Capsaicin	Postherpetic neuralgia	Qutenza®

Goals of Transdermal Drug Delivery

1) Minimal invasiveness or non-invasiveness of an application, including injection pain reduction;
2) Improved drug pharmacokinetics;
3) Targeted drug delivery.

Potential Benefits

Transdermal delivery has a variety of advantages compared with the oral route. Particularly it is used when there is a significant first-pass effect that can prematurely metabolize drugs. This delivery also has advantages over hypodermic injections, which are painful, generate dangerous medical waste and pose the risk of disease transmission by needle re-use, especially in developing countries. In addition to this noninvasive system, patients can self-administer the

drugs (Prausnitz and Langer, 2008). Also transdermal systems are targeted to provide small dosages, long periods of time of drug release, thus a safer pharmacokinetic profile can be maintained with less drug concentration fluctuation.

Permeation Enhancement

The greatest challenge for transdermal delivery is that only a limited number of drugs are amenable to administration by this route (Prausnitz and Langer, 2008). Current methods can transfer molecular masses that are only up to a few hundred Daltons. Therefore, skin permeation enhancement has been studied, and there are mainly three methods, which are physical, chemical, and biochemical approaches. Physical approach includes stripping or hydration of stratum corneum, iontophoresis or phonophoresis, heat or thermal energy abrasion, and microneedle applications. Chemical enhancement involves systhesis of lipophilic analogues, delipidization of stratum corneum, and coadministration of many skin permeation enhancers. Biochemical approach contains synthesis of bioconvertible prodrugs and coadministration of skin metabolism inhibitors. Generally these penetration enhancement methods can disrupt the highly ordered stratum corneum lipids, interact with cellular proteins and improve partitioning of drug co-enhancer or co-solvent into the stratum corneum and assist drug permeation into the skin.

In this investigation the physical penetration enhancement techniques of iontophoresis and microneedles are utilized for delivering two poorly permeable model drugs (prochlorperazine edisylate and selegiline hydrochloride).

Iontophoresis

As mentioned in previous chapter, the skin restricts the permeation of drugs through the stratum corneum into the systematic circulation. The currently available transdermal drugs (clonidine, estradiol, fentanyl, nicotine, nitroglycerin, scopolamine, testosterone, oxybutynin and

so on) are all potent low molecular weight molecules which are active at blood concentrations on the order of a few ng/ml or less (Kalia et al., 1998; Kalia et al., 2004). In order to increase the range of drugs available for transdrmal delivery, a number of chemical and physical enhancers have been developed in an attempt to overcome skin barrier function without concomitant skin irritation. Iontophoresis is one of the physical enhancement techniques and one of attractive alternatives to the second-generation drug delivery system in transdermal delivery. It is defined as the application of an electrical potential that maintains a constant, very low electric current across the skin and enhances the delivery of ionized as well as unionized moieties. As many therapeutically active drugs are hydrophilic, high molecular weight and charged compounds (Wang et al., 2005), iontophoresis technique is capable of expanding the range of compounds that can be delivered transdermally.

Iontophoresis provides the usual advantages of a transdermal route like therapeutic efficacy improvement by bypassing the hepatic "first pass" metabolism, avoidance of inconvenience caused by parenteral drug delivery and prevention of variation in the absorption seen with oral administration (Dixit et al., 2007). Beside these, it also has the advantages of delivering both ionized and unionized drugs, enabling continuous or pulsatile delivery of drug, permitting an easier termination of the delivery, offering better control over the amount of drug administered since it is limited by the applied current, improving the delivery of polar as well as high molecular weight molecules. Iontophoresis allows either systemic or local delivery of drugs, reduces the chance of dosing variation by providing programmed delivery of the drug (Williams and Barry, 1991; Williams and Barry, 1992). It also provides a therapeutic regimen leading to better patient compliance (Wang et al., 2005).

Iontophoretic Electrochemistry

An iontophoretic device includes a power source and two electrode compartments (Fig. 1.1). The drug is often in its salt formulation (D^+A^-). The ionized molecule (D^+) and its counter ion (A^-) are placed in the electrode compartment bearing the same charge (e.g. a positively charged drug such as lidocaine would be placed in the anodal compartment). The opposite electrode compartment like the cathodic counter reservoir, contains biologically acceptable cations (C^+) and anions (A^-), and is placed at a distal site on the skin (Kalia et al., 2003; Phipps et al., 2002). Of all the types of electrode, the best-suited to iontophoresis is the Ag/AgCl couple, because of many reasons that would be stated later in this article. Once the current is applied, the electric field imposes a direction on the movements of the ions present (as shown in Fig. 1.1): positive charges in the anodal compartment move towards the cathode, anions move in the opposite direction. The drug ions migrate together with the cationic ions into the skin, endogenous anions, mostly chloride, migrate from the body into the donor reservoir (Kalia et al., 2003, Wang et al., 2005). In the cathodal chamber, Cl^- ions are released from the electrode and electroneutrality requires that either an anion is lost from the cathodal chamber or that a cation enters the chamber from the skin.

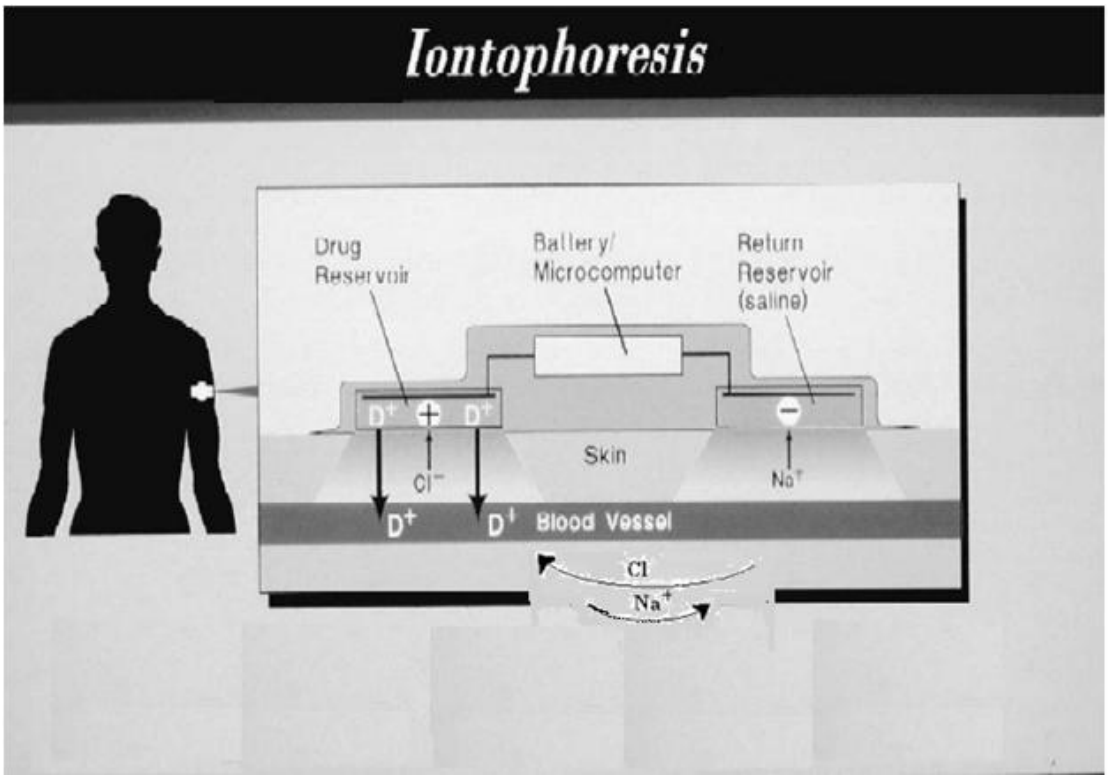


Fig. 1.1: Iontophoresis Electrochemistry (Junginger, 2002)

Iontophoretic Transport Mechanisms

The iontophoretic technique generally is based on the principles that like charges repel and opposite charges attract each other. Thus, during iontophoresis, the mechanism comprises several processes for moving molecules across the skin: molecular diffusion, electromigration and electroosmosis. Electromigration is the movement of charged ionic species in response to an applied electric field. This is the primary important process in delivering charged drugs. The movement of charged species within a solvent can induce solvent flow by a process known as electroosmosis. This process is useful for delivering both charged and neutral drugs (Phipps et al., 2002). For any given iontophoretic treatment, one or more of the processes may occur simultaneously and the varying extent depends on the magnitude and duration of the current applied, the composition of the donor reservoir and the type of the tissue being treated.

There is growing evidence that the preferred path for ionic species across the stratum corneum is not spatially homogenous but rather consists of a distribution of localized regions. These regions include endogenous shunt-like structures like sweat ducts and hair follicles (Burnette, 1988), but might also be pathways not associated with natural shunts (Cullander, 1992; Guy and Cullander, 1991). Direct physical measurements of the transport of model permeants through the skin of hairless mice have been made and showed between 60%~90% of the overall flux can be explained by such regions (Scott et al., 1995).

Due to the complex nature of iontophoretic delivery, transport mechanisms were elucidated to quantify the rate of this delivery. The observed iontophoresis flux of a charged species X, can be considered as the sum of two separate transport mechanisms at steady state, assuming the passive permeation is negligible:

$$J^{T}x = J^{EM}x + J^{EO}x.$$

In the above equation, $J^{EM}x$ represents electromigration and $J^{EO}x$ stands for electroosmosis.

The rate of transport of a charged drug species across a biological membrane is generally modeled by the Nernst-Planck equation. This equation contains terms for diffusion, electromigration and bulk convection. However, the electromigration contribution is often much greater than the others. Electromigration refers to the ordered movement of the ions in the presence of the applied electric field. The electromigratory flux of an ion X is related to the component current flow Ix due to its transport by Faraday's constant F, so we have:

$$J^{EM}x = \frac{1}{ZxAF} \cdot Ix$$

Where A represents the cross-sectional area for transport across the skin and Zx is the charge. The ionic current flow Ix, due to the movement of X can be related to the applied current

I, by a proportionality constant Tx. Tx is the transport number that describes the fraction of the total current transferred by X (0<Tx<1).

$$J^{EM}X = \frac{1}{ZxAF} \cdot Tx \cdot I$$

The transport number of X depends on the physicochemical properties of X and how these compare with the corresponding properties of the other charge carriers present in the system. Specifically, $Tx = \frac{ZxUxCx}{\sum_{n=o}^{i} ZiUiCi}$ (Phipps et al., 2002), where Zx, Ux and Cx refer to the charge, mobility and concentration of the drug in the membrane respectively. Then $J^{EM}X = \frac{1}{ZxF} \cdot \frac{ZxUxCx}{\sum_{n=o}^{i} ZiUiCi} \cdot I_D$, where I_D is the applied current density (=I/A). The denominator is the sum of the products of these parameters for each ion in the system contributing to charge transfer across the membrane. This formula also explains why the presence of competing ions can reduce the drug flux and the delivery efficiency (Kalia et al., 2003). Because the extent of competition and attenuation of drug transport will depend on the products of the respective electrical mobilities and concentrations in the membrane, increasing the concentration of a less mobile drug species can improve delivery efficiency in the presence of a small highly mobile competing ion.

Electroosmosis can be explained as the volume flow induced by the current flow, in other words the movement of charge, across a membrane. At a molecular level, electroosmosis can be viewed as resulting from the fact that the skin has a pI 4 ~4.5 (Marro et al., 2001), above which the carboxylate groups present in the membrane become ionized. Application of an electric field across a charged membrane favors the movement of counter-ions that try to neutralize the membrane charge and, in the case of the skin, gives rise to its cation permselectivity. The

existence of this solvent flow in the anode-to-cathode direction means that

1) Neutral molecules can be delivered by anodal iontophoresis.

2) Cations will benefit from a second driving force in addition to electromigration.

However, electroosmotic component is less intuitive and non-equilibrium thermo-dynamics can be used to explain how seemingly unrelated flow processes can interact and impact each other. The application of pressure and a potential difference across a membrane can be used to generate current and volume flows respectively (Pikal, 1992; Pikal and Shah, 1990; Kalia et al., 2003).

Factors Affecting Iontophoresis

1. Effect of Drug Concentration

Concentration of the drug is one of the most important factors affecting iontophoresis. Studies showed as concentration in the donor compartment increased, the transdermal apparent steady flux of a number of drugs would increase, such as metoprolol (Thysman et al., 1992), butyrate (Behl et al., 1989), diclofenac sodium (Koizumi et al., 1990), rotigotine (Nugroho et al., 2004) and ketorolac (Tiwari and Udupa, 2003). Although the equations described above suggest a fairly straightforward correlation between the amount of drug in the formulation and the observed flux, more experimental results demonstrated more complicated cases that some drugs do not have an increase in flux when the drug concentration is increased. Sometimes a simply increase in the amount of drug in the formulation may not increase the number of molecules in the membrane, and the concentrations and mobilities of competing ions play an important role, and the physicochemical properties of the drug should be take into account (Kalia et al., 2003). The general observation is an increase in concentration increases flux up to a point, the flux plateaus as concentration increases (Marro et al., 2001; Van Der Geest et al., 1997) independent

of the donor concentration. The reason for this is probably due to the charge saturation of the aqueous conducting pathways of skin (Sanderson et al., 1989). More complex relationship between the iontophoretic flux and drug concentration has been observed for some lipophilic cations like nafarelin and leuprolide, which showed a reduction in drug delivery when concentration is increased (Delgado-Charro and Guy, 1994; Delgado-Charro and Guy, 1995; Hirvonen and Guy, 1997; Lu et al., 1993; Rodriguez Bayon and Guy, 1996). In the absence of competing ions, like in the iontophoresis of lidocaine hydrochloride (Marro et al., 2001) or hydromorphone hydrochloride (R. van der Geest et al., 1997), studies showed that the iontophoretic flux is independent of the donor drug concentration.

2. Ionic Strength and Presence of Other Ions

The main purpose in iontophoresis is the drug ion should carry maximum charge across the membrane. An increase in ionic strength will decrease drug delivery, because extraneous ions compete with the drug ions (Dixit et al., 2007). The buffering agents used in iontophoresis is a source of co-ions, that are generally more mobile and smaller in size than the drug ions, so they dominate the penetration into the skin and cause a decrease in transdermal flux of the drug. Ketorolac showed increased flux with decrease in ionic strength (Tiwari and Udupa, 2003). Salicylic acid flux was found to decrease with the increase in concentration of HEPES buffer (Yoshida et al., 1995) and similarly many peptides widely studied for ionic strength showed a higher flux occurring at low electrolyte concentration (Chien et al., 1989; Morimoto et al., 1992; Craane-Van et al., 1994; Fu et al., 1993). Thus the extraneous ions and drug ions should maintain a balance to reach the maximum drug delivery.

3. Effect of Current

The current could be easily controlled by the electronics, and it is a convenient way to control delivery of drugs to the body. In general, it is true for small molecules and peptides that the electromigratory contribution to iontophoretic flux should be linearly proportional to the applied current provided that the respective ion concentrations are kept constant (Kalia et al., 2003). Although there is a linear relationship between the apparent flux and current for a number of drugs, the current cannot be too much as it may cause irritation and damage to the skin. Generally the maximum amount of current is usually 0.5mA/cm^2 on human skin.

4. Effect of pH

Many drugs have a broad pH range when drugs are soluble and stable for transdermal delivery by iontophoresis. But optimal drug delivery and biocompatibility are usually limited to a narrow range of formulation pH. The skin has an iso-electric point at pH 4 (Phipps et al., 2002), the pH of the donor solution influences the pH of the skin and makes the skin a permselective membrane especially if the pH of the skin rises above 4, because this causes the carboxylic acid moieties in the skin to become ionized and then the anodal iontophoresis promotes the permeation of cationic drugs. The pH of the donor solution also affects the ionization of the drug itself and a weakly basic drug will be ionized to a lower extent at pH higher than its pKa and will not permeate by iontophoresis (Subedi et al., 2010).

The biocompatibility has been investigated by Cormier and Johnson (Cormier and Johnson, 1997) and they found that formulation pH can have a substantial effect on skin irritation (Sanderson et al., 1989; Cormier et al., 1997; Phipps et al., 1997). The skin irritation was reduced by choosing different pH ranges for the anode and cathode formulations, like for anode formulations, pH values between 4 and 10 produced the lowest skin irritation and pH values between 2 and 4 were least irritating for cathode formulations.

5. Type of Electrodes Used

Electrodes serve as the bridge between the electric circuit and the two reservoirs, and perform both electrical and chemical functions. A practical electrode system must meet a variety of performance, compatibility and physical requirements. The type of electrodes used could be non-consumable electrodes or consumable electrodes. Some electrodes like stainless steel can release metal ions through direct oxidation at the anode or indirectly by the creation of a caustic environment at the cathode. The preferred electrodes are silver as an anode and silver-chloride as a consumable cathode, as they resist the changes in pH (like sharp decreases in pH have been seen with Pt-metal electrodes). The electrochemistry reaction occurs at voltages lower than those necessary for the electrolysis of water, and will not in turn react with water causing some side effects, like proton created at the anode site would compete with drug ions and lead to acid-induced skin burns. The ions migrated to the donor skin do not compete with charged drugs. The following reactions occur at the anode (Phipps et al., 2002).

$$Ag + Cl^- \longrightarrow AgCl + e^-$$

The final product is silver chloride, it forms on the surface of the silver anode and is electrically neutral and practically insoluble, which does not generate species that compete with cationic drugs for delivery.

6. Type of Skin

Skin condition could also affect the penetration properties in iontophoresis. In vivo studies showed that skin from different areas of human body have different effects in passive diffusion of some drugs. The rank order is abdomen> forearm> instep> heel> planter for all subjects. Also there is evidence that passive diffusion occurred maximally from the area with numerous hair follicle and lesser in area with thickest stratum corneum.

Iontophoresis Applications

1. Short Term Iontophoretic and Post-iontophoretic Transport of Drugs

Akomeah et al. investigated the effect of short-term current application (0.4mA for 10 min) on the epidermal transport of two model penetrants (butyl paraben, BP; caffeine, CF) of differing lipohilicity (Akomeah et al., 2009). Franz cell diffusion experiments were conducted using human epidermal sheets and a saturated buffer solution of the respective penetrant. The effects of electrode type (anodal or cathodal) and current treatment protocol (iontophoresis or post-iontophoresis) on solute permeation was found not to be significantly different ($p > 0.05$). However, a significant increase in CF transport (3~5 fold) relative to untreated skin was observed when iontophoretic/post-iontophoretic treatment protocols were employed. Results from this study suggest that the permeation of the more hydrophilic CF across the skin may involve multiple pathways. Electroperturbation of the epidermis was confirmed as the mechanism responsible for enhancing CF transport when electrical current was applied. Thus, iontophoretic and post-iontophoretic enhancement may serve as a potential approach to enhance the topical delivery of CF in cosmetic or dermatological treatments.

Because the limitations of most iontophoretic experiments include the use of current with long treatment times (often over 2 h) and an infinite dose method to assess solute permeability across skin, and an infinite dose application may not mimic in-use conditions, where a thin film (finite dose) of the drug product is usually applied to the skin, a finite dose methodology and a shorter iontophoretic treatment are planned to be employed in this study. Short-term iontophoresis would also be expected to reduce the likelihood of potential damage to the skin and or irritation induced by long-term iontophoresis (Cullander, 1992). This condition may also

be advantageous when a localized effect is desirable due to increased drug penetration, large skin drug depot, short lag times and ease of compliance (Sintov and Brandys-Sitton, 2006).

2. Reverse Iontophoresis

Reverse iontophoresis is a technique in which low electric current is applied to draw body fluid from the body to the outside through the skin, this is widely applied in devices meant for diagnostic purposes.

Reverse iontophoresis provides a non-invasive method for sampling of body fluids so as to permit simultaneous measurement of the desired substance in the body fluid and thus efficient monitoring could be achieved. Glucowatch uses the reverse iontophoretic process to continuously monitor the glucose level in the blood of diabetic patients, and uses an electrical signal that is proportional to the amount of glucose in the extracellular fluid (Potts et al., 2002; Tierney et al., 2002). Also this technique allows a feasible method for rapid, linear extraction of phenylalanine and for easy monitoring of diseases like phenylketonuria (Merino et al., 1999). One thing needed to be mentioned is that though very successful, this method needs a very sensitive analytical means since the amount extracted is very low (Dixit et al., 2007).

3. Other Applications

Iontophoresis has been used in a variety of biomedical fields like dermatology (palmar hyperhydrosis, male contraception, ulcers, allergy testing, cystic fibrosis, scleroderma), ophthalmology (atropine, scopolamine, gentamycin, fluorescein delivery), ENT (providing anaesthesia of external ear canal in facial prosthetic surgeries), dentistry (local anaesthesia for multiple tooth extraction), neuropsychological (for studying neuromuscular junction, peripheral and central nervous system), muscle skeletal disorders (Mg for bursitis, Ca for myopathy, Ag for osteomylitis, local anaesthetics and steroids into elbow, shoulder and knee joints), and drug

delivery for counterirritants, antihypertensives, antidiabetic, antirheumatoids, hormones and so on (Dixit et al., 2007). For example, iontophoresis is currently used clinically to deliver lidocaine for local anesthesia, pilocarpine to induce sweating as part of a cystic fibrosis diagnostic test and tap water to treat hyperhydrosis (excessive sweating), glucose monitoring to extract glucose from the skin, as well as to periodically activate the iontophoretic patch to administer a bolus of fentanyl based on their need for pain relief (Prausnitz MR and Langer R, 2008).

Microneedles for Enhanced Transdermal Delivery

An idea to selectively permeablilize the stratum corneum straightforwardly is to pierce it with very short needles. The first microneedle arrays reported in the literature were etched into a silicon wafer and developed for intracellular delivery in vitro by Hashmi et al. (Hashmi et al., 1995). Over the past decade, microneedles have been developed as a means to deliver drugs into the skin in a minimally invasive manner (Sivamani et al., 2007; Prausnitz et al., 2008). In general, microneedles increase skin permeability by creating micron-scale pathways into the skin, actively drive drugs into the skin either as coated or encapsulated cargo introduced during microneedle insertion or via convective flow through hollow microneedles and target their effects to the stratum corneum, although microneedles typically pierce across the epidermis and into the superficial dermis too (Lee et al., 2008).

Because the skin's statum corneum barrier has no nerves, it provides an opportunity to piece needles across the statum corneum without stimulating nerves. Although there is evidence of microneedles penetrating not just 10-20μm across stratum corneum but also the viable epidermis, where nerves are found, microneedles are still reported as painless, probably because

their small size reduces the odds of encountering a nerve or of stimulating it to produce a painful sensation (Prausnitz, 2004; Wermeling et al., 2008).

Currently four different modes of microneedle-based drug delivery have been investigated (Prausnitz 2004; Prausnitz et al., 2005). They are:

1) Piercing an array of solid microneedles into the skin followed by application of a drug patch at the treated site (Henry et al., 1998; Martanto et al., 2004). They have been shown to painlessly pierce the skin to increase skin permeability of a variety of small molecules, proteins and nanoparticles from an extended-release patch;

2) Coating or encapsulating drug formulations onto microneedles and inserting them into the skin for subsequent dissolution of the coated drug within the skin (Cormier et al., 2004);

3) Encapsulating drug within biodegradable, polymeric microneedles followed by insertion into skin for rapid or controlled release of peptides and vaccines (Park et al., 2006);

4) Injecting drug through hollow microneedles, they have been used to deliver insulin and vaccines by infusion (Zahn et al., 2000; Prausnitz MR and Langer R, 2008; Harvinder and Prausnitz, 2007).

Micro-scale needles assembled on a transdermal patch have been proposed as a hybrid between hypodermic needles and transdermal patches to overcome the individual limitations of both injections and patches (Prausnitz, 2004; Prausnitz et al., 2005). The first study to determine if microneedles could be used to increase transdermal drug delivery was conducted by Henry et al. (Henry et al., 1998), where an array of solid microneedles was embeded in cadaver skin, and caused skin permeability to a small model compound, calcein, to increase by three orders of magnitude. Microneedles have been shown to be painless in human subjects relative to hypodermic needles (Kaushik et al., 2001; Mikszta et al., 2002). They have the advantages over

transdermal patches that they can successfully deliver variety of large and hydrophilic compounds into the skin including proteins and DNA (Li et al., 2010). In vitro skin permeability enhancement was observed for small molecules like calcein and large compounds like proteins or nanoparticles (Henry et al., 1998; McAllister et al., 2003). In vivo delivery has been shown for peptides such as insulin and desmopressin (Martanto et al., 2004; Cormier et al., 2004); genetic material including plasmid DNA (Lin et al., 2001; Chabri et al., 2004) and vaccines directed against hepatitis B, anthrax and Japanese encephalitis (Mikszta et al., 2005; Dean et al., 2005; Mikszta, et al., 2002).

The microelectronics revolution has provided tools for highly precise, reproducible and scalable methods to fabricate structures of micrometer dimensions (Madou, 1997). This lithography-based approach can produce large arrays of microneedles that can be inserted into cells, skin or other tissues. The increased importance of macromolecular therapeutics, combined with the newly acquired power of microfabrication, has recently prompted interest in fabricating (Chen et al., 1997; Lin et al., 1999; Stoeber et al., 2000) and testing (Matriano et al., 2002; Mikszta et al., 2002) microneedles for drug delivery.

Solid microneedles were etched from silicon substrates (Henry et al., 1998). Briefly, chromium was sputter deposited and then lithographically patterned onto 2-inch, 100 oriented silicon wafers. Reactive ion etching was then carried out and microneedle fabrication was finished when the chromium masks became fully undercut and fell off the needle tips (McAllister, et al., 2003). During experiments, forces of insertion of these needles varied from 0.1 to 3.0 N and showed an approximately linear dependence on the area of the needle tip (Prausnitz, 2004).

Several recent advances in microneedle design and formulation are worthy of notice. Original fabrication methods involving clean room-based sculpting of silicon-based structures have moved to low-cost manufacturing methods to make microneedles out of metals and polymers commonly found in FDA-approved devices and parenteral formulations. Dip-coated microneedles with a variety of compounds including small molecules, proteins, DNA and virus particles have been developed (Harvinder and Prausnitz, 2007). Microneedles of water-soluble polymers that encapsulate various compounds within the needle matrix have been made as well (Lee et al., 2008).

Microneedles in Vitro and in Vivo Transdermal Transport Studies

In a recent interesting study, advances have been made in delivery to humans using microneedles. Naltrexone was administered to healthy volunteers whose skin was pretreated with microneedles (Wermeling et al., 2008). After applying a naltrexone patch, blood levels of naltrexone reached the therapeutic range. Transdermal delivery without microneedle pretreatment yielded naltrexone levels below detection. Microneedle treatment was reported to be painless by the volunteers and was generally well tolerated.

In another study microchannels created by sugar and metal microneedles were reviewed by many techniques (Li et al., 2010). Methylene blue staining, calcein imaging, confocal microscopy and histology studies were performed to characterize the microchannels created by maltose microneedles and DermaRoller^{TM} (a commercially available cosmetic handheld device). Each array of maltose microneedles created microchannels of 125/cm^2 (153.33±20.82 μm in depth, and 58.16±6.23μm in surface diameter by fluorescence imaging) while DermaRoller^{TM} created much bigger 16/cm^2 microchannels (146.67±5.77 μm in depth and 82.49±9.97 μm in surface diameter). Both types of microneedles can work in a reproducible manner, and

histological sectioning of skin samples confirmed the disruption of the SC barrier by both. Though having fewer microchannels, DermaRoller^{TM} tends to have a much better effect in vivo as shown by transdermal permeation of human IgG. Steady-state fluxes of 45.96 and 353.17ng/cm^2/h were reported for maltose microneedles and DermaRoller^{TM}. The reason for this might be the creation of bigger microchannels with DermaRoller^{TM}. Another important observation is that microneedles could disrupt the integrity of stratum corneum, and the duration the channels created by microneedles remain open after 24h in vivo by methylene blue staining method.

Iontophoresis in Conjunction with Microneedles

Micro-structured transdermal systems are micro-needle patches that have needles or projections on the surface of patches which are sufficiently long to penetrate through the stratum corneum but short enough not to stimulate nerves to cause pain in the deeper tissues. This method is favored for delivery of vaccines, proteins or peptide drugs. The combination of iontophoresis with micro-needle technology could provide macromolecule transdermal delivery with precise electronic control. The rate of delivery can be controlled by duration of the patch application, donor drug concentration, current density and active patch area (Wang et al., 2005; Lin et al., 2001).

II. TRANSDERMAL IONTOPHORETIC DELIVERY OF SELEGILINE HYDROCLORIDE

Abstract

Transdermal iontophoretic delivery of selegiline hydrochloride (SH) across dermatomed human skin was studied. Electrochemical stability and various factors affecting the skin permeation were investigated. SH was stable under the influence of electrical field. The permeation of SH was very low by passive delivery (2.29 ± 0.05 μg/cm^2/h) as compared to iontophoresis at 0.5 mA/cm^2 (65.10 ± 5.04 μg/cm^2/h). Increase in drug concentration from 1 to 20 mg/ml, increased the iontophoretic flux by 13 fold. Optimal pH and salt (NaCl) concentration for iontophoretic delivery of SH were found to be pH 5 and 100 mM, respectively. Overall, with 20 mg/ml of SH, and at a current density of 0.4 mA/ cm^2, a maximum flux of 305.5 μg/ cm^2/hr was obtained. Based on the reported pharmacokinetic parameters, input target delivery rate to achieve effective plasma concentration of SH (2.2 ng/ ml) was calculated. With a surface area of 40 cm^2, Iontophoretic delivery can provide 6-7 times higher levels of SH than the target delivery rate, which enables lowering of dose and/or patch surface area. Further in vivo studies will prove the efficacy of ionophoresis for enhanced delivery of SH.

Introduction

Selegiline Hydrochloride (SH) has a chemical name (R)-(-)-N,2-dimethyl-N-2-propynylphenethylamine hydrochloride. It is a weak base with a pKa of 7.7 and it is more cationic (+) under a lower pH condition. Selegiline is an irreversible inhibitor of MAO that is administered orally as an adjunct to levodopa in the treatment of late-stage Parkinson's disease (Knoll, 1986; Chrisp et al., 1991). At these doses for this disease, selegiline is a preferential inhibitor of MAO-B and does not require tyramine diet restrictions (Elsworth et al., 1978; Pickar et al., 1981). However, the antidepressant doses of selegiline are 3 to 6 times higher than those approved for the treatment of Parkinson's disease. These higher doses can cause the loss of MAO-B selectivity that might result in serious side effects such as hypertensive crisis or tachycardia (Blackwell et al., 1967; Anderson et al., 1993). It is believed that this effect is the result of the inhibition of MAO-A in the intestine (Azzaro et al., 2007). MAO-A acts as a barrier and restricts the entry of tyramine into the systemic circulation. It is for this reason that many psychiatrists have been reluctant to prescribe MAO inhibitors including selegiline orally.

Recently alternate methods such as the transmucosal and transdermal routes have been investigated for the delivery of selegiline (Tetrud and Koller, 2004; Pae et al., 2007). These delivery systems possess several advantages over the conventional oral form of the drug such as avoidance of hepatic first pass metabolism and prolonged delivery. A transdermal patch formulation of selegiline has been approved as a once-daily treatment of major depressive disorder (MDD) and is being manufactured in doses of 6, 9, and 12 mg/day. Transdermal

selegiline also permits administration of the required high antidepressant concentrations of drug to the CNS while maintaining the intestinal barrier to dietary tyramine, thus avoiding the risk of tyramine induced hypertensive crisis. Selegiline has been approved as a 6 mg per day transdermal system for the treatment of MDD without dietary restrictions. However, because of limited safety data, the 9 and 12 mg per day doses of the selegiline transdermal system require the adherence to a modified tyramine diet. An important concern for the transdermal selegiline patch is skin reactions. The most common side effects include mild skin rash, itching, redness and irritation. Published clinical trials have shown that about 24~40% of patients that used the transdermal patch developed a skin reaction (Feiger et al., 2006; Howland, 2006; Patkar et al., 2006). These skin reactions can occur due to components of the transdermal patch and can be reduced by the modification of the formulation (Fang et al., 2009) or delivery method. Another limitation is that the lag time to the onset of action is typically longer with transdermal systems. Use of physical penetration enhancement strategies such as iontophoresis can push the molecules into the skin resulting in an enhanced delivery rate with a minimal lag time. Due to enhanced drug delivery, the patch size can be reduced considerably resulting in less skin reactions and increased patient compliance.

Transdermal iontophoresis is an attractive approach for the treatment of Parkinsonism or depression, because of its non-invasive feature which can deliver drug ions into or through skin continuously. In addition, this method offers the possibility of an individualized dose titration by modulation of the current density. This is an important advantage in treating Parkinson's disease for which individual dose escalation is usually required to obtain optimum therapy while minimizing side effects (Wong et al., 2003). The objective of this study is to enhance the delivery of SH through skin by the use of iontophoresis so that the disadvantages of the

selegiline transdermal patch can be minimized. Various formulation and iontophoretic factors affecting the skin permeation of SH were investigated.

Materials and Methods

1. Materials

Selegiline hydrochloride, silver wire and silver chloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium phosphate monobasic, sodium phosphate dibasic, sodium chloride, methanol, acetonitrile, citric acid and sodium citrate were obtained from Fisher Scientific (Pittsburgh, PA, USA). Dermatomed human skin (thickness ~ 0.35 mm) was obtained from the International Institute for Advancement of Medicine (Exton, PA). The skin was collected within 8 h of donor death and frozen at -70^{o}C until use.

2. Electrochemical Stability

Electrochemical stability of SH was studied using a computer controlled potentiostat (66-CS1200) from Cypress Systems (Chelmsford, MA, USA). The data acquisition was performed using custom build software from Cypress Systems. All electrochemical measurements were performed in a conventional three-electrode cell at room temperature, with Ag/AgCl as the reference electrode, a platinum wire as the working electrode and Ag/AgCl served as an auxiliary electrode. Experiments of cyclic voltammetry were performed in 10 ml of pH 5 citrate buffer. Cyclic voltammograms of SH (10 mg/ml) were obtained by scanning the potential range from -800 up to 1200 mV at 200 mV/s. Ten scans were performed for each condition.

3. Permeation Studies

In vitro studies were performed with dermatomed human skin using vertical static Franz diffusion cells (PermeGear, Hellertown, PA, USA). The frozen skin was thawed at ambient temperature for about 20 minutes. It was then mounted between donor and receptor cells with the

epidermis facing the donor. The receiver chamber contained 5 ml of citrate buffer (pH 4, 5 or 6) and was maintained at 37^{o}C with a water circulation jacket that surrounded the lower part of the cell. The diffusion area of the skin was 0.64 cm^{2}. The donor compartment was loaded with 0.5 ml of SH solution in citrate buffer (pH 4, 5 or 6) and covered with parafilm to prevent evaporation. At regular intervals, 400 µl aliquots from the receptor were collected and replenished with fresh receptor solution. The samples were stored in a refrigerator until analyzed. All experiments were carried out in triplicate.

4. Iontophoresis

Iontophoretic protocols involved application of direct current (Keithley Instruments, Inc, Cleveland, Ohio, USA) using Ag as an anode and Ag/AgCl as a cathode. All experiments were conducted using static Franz diffusion cells as described in the previous section.

5. Influence of Current Density

The influence of applied current on in vitro iontophoretic transport of SH was investigated. The current density applied was 0.05, 0.1, 0.3, 0.4 or 0.5 mA cm^{-2}. The donor solution contained 10 mg/ml SH and 100 mM NaCl in citrate buffer (25 mM, pH 5). The receptor cell was filled with pH 5 citrate buffer, but without NaCl, and was maintained at a temperature of 37^{o}C.

6. Influence of SH Concentration

The influence of SH concentration on iontophoretic transport was investigated by varying the donor concentration of SH over a twenty-fold range. SH concentration of the donor solution was 1, 5, 10 or 20 mg/ml. Citrate buffer (25 mM, pH 5) containing 100 mM NaCl was chosen as the donor phase. The receptor buffer was the same as described under the "Influence of current density" section.

7. Influence of Donor pH on SH Transport

The pH of the donor solution was maintained at 4, 5, or 6 using citrate buffer (25 mM). The concentration of NaCl in the donor compartment was 100 mM and the concentration of SH was 10 mg/ml for each of the pH conditions investigated. In each of these cases, the receptor contained the same buffer as the donor, but without NaCl.

8. Influence of Donor NaCl Concentration

The iontophoretic transport of SH was studied in the presence of various concentrations of NaCl while holding all other diffusion variables constant. NaCl (0, 100, or 200 mM) was added to the donor solution containing 10 mg/ml SH in citrate buffer (25 mM, pH 5). In all of these experiments, pH 5 citrate buffer without NaCl was used as a receptor phase.

9. Analytical Method

Analysis of SH was performed using HPLC method. The system (Waters Corp., MA, USA) equipped with an autosampler (model 717 plus), an isocratic pump (model 1525) and a PDA UV detector (model 998) was used. The system was interfaced with Empower 2 software for data collection and processing. Samples were eluted on a C8 analytical column, 5μm silica particles, 4.6 mm x 250 mm (Betasil, Thermo Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of an 80:20 mixture of 0.1 M ammonium phosphate (pH 3.1) and acetonitrile. A flow rate of 1.0 ml / min was maintained and 60 μl of the sample was spiked onto the column and detected at a wavelength of 205 nm.

10. Data Analysis

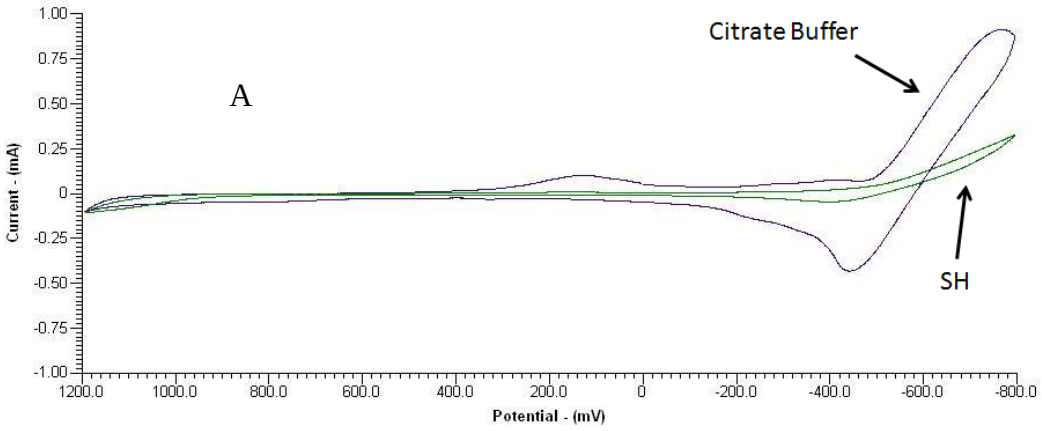
Results are presented as means with their respective standard error. The cumulative amount of drug permeated through a unit area of skin was plotted as a function of time. The in vitro steady-state permeation flux was calculated from the slope of the linear portion of the plot.

Analysis of variance was used to assess the statistical significance of the observations. For all statistical analysis, $P < 0.05$ was considered to be significant.

Results and Discussions

1. Electrochemical Stability

Fig. 2.1A is a cyclic voltammogram of a blank citrate buffer solution (25 mM, pH 5). The voltage was swept from -800 mV to 1200 mV. The same figure also shows the cyclic voltammogram of SH. There is no oxidation or reduction of SH that can be attributed to SH alone. The citrate buffer alone undergoes oxidation and reduction. Similar observations were noted (Fig. 2.1B) when 100 mM NaCl was included in the solution. No oxidation or reduction that could be attributed to SH alone was observed.



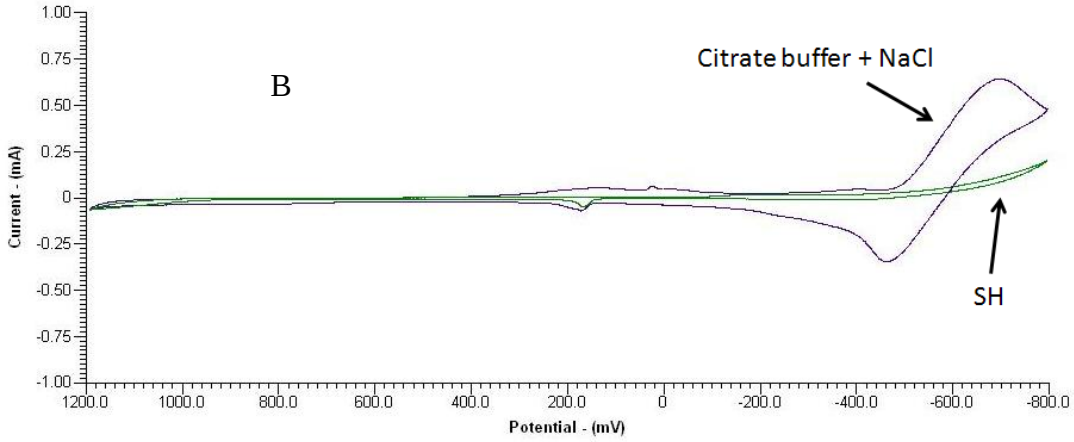


Fig. 2.1: Cyclic voltammogram of A) 25 mM citrate buffer and SH (10mg/ml) B) 25 mM citrate buffer with 100 mM NaCl and selegiline hydrochloride (10mg/ml).

2. Cumulative Amount Permeated Versus Time Profile for SH Transport

The cumulative amount of SH (10 mg/ml and 100 mM NaCl) permeated versus time as a function of current density is shown in Fig. 2.2A. In the absence of any externally applied current (passive delivery), the flux of SH is very low (2.29 ± 0.05 µg/cm^2/h). By the application of direct current (0.5 mA/cm^2), the SH permeated consistently increased (flux, 165.10 ± 5.04 µg/cm^2/h) with time during the current application period of 6 h. Removal of current after 6 h resulted in a gradual decrease in the rate of SH permeation. However, even after 2 h post-iontophoresis, the flux (28.29 ± 3.59 µg/cm^2/h) was still higher than the value observed during passive delivery (2.29 ± 0.05 µg/cm^2/h). There is a decrease in the lag time from 0.8 h to 0.3 h following iontophoresis. The iontophoretic flux values for SH obtained with a current density of 0.05, 0.1, 0.3, 0.4 and 0.5 mA/cm^2 were 2, 19, 48, 66 and 72 fold higher, respectively, than for passive delivery. As shown in Fig. 2.2B, there is a linear relationship (R^2=0.98) between current density and flux (in the range of 0.1 ~ 0.4 mA/cm^2). The increase in the flux of SH beyond 0.4 mA/cm^2 was not linear. The post iontophoretic flux values of SH with a current density of 0.05,

0.1, 0.3, 0.4 and 0.5 mA/cm^2 were 1.4, 4.6, 10, 10 and 12 fold higher, respectively, than passive delivery.

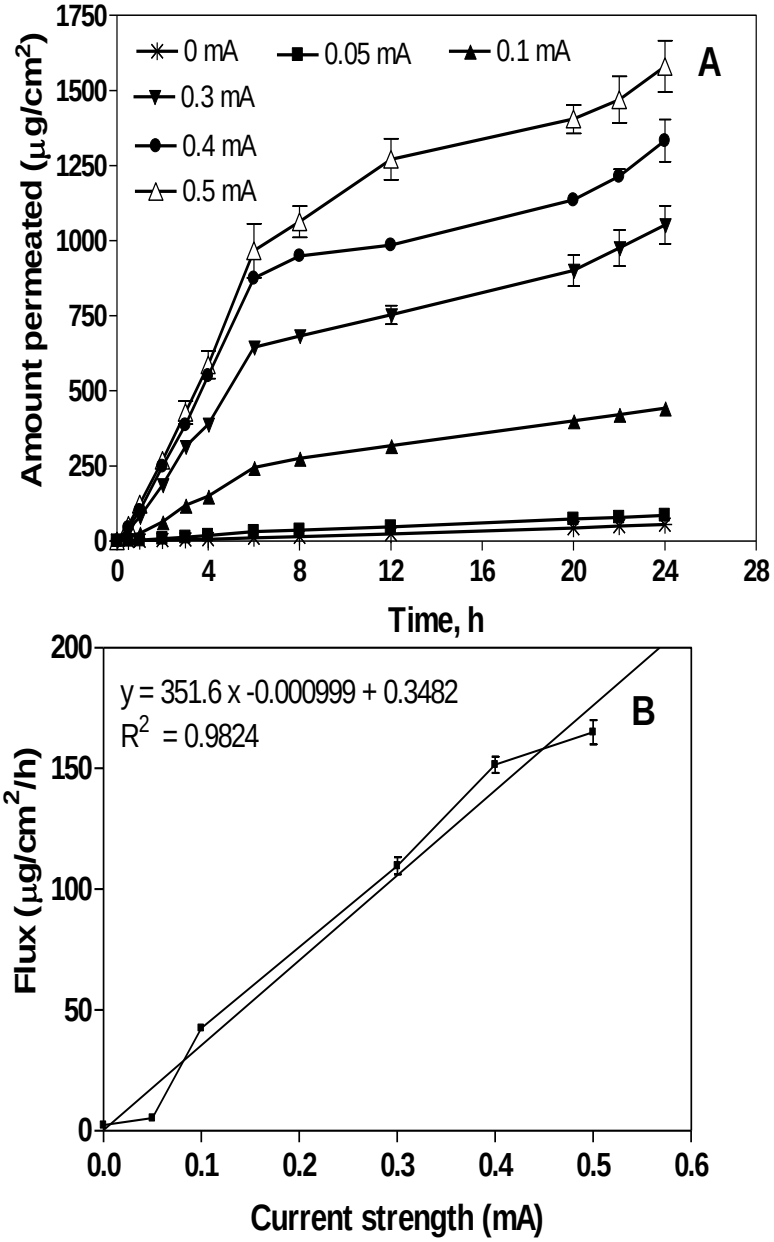


Fig. 2.2: Effect of current density on the iontophoretic delivery of SH (10 mg/ml) across dermatomed human skin. A) Iontophoresis and Passive Delivery, B) Flux versus Current Density Plot. Values represent mean ± SEM; n= 3, control n=10.

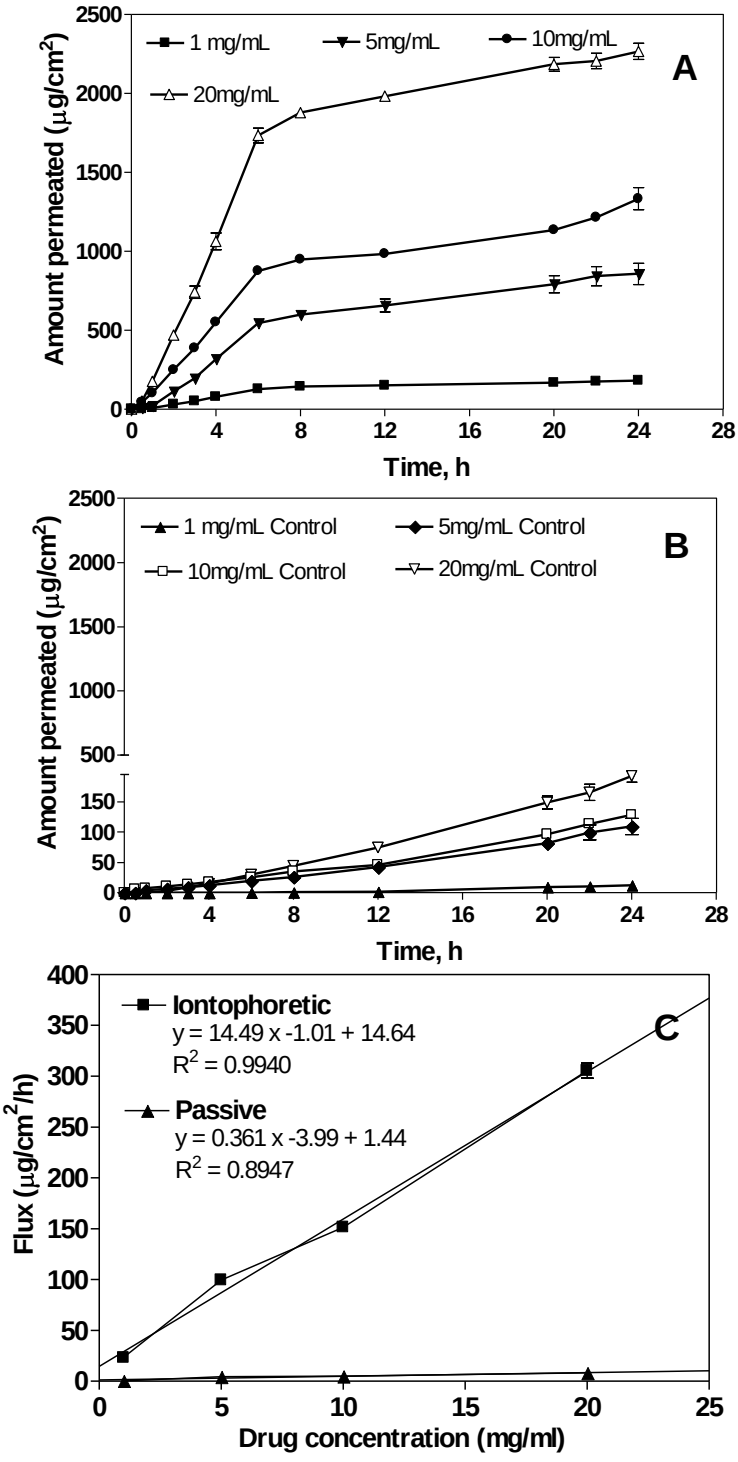


Fig. 2.3: Effect of drug concentration on the iontophoretic delivery of SH across dermatomed human skin (current density: 0.4 mA/cm^2). A) Iontophoresis, B) Passive Delivery, C) Flux versus Drug Concentration Plot. Values represent mean ± SEM; n= 3

3. Influence of Drug Concentration

The cumulative amount of SH permeated versus time as a function of drug concentration is shown in Fig. 2.3A.The data show an increase in SH transport with increased concentration of the drug in the donor within the concentration range investigated. There was a 13 fold increase in the iontophoretic flux with an increase in the drug concentration from 1 to 20 mg/ml. Passive delivery, as predicted, showed an increase in the permeation with an increased donor concentration, but at all drug concentrations the permeation was several fold lower than with iontophoresis (Fig 2.3B). A maximum flux of 305.50 ± 7.46 μg/cm^2/h was achieved at the 20 mg/ml concentration and the least flux (23.39 ± 0.63 μg/cm^2/h) with the 1 mg/ml concentration. The steady state flux values were significantly different for the different donor concentrations ($p < 0.05$) and a linear correlation between donor concentration and SH steady state flux was observed with $R^2 = 0.99$ (Fig. 2.3C). This increase was observed in post-iontophoretic flux values as well. At each drug concentration examined, there was an increase in iontophoretic flux by at least 22 fold as compared to passive delivery.

4. Influence of Donor pH

The influence of donor chamber pH on the efficiency of passive and iontophoretic delivery of SH is presented in Fig. 2.4. Flux was determined at pH 4, 5 and 6 using an appropriate combination of citric acid and sodium citrate while maintaining the same molar concentration of the buffer. As shown in Fig. 2.4, SH flux was pH sensitive with the greatest transport occurring at pH 5, followed by pH 4, and the least flux was observed at pH 6. The flux values at pH 4 and pH 5 were not statistically significantly different. In comparison to pH 6, either of these results was statistically significantly different. Termination of current flow after 6 h caused the drug transport rate at pH 4, 5, and 6 to decrease quickly. At pH 5, however, while

transport slowed, it continued at a relatively elevated level as compared to the other pH conditions. The iontophoretic flux at pH 4, 5 and 6 was, respectively, 90, 30 and 13 fold higher than the respective flux obtained by the corresponding passive delivery. Iontophoretic flux is highest at pH 5 (151.6 ± 3.39 μg/cm^2/h), followed by pH 4 (142.7 ± 2.94 μg/cm^2/h) and pH 6 (15.05 ± 0.14 μg/cm^2/h). Similarly, post-iontophoretic flux is highest at pH 5 (22.37 ± 3.65 μg/cm^2/h) followed by pH 4 (10.08 ± 0.36 μg/cm^2/h) and pH 6 (1.43 ± 0.07 μg/cm^2/h).

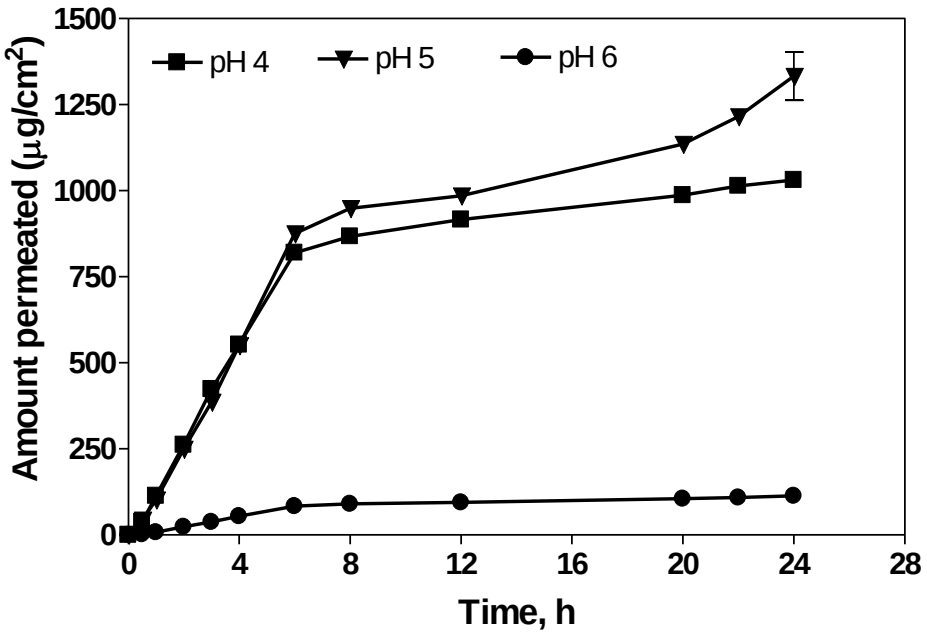


Fig. 2.4: Effect of pH on the iontophoretic delivery of SH (10 mg/ml) across dermatomed human skin (current density: 0.4 mA/cm^2). Values represent mean ± SEM; n= 3.

5. Influence of NaCl Concentration

Ion competition can affect iontophoretic flux. Therefore, the effect of NaCl concentration on SH transport was studied (Fig. 2.5). An increase in the NaCl concentration in the donor compartment resulted in decreased flux of SH. In the study where no NaCl was used, the electrochemistry was only sustained for 3.5 hr before precipitation of the anode was observed. With 100 mM and 200 mM NaCl, the current was maintained for the duration of the experiment (6 hr). The iontophoretic flux (calculated for 4 h) for SH was highest from the donor solution

that contained no NaCl. As the salt concentration increased, the flux of SH decreased. However, the current (with no NaCl in the donor) could not last for the duration of the experiment. The flux observed was least (56.06 ± 0.80 µg/cm^2/h) with 200 mM NaCl in the donor. Overall, the flux decreased with an increase in the salt concentration in the donor.

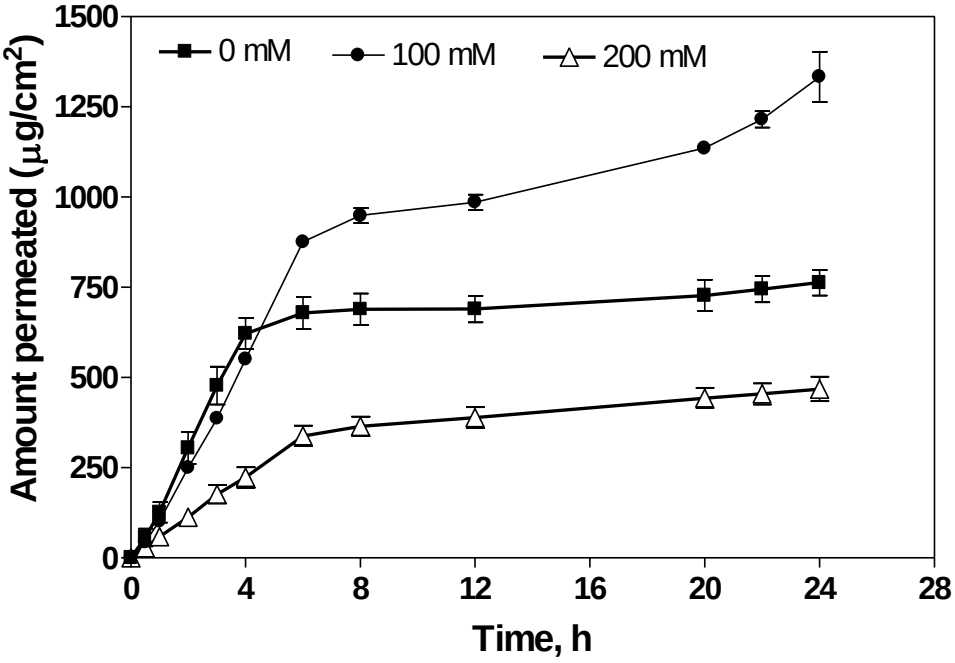


Fig. 2.5: Effect of NaCl concentration on the iontophoretic delivery of SH (10 mg/ml) across dermatomed human skin (current density: 0.4 mA/cm^2). Values represent mean ± SEM; n= 3.

Discussion

Selegiline hydrochloride is a weak base with a pKa of 7.5 (Pae et al., 2007). It has a low molecular weight of 187.3, solubility of about 33 mg/ml and a calculated partition coefficient (octanol/water) of 0.04 (Barrett et al., 1997). Also, it is extensively metabolized through first-pass metabolism resulting in a bioavailability of about 4.4 % when administered orally (Patkar et al., 2006). All of these properties make SH an ideal candidate for transdermal delivery. For

iontophoresis, we have taken advantage of the fact that SH acquires a positive charge at neutral pH and below; and is therefore suitable for anodic drug delivery.

The currently available selegiline transdermal delivery system provides cumulative daily doses of 6, 9 or 12 mg per day with respective patch sizes of 20, 30 and 40 cm^2 (Patkar et al., 2006). This mode of delivery has advantages over the oral route, yet suffers from two limitations: (a) there is a significant time-lag (Azzaro et al., 2007) and (b) a need for a relatively large patch for higher doses. Transdermal iontophoretic delivery of SH could overcome the limitations of passive transdermal delivery.

Cyclic voltammetry is a common electrochemical technique that can be used to study redox states of molecules. Cyclic voltammograms (Fig. 2.1A, B) show that SH is neither oxidized nor reduced under the influence of an electrical field. These observations were substantiated from the fact that no additional degradation peaks were found when the samples were analyzed using HPLC.

Fig. 2.2A provides compelling evidence that the iontophoretic delivery of SH is significantly higher than passive delivery. Anodal iontophoresis was very efficient and resulted in a considerable increase in SH delivery during the current application period. The lag time was much shorter with iontophoresis as compared to passive delivery indicating the possibility of a quick onset of action. Further, the low passive flux indicates that passive transport contributes negligibly to the overall SH transport during iontophoresis. Following termination of the current (6 h), the flux decreased, but was always higher than that observed with passive permeation under similar conditions. The post iontophoretic fluxes were significantly greater than passive delivery because during iontophoresis, a significant amount of drug enters the skin and forms a

depot. Once the iontophoresis is stopped, drug in the depot along with drug from the donor contribute to the enhanced delivery as compared to passive diffusion.

A near linear relationship was observed between applied current and SH flux values (Fig. 2.2B). Generally, a current density of less than or equal to 0.5 mA/cm^2 is considered for transdermal iontophoresis and any density above this value has the potential to precipitate vascular reactions and skin irritation. In the present study, a current density of 0.05 mA/cm^2 was not sufficient to significantly affect SH penetration into the skin. However, as the current density was increased, a linear increase in the SH flux was observed up to 0.4 mA/cm^2, and 0.5 mA/cm^2 offered the highest SH flux though the increase was no longer linear. Many vitro and in vivo studies have been used to demonstrate that the rate of drug delivery is linearly proportional to the applied current over a wide range. However, although increasing the current produces an increase in iontophoretic transport, the response can plateau at higher current levels, suggesting the presence of a saturation phenomenon. Once a limiting transport number is achieved, a further increase in current has no effect (Kasting and Keister 1989). The iontophoretic transport of chlorpromazine increased with current density up to 0.35 mA, but this effect was not statistically significant between 0.35 and 0.5 mA/cm^2 (Alvarez-Figueroa and González-Aramundiz 2008). Similarly, the steady state flux of metopimazine doubles with a current density increase from 0.125 to 0.25 mA/cm^2, but the flux increase was marginal at 0.5 mA/cm^2 (Bounoure et al 2008).

When the donor concentration of the drug is increased (Fig. 2.3A), the drug transport number increases according to the Phipps and Gyory equation (Phipps and Gyory, 1992):

$$J_d = (t_d.I/F.Z_d).$$

J_d, t_d and Z_d, respectively, are the flux, the transport number and the valence of the selegiline ion, I is the current density and F is the Faraday constant. If electro-repulsion plays a

significant role in iontophoretic drug transport, the flux should increase with concentration. This may not be true when electro-osmosis is the primary contributor for drug transport across the skin as seen with certain drugs like naferelin (Delgado-Charro et al., 1995) and propranolol (Hirvonen and Guy, 1997). Further, a linear relationship of flux with donor concentration indicates that the solubility limits of the compound have not been reached and that the ion conducting pathways of the skin have not reached saturation (Brand and Guy, 1995b).

One of the important factors that govern iontophoretic delivery is the pH of the donor solution. An optimum pH for iontophoretic delivery is one where the compound remains predominantly ionized. We have studied delivery at pH 4, pH 5 and pH 6 (Fig. 2.4). Since the pKa of selegiline is 7.5, the ionization of SH is highest at pH 4, followed by pH 5, and least at pH 6, where the drug is 96.9% ionized. In the first two instances also, more than 99% of SH is ionized in solution.

The iontophoretic flux at pH 4, where SH is most ionized, is less than (though not significantly) that at pH 5. This phenomenon has been reported previously for lysine (Green et al., 1991) and nicotine (Brand and Guy, 1995a) and the changes could be attributed to a change in the permselectivity of the skin at lower pH values. Furthermore, at pH 4 and lower, water becomes ionized and the concentration of hydronium ions increases. These ions will compete with selegiline ions to carry the charge across the skin. The mobility of hydronium ions is approximately 7 times greater than that of sodium ions in free solution (Burnette and Ongpipattanakul, 1987) and it will be even greater compared to selegiline ions. Therefore, any factor that increases the hydronium ions (or other smaller ions) would significantly affect the iontophoretic flux of SH. From these observations, it was concluded that pH 5 would be optimal for iontophoresis of SH. In the present study, we did not compare different buffer species and

therefore we do not know the influence of competing buffer ion species on the transport number of selegiline. However, all experiments were conducted at a fixed molar concentration of citrate buffer; therefore the citrate ion should not have big influence on the transport number of selegiline. Furthermore, the flux of selegiline generally cannot be addressed based the transport number alone because the percent ionized and unionized drug species differs at various pH; the unionized species is absorbed better by passive diffusion process.

The use of Ag/AgCl electrodes can surmount the problem of hydrolysis that is frequently encountered with platinum electrodes (Phipps et al., 2002). These electrodes, in addition to preventing hydrolysis, take part in the electrochemistry resulting in the net flow of current. Anodal reaction, while working with Ag/AgCl electrodes, involves oxidation of the silver wire at the anode releasing Ag^+ which associates with Cl^- to precipitate as AgCl on the electrode (Kasha and Banga, 2008). Therefore, the presence of Cl^- ions in sufficient amounts is necessary to continue the electrochemistry. Lack of an adequate supply of Cl^- ions results in hydrolysis of the anode and subsequent electrode consumption. In certain instances, a fraction of the Cl^- ions are provided by the drug itself (as hydrohalide salts of cationic drugs) and further Cl^- ions, if required, are supplemented by the additional of NaCl or other halide salt.

When NaCl was added to the donor solution, Na^+ ions were generated which compete with the positively charged selegiline ions to cross the skin. Since the Na^+ ions are small compared to the selegiline ions, they have a higher mobility and are preferentially transported across the skin. Chloride ions inherently present in SH solutions could maintain the electrochemistry for 3.5 h and a donor with 50 mM NaCl could maintain the electrochemistry for 5 h (data not shown). Higher concentrations of NaCl could maintain the electrochemistry for the duration of the experiment. The decrease in flux with an increase in NaCl concentration could be

attributed to ionic competition between selegiline and sodium ions. The transport number for selegiline at 10 mg/ml selegiline HCl concentration, current strength of 0.4 mA/sq.cm, pH 5 and 100 mM NaCl, was found to be 0.28, which indicates that only a small fraction of charge is carried out by the selegiline ion. At higher NaCl concentrations (200mM NaCl) the transport number further decreases to 0.10, which clearly indicates that majority of the charge is carried out by highly mobile sodium ions. This explains why there was a decrease in flux at higher NaCl concentrations. A minimum of 100 mM NaCl was therefore added to the donor for all experiments.

Based on the reported pharmacokinetic parameters, target delivery rate (K_o) to achieve effective plasma concentration of selegiline (2.2 ng/ml) (Azzaro et al 2007) was calculated according to the following equation (Kolli and Banga, 2008):

$$AK_o = CL_T \,.\, C_{SS}$$

Where is CL_T is plasma clearance of SH, which is 845.6 L/h (Azzaro et al., 2007). With a surface area (A) of 40 cm^2, iontophoretic delivery can provide a 6-7 times higher level of selegiline than the target delivery rate. This enables lowering of dose and/or patch surface area. Further in vivo studies may prove the efficacy of iontophoresis for enhanced delivery of selegiline. In conclusion, this study demonstrates that the delivery of SH across the skin via iontophoresis is possible. A significant reduction in dose or patch size of SH as compared to the conventional passive transdermal patch may be achieved.

III. MICRONEEDLE ASSISTED IONTOPHORETIC TRANSDERMAL DELIVERY OF PROCHLORPERAZINE

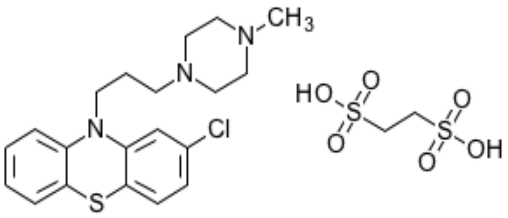
Abstract

Prochlorperazine edisylate (PE) is in the phenothiazine class of antipsychotic agents. It has a prominent antiemetic/antivertiginoic activity and is most often used for the treatment of nausea, vomiting and vertigo. A transdermal dosage form would be preferable because it avoids the inconveniences of oral dosage forms in nauseated patients as well as avoiding hepatic first pass metabolism. Permeation studies of PE across dermatomed human skin were conducted using Franz diffusion cells. The skin was microporated using a Dermaroller™ to study the effect of solid microneedles on the transport of PE with and without the influence of an electric current. Iontophoretic protocols involved application of direct current at a density of 0.4 mA/cm^2 using Ag as an anode and Ag/AgCl as a cathode. The effect of PE concentration (20, 50 and 100 mg/ml), number of passes of the Dermaroller ^{TM} (0, 5, 10 and 20) and the combined effect of microneedle and iontophoresis on the transport of PE across the skin was determined. Samples were assayed by HPLC. PE was stable under the influence of an electrical field. An increase in PE concentration in the donor increased the passive and iontophoretic flux in a dose dependent manner. The permeation of PE was very low by passive delivery ($0.047 \pm 0.003 \mu g/cm^2/h$) as compared to iontophoresis (5.216 ± 0.567 $\mu g/cm^2/h$); which increased the flux by 53 fold. When the skin is microporated (5 passes) the total iontophoretic flux was increased by 92 fold as compared to the control (passive delivery). A synergistic increase in the transdermal transport of

PE was observed when iontophoresis (0.4 mA/cm^2) was used in conjunction with microporation (5 passes). The combined system can provide a flux of 4 $\mu g/cm^2/h$, which is 2 fold higher than the target delivery rate (2 $\mu g/cm^2/h$), to provide effective plasma concentrations.

Introduction

Prochlorperazine edisylate is 2-chloro-10-[3-(4-methyl-1-piperazinyl) propyl] phenothiazine 1,2-ethanedisulfonate (1:1) and its structure is shown below. It is a highly lipid soluble base with a pKa of 8.1 (Ward et al., 1988). It is a weak dopamine receptor blocker. It blocks the chemoreceptor trigger zone (CTZ) in the brain, which is responsible for causing severe nausea and vomiting (Kwon, et al., 2002; Obata et al., 2010). Prochlorperazine has a prominent antiemetic activity and is most often used for the short-term treatment of nausea, vomiting and vertigo (Dhandapani et al., 2009). The most common concern regarding antiemetic use is the potential for side effects, which include dystonic reactions, akathisia, sedation, and postural hypotension (Carr et al., 1987; Saller et al., 1986). The use of prochlorperazine is strictly restricted to a maximum of 2 days because the drug can cause tardive dyskinesia, a condition involving unusual, uncontrollable body or face movements (including abnormal movements of the tongue). The condition can become permanent even if prochlorperazine is stopped (Alberts et al., 1996). This drug can cause severe circulatory damage when used intravenously in emergency rooms. Due to high intestinal and hepatic extraction, the value of oral prochlorperazine is limited. This drug also has slow absorption, very low oral bioavailability (15%), and a short biological half-life (8 h) (Taylor and Bateman, 1987; Isah et al., 1991). Also, the oral route is impractical in patients with nausea and vestibular disturbance, especially since they have also been demonstrated to have impaired gastric emptying (Bond, 1998).



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Clinical studies demonstrated that buccal administration of prochlorperazine produces a plasma concentration more than twice as high as an oral tablet with less than half the variability

(Finn et al., 2005). Exposure to metabolites after buccal administration was approximately half that observed after the oral tablet. The buccal preparation achieves higher plasma concentrations because of its direct absorption into the systemic circulation (Bond, 1998; Singh et al., 1999). In addition, buccal prochlorperazine achieves a significantly faster onset of action compared with oral use and was significantly better in reducing the frequency of nausea and severity of vomiting (Bond, 1998). Another study indicated that 3 mg twice daily buccal delivery is equal to a 5 mg three times daily oral dose (Hessell et al., 1989). Therefore, buccal prochlorperazine is safe, effective, and suitable for treating dizziness associated with nausea and/or vomiting in patients suffering from vertiginous disorders.

The transdermal route for prochlorperazine has not been prospectively evaluated. A transdermal system provides several advantages for prochlorperazine over conventional dosage forms. These include prolonged delivery, by-passing hepatic first-pass metabolism, and avoiding risks and inconveniences of parenteral therapy. Transdermal prochlorperazine should permit delivery of the required antiemetic concentrations of drug to the CTZ while avoiding the circulatory damage of intravenously administered drug.

In this study, the physical penetration enhancement methods of iontophoresis and solid microneedles, and their combination, have been utilized for transdermal delivery of prochlorperazine. Transdermal iontophoresis is the use of a low voltage electric current to accelerate transdermal permeation of charged molecules, as a noninvasive enhancement technique (Kalia et al., 2004). Iontophoresis is a favorable delivery method because the current applied can be modulated to deliver a desired dose of the drug. Another strategy to enhance the skin permeation of drugs is the use of microneedles (Prausnitz, 2004). These needles do not induce any pain sensation, but provide micro pores in the stratum corneum, the superficial layer

of skin which is the formidable barrier to transdermal drug delivery. The delivery of drugs to skin can be regulated by the number of micro pores created on the skin. The number is regulated through the design and number of passes of the roller. This method is proposed to be safe and increased transdermal transport has been demonstrated by several studies (McAllister et al., 2004, Li et al., 2009; Mikstza et al., 2002). However, there are no reports on the iontophoretic delivery or microneedle approach to enhance the delivery of prochlorperazine across skin. The objective of this study was to investigate the administration of prochlorperazine edisylate by iontophoretic and microneedle treatments alone and in combination to synergistically enhance the transdermal transport of the drug across dermatomed human skin.

Materials and Methods

1. Materials

PE was obtained from PCCA (Houston, TX, USA). Silver wire and silver chloride electrodes were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium chloride, acetic acid and HPLC grade triethylamine were purchased from Fisher Scientific (Fair Lawn, NJ, USA). HPLC grade methanol was procured from EMC Chemicals Inc. (Gibbstown, NJ, USA). Citric acid was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ, USA), and sodium citrate was obtained from Amend Drug and Chemicals Co. (Irvington, NJ, USA).

2. Skin Permeation Studies

Dermatomed human skin (thickness ~ 0.35 mm) was obtained from the International Institute for Advancement of Medicine (Exton, PA, USA). The skin was collected within 8 h of donor death and frozen at -70^{o}C until use. In vitro studies were performed using vertical static Franz diffusion cells (PermeGear, Hellertown, PA, USA). The frozen skin was thawed at ambient temperature for about 30 minutes. It was then cut and sandwiched between donor and

receptor cells with the epidermis facing the donor. The receiver chamber contained citrate buffer (5 ml, pH 5, 25 mM) and was maintained at 37^{o}C with a water circulation jacket that surrounded the lower part of the cell. The available diffusion area of the skin was 0.64 cm^{2}. The donor compartment was filled with PE solution in citrate buffer (0.5 ml, pH 5, 25 mM) with 100 mM sodium chloride, and covered with parafilm to prevent evaporation. The receptor cells were stirred with magnetic bars during the permeation study. At regular intervals, 400 µl aliquots from the receptor were collected and replaced with fresh receptor solution. The samples were stored in a refrigerator and analyzed within 24 h. All experiments were performed in triplicate.

3. Iontophoresis

Iontophoretic protocols involved application of a direct current (Keithley Instruments, Inc, Cleveland, Ohio, USA) of 0.4 mA/cm^{2} using Ag as an anode and Ag/AgCl as a cathode. The duration for iontophoresis treatment was 6 h. All experiments were conducted using static Franz diffusion cells as described in the previous section.

4. Microporation Studies

Microporation studies employed human skin pretreated by solid microneedle passes after thawing using a Demoroller™ (Dermaoller Deutschland S.a.r.l., Germany). The skin was then cut and mounted between donor and receptor cells. All experiments were conducted using static Franz diffusion cells as described in the section on skin permeation studies.

5. HPLC Analytical Method

The HPLC method of PE analysis employed a system (Waters Corp., MA, USA) equipped with an autosampler (model 717 plus), an isocratic pump (model 1525) and a PDA UV detector (model 998). The system was interfaced with Empower software for data collection and processing. Samples were eluted on a C18 analytical column, 5µm silica particles, 4.6 mm x 250

mm (Bellefonte, Thermo Electron Corporation, Bellefonte, PA, USA). The mobile phase consisted of a mixture of 70:28:1.5:0.5 methanol: water: acetic acid: triethylamine and was delivered at a flow rate of 1.0 ml / min at ambient temperature. The sample injection volume was 40 µl and PE was detected at a wavelength of 254 nm.

6. Scanning Electron Microscopy Studies

Dermatomed human skin was treated with passes of Dermaroller™ microneedles. The samples were mounted on a metal stub using double-sided carbon tape and sputter coated using a Hummer VI Sputtering System (Anatech, USA) with Au/Pd target. Skin samples were examined using a field emission scanning electron microscope (S4100 Hitachi, Tokyo, Japan) with a critical dimension measurement system. Primary beam accelerating voltage was 15 kV and secondary ion images were collected.

7. Data Analysis

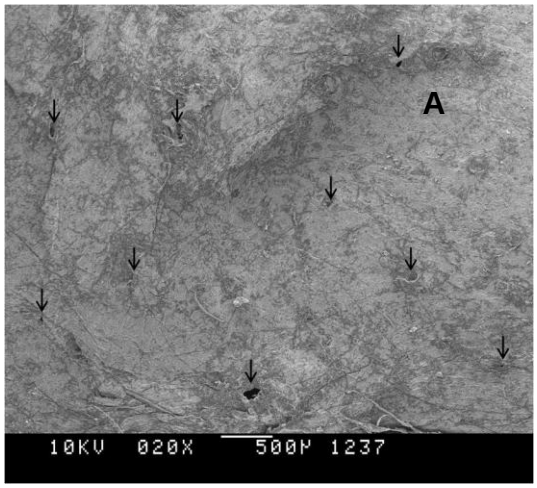
All results are presented as means with their respective SEM. The cumulative amount of drug permeated through a unit area of skin was plotted as a function of time. The in vitro steady-state permeation flux was calculated from the slope of the linear portion of the plot. Analysis of variance was performed to determine the level of significance between the means. Mean differences with $P < 0.05$ were considered to be significant.

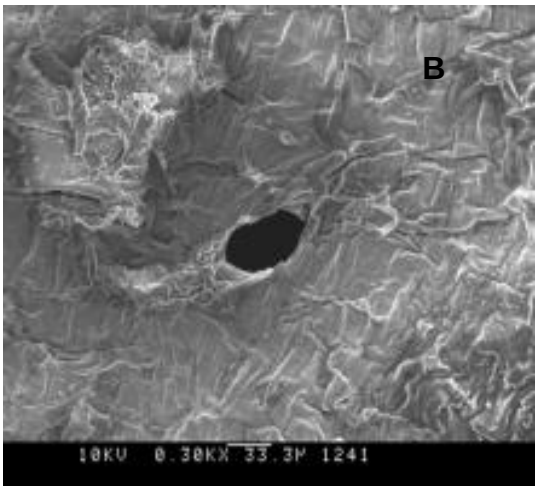
Results and Discussions

1. Visualization of Microchannels

Microchannels created by solid metallic microneedles (present on the dermaroller) were visualized using scanning electron microscopy. The pictures (Fig 3.1A and B) indicate that the microneedles effectively breached the epidermis of the skin to create microchannels. The diameter of the channel was approximately (120 microns) as visualized from the SEM pictures.

The created microchannels were further stained and visualized using light microscopy. These light microscopic pictures consolidated our earlier findings that microchannels were created upon pretreatment with dermaroller. In addition, with an increase in number of passes, there was a linear increase in the number of microchannels created. It was also evident from scanning electron and light microscopic studies that repeated passes of the dermaroller did not result in overlapping of the microchannels.





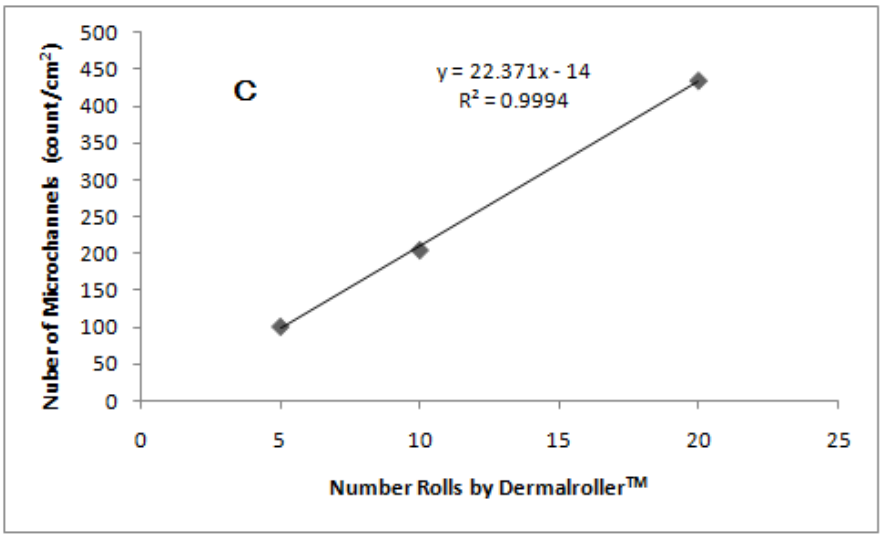


Fig. 3.1: Scanning electron micrograph image of surface morphology of dermatomed human skin treated with Dermaroller™ microneedles. A) low magnification B) high magnification C) effect of the number of Dermaroller™ passes on the number of micro-channels formed.

2. Influence of Drug Concentration

The cumulative amount of PE permeated versus time as a function of drug concentration with and without iontophoresis is shown in Fig. 3.2. For passive diffusion, the amount of PE permeated and the corresponding flux are very small. There was no statistically significant increase in permeation with increases in donor drug concentration. With iontophoresis, there was an increase in PE transport with an increase in the concentration of the drug in the donor compartment within the concentration range investigated. There was a 6.5 fold increase in the iontophoretic flux with an increase in the drug concentration from 20 to 100 mg/ml. A maximum flux of 7.7 ± 0.36 µg/cm^2/h was achieved at 100 mg/ml. The steady state flux values were significantly different for the different donor concentrations ($p < 0.05$) and a linear correlation between donor concentration and iontophoretic PE steady state flux was observed with R^2 = 0.999 (Fig. 3.2). This increase was observed in post-iontophoretic flux values as well. At each

drug concentration examined, there was an increase in iontophoretic flux by at least 15 fold as compared to passive delivery.

3. Influence of Microneedles on the Transdermal Delivery of PE

The influence of microneedles pretreatment on the skin permeation of PE across dermatomed human skin is shown in Fig. 3.3 and 3.4A. A drug concentration of 50 mg/ml and a current density of 0.4 mA/cm^2 were used in these experiments. The skin permeation of PE due to passive diffusion was minimal in the absence of microneedles (0.047 ± 0.003 $\mu g/cm^2/h$). While the skin permeation increased by 1.5 fold due to 5 passes of microneedles on the skin (Fig. 3.4A), this increase was not statistically significant ($P > 0.05$). The skin permeation of PE, however, was significantly increased when 10 or 20 passes of microneedles were applied on the skin (more than two fold increase, $P < 0.05$). The skin permeation of PE due to 20 Dermaroller™ passes is not significantly different from that of 10 passes ($P > 0.05$). From these results it is clear that 5 or 10 passes of microneedles was optimum since additional passes did not further increase the skin permeation of PE. The probable reason could be due to the saturation of the microchannels with PE. From these observations, we decided to proceed with 10 passes of MN as it was considered optimum and beyond 10 passes may result in no additional benefit.

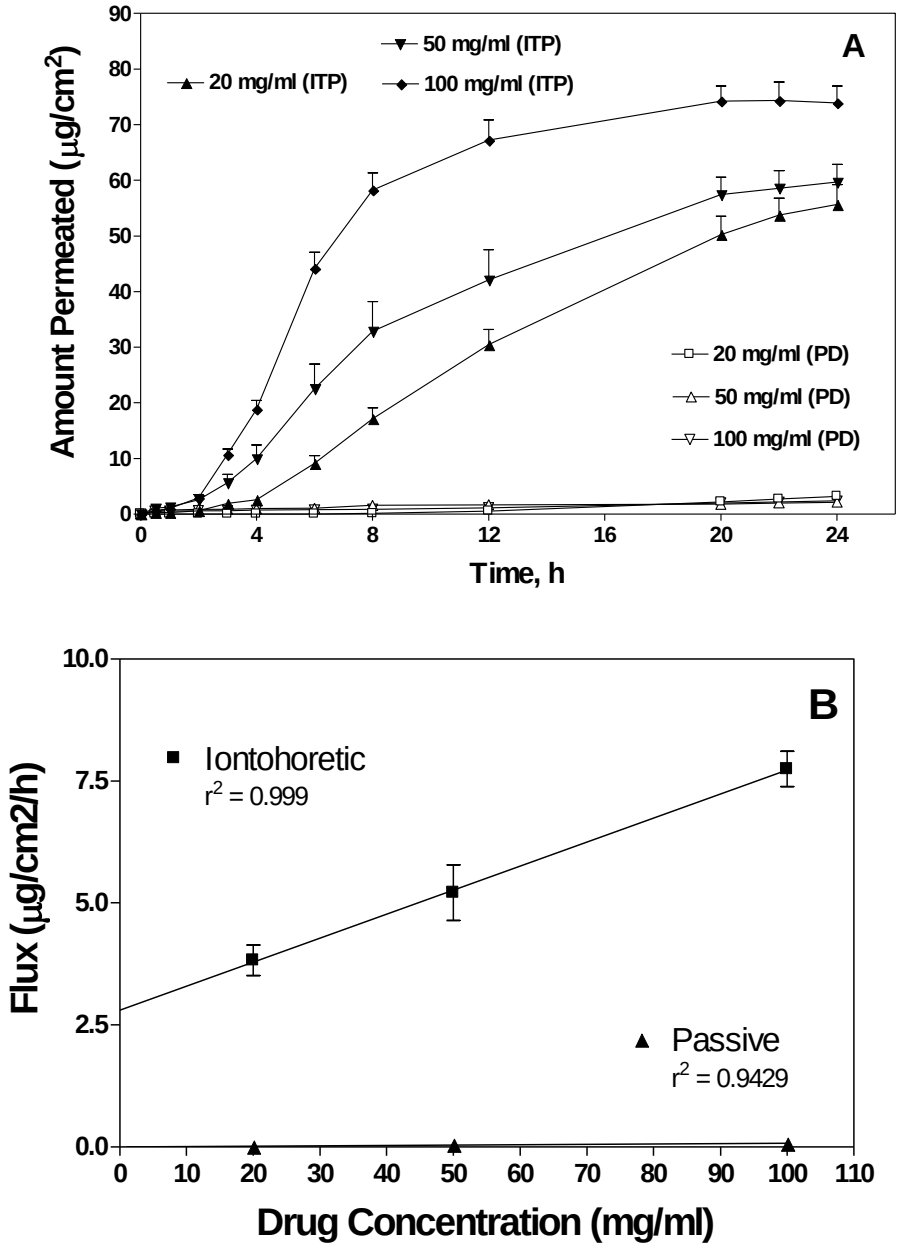


Fig. 3.2: Effect of drug concentration on the passive (PD) and iontophoretic (ITP) (current density: 0.4 mA/cm^2) delivery of PE across dermatomed human skin. A) Skin permeation profiles, B) Steady state flux data calculated from iontophoretic skin permeation profiles. Values represent mean ± SEM; n= 3

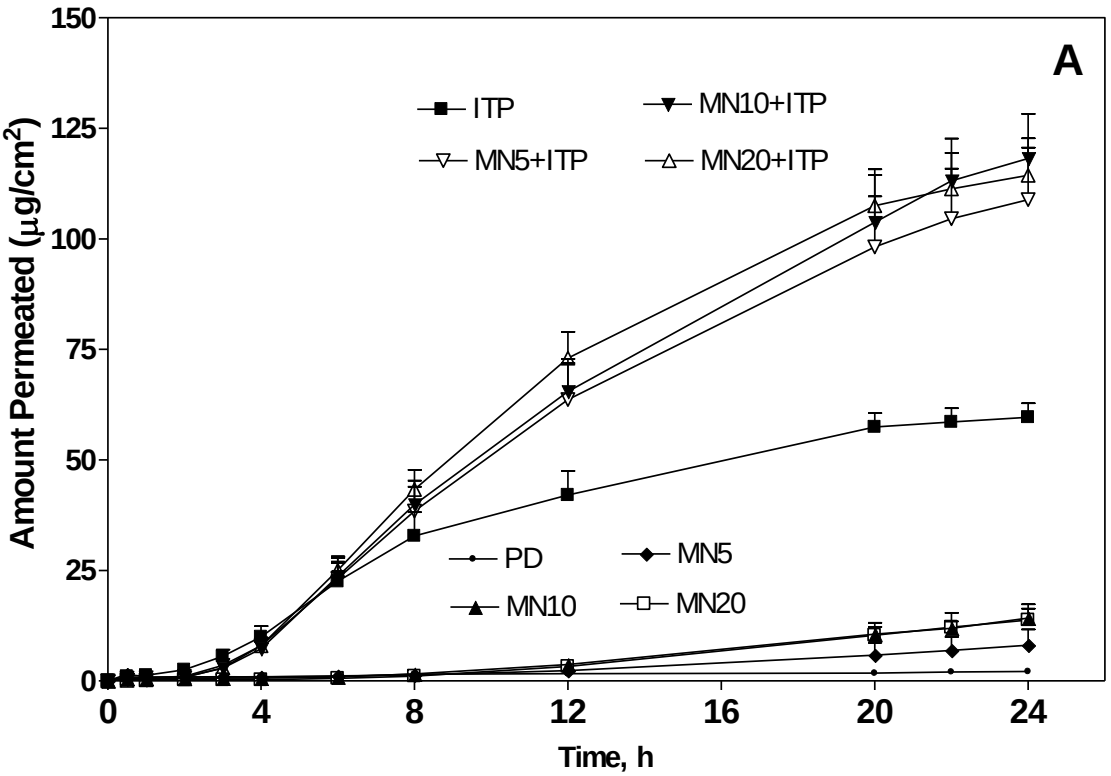


Fig. 3.3: Influence of microneedles (MN) alone and in combination with iontophoresis (ITP) (drug concentration: 50 mg/ml; current density: 0.4 mA/cm^2) on the permeation of PE across dermatomed human skin. MN 5, MN 10 and MN 20 represent 5, 10 and 20 MN passes.

4. Combination of microneedle and Iontophoresis on the Transdermal Delivery of PE

The effect of the combination of microneedle and iontophoresis on PE delivery is shown in Fig. 3.3 and 3.4B. A drug concentration of 50 mg/ml and a current density of 0.4 mA/cm^2 were used in these experiments. The permeation of PE was very low by passive delivery (0.047 ± 0.003 µg/cm^2/h) as compared to iontophoresis (3.24 ± 0.267 µg/cm^2/h) which increased the flux by 68 fold. When the skin was microporated (5 passes of Dermaroller™) the total iontophoretic flux was increased by 92 fold as compared to the control (passive delivery). When the skin was microporated with 10 or 20 Dermaroller™ passes, the total iontophoretic flux was increased by 95 and 98 fold, respectively, as compared to the control (passive delivery). There was no

significant difference in flux between 5, 10 or 20 Dermaroller™ passes when combined with iontophoresis.

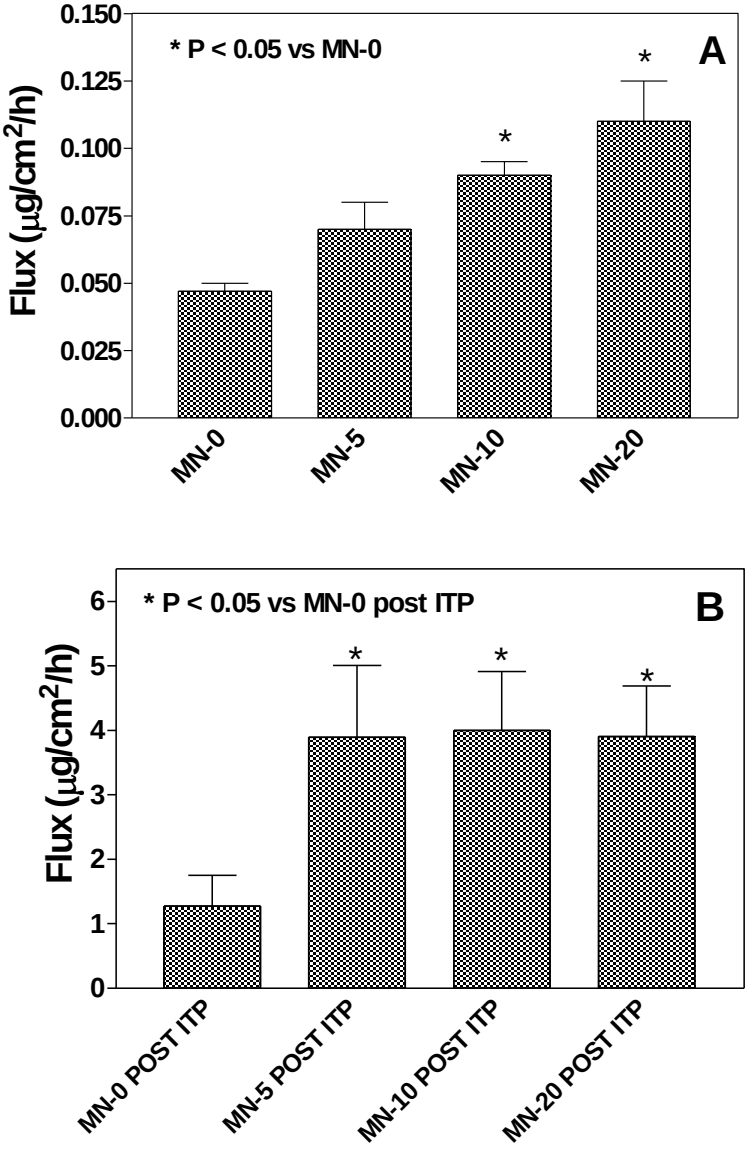


Fig. 3.4: Influence of microneedles alone (A) and in combination with iontophoresis (B) on the steady state flux of PE across dermatomed human skin. The number following microneedles is the number of Dermaroller™ passes.

Based on the reported pharmacokinetic parameters below, the target delivery rate (K_o) to achieve a therapeutic plasma concentration of prochlorperazine (Css of 0.54 ng/ml; Taylor and Bateman 1987) was calculated according to the following equation (Kolli and Banga, 2008):

$$AKo = CL_T \,.\, C_{SS}$$

where CL_T is the total plasma clearance of PE, which is 148 L/h (Taylor and Bateman 1987). With a surface area (A) of 40 cm^2, iontophoretic delivery can provide 2 times higher flux of prochlorperazine than the target delivery rate (2.0 $\mu g/cm^2/h$). This enables a lowering of dose and/or patch surface area. For example, at the maximum delivery rate of 4 $\mu g/cm^2/h$, a patch size of only 20 cm^2 is required. Further in vivo studies are needed to prove the efficacy of iontophoresis combined with microneedles for enhanced delivery of prochlorperazine.

In conclusion, this study demonstrates that the delivery of prochlorperazine across the skin via iontophoresis and microneedles is possible. A combination of microneedle (5 passes) and iontophoresis (0.4 mA/cm^2) synergistically increased the total transdermal transport of PE across human skin. The iontophoretic system can provide a flux of 4 $\mu g/cm^2/h$ which is 2 fold higher than the target delivery rate (2.0 $\mu g/cm^2/h$ for a 40 cm^2 patch) to provide theraputic plasma concentrations.

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