TOPICAL AND TRANSDERMAL IONTOPHORETIC DELIVERY OF THERAPEUTIC AGENTS USING SELF-CONTAINED PATCHES

by

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DEDICATED TO MY BELOVED PARENTS

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ABSTRACT

PURNACHANDAR KASHA

Topical and Transdermal Iontophoretic Delivery of Therapeutic Agents Using Self-contained Patches
(Under the direction of AJAY K. BANGA)

Skin forms a protective barrier to the entry of foreign molecules, especially water soluble compounds. Iontophoresis, one of the promising approaches to deliver water soluble drugs through the skin, has already been approved by FDA to deliver lidocaine and fentanyl citrate. Iontophoresis involves application of current (< 0.5 mA/cm^2) to push charged molecules into, or across the body tissue and offers programmable delivery. The major goal of this research project is to evaluate the feasibility of using self-contained patches in topical and transdermal iontophoretic delivery of therapeutic agents. Wearable Electronic Disposable Drug Delivery (WEDD®) patches were used; these are custom designed, consisting of a 1-volt Zn anode and Ag/AgCl cathode, connected in series to a 3-volt lithium button cell battery.

This makes the patch self-contained and devoid of external power source connections. Sumatriptan succinate and tizanidine HCl have been chosen as transdermal delivery candidates while diclofenac potassium and lidocaine HCl have been chosen for topical delivery. Dermal and/or subcutaneous microdialysis are used to assess the relationship between subdermal and blood concentration of drugs or to compare techniques used to characterize dermatopharmacokinetics or to check the linear diffusion in the skin. Non compartmental analysis has been used to characterize pharmacokinetics using data analysis software, WinNonlin.

CHAPTER 1

INTRODUCTION

Delivery of drugs through the skin has been actively pursued for several years, partly due to the benefits of this route over other routes of delivery, such as avoiding first pass effects as compared to oral delivery. Patent expiration of blockbuster drugs during last decade renewed interest of many pharmaceutical companies to seek product life extensions for these drugs by developing drug delivery systems. Transdermal delivery has come to limelight in recent years, partly due to advertisement of nicotine patches, though the first patch was introduced almost three decades ago (Banga, 2006). Iontophoresis is one of the many promising approaches to deliver water soluble drugs as well as therapeutic proteins and peptides (Banga, 1998; Banga, 2005; Banga, 2006). Iontophoresis involves application of current to push charged drug molecules by electrorepulsion wherein the ionized drug solution is placed under corresponding electrode.

It can also be used to deliver unionized drugs by electroosmosis. Typical iontophoretic drug delivery system contains an iontophoretic patch consisting of two reservoirs, one is called an active reservoir (donor) wherein the charged drug solution is placed and the other one is called a return electrode wherein electrolyte solution is placed. Electrodes are enclosed above the reservoir in contact with reservoir solutions. These electrodes are connected to a controller, which mainly consists of a battery and electrical circuits (Fig. 1.1). Iontophoretic delivery offers better patient compliance as it can be programmable.

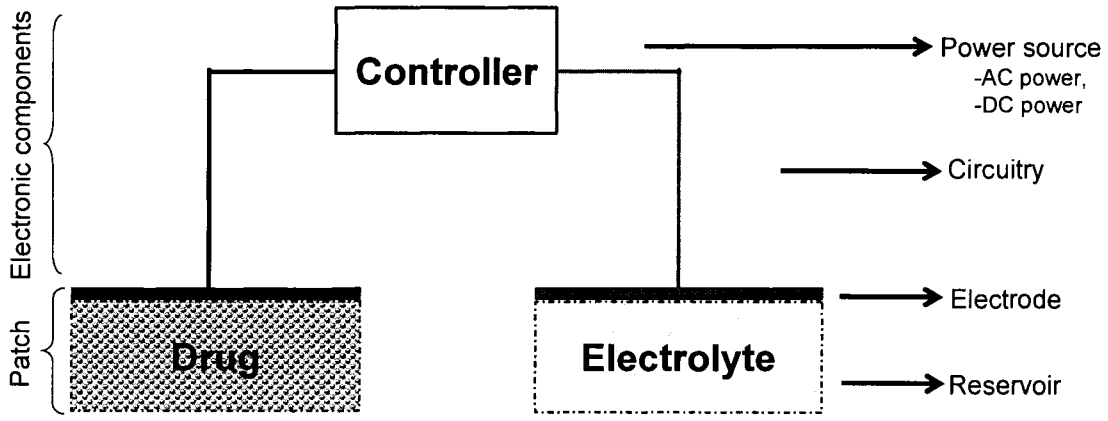

Figure 1.1 Components of an iontophoretic drug delivery system. The drug containing chamber is called donor electrode, connected to anode or cathode, while electrolyte containing chamber is called return electrode or counter electrode connected to corresponding polarity.

Topical Drug Delivery

Skin is the largest organ in the body consisting of three main layers, epidermis, dermis and subcutaneous tissue. The stratum corneum (SC) is the outermost layer of epidermis and is about 10-20 μm thick. It is composed of 15-25 flattened, stacked, hexagonal and cornified cells, embedded in intercellular lipid. Each cell in SC is approximately 40 μm in diameter and 0.5 μm in thickness. Dermis is about 0.1-0.5 cm thick and composed of collagenous fibers and elastic connective tissue in a matrix of mucopolysaccharides. Dermal cells are involved in immune and inflammatory responses. Topical delivery has been used for medicinal delivery purposes for many thousands of years. There has been a tremendous growth in the field of topical drug delivery for the past six decades in understanding fundamental concepts of topical delivery. Passive delivery is known to be highly variable due to various biological confounders of the skin. Several alternative approaches such as penetration enhancers, mechanical disruption of the SC, iontophoresis, ultrasound, and thermal microporation have been investigated alone or in combination to enhance topical delivery of drugs.

Transdermal Drug Delivery (TDD)

The outermost layer of skin, stratum corneum, is considered as a formidable and rate limiting barrier for transdermal delivery. Molecules cross the skin through intercellular, intracellular and transappendageal pathways, depending on the mode of transdermal delivery (Banga et al., 1999; Conjeevaram et al., 2003). Low dose lipophilic drugs with high first pass metabolism upon oral administration have been the ideal candidates for transdermal delivery. Skin penetration enhancement is desirable to expand the scope of transdermal delivery to hydrophilic drugs. Currently, several alternative approaches exist to breach the stratum corneum such as chemical enhancers, ultrasound, light, heat, microneedles, micro blades, and electrical current.

Iontophoretic Drug Delivery

Iontophoresis utilizes small amount of current to push charged drug molecules through the skin and has been investigated as a promising transdermal delivery technology for various drugs, therapeutic proteins and peptides (Banga, 1998; Banga et al., 1999; Banga, 2005; Banga, 2006). In the past, iontophoresis has been carried out in clinical medicine using battery operated, external palm

sized devices hooked up to a drug reservoir electrode and an inactive electrode using clips and wires. This approach can be inconvenient for patients, and therefore limits commercial applications. Wearable and disposable devices have been introduced for the localized treatment of near surface inflammations which are cost effective. More powerful versions of the disposable patches, which include faster onset and rate control mechanisms for systemic delivery, are in various stages of development. Many promising results have been reported for iontophoresis of non-peptide therapeutic agents such as opioids, local anaesthetics, β-blockers, antiviral agents, NSAIDs, anti-migraine agents, and anti alzheimer agents etc. (Banga, 1998; Kalia et al., 2004). The main factors influencing iontophoretic flux are drug concentration, surface area of the patch, pH of the environment, and counter ion concentration. Iontophoretic flux ($J^X{}_I$) is a composite of two primary mechanisms namely, electromigration (EM), electrorepulsion) and electrosmosis (EO).

$$J^X{}_I = J^X{}_{EM} + J^X{}_{EO} \qquad (1.1)$$

Electromigration is the transport of charged ions with the direction of current flow ($J^X{}_{EM}$). Electroosmosis is the convective solvent flow and generally from anode to cathode

(J^{X}_{EO}). For anodic delivery, the electroosmosis is in favor of iontophoresis and most of the therapeutic agents are cationic at physiological pH which enables them to be delivered by anodic iontophoresis.

Wearable Electronic Disposable Drug Delivery (WEDD®)

The Iontophoretic patches were obtained from Travanti Pharma (formerly known as Birch Point Medical Inc.,) and were constructed of electrodes, 3 V lithium battery, internal resistance, absorbent pads and medical adhesive tapes. For the electrodes, specific amounts of Zn and AgCl were coated on the anodes and cathodes respectively, and these electrodes were connected through an electrically conductive material. Combination of Zn/AgCl results in net potential of 1 volt making total voltage of 4 volt system in the patch.

The electrochemistry involved at the electrode interface is shown below:

$Zn \rightarrow Zn^{2+} + 2e^{-}$ $E = -0.76$ (Anode) (1.2)

$AgCl + e^{-} \rightarrow Ag + Cl^{-}$ $E = 0.22$ (Cathode) (1.3)

Clinical Significance

Iontophoresis has been well accepted for topical drug delivery with the successful launch of Iontocaine® and LidoSite® (Banga, 2006). It is currently gaining acceptance

for systemic drug delivery with the approval of fentanyl patch, IONSYS® (Banga, 2006; Mayes and Ferrone, 2006). Extensive literature is found in the application of iontophoresis to deliver drugs to treat several disorders such as psoriasis, contact dermatitis and basal cell carcinoma. The transdermal iontophoretic delivery of therapeutic agents will continue to be an area of high growth in addressing the unmet needs of drug delivery methods particularly in the areas of chronic CNS disorders, pain management and women's health.

Pain Management - Diclofenac Potassium

Pain is one of the many health care crisis affecting people and causes significant burden on society. Pain can be classified as nociceptive and neuropathic pain. Pain stimuli are transferred to the brain through specialized neurons and nociceptors causing nociceptive pain. Damage to the nerve itself can cause neuropathic pain. Any form of pain can affect individual's performance at work or home (Katz, 2002). Untreated postoperative pain may impair healing due to physical and psychological consequences. Postoperative pain management currently includes opioids, local anaesthetics and Non Steroidal Anti Inflammatory Drugs (NSAID). Opioids can be effective for chronic pain

but may lead to dependency. On the other hand, there has been an increased concern over the cardiovascular risks in addition to gastric side effects associated with oral delivery of NSAIDs. Topical delivery in the form of ointments, gels and sprays has been preferred in recent years to avoid any systemic side effects and drug-drug interactions. However, it still suffers from many limitations such as high inter individual variability and irritation at the site of application (Stanos, 2007).

Wound Healing Model for Drug Delivery Studies

Wound is an injury in which the skin or another external body surface is torn, pierced or cut. Wound management primarily involves management of pain for prolonged period of time, preventing wound flora by antibiotics and application of wound healing agents (Drucker et al., 1998). Recent findings reveal that topical application of therapeutic agents may not effectively benefit patients (Dixon et al., 2006).

Migraine - Systemic Delivery of Sumatriptan in hairless rats using iontophoresis

Migraine is a chronic condition that affects 12% of the population occurring in all races, cultures and geographical locations (Arulmozhi et al., 2005; Arulmozhi

et al., 2006). This is characterized by unilateral throbbing headache with nausea, vomiting, and sensitivity to light, sound and head movements. This episodic disorder may last from 2- 4 days indirectly affecting the productiveness at work. Triptans have been prescribed for treating migraine for many years. Current treatment strategies include oral, nasal, rectal delivery and subcutaneous injections. However, patients complain about limited efficacy, slow onset and incomplete prevention of recurrence as well as unwillingness to take oral medication during migraine symptoms. Though the subcutaneous injection of sumatriptan provides quick onset, it is least preferable due to irritation at the site of injection and low patient compliance. Sumatriptan is proved to be very effective in the treatment of migraine (Femenia-Font et al., 2005b; Femenia-Font et al., 2005a). Therapeutic effect results from its selective stimulation of 5-HT_{1D} receptors present in the human basilar artery and in the vasculature of human duramater, which mediates the vasoconstriction.

The absolute bioavailability of sumatriptan is reported as 15, 14 and 96% after intranasal, oral and subcutaneous administration respectively due to incomplete absorption or pre-systemic metabolism (Duquesnoy et al.,

1998; Femenia-Font et al., 2005b). Sumatriptan is a good candidate for iontophoresis due to its positive charge at pH 7.5. Transdermal delivery of sumatriptan succinate (pKa 9.6 & 12, logP1.05) would be an effective alternate approach for systemic delivery to improve the patient compliance.

Spasticity - Single-Dose Pharmacokinetic Study of Tizanidine when Administered *via* an Iontophoretic Patch to the Rat

Spasticity is a disorder of the body's motor system in which certain muscles are continuously contracted. This may be caused by lesions of the central nervous system (CNS). This contraction causes stiffness or tightness of the muscles and may interfere with gait, movement, and speech. Tizanidine hydrochloride (MW: 290.2; pKa: 9) is a centrally acting skeletal muscle relaxant (α_2-adrenoceptor agonist). Tizanidine is well absorbed from the gastrointestinal tract and peak plasma concentrations occur about 1 to 2 hours after doses by mouth. It is about 30% bound to plasma proteins. Tizanidine undergoes extensive first-pass metabolism in the liver. Positive charge at physiological pH and solubility of 29 mg/mL in water makes it an ideal candidate for iontophoretic delivery under anode. It is

believed that a constant transdermal delivery of tizanidine may avoid deleterious side effects that may be associated with oral delivery.

The specific aims are broadly summarized below:

I. To evaluate the feasibility of topical iontophoretic delivery of therapeutic agents using self-contained iontophoretic patches
   1. Diclofenac in pain management
   2. Lidocaine in surgical pain

II. To evaluate the feasibility of transdermal iontophoretic delivery of therapeutic agents using self-contained iontophoretic patches
   1. Sumatriptan for migraine
   2. Tizanidine for treating spasticity

CHAPTER 2

LITERATURE REVIEW

Skin

Skin is the largest organ of the body and acts as a two-way barrier to prevent the entry of foreign molecules or outward passage of water and electrolytes. It accounts for more than 10% of body mass.

Structure of the Skin

The skin comprises of three layers: epidermis, dermis, and subcutaneous tissue. The cellular, stratified, avascular and multilayered outer portion of the skin is called epidermis. The epidermis renews continuously and forms structures like hair, sebaceous glands, apocrine glands, sweat glands, and nails. The epidermis is further divided into distinct layers (Barry, 1983; McGrath et al., 2004):

Stratum germinativum (the basal layer)

The basal cells are nucleated, columnar, about 6 μm wide. This layer constantly undergoes mitosis. Thus it

renews the epidermis, balancing the loss of dead cells from the surface. It acts as mechanical support for the epidermis and serves as a junction between epidermis and dermis. It controls the passage of cells and molecules across the junction as the basal cell layer proliferates and moves outward. These particular cells maintain the integrity of the epidermis by forming inter cellular bridges-desmosomes.

Stratum spinosum

This layer is also called prickle cell layer, consisting of 8- 10 layers of cells. These cells are named since they appear spine like in histological sections. These cells have numerous desmosomes that connect adjacent keratinocytes providing a network for stability.

Stratum granulosum (the granular layer)

The stratum granulosum is a transition zone between living cells and dead cells. This layer consists of 2-5 cell layers. It is called granular due to the presence of keratohyaline granules.

Stratum lucidum

This layer is a translucent layer immediately above the stratum granulosum. This layer is seen in palms and soles.

Stratum corneum

The stratum corneum (SC) is the outermost non-viable cell layer of epidermis and is approximately 10-20 µm thick and composed of 15-25 flattened, stacked, hexagonal and cornified cells (corneocytes) embedded in intercellular lipid. Each cell in SC is approximately 40 µm in diameter and 0.5 µm in thickness. This layer continuously sheds off.

The dermis is about 0.1-0.5 cm thick and composed of collagenous fibers and elastic connective tissue in a matrix of mucopolysaccharides. This layer consists of blood vessels, lymphatics, and nerves along with the hair follicles and sweat glands. Dermis is divided into two layers, the superficial papillary dermis and the denser reticular dermis (Rolland, 1993). Dermis contains an extensive vascular network with a blood flow rate of 0.05 mL/min and is the layer responsible for systemic absorption of drugs delivered through skin (Knepp et al., 1987; Moore and Chien, 1988; Banga, 1998; Hadgraft, 1999). Appendages such as nail and hair follicles lie deep in the dermis.

Hair follicles extend into the deeper dermis facing towards hypodermis. The shaft of the hair follicles is composed of epithelial lining and sebaceous glands open into the hair follicles. Subcutaneous tissue or hypodermis

consists of a network of fat cells arranged in lobules and carries vascular and neural systems for the skin. There are also several associated appendages such as hair follicles, sweat ducts, apocrine glands and nails.

Functions of the Skin

The skin is the outermost barrier between the human body and environment. It performs multiple functions, primarily to protect the bulk of body mass from a relatively hostile environment. The main functions of the skin can be classified as protective, maintaining homeostasis and sensing (Menon, 2002). In addition, it acts as the largest immunologically active organ in the body (Archer, 2004).

Barrier properties of the skin

The skin acts as two way barrier by controlling the inward and outward diffusion of molecules across it. The outermost layer, stratum corneum forms a formidable barrier to the entry of foreign molecules with protein rich cells embedded in the lipid matrix. Because of the lipid nature of the stratum corneum, highly lipophilic drugs can easily partition into the skin. However, water soluble (hydrophilic) molecules do not easily permeate across the stratum corneum. For example, the percutaneous absorption

of water soluble drugs may be 1000 times rapid when the SC is removed, compared to when it is intact (Bronaugh and Stewart, 1985b). Polar channels exist within the intercorneocyte spaces which allow the diffusion of hydrophilic molecules. Thus, drugs can diffuse through the SC via intercellular, intracellular, and/or transappendageal pathway (Fig. 2.1). Physicochemical properties of the drug molecule such as solubility, partition coefficient and diffusion coefficient will dictate the possible permeation through skin through different routes (Green et al., 1989; Phipps et al., 1989). The rate of delivery of molecules across the skin is highly dependent on the physicochemical properties of the drug molecule (penetrant). Drug molecules with strong charges do not yield good passive transdermal delivery due to ionic/electrostatic interaction with the charged sites present in the skin (Phipps et al., 1989). Thus, the SC forms the rate limiting step for drug delivery across the skin. The lipophilic SC represents the primary barrier for charged hydrophilic molecules, while partitioning out of the SC would be the rate limiting step for the charged relatively lipophilic molecules (Phipps et al., 1989).

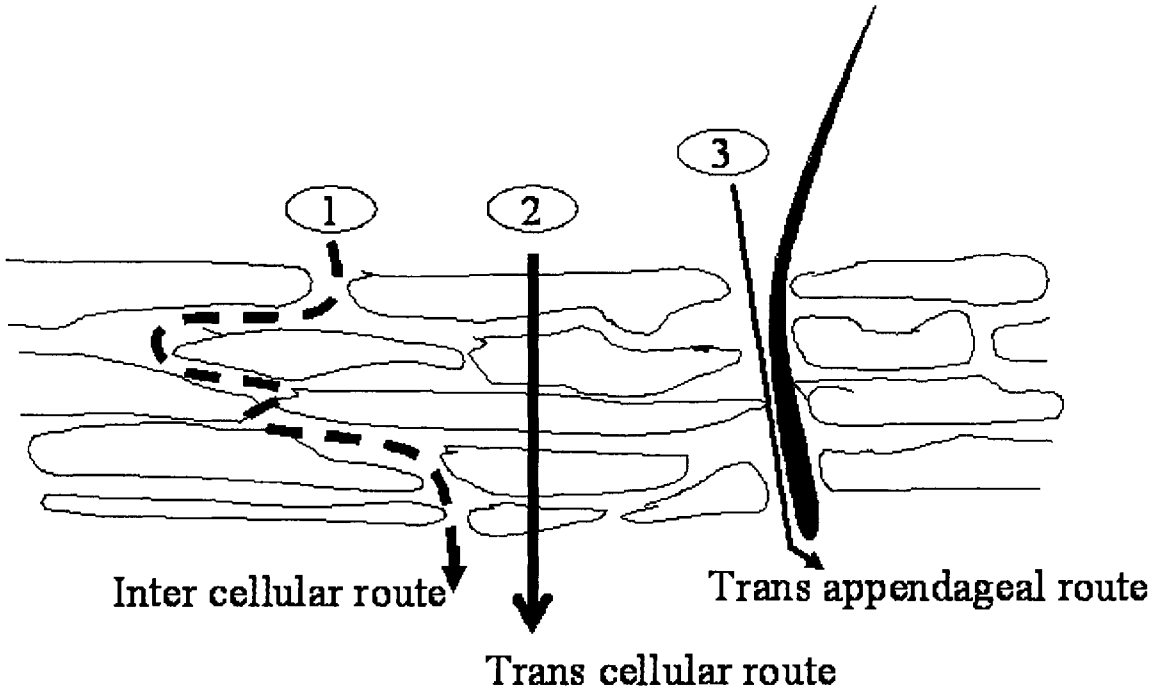

Figure 2.1 Mechanism of drug transport across the skin. Transepidermal (1&2) pathways include intercellular and transcellular route. Transappendageal route (3) includes pilosebaceous unit and eccrine gland.

Immediately after penetrating through the SC, the drug has to partition into the viable epidermis. Drugs with suitable octanol-water partition coefficient(log P)readily diffuse into the epidermis (Green et al., 1989). Drugs with low log P exhibit low permeability due to low partitioning into the skin lipids. However, drugs with high log P tend to form a depot in the SC. In general, parabolic relationship exists between log P and flux (Kim et al., 2000). Thus, ideal log P value for a maximum permeability is between 1 and 3 (Guy and Hadgraft, 1988). The diffusion

of molecules across the skin takes place in accordance with Fick's first law (Flynn et al., 1974). Delivery of hydrophilic and larger molecules has not been promising until the invention of novel alternate transdermal technologies (Banga, 2006; Brown et al., 2006).

Topical and Transdermal Drug Delivery

Skin being the largest organ of the body consists of different layers. Drugs can be delivered either to skin alone or to the systemic circulation. Therapeutic goal is an important aspect in distinguishing between topical or transdermal therapeutic agents. Disorders of the skin are broadly summarized below (Barry, 1983):

1. External trauma,
2. Abnormalities of the epidermis,
3. Abnormalities of dermoepidermal junction,
4. Disorders of the dermis,
5. Disorders of the appendages,
6. Infective conditions.

In addition, the rich blood supply of the dermis has been utilized for delivering molecules through the skin into systemic circulation. Thus, the term 'topical' is defined as targeting pathological sites within the skin, which involves minimal systemic exposure, e.g., lidocaine

as local anesthetic; 'transdermal' is defined as delivering therapeutic molecules across the skin into the systemic circulation, e.g., treatment of withdrawal symptoms using nicotine (Brown et al., 2006).

Topical/Transdermal delivery systems can be divided into two main categories, passive and active systems based on the mechanism of absorption. Typically drugs have been delivered to the skin in the form of ointments, creams, lotions, and gels etc. In recent year passive patches appeared in the market to deliver medication to the skin as well as to the systemic circulation, e.g., lidocaine, estradiol, nitroglycerin, scopolamine, nicotine, clonidine, oxybutynin, and fentanyl (Brown et al., 2006). Several approaches have been used to enhance the delivery across the skin such as chemical enhancers (Williams and Barry, 2004), liposomes, supersaturated systems, prodrugs, skin metabolism modifiers, heat, microneedles, and electrical methods (Barry, 1983; Asbill and Michniak, 2000; Brown et al., 2006; Kasha and Banga, 2008). Patches are based on either membrane controlled or matrix formulations which can provide prolonged drug release. The major disadvantage of this mode of delivery is the lag time associated with the patches for the drug release and also very few drugs can be

delivered by this method. The limitation associated with patch size has been overcome by the matrix technology in which the high concentration of drug is put into the patch (Brown et al., 2006).

Physicochemical properties of drugs limit the transdermal drug delivery, for example, water soluble hydrophilic drugs and protein molecules come under 'difficult-to-deliver' drugs (Kasha and Banga, 2008). Active transdermal delivery methods involve barrier perturbation methods (microneedles, microblades, dermabrasion, and needleless injection), electrical methods (iontophoresis, electroporation, phonophoresis,and magnetoporesis) and miscellaneous methods (laser assisted delivery, and radiofrequency)(Guy, 1996; Banga, 2006; Brown et al., 2006) .

Advantages of Topical and Transdermal Drug Delivery

1. It avoids the first pass metabolism.
2. Better patient compliance compared to conventional routes of drug administration.
3. Sustained and controlled delivery over a prolonged period of time.
4. Reduced risk of dose dumping compared to other controlled drug release formulations.

5. Convenient and painless administration.
6. Ease of termination of medication by removing the patch if any side effects occur.
7. Direct access to the dermatological conditions, e.g., psoriasis, acne, fungal infections, nociceptive pain etc.
8. The dose can be tailor made for an individual patient.

Limitations of the Topical/Transdermal Drug Delivery

1. Large molecules are difficult-to-deliver.
2. Molecules only with a log P (octanol/water) between 1-3 are considered as 'easy-to-deliver drugs'.
3. High variability is associated with biological confounders such as age, sex, race, skin thickness, blood flow etc.
4. Difficult to correlate between normal skin and disease skin.
5. Skin metabolism.
6. Skin irritation and sensitization.

Iontophoresis

Iontophoresis involves the application of electric current (<0.5 mA/cm^2) to push charged molecules into or across the skin.

Principles of Iontophoretic Drug Delivery

According to the principle, the molecules are transported by electrochemical potential gradient under applied electric field. Iontophoretic transport takes place by two different mechanisms:

1. Electrorepulsion
2. Electroosmosis

Electrorepulsion/electromigration

Electrorepulsion is the simple repulsive force between similar charges. This is the major driving force for delivering charged molecules. The flux due to electrotransport takes place when concentration gradient and potential gradient both exist. This is defined by Nernst-Planck equation (Riviere and Heit, 1997):

$$J = -D\,(dc/dx) - (D.C.Ze.F/RT)(dE/dx) \qquad (2.1)$$

Where D is diffusion coefficient of solute across a membrane, C is the concentration of ions with valency Z and electron charge e, F is the Faraday's constant, R is the gas constant, T is the absolute temperature, 'dE/dx' is electrical potential difference, and 'dc/dx' is the concentration gradient.

Electroosmosis

Electroosmosis can be explained as volume flow induced by current flow when electric potential difference is applied across a porous membrane containing fixed charges. The skin becomes negatively charged due to its isoelectric point of ~4 - 4.5 (Kalia et al., 2004). Skin becomes cation permselective upon application of potential difference across it. This electroosmosis can be utilized to deliver neutral molecules from anode. Positively charged molecules benefit from additional driving force besides electrorepulsion. The relative importance of electrorepulsion and electroosmosis depends on the physicochemical and electrical characteristics of the membrane (Guy et al., 2000; Kalia et al., 2004). Mannitol flux was conventionally used as a marker for electroosmotic flow (Kalia et al., 2004).

Modified Nernest-Planck model

Iontophoretic transport of monovalent cations and anions can be approximately predicted by the modified Nernst-Planck model which is modified to account for the influence of electroosmotic flow. The steady state flux

($J_{\Delta\psi}$) during iontophoresis in a porous membrane can be written as follows:

$$J_{\Delta\psi} = \varepsilon\left\{-\left(D\frac{dC}{dX}+\frac{CzF}{RT}\frac{d\psi}{dx}\right)\pm vC\right\} \qquad (2.2)$$

where ψ is electric potential in the membrane, F is Faraday constant, R is gas constant, T is absolute temperature, v is the average velocity of the convective flow, ε is combined porosity and tortuosity factor of the membrane, and C is the concentration, x is the position in the membrane, z is the charge number, and D is the diffusion coefficient.

The predictions of the fluxes from this equation are consistent with the induction of pores in iontophoresis. If the permeant molecular radius is similar to the magnitude of the membrane pore radius, hindrance considerations must be included and Equation (2.2) can be rewritten as following:

$$J_{\Delta\psi} = \varepsilon\left\{-\left(HD\frac{dC}{dX}+\frac{CzF}{RgasT}\frac{d\psi}{dx}\right)\pm WvC\right\} \qquad (2.3)$$

Where, H is the hindrance factor for simultaneous Brownian diffusion and migration driven by electric field, and W is the hindrance factor for permeant transport *via* convective solvent flow. Equation (2.3) is called modified Nernst-Plank equation(Li et al., 2001). The steady state flux for

passive transport ($J_{passive}$) across a porous membrane can be expressed as:

$$(J_{passive}) = \frac{DC_D \varepsilon H}{\Delta x} \qquad (2.4)$$

where, H = hindrance factor for passive diffusion, C_D =concentration of the solute on the donor side, and Δx =effective thickness of the membrane.

Li *et al.* showed the correlation between the predicted and observed electromobilities. It was suggested that the modified Nernst-Planck model predictions were satisfactory only when the electromobilities and the effective molecular size of the molecule were known (Li et al., 2001).

Parallel permeation pathways, pore and lipid pathways, were assumed according to hydrodynamic pore theory. Pore pathways are the main routes for hydrophilic drugs, as explained by the pore theory, and the net flux of a drug at steady state (J) can be described as the sum of individual fluxes:

$$J = J_L + J_P \qquad (2.5)$$

Where, J_L and J_P represent the flux through lipid and pore pathways respectively (Manabe et al., 2000).

Factors Affecting Iontophoretic Drug Delivery

Molecular size

The iontophoretic flux decreases with increase in molecular size as the diffusion of molecules will be affected by the size. The molecular weight and molecular volume have been shown to be inversely related to iontophoretic flux (Riviere and Heit, 1997; Banga, 1998).

Drug concentration

This is one of the important factors to be considered in iontophoretic experiments. It is implied from the principles of iontophoresis; the delivery should linearly be increased with increase in concentration. In contrast to conventional passive transdermal delivery, iontophoretic flux generally increases with the increase in the drug concentration only up to a certain drug concentration (Riviere and Heit, 1997; Kalia et al., 2004; Femenia-Font et al., 2005). This could probably be due to many reasons:

1. The increase in drug concentration may not increase the number of molecules in the skin.
2. Mobility of the ions within the skin which is also charged.
3. Physicochemical properties of the drugs which in turn affect interaction with the skin structures.

Marro et al. investigated the effect of donor concentration on the iontophoretic flux of lidocaine, propranolol, and quinine in the presence and absence of background electrolyte. They observed that in the presence of background electrolyte (NaCl), flux was linearly increased with the donor concentrations for the least hydrophobic lidocaine and the lidocaine HCl flux was unaffected by the concentration in the absence of NaCl. In contrast, flux-concentration profiles of propranolol and quinine showed curvilinear shape and reached a plateau. This was explained by the more competing tendency of lidocaine with the background ion to transport the current with increasing concentration. In the absence of background electrolyte, there was no effect of concentration on the iontophoretic flux of any of the three drugs used (Marro et al., 2001; Kalia et al., 2004).

Effect of electric current

The total iontophoretic flux is the sum of flux due to electrorepulsion, flux due to electroosmosis and flux due to passive delivery. Electrorepulsion contributes almost 90% of the iontophoretic flux which is directly related to the current applied. Apparently, increase in the current will increase the electromigration but it reaches a

saturation point after which the current has no influence on the overall flux (Kalia et al., 2004). Though the applied electric current increases the delivery, the maximum tolerable current has been suggested to be 0.5 mA/cm^2 for iontophoresis (Banga, 1998; Kalia et al., 2004; Wang et al., 2005).

Effect of pH

The pH of the drug solution affects iontophoretic transport in two ways:

1. Degree of ionization of the molecules based on their pKa.
2. Modification of the electrical properties of the skin.

Charge on any molecule or a membrane is a function of pH of the environment. Drugs will be 50% ionized at its pKa and for weak bases increase in pH will decrease the ionization as result decreased iontophoretic transport. The skin charge depends on the pH of the environment due to its isoelectric point (pI) of 4-4.5 (Kalia et al., 2004). The skin becomes negatively charged at higher pH values due to carboxylic acid groups. Therefore, the skin becomes cation permselective. In addition, it favors the electroosmotic flow from anode to cathode. At pH < pI, the skin becomes positively charged, hence, electroosmosis is

in the cathode-to-anode direction suggesting cathodal iontophoresis. Thus, the direction of the electroosmotic flow can be altered by changing the electrical properties of the membrane (Marro et al., 2001). The charge of proteins and peptides will be dictated by their isoelectric point (pI) and pH of the formulation, for example, the iontophoretic flux of lysine (pKa, 10.8) was higher at pH 7.4 than pH 4.0 because of the contribution of electroosmosis(Green et al., 1989; Green et al., 1991). But, extreme pH values of the formulation can produce undesirable hydroxonium or hydroxide ions with high electrical mobilities, which can compete with the ionized drug molecules affecting the iontophoretic flux (Kalia et al., 2004).

The type of electrode

The choice of an electrode is an important aspect in iontophoresis. The rule of thumb is to avoid toxic effects due to generated metal ions during iontophoresis (Kasha and Banga, 2008). Electrodes made of Ag/AgCl are more preferred combination as they resist changes in pH which is seen with other electrodes such as platinum, Carbon, and Zinc/Zinc chloride (Wang et al., 2005).

Iontophoretic Patch Design and Electrochemistry

A typical iontophoretic drug delivery system contains an iontophoretic patch consisting of two reservoirs, one of which is called an active reservoir (donor) wherein the charged drug solution is placed and the other one is called a return electrode wherein electrolyte solution is placed. Polarity of the corresponding electrode is chosen based on the charge of the drug in solution. Electrodes are enclosed above the reservoir in contact with reservoir solution. These electrodes are connected to a controller, which mainly consists of a battery and electrical circuits (Fig. 2.1).

Electrode material and design

Evaluation of suitable electrode material is an important step for proper electrochemistry of iontophoresis. A typical iontophoretic controller includes metals and/or metal halides as anode or cathode to achieve corresponding polarity. Early in 1972, Reeves described a disposable electrode system to induce localized sweat by administering pilocarpine for subsequent diagnostic testing. The electrodes can be classified as shown below (Kasha and Banga, 2008):

a) Inert electrodes: e.g., carbon or platinum electrodes

$$2H_2O \rightarrow 4H^+ + O_2 + 4e^- \text{ (at anode)} \quad (2.6)$$

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \text{ (at cathode)} \quad (2.7)$$

b) Reversible electrode: e.g., Ag/AgCl electrodes etc.

$$Ag \longrightarrow Ag^+ + e^- \xrightarrow{+Cl^-} AgCl + e^- \text{ (at anode)} \quad (2.8)$$

$$AgCl + e^- \longrightarrow Ag + Cl \text{ (at cathode)} \quad (2.9)$$

c) Organic oxidation-reduction electrodes: e.g., quinine, aminal, etc.

Even though iontophoresis is realized as a well known art, many problems remain to be addressed to improve its performance. Electrolysis of water during iontophoresis has been one of such problems in selecting electrode materials, though it has been solved now by using Ag/AgCl electrodes or other approaches. Electrolysis of water, as shown in equation (2.6) and (2.7), causes the pH to drop/increase at anode/cathode resulting in skin burns and/or significant decrease in delivery due to generation of competitive ions. Skin burns due to pH fluctuations can be avoided by including buffer system in solution with the medicament. However, addition of buffer species will introduce undesirable competing ions, leading to loss of efficiency. The efficiency of iontophoretic delivery is affected by generation of electrode ions and/or H^+/OH^-. In addition,

degradation or adsorption of drug under electrode has been a major concern. Several researchers have addressed this problem. Usually four approaches have been mentioned (Banga, 1998; Kasha and Banga, 2008):

1. Salt bridges to separate electrode from the donor and receptor solution,
2. Ion exchange membrane to separate the electrode and drug compartment,
3. Ion exchange resin to exchange with drug ion and then with H^+/OH^-,
4. Formation of precipitation by combination of special metallic electrodes.

Delivery Efficiency

Iontophoresis involves the application of electric field across two electrodes. The number of molecules delivered is proportional to the number of electrons flowing in the external circuit (Phipps et al., 1989). This can be calculated by following equation:

$$E = f_i . F/MW \qquad (2.10)$$

Where, f_i is the slope of the plot between steady state flux versus current density, F is Faraday's constant (96500 coulomb/mole; 1 coulomb = 1 amp-sec), MW is molecular weight of the ion.

Transport Number

A transport number is defined as the fraction of total current carried by a given ionic species, which can be regarded as the efficiency of the electrophoretic transport of that species (Kalia et al., 2004):

$$t_n = J.z.A.F/I \quad (2.11)$$

Where, t_n is the transport number, J is the transdermal flux, z is the valence of the ionic species, F is Faraday's constant, A is the cross sectional area for transport across the skin, and I is the applied current.

Iontophoretic Transport Pathways

Transdermal delivery pathways include intercellular, intracellular and transappendageal routes. Largest currents were observed near the vicinity of residual hair using a vibrating probe electrode which can measure the currents on the skin surface (Cullander and Guy, 1991). It was suggested that the electroporation of the hair follicles at the epidermal cells allow the transport of drug molecules from the appendages (Banga et al., 1999). Monteiro-Riviere et al. investigated the iontophoretic delivery of mercuric chloride to identify the pathways of transport by microscopy. They concluded that intercellular routes were predominantly responsible for the iontophoretic transport.

Even in the follicular transport, final pathway will still be intercellular between hair follicle epidermal cells (Monteiro-Riviere et al., 1994). Hinsberg et al. investigated the role of appendages on iontophoretic flux by comparing the flux through human stratum corneum (consists appendages) and shed snake skin (lacks skin appendages). They concluded that the appendageal pathways contribute less for the flux for relatively small ionic species with high mobility. But, larger molecules such as peptides follow appendageal channels. Turner and Guy showed the dependence of iontophoretic transport pathways on the penetrant physicochemical properties using confocal microscopy. Iontophoretic delivery of calcein for 1 hour, resulted in the delivery of fluorophore deep (~ 20 μm below the skin surface) into the piliary canal of the hairfollicle and intercellular penetration was also observed (Turner and Guy, 1997). Uitto and White et al. have showed that the electroosmotic transport of neutral molecules in human skin occurs via shunt pathways identified as hair follicles or sweat glands. They speculated the possible involvement of gap functions in epidermis to induce electroosmotic flow (Uitto and White, 2003).

Iontophoretic Sampling by Reverse Iontophoresis

Iontophoretic sampling involves application of iontophoretic electrodes on skin/other biological membrane to extract ions/molecules from the body by electrorepulsion/electroosmosis. This process of sampling is called as reverse iontophoresis (Banga et al., 1999; Leboulanger et al., 2004a; Leboulanger et al., 2004b; Wascotte et al., 2005; Nixon et al., 2007). Reverse iontophoresis further implies the possibility of any alternative transdermal delivery wherein the delivery of medicament in response to physiological response is anticipated. Rao et al., in their pioneering work, developed and patented a method for the iontophoretic non-invasive sampling of in vivo concentration levels of glucose and modeling the iontophoretic sampling or delivery in vitro using excised skin of a mammal (Rao et al., 1993; Rao et al., 1995). They patented the device for sampling the diagnostic charged molecules and deliver appropriate levels of therapeutic substances by a biofeedback mechanism. As per the principles of iontophoresis, glucose should be collected in cathode chamber. However, it was found that glucose was converted into negatively charged metabolites, pyruvic acid, lactic acid, etc, in the skin,

which were then collected in anode chamber. The relative high concentrations of metabolites in anode chamber called for biosensors to detect metabolites with better efficiency (Rao et al., 1993; Rao et al., 1995).

Pharmacokinetics of Topical and Transdermal Drug Delivery

Drug delivery through skin is a complex phenomenon due to the complexity involved in the skin structure. Percutaneous absorption is a process in which the drug moves through the skin layers from the site of application and finally is taken up by blood capillaries in the dermis. The stratum corneum is the rate limiting step for hydrophilic drugs and dermis is the rate limiting step for hydrophobic drugs. Pharmacokinetics is a subject which deals with absorption, distribution, metabolism, and excretion of drug delivery systems. The ultimate aim of the pharmacokinetic evaluation is to predict the future state of a system which plays a major role in optimizing the dosing regimens and toxicological consequences (Berner and John, 1994). The drug undergoes different phases after absorption through topical and transdermal drug delivery:

1. Lag Phase - Initial period where there is no absorption.

2. Rising Phase - The drug uptake into systemic circulation increases as the drug moves from the skin layers into dermis.
3. Falling Phase - The drug levels decline as the elimination dominates the rate of absorption.

Pharmacokinetic evaluation of transdermal drug delivery system involves comparison of its plasma concentration-time profile with that of IV administration. IV data can be used to characterize steady state transdermal delivery rate (TDR) based on the assumption that the total body clearance is linear. Thus, the value of TDR can be calculated from the following equation (Berner and John, 1994):

$$TDR = C_{ss} . CL_{IV} \quad (2.12)$$

Where, C_{ss} steady state plasma concentration, and CL_{IV} is total systemic clearance obtained from IV administration. Cumulative amount delivered (dose delivered) using transdermal drug delivery system can be calculated by following equation:

$$\text{Dose delivered (TDD)} = AUC_{TDR} . CL_{IV} \quad (2.13)$$

Where, AUC_{TDR} is area under the plasma concentration-time curves of transdermal drug delivery, and CL_{IV} is total systemic clearance obtained from IV administration.

Predictions Using Compartmental Assumptions

In this method a reference IV administration of the drug is required, in addition to the *in vitro* drug permeation data. Basic pharmacokinetic parameters of the drug will be calculated from the concentration versus time data obtained after IV administration by fitting to the appropriate compartment model. Assuming transdermal administration provides zero-order release, plasma concentration (C) can be represented as below:

For a drug following one compartment model:

$$C = \frac{J_{ss}S}{VK}(1-e^{-kt})(e^{-kT}) \qquad (2.14)$$

Where, J_{ss} is steady-state flux *in vitro,* S is surface area of the patch, V is volume of distribution, K is elimination rate constant, T is duration of patch application, and t is time course of drug administration.

For a drug following two compartment model:

$$C = \frac{J_{ss}S}{VK_E}\left[1+\frac{\beta-K_E}{\alpha-\beta}\exp(-\alpha t)+\frac{K_E-\alpha}{\alpha-\beta}\exp(-\beta t)\right] \qquad (2.15)$$

Where, α and β are the first order rate constants of drug transfer between compartments, and K_E is the elimination rate constant. For both one and two compartment model drugs, steady state plasma concentrations (C_{ss}) can be calculated as:

$$C_{ss} = \frac{J_{ss} \cdot S}{V \cdot K_E} \qquad (2.16)$$

Where, Css is the steady state plasma concentration, S is the surface area of the patch, V is volume of distribution, K is elimination rate constant, and Jss is the steady state flux *in vitro.*

Convolution/Deconvolution Methods

Deconvolution is used to determine the input function. This function reveals the information about multiple peaks. The integral of input function gives bioavailability. Convolution can be considered opposite phenomenon to deconvolution. These methods also require a reference intravenous administration.

If the disposition of the drug after intravenous bolus administration is w(t) and input rate is I(t), the response function r(t) can be considered as a cumulative of various intravenous doses. Thus the relation between the input rate and response function can be written as:

$$I(t) = r(t) // w(t) \qquad (2.17)$$

Where, // is the deconvolution operator.

The plasma concentrations (C) can be calculated as

$$C = r(t) = I(t) * w(t) \qquad (2.18)$$

Where, * is the convolution operator. There are few reports in the application of IVIVC for transdermal drug delivery.

IVIVC is usually applicable for passive transdermal systems but not for the iontophoretic delivery systems. This could be due to many reasons such as lack of microvasculature in the skin *in vitro* to clear the higher flux of drug by iontophoresis, skin depot formation in the skin and effects of donor and receptor buffers used (Kankkunen et al, 2002; Qi et al, 2003, Luzardo-Alvarez et al, 2003; Valiveti et al, 2004).

Methods for Assessing Topical and Transdermal Drug Delivery

In vitro Methods for Assessing Topical/TDD

Topical and transdermal absorption studies include *in vitro* evaluation of absorption using Franz diffusion cells which consists of a donor and receptor compartment separated by a membrane of interest clamped between the two compartments. They provide cost-effective evaluation over the other methods. But the inherent problem with static Franz diffusion cells is the lack of microvasculature which will be present in the *in vivo* situation and helps in rapid clearance of the drug. It is particularly a problem when poorly soluble drugs are tested by these methods. For poorly soluble drugs, the drug concentration may reach closer to the saturation solubility and thus assumption of sink conditions is no more valid. To avoid these problems,

flow-through cells were designed in such a way that the receptor buffer would be continuously removed (Bronaugh and Stewart, 1985a). Flux is calculated as the linear slope of cumulative amount delivered versus time profile in standard Franz diffusion cell experiments. Permeability coefficient (P) can be derived by the following equation:

$$J = A \cdot P \cdot \Delta C \qquad (2.19)$$

Where, A is the surface area, J is steady state flux, and ΔC is the difference in the donor and receptor concentration.

The collector volume, receiver cell volume, flow rate, and sampling interval could modify the apparent flux data when flow through cell was employed. Additionally, these parameters may influence the finite dose flux profiles and the infinite dose diffusion lag times. In general, concentration in the receiver (C_r) of a flow-through diffusion cell versus time (t) in case of infinite dose can be studied by the following equation (Cordoba-Diaz et al., 2000):

$$V_{rec} \bullet \frac{dC_{rec}}{dt} = J \bullet A - F_{rec} \bullet C_{rec} \qquad (2.20)$$

Where, V_{rec} = volume of receiver chamber, J = Flux of drug out of the skin, A = Diffusional area, F_{rec}= Flow rate of receptor fluid.

Tanojo et al. proposed a modification to the flow-through diffusion cells. The contact between the membrane and the receptor chamber was optimized by a spiral channel when the membrane was clamped between the donor and receptor compartments. Thus, the concentration of donor can be ensured through out the experimental period by separate flow-through system. These cells give a unique advantage of using both finite and infinite doses in the donor. This design would eliminate any stagnant domains in the compartment and sink conditions and proper mixing in the receptor compartment was assured. It was noticed that the cumulative amounts reached plateau for p-amino benzoic acid through human stratum corneum in case of finite dose due to the depletion of the drug in 3 hours, whereas it increased through out 16 hours when the constant donor concentrations were maintained (Tanojo et al., 1997).

Bosman et al. developed Kedler cells which can be used in combination with ASPEC system (Automatic Sample Preparation with Extraction Columns). These are the automated alternative to the static Franz cells. These cells consist of inlet, donor and receptor compartments. This design has advantages of automated sampling, reduction of air bubble entrapment, mimicking the *in vivo* situation

by the continuous flow of receptor buffer and smaller areas of skin can be used for experiments. They showed the comparative results of Kedler cells with Franz cells for the permeation of dexetimide (Bosman et al., 1996; Bosman et al., 1998).

Reviere et al. developed an Isolated Perfused Porcine Skin Flap model (IPPSF) which consists of porcine skin isolated with the microcirculation. This method can be used to determine *in vitro* absorption and metabolism within the skin. Pig skin has a potential disadvantage of excessive fat and this makes the drug distribution into the fat than the actual sampling compartment (Riviere et al., 1992).

Microdialysis

'Microdialysis' is a sampling technique for measuring endogenous or exogenous substances in the extracellular fluid. This technique was originally developed for neurotransmitter sampling in CNS studies (Ault et al., 1992; Benfeldt, 1999). The use of microdialysis sampling has been widely used in pharmacokinetic and pharmacodynamic studies (Elmquist and Sawchuk, 2000).

Microdialysis Principle and Applications

Microdialysis is a novel sampling technique based on the principle of passive diffusion across dialysis

membrane. Microdialysis probe consist of a narrow hollow inlet tube, semi permeable membrane, and an outlet tube. In this method, a microdialysis probe with dialysis membrane is implanted into the tissue of interest and perfused with a solution similar in composition to the extracellular fluid (ECF) of the tissue. The hollow microdialysis probe is analogous to a capillary of the tissue. The solution which is perfused through the microdialysate is called 'perfusate'. The solution which is collected from outlet tube is called 'dialysate'. Drug molecules will exchange between the ECF and perfusion fluid according to Fick's first law of diffusion. However, a non-equilibrium gradient exists as the perfusate is perfused continuously. So, the concentration of dialysate is less than the actual concentration in the ECF surrounding the membrane.

Advantages of microdialysis sampling

1. Unbound drug concentrations can be determined by this technique which truly represents the drug at the effector site.
2. High temporal resolution of the data (many data points).
3. Large molecules such as proteins cannot pass through the dialysis membrane depending on the membrane cut-

off value. Thus, the microdialysate sample is free of interfering substances for HPLC assays which reduces the longer sample preparation times during analysis.

4. Reduction in the number of animals required especially when pharmacokinetics studies involve destructive sampling.

Disadvantages of microdialysis sampling

1. It needs specialized personnel and surgical methods.
2. Sensitive analytical methods are required to work with smaller volumes.
3. The concentration of the dialysate will be affected by the evaporation of perfusate from the relatively smaller volume.
4. Physicochemical properties of the drugs will limit its application.
5. Variability associated with microdialysis sampling has to be considered.

Microdialysis Probes

Microdialysis probes are made of various materials such as polycarbonate, regenerated cellulose, polyacrylonitrile, and polyethersulfone etc. with 6 -100 kDa molecular weight cut-off (Plock and Kloft, 2005). Microdialysis probes are commercially available as linear,

loop, side-by-side, and concentric probes with different membrane lengths. The choice of the probe depends on the site of implantation (Davies et al., 2000). The linear probes are well suited for the implantation in peripheral tissues in which the perfusion fluid enters and exits through the membrane in a linear fashion. Linear probes have been used to sample drug levels in muscle, skin, liver, and tumors (Davies et al., 2000). A shunt probe consists of a linear dialysis probe inside a piece of plastic tubing. This probe has been used for sampling from bile while maintaining enterohepatic circulation (Davies et al., 2000). Concentric probes have been widely used to monitor neurochemistry of brain which consists of an inlet tube placed inside an outlet tube. Dialysis membrane is located at the end of the outer tubing. Concentric probes have been employed in sampling subcutaneous tissue (Chaurasia, 1999; Davies et al., 2000; Chaturvedula et al., 2005). Side-by-side probe design is a modification of concentric probe design with added flexibility and useful in awake animals (Plock and Kloft, 2005).

Selection of Perfusion Fluid

The perfusion fluid used in microdialysis varies in composition and pH (Davies et al., 2000). It should be as

close as possible in ionic strength, osmotic value and pH with the ECF of the dialyzed tissue (Plock and Kloft, 2005). Commonly used perfusion fluids include normal saline solution, Ringers solution, Krebs Ringer solution, Krebs-Henseleit bicarbonate buffer and mock-cerebrospinal fluids (Davies et al., 2000; Plock and Kloft, 2005). However, relative recoveries of lipophilic substances by conventional microdialysis method perfused with Ringers solution are generally too low. In order to improve the relative recovery, perfusion with lipid emulsion has been reported in the literature and is called 'Lipo-microdialysis'. This technique is yet to be evaluated more in animal models (Kurosaki et al., 1998).

Microdialysis Probe Calibration

It is crucial to determine the relative fraction of analyte coming into the perfusion fluid due to the non-equilibrium conditions in the microdialysis process. This is termed as 'probe recovery'. Probe recovery can be divided as relative and absolute recovery. Relative recovery reflects the gradient in the concentration of ECF to the perfusate for an analyte and absolute recovery is the actual amount (mass) of the analyte that is collected

from the probe during a defined time. Relative and absolute recovery can be estimated by the following equations:

$$R = \frac{C_{pf}}{C_{ef}} \quad (2.21)$$

$$A = C_{ef} \times F_v \times R \quad (2.22)$$

Where, R = Relative recovery, A = Absolute recovery, C_{pf}= Concentration in the perfused sample, C_{ef}= Concentration in the external solution, and F_v = Perfusion flow rate.
The microdialysate concentrations will be converted into the actual tissue concentration by the following equation:

$$C_{tissue} = \frac{C_{microdialysate}}{R} \quad (2.23)$$

where, C_{tissue} = Actual concentration of the tissue, $C_{microdialysate}$ = Microdialysate concentration, and R = Relative recovery of the analyte

Absolute recovery relates the perturbation of the physiological dynamics of the sample tissue. A higher absolute recovery indicates a higher perturbation in the system.

Factors Affecting Probe Recovery

Probe recovery is influenced by variety of factors (Chaurasia, 1999; Davies et al., 2000; Plock and Kloft, 2005):

1. Physicochemical properties of the drug,

2. Flow rate,
3. Membrane material and length,
4. Concentration of the analyte,
5. The composition of the perfusion fluid,
6. Tortuosity factor of the tissue,
7. Blood flow within the tissue,
8. Temperature of the tissue etc.

Depending on the sensitivity of the analyte the flow rate is set in a microdialysate experiment. Higher the flow rate lower the relative recovery (de Lange et al., 2000).

Methods to Calculate Probe Recovery

Probe calibration is an important step in microdialysis. This significantly increases the actual time of a typical experiment. Moreover, there is poor correlation between *in vitro* and *in vivo* probe recoveries (de Lange et al., 2000). It could be due to difference in tortuosity and nonhydrodynamic nature of many tissues. Thus, it may be inappropriate to infer *in vivo* recovery from *in vitro* recovery study. However, a good correlation was reported between recoveries *in vitro* and *in vivo* during subcutaneous microdialysis experiments (Mathy et al., 2003).

Flow rate variation method

The probe recovery is calculated by varying the perfusate flow rate. The relation between the dialysate concentration (C_{dial}) and actual tissue concentration (C_0) can be written as (Plock and Kloft, 2005):

$$C_{dial} = C_0 \ (1-exp^{-rA/F}) \qquad (2.24)$$

Where, F is the perfusion flow rate, r is the mass transport coefficient, and A is the surface area of the dialysis membrane. The change in the analyte concentration is plotted as a function of perfusate flow rate with the actual sample concentration determined by extrapolating to zero flow, where equilibrium dialysis conditions exist. The main drawback of this method is the lack of temporal resolution of the time points and sensitive analytical methods to handle very low sample volumes from very low flow rates.

Zero-net flux method

In this method, a series of analyte concentrations will be perfused through the membrane at a constant flow rate. The series of concentrations should bracket the expected ECF concentrations. The difference between C_{in} and C_{out} (C_{in}-C_{out}) will be calculated and plotted as a function of C_{in}. This function crosses the x-axis at the value where

C_{in} equals the periprobe concentration in the ECF which is called zero-net flux. The slope of the linear part of this line gives the relative recovery. This method is not suitable for monitoring ECF concentrations as a function of time. This method is very time consuming.

Dynamic no net-flux method

In this method, three or four animals are continuously perfused with a single perfusion concentration and finally the data is pooled, rather than perfusing different concentrations in single animal as mentioned in zero-net flux method. Regression of the mean data at one time point gives the recovery at that point. This method is limited by the number of animals required for calibration alone. This method also suffers from possible interprobe variability.

Retrodialysis method

It is widely utilized method to calculate the relative recovery. The basic assumption in this method is the relative loss of the analyte from the microdialysis membrane equals the relative gain (diffusion process is equal in both directions). In this method, the drug of interest will be perfused through the microdialysis membrane and relative loss of the analyte is estimated. Relative recovery R can be estimated by this method as:

$$R = \frac{Cin - Cout}{Cin} \qquad (2.25)$$

In the modified retrodialysis method, an internal standard is used during an experiment which is called 'retrodialysis by internal standard' calibration method. The internal standard should have similar characteristics such as diffusivity with the drug of interest. Retrodialysis by internal standard allows us to continuously monitor the recovery during an experiment(Bouw and Hammarlund-Udenaes, 1998). Ritzell et al. have validated the internal standard method using no-netflux method for microdialysis measurements of interstitial histamine in rats (Rizell et al., 1999).

Cutaneous Microdialysis

Application of microdialysis technique to measure skin concentrations of drugs has been widely reported in the literature (Nakashima et al., 1996; Cross et al., 1998; Hashimoto et al., 1998; Murakami et al., 1998; Stagni et al., 1999; Benfeldt and Serup, 1999; Morgan et al., 2003; Simonsen et al., 2004; Chaturvedula et al., 2005). It has been stated as a minimally invasive method and more suitable than the current methods of drug assessment in the skin (Ault et al., 1992; Ault et al., 1994; Benfeldt et al., 1999). Probe insertion into the dermis can cause

trauma to the tissue. In a study conducted by Ault et al., the effect of probe implantation did not result in an inflammation or edema immediately after the probe insertion (Ault et al., 1992; Ault et al., 1994). However, increased infiltration of lymphocytes was observed after 6 hours of implantation and during the entire experiment (72 hours). Noticeable changes in the cells surrounding the probe were observed 32 hours of probe implantation (Ault et al., 1994). Groth and Serup investigated the probe insertion trauma in humans. They reported that the insertion of 21G needle increased blood flow, skin colour, skin thickness, and erythema. But the blood flow came back to normal after 90-120 minutes (Ault et al., 1992; Ault et al., 1994; Groth and Serup, 1998). Ultrasound imaging studies showed a 38% relative skin thickening due to the probe (Groth and Serup, 1998). Mathy *et al.* investigated the *in vivo* tolerance of skin after subcutaneous and dermal microdialysis probes in rat using transepidermal water loss (TEWL), Laser Doppler Velocity meter (LDV), and Chromameter. There was no evidence of physical damage observed when 26 G needle was used as a guiding cannula to insert dermal and subcutaneous probes. But, elongation of the cells surrounding the probe

was noticed after 24 hours of probe insertion (Mathy et al., 2003).

Tape Stripping

Tape stripping is relatively painless and non-invasive technique for estimating *in vivo* drug absorption through skin which involves sequential removal of microscopic layers of stratum corneum (Herkenne et al., 2008). It can be used for measuring the rate and extent of cutaneous drug absorption. It has been widely used in various fields to evaluate the barrier function, to investigate dermatophathologies, and to evaluate animal skin as an alternate for human skin (Herkenne et al., 2008). This technique is particularly useful for agents whose target site is upper skin layers, for example, antifungal agents. But, there are some limitations associated with this method (Herkenne et al., 2008):

1. Concentrations in the tape strips can be affected by the diffusion during the tape stripping (Reddy et al., 2002).
2. Applicability of tape stripping data from normal skin to predict its performance in the diseased skin.
3. This method further needs to be standardized because of variability associated with tape stripping.

CHAPTER 3

TISSUE CONCENTRATIONS FOLLOWING TOPICAL IONTOPHORETIC DELIVERY OF DICLOFENAC

Abstract

Purpose. A self-contained Wearable Electronic Disposable Drug Delivery (WEDD®) patch was used to demonstrate that subcutaneous diclofenac levels delivered *via* iontophoresis are greater than estimated minimal effective concentrations (MEC) and are also greater than either passive or intravenous (IV) administration using hairless rats.

Methods. *In vivo* animal studies were done using patches powered with a 4-volt system, consisting of a 1-volt Zn anode and Ag/AgCl cathode with built in 3-volt lithium battery. The anode absorbent pad was loaded with normal saline solution while cathode pad was loaded with 20 mg/mL diclofenac potassium prior to the experiment. Current was monitored during the delivery period to ensure proper electrical connections.

Serum samples were collected at 0, 1, 2, 4, 6, 8, and 10 hours with simultaneous microdialysis sampling. Subcutaneous microdialysis sampling was performed in the IV administration using the same time points and methodology as in the patch testing.

Results. Current levels increased to 1.0 mA at 30 minutes, then fell to a steady state of ~0.4 mA. Both passive and WEDD® patches produced measurable levels of diclofenac in the subcutaneous region below the application site (Cmax = 1.143 ± 0.354 µg/ml and 1.974 ± 0.166 µg/ml, respectively). The dose delivered in 6 hrs was calculated to be 0.226 ± 0.072 mg and 0.430 ± 0.048 mg in passive and iontophoretic delivery, respectively. Diclofenac was not detected in the subcutaneous tissue after IV (1.5 mg/kg) administration.

Conclusion. The trend indicates that self contained patch, WEDD®, can be used to successfully deliver therapeutic levels of diclofenac to the subcutaneous tissue. The subcutaneous levels were found to be higher when compared to either passive delivery or IV bolus administration.

Introduction

Diclofenac is a potent and effective non-steroidal anti-inflammatory drug (NSAID), and is commercially available in the market as tablets, injections, and topical formulations for the treatment of rheumatoid arthritis, osteoarthritis and non articular rheumatic conditions such as myositis and periarthritis(Dehghanyar et al., 2004). It was also approved for use in local treatment of actinic keratosis, a relatively common pre malignant skin lesion seen on areas of skin exposed to sun (Peters and Foster, 1999). Diclofenac is widely prescribed as an oral medication, but due to its gastrointestinal (GI) side effects such as gastric ulcers and GI bleeding, extensive first pass metabolism (~ 50%) and short biological half life, an alternative delivery approach is desirable. One such approach, iontophoretic delivery, can potentially provide deeper tissue penetration without compromising target tissue concentrations (Boinpally et al., 2003). Diclofenac potassium (pKa 4.0) has partition coefficient (Raz et al., 1988) of 13.4 at pH 7.4. The presence of salt forms in topical formulation with increased solubility (1% w/v in water), and their ability to dissociate and to form ion pairs offer pathways across skin either through

hydrophilic pores or passive diffusion across lipid matrix (Fini et al., 1999). One aim of the present study is to demonstrate at least two times greater concentrations than the estimated minimally effective tissue concentration (MEC) in the subcutaneous tissue below the electrode, for a period of at least two consecutive hours.

Iontophoresis is a technique where ions are transferred into the body using an applied electric field (Banga, 1998). This technique offers an advantage of delivering larger quantities of a given drug when compared to passive delivery (Hui et al., 2001). In a previous study by Hui et al, transdermal iontophoresis was found to facilitate the direct penetration of diclofenac sodium to deeper tissue, as measured by radiolabeling techniques in extracted tissue samples (Hui et al., 2001). However, this study employed reusable tabletop power sources connected by wire to electrodes, which are cumbersome to use and commercially unacceptable. Additionally, the study also used relatively high delivery current levels of 2-4 mA, which many patients would find uncomfortable to wear in a home setting. Also, measurements were indirect, using radio labeled diclofenac.

Microdialysis is a sampling technique used to measure tissue concentrations of drugs in pharmacokinetic and metabolism studies. The simultaneous estimation of the pharmacokinetics in skin and plasma helps in understanding the kinetic relationship between absorption at the two sites (Stagni et al., 2004; Stagni and Shukla, 2003) and gives an understanding of drug permeation before entering the systemic circulation. Dermal absorption due to iontophoresis was previously investigated using microdialysis for the evaluation of dermal kinetics of various drugs including anoxacin, diclofenac and acyclovir (Fang et al., 1999a; Fang et al., 1999b; Stagni et al., 2004). Subcutaneous microdialysis has been reported in various pharmacokinetic studies to measure drug kinetics in extra cellular fluid (ECF). Subcutaneous tissue is homogenous in nature and the ECF is in constant equilibrium with the systemic circulation. The subcutaneous microdialysis technique is also considered to be easier than dermal microdialysis (Graumlich et al., 2000). With dermal microdialysis, probe insertion and control over insertion depth are difficult, and the probe itself can increase skin thickness (Groth et al., 1998; Groth and Serup, 1998).

The objective of this present investigation was to use custom-designed wearable disposable drug delivery (WEDD®) patches to deliver therapeutic levels of diclofenac to the subcutaneous tissue. Microdialysis (MD) was used as a sampling technique to measure the effectiveness of delivery. This study also served to evaluate passive delivery and IV administered drug, in order to compare both localized and systemic levels measured to those obtained from iontophoresis.

Materials

Animals

Male CD-hairless rats (Charles River, Wilmington, MA) weighing 290-350 g were used. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). Food and water were provided *ad libitum.* The average number of replicates for each study was four.

Chemicals

Diclofenac potassium was purchased from Exim-Pharm International (Mumbai, India). Naproxen was purchased from Sigma Aldrich (St. Louis, MO, USA), Sterile and pyrogen-free 0.9 % w/v sodium chloride USP was purchased from Baxter Healthcare Corporation (Deerfield, IL). Water,

acetonitrile, glacial acetic acid and sodium acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). All solvents used were of HPLC grade.

Cyclic Voltammetry System

Potentiostat (Cypress Systems, ESA Inc., KS) using platinum working electrode, silver-silver chloride reference electrode, and silver counter electrode was used for cyclic voltammetry.

Microdialysis System

A CMA102 microdialysis pump with CMA142 micro fraction collector (CMA/Microdialysis AB, Stockholm, Sweden) was used. CMA 20 microdialysis probes (CMA/Microdialysis AB, Stockholm, Sweden) with 10 mm polycarbonate membrane, 20 kDa molecular weight cut off were used for subcutaneous insertion.

Methods

In vivo Iontophoretic Delivery of Diclofenac Potassium

Iontophoretic patch - principle of operation

The proprietary WEDD® patches were custom designed to fit the animal. Each was powered by a 4-volt system, consisting of a 1-volt Zn anode and Ag/AgCl cathode connected in series to a 3-volt lithium button cell battery. Prior to placing the patch on the animal, an anode

absorbent pad was loaded with normal saline solution and a cathode pad was loaded with 20 mg/mL diclofenac potassium. Current was monitored during the delivery period to ensure proper electrical connections.

The electrochemistry involved at the electrode interfaces is shown below:

$$Zn \rightarrow Zn^{++} + 2e^{-} (Anode)$$
$$AgCl + e^{-} \rightarrow Ag + Cl^{-} (Cathode)$$

Male CD hairless rats weighing between 250-400 g were used in the study. Each was anesthetized by intraperitonial injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). A CMA microdialysis probe (polycarbonate membrane, 10x0.5mm, 20kDa cut-off) was inserted subcutaneously into the abdominal area of the rat using a guide cannula, and sutured into the skin (Chaturvedula et al., 2005). Microdialysis probes were perfused at a flow rate of 2 µL/min (CMA Microdialysis pump) using sterile 0.9% NaCl as perfusion fluid. Samples were collected every 30 min for 6 hours. For iontophoretic and passive patch testing, serum samples were collected at 0, 1, 2, 4, 6, 8, and 10 hours. For IV testing, a microdialysis analysis was performed in the same abdominal region using the same time points and methodology as in the patch testing. Blood samples (300 µL)

were also collected at different time intervals from the tail vein and serum was collected after clotting. Serum samples were stored at -20°C until analyzed by HPLC. At the end of each experiment, animals were sacrificed using a CO_2 chamber. The skin was cut and visually observed for the probe placement after sacrificing the animal. Passive (control) experiments were also done following the same protocol, but the patches used were without the electrodes or power.

Microdialysis Probe Recovery

After probe implantation, a one hour recovery period was given before starting the calibration by perfusing 0.9 % w/v NaCl solution at 2 µL/min flow rate. *In vivo* probe recovery was determined by retrodialysis method in which probes were perfused with 500 ng/mL of diclofenac in 0.9 % w/v NaCl solution at 2 µL/min flow rate. Microdialysate samples were collected every 30 minutes and analyzed by HPLC. The recovery was performed until the levels in the dialysate reached a steady state. Then, the recovery factor (RF) was calculated by the following formula:

$$RF = \frac{C_P - C_d}{C_p} \qquad (3.1)$$

Where, C_p = Concentration of perfusate; C_d = Concentration of microdialysate. The average recovery factor was found to be 0.75 ± 0.05.

Intravenous Administration of Diclofenac Potassium

Animals were anesthetized as described previously. Diclofenac in water for injection was injected (1.5 mg/kg dose) into the femoral vein of the animal and blood samples were collected from tail vein at different time intervals (0, 5, 10, 15, 30, 45, 60, 120, 240, 360, 480, and 600 minutes.) Serum was collected after clotting and stored at -20°C until analyzed by HPLC (Fig. 3.1). Pharmacokinetic parameters are shown in Table 3-1.

HPLC Analysis

Waters® Alliance HPLC system with Empower® software and a Waters® 2475 fluorescence detector were used. Several methods have been reported to analyze diclofenac levels in biological fluids(Avgerinos et al., 1993; Blagbrough et al., 1992; Godbillon et al., 1985; Lee et al., 1989; Millership et al., 2001; Rbeida et al., 2004; Riegel and Ellis, 1994; Roskar and Kmetec, 2003).

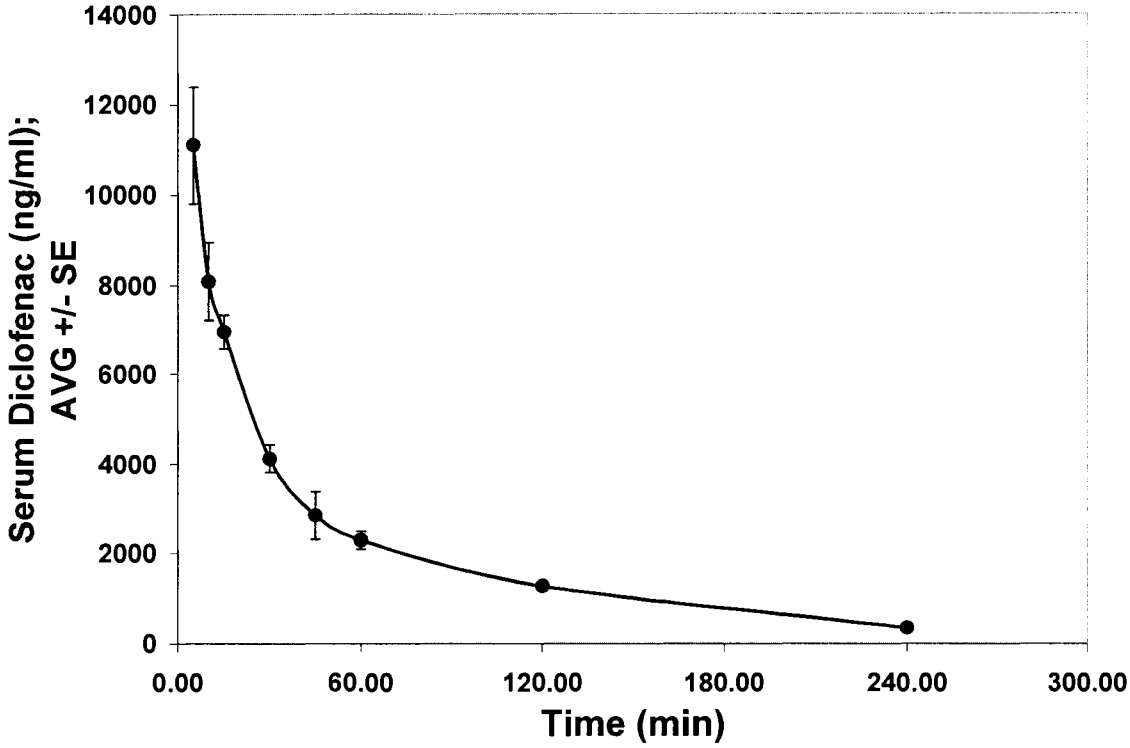

Figure 3.1 Serum diclofenac levels after IV bolus (Dose: 1.5 mg/kg) administration.

Table 3-1 Pharmacokinetic parameters after IV bolus administration (n=3)

Parameter	*Units*	*Estimate ± SE*
Elim. rate const.(λz)	1/min	0.01 ± 0.002
Half life	min	74.2 ± 14.9
Clearance	ml/min	0.87 ± 0.09
Vol. of dist. Vz)	mL	97.5 ± 28.7
$AUC_{0-\alpha}$	min*µg/mL	573.4 ± 30.7

The HPLC conditions were adopted from literature and the simplified HPLC assay was validated for intraday and interday variations. All *in vivo* sample analyses were performed by HPLC using a C18, Varian microsorb-MV (250*4.6 mm, 5µ) column with fluorescence detection at 282 nm and 365 nm as excitation and emission wavelengths, respectively. The mobile phase was Sodium acetate (0.075 M; pH 5) and acetonitrile (55:45). Serum samples were extracted by adding 50 µL of diclofenac standards to 100 µL of serum and boiled on a water bath for 10 min at 85°C followed by adding 200 µL of methanol. This mixture was mixed by pipetting up and down. After cooling for 2 minutes, 50 µL of 560 ng/mL of internal standard (naproxen) was added and vortexed for few seconds. Supernatant was obtained after centrifugation at 3500 rpm for 3 minutes and injected into HPLC for analysis.

Cyclic Voltammetry (CV)

Cyclic voltammetry (Huang et al., 2004) is a unique technique for the electrochemical study of the redox systems. The stability of diclofenac in an applied electric field was tested using cyclic voltammetry (Pt electrode/ Ag-AgCl Reference/Ag counter electrode). With this method, the voltage of an electrode immersed in a test solution is

increased linearly and then decreased to its starting point, while current flow is monitored for evidence of oxidation or reduction reactions.

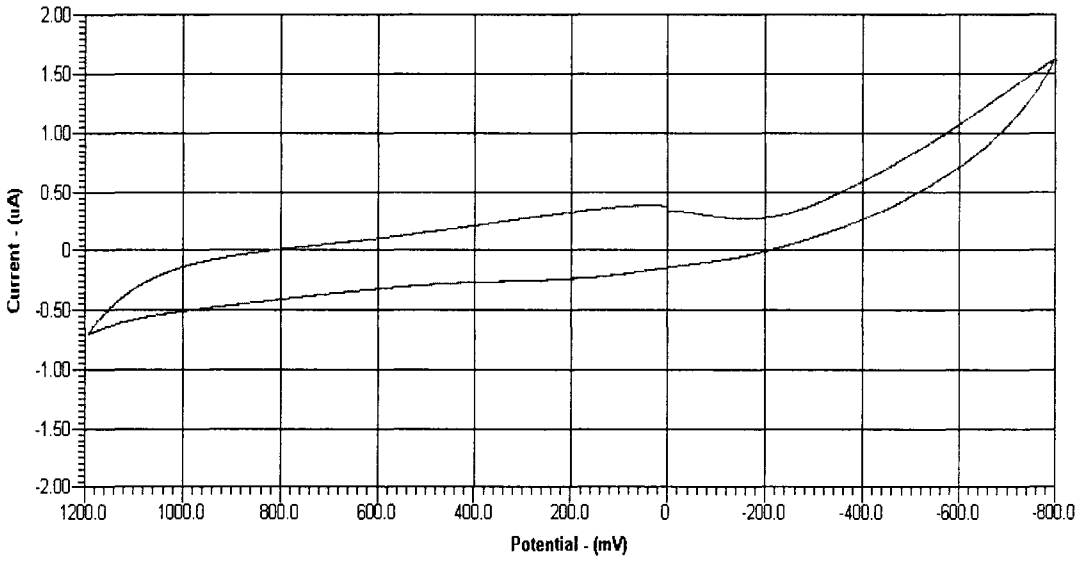

Figure 3.2 Cyclic Voltammogram of phosphate buffer (pH 7.4); initial potential = 0, E1 = -800 mV, E1 = 1200 mV, final potential = 0, scan rate = 200 mV/sec, and number of scans = 10.

Pharmacokinetic Data Analysis

Serum concentration versus time profiles from IV injection and iontophoretic delivery of diclofenac potassium were analyzed using non-compartmental analysis (NCA) by WinNonlin (5.0.1). Pharmacokinetic parameters such as AUC_{0-inf}, terminal elimination rate constant (λz), clearance/F and C_{max} were calculated (Table 3-1 & 3-2).

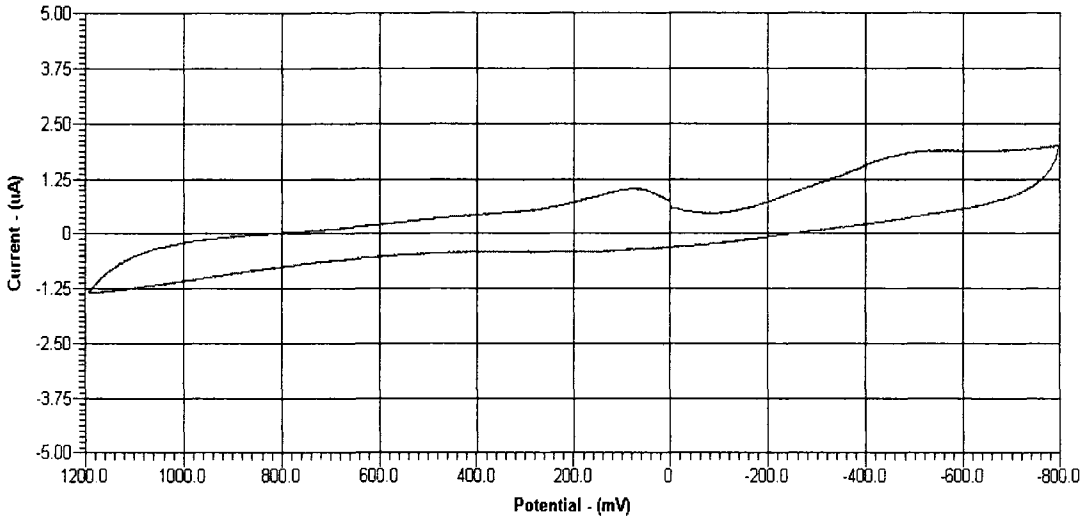

Figure 3.3 Cyclic Voltammogram of diclofenac in phosphate buffer (pH 7.4); initial potential = 0, E1 = -800 mV, E1 = 1200 mV, final potential = 0, scan rate = 200 mV/sec, and number of scans = 10.

Clearance obtained from IV data was used to calculate the dose delivered during iontophoresis by the following equation, with the assumption that iontophoretic delivery provides a zero order infusion and linear pharmacokinetics in from both delivery routes:

$$F^{*}\text{Dose delivered} = AUC_{\text{iontophoretic}} \times \text{Clearance}_{IV} \qquad (3.2)$$

Rate of infusion (R_0) at steady state was calculated by the following equation:

$$R_0 = F^{*}\text{Dose delivered}/\text{Duration of patch application} \qquad (3.3)$$

Where, F represents the fraction of dose absorbed into systemic circulation. It represents the drug loss in the

skin and subdermal layers. F*Dose delivered is calculated as a single function from Equation (3.2).

Statistical Analysis

The data is shown as mean ± SE. Pharmacokinetic parameters were obtained for individual rat and then averaged. Student's t-test at $\alpha = 0.05$ was performed for comparison.

Results

The linear range of the HPLC assay was obtained between 10 - 1000 ng/mL with extraction efficiency of 90 %. The mean recovery factor from microdialysis calibration was calculated to be 0.75. Recovery factor was later used to calculate actual concentrations surrounding the microdialysis probe. Similarities between scans in blank buffer and diclofenac solutions indicate electroactive stability of the drug (Figs. 3.2 &3.3).

In vivo Iontophoretic Delivery of Diclofenac Potassium

Current levels peaked at approximately 1.0 mA at 30 minutes, and then fell to a steady rate of approximately 0.4 mA. Both passive and WEDD® patches produced measurable levels of diclofenac in the subcutaneous region below the application site (Fig. 3.4). In comparing WEDD® active delivery with passive, the trend indicates that active

delivery produces tissue concentrations more than two-fold higher, and faster, than passive delivery. The data from the subcutaneous microdialysis probe was found to be highly variable when compared to serum levels in the same rat. The calculated mean clearance value from IV bolus (1.5 mg/kg) administration was 0.87 ± 0.09 mL/min (Table 3-1). Pharmacokinetic parameters are summarized in Table 3-1 and 3-2.

Discussion

It was inferred from previous *in vitro* studies that there is a substantial benefit in using the potassium salt of diclofenac, with efficiency nearly two-fold higher than that found using a comparable concentration of the sodium salt. Therefore, by using the diclofenac potassium, the total dosage of the Hui study (Hui et al., 2001) could be duplicated at lower current density.

Table 3-2 Pharmacokinetic parameters of diclofenac

Parameter	Units	Estimate ± SE	
		Passive	Iontophoresis
Cmax	µg/mL	1.143 ± 0.354	1.974 ± 0.166
AUC_{0-6}	hr*µg/mL	4.326 ± 1.372	8.187 ± 0.927
F*Dose (6 hours)	mg	0.226 ± 0.072	0.430 ± 0.048

It was surprising to see very low levels, 30-120 ng/mL, in microdialysate during iontophoresis. Similar

studies when conducted with granisetron (Chaturvedula et al., 2005) and another study done with diclofenac (Hui et al., 2001) led us to anticipate levels on the order of 5,000-10,000 ng/mL. The difference seen is most likely a function of the drug and microdialysis measurement technique used in this study. Diclofenac is known to extensively bind with proteins, approximately 99.7% in plasma, and approximately 99.5% in synovial fluid (Radermacher et al., 1991).

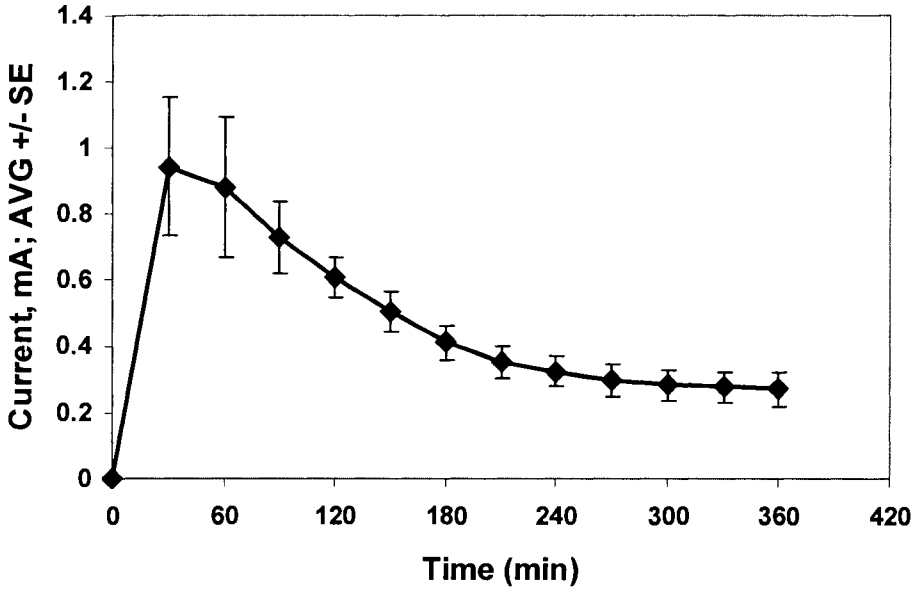

Figure 3.4 Iontophoretic current during 6 hours of patch application.

The protein-bound form of diclofenac does not diffuse through the dialysis membrane of the microdialysis probe

because of 20 kDa molecular weight cut off. Moreover, significant protein levels are found in the interstitial fluid of rats (Wiig et al., 2000) and humans (Fogh-Andersen et al., 1995). Thus, binding effects should be anticipated. Therefore, microdialysis measurements of diclofenac penetration should be interpreted on the assumption that it is an unbound fraction of a much higher total concentration delivered. In addition, an increase in the number of HPLC peaks over time, at elution times slightly different from the diclofenac peak was noticed. These peaks were found in active, passive and in vivo calibration data sets. More over, it was not an indicative of diclofenac degradation from an electric field as the stability of diclofenac under applied electric field was confirmed by investigation using cyclic voltammetry (Figs. 3.2 & 3.3). With this method, the voltage of an electrode immersed in a test solution is increased linearly and then decreased to its starting point, while current flow is monitored for evidence of oxidation or reduction reactions. Similarities between scans in blank buffer and diclofenac solutions indicate electroactive stability of the drug (Figs. 3.2 & 3.3). It was hypothesized that these peaks were related to diclofenac (because of the fluorescence sensitivity), and

were perhaps a percentage of diclofenac that is bound to smaller proteins capable of entering the microdialysis probe. A high degree of variability was also noted; with approximately 100 % CV's seen in both active and passive data sets. This is apparently not unusual, since variability as high as 146 - 215 % has been noted in numerous other studies with transdermal delivery of diclofenac (Brunner et al., 2005; Dehghanyar et al., 2004; Muller et al., 1997). Some have speculated that high variability may be due to biologic confounders such as skin thickness, local blood flow, lipid content, and the presence of hair follicles (Brunner et al., 2005; Dehghanyar et al., 2004; Muller et al., 1997).

Both passive and active WEDD® patches produced measurable levels of diclofenac in the serum (Fig. 3.5). In comparing WEDD® active delivery with passive, a trend very similar to our microdialysis results indicates that active delivery results in serum concentrations more than two-fold higher, and faster than passive delivery (Figs. 3.6 & 3.7).

Given the discussion above, it is important to recognize that the serum results are for total diclofenac. The higher concentrations seen in serum (when compared to the unbound microdialysis concentrations in the

subcutaneous space) are consistent with the hypothesis of protein binding effects associated with the microdialysis measurement technique.

Microdialysis probe was inserted into the same subcutaneous space as in the passive and WEDD® patch testing during IV bolus injection. This was performed to measure the amount reached to subcutaneous region *via* IV compared to the amounts reached *via* transdermal patches. The MD results after IV bolus dosing for the animal were below the detection limits at all time points (Fig. 3.6). The substantially higher subcutaneous levels found in the iontophoresis and passive testing supports the potential advantage of localized delivery to tissue when compared to systemic dosing (Fig. 3.7).

It was estimated that the minimal effective concentration (MEC) of unbound diclofenac to be between 0.5 to 2.5 ng/mL based on the following assumptions: Chlud and Wagener (Chlud K, 1987) estimated MEC of total diclofenac in synovial fluid to be 100-500 ng/mL. Protein binding has been measured to be 99.5% in synovial fluid (Radermacher et al., 1991). Therefore, a 0.5% unbound fraction of 100-500 ng/mL total diclofenac is 0.5 to 2.5 ng/mL. Similarly, to compare the unbound measurement of microdialysis with the

total measurement made in serum, 99.5% binding concentration was assumed.

Thus, unbound concentration was multiplied by factor 200 to compare with our serum results. This factor was supported, as our estimated total subcutaneous concentration of 18,840 ng/mL was comparable to a previous study with direct measurement of ~11,000 ng/mL subcutaneous diclofenac after iontophoresis using radiolabeled diclofenac (Hui et al., 2001).

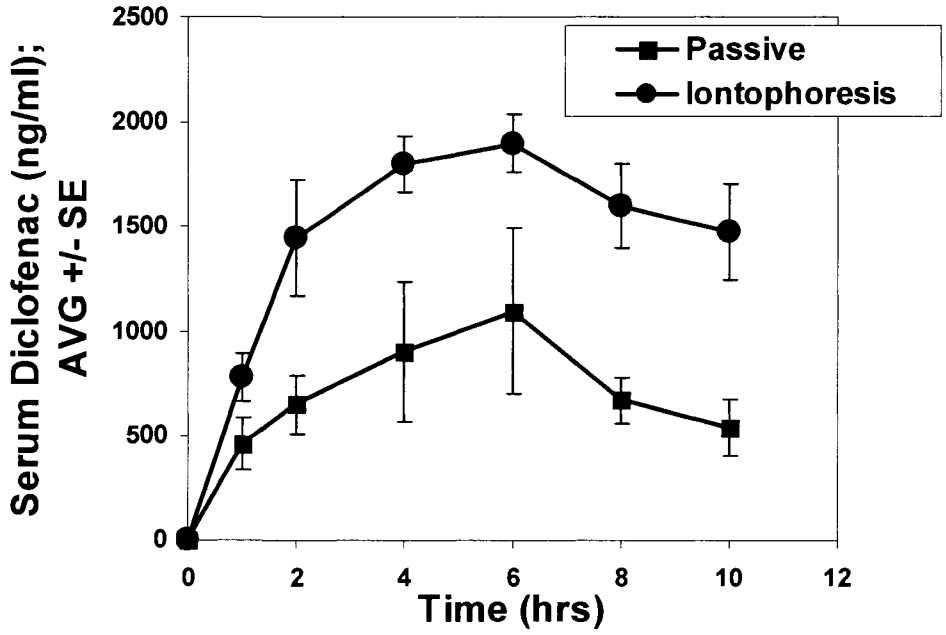

Figure 3.5 Serum diclofenac levels after passive and iontophoretic delivery.

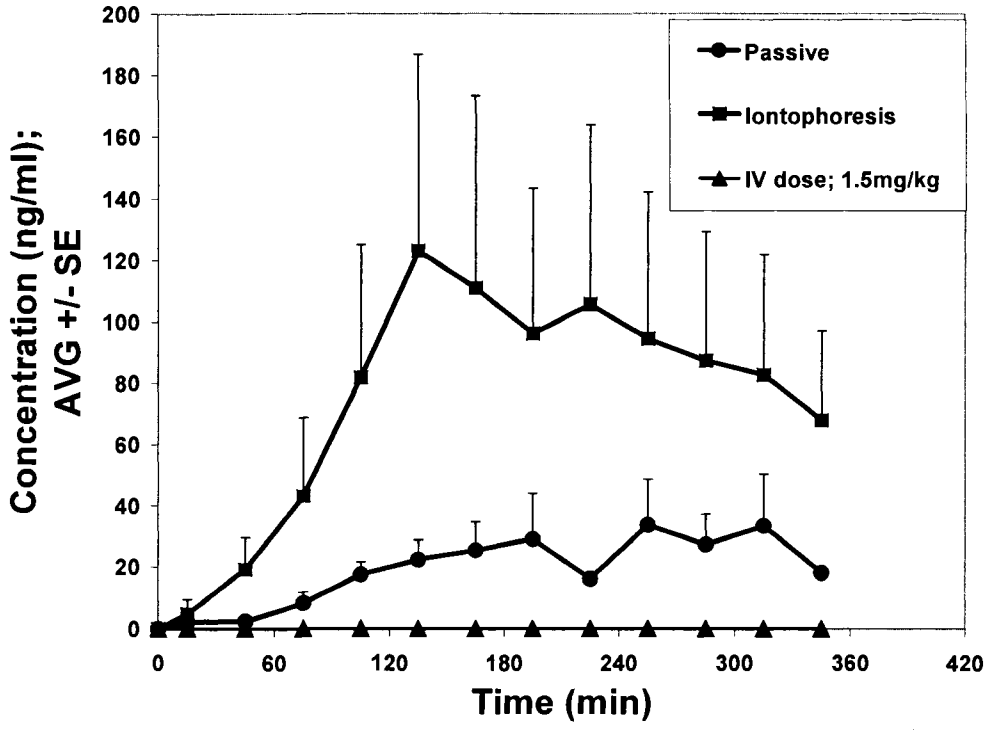

Figure 3.6 Microdialysate levels in subcutaneous tissue.

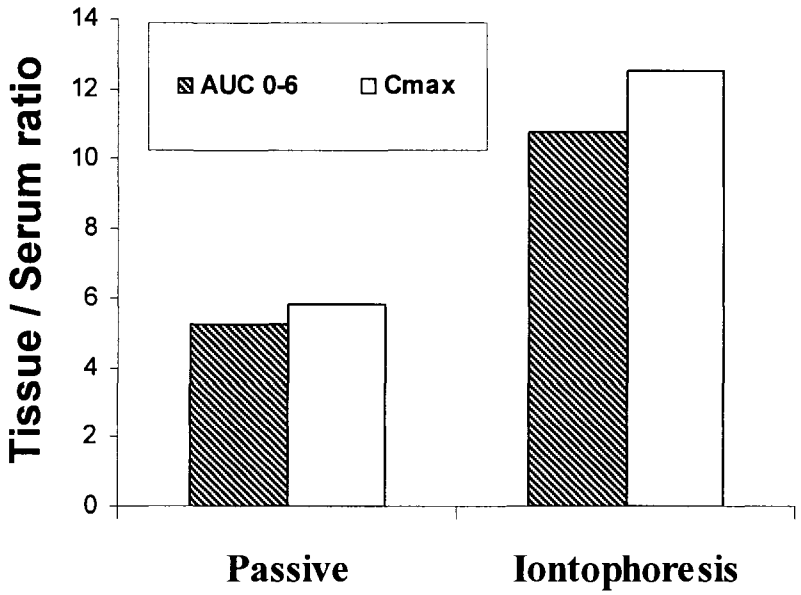

Figure 3.7 Comparison of tissue-serum concentration ratios for diclofenac after passive and iontophoretic delivery (tissue concentration was corrected for protein binding)

Conclusion

The results of this study suggest that a user-friendly, disposable WEDD® (Wearable, Electronic, and Disposable Drug Delivery) patch may be used to deliver diclofenac in amounts and rates that can exceed passive delivery. Further, evidence is presented that suggests the delivered amounts may be efficacious on a localized basis at a subcutaneous tissue depth. Microdialysis has been confirmed to be a useful measurement for localized concentrations, although with limitations hypothesized to be associated with protein binding.

Acknowledgements

This project was funded by Travanti Pharma Inc., Mendota Heights, MN 55120. We would like to thank Pharsight Inc., Mountain View, CA, for providing us the academic license for WinNonlin Pro software.

Abbreviations

WEDD® - Wearable Electronic Drug Delivery, ECF - Extra cellular fluid, AUC - Area under the curve, HPLC- High performance liquid chromatography, CV - Cyclic voltammeter, RF - Recovery factor, NCA - Non compartment analysis, PVA - Polyvinyl alcohol, MD - Microdialysis, and MEC- minimum effective concentration.

CHAPTER 4

IN VITRO MODEL FOR DRUG DELIVERY STUDIES INTO SUTURED SURGICAL INCISION

Abstract

Purpose. To develop an *in vitro* model for drug delivery into sutured surgical incision and test it for iontophoretic delivery of lidocaine relative to delivery through intact skin.

Methods. A one-inch incision was made on full thickness human skin with a surgical blade and two edges were put together with simple interrupted sutures. A simple linear probe (L1) was inserted into the incision after suturing. A second linear probe (L2) was placed intradermally next to the incision, into the intact skin. The skin was placed over a mold having agarose gel and two concentric probes (C1 and C2) placed in the gel 1 cm and 4 cm below the surface of the skin. A cotton pad with Zn electrode was used as anode and was filled with 500 µL of 4% lidocaine HCl injection USP. A AgCl electrode was placed at the bottom of the gel.

A current of 0.12 mA, 0.33 mA, and 0.54 mA was applied over 6 hours, with each setting being used for 2-hour periods. Microdialysis was performed at 2 µL/min flow rate for 6 hours. The dialysate samples were collected every 30 minutes and analyzed using HPLC immediately after collection.

Results. Iontophoresis (6 hours) increased levels of lidocaine in L1 probes 3 folds within 1 hour and 5 folds within 6 hours compared to passive delivery. The lidocaine levels inside the incision reached 114 µg/mL, 181 µg/mL and 196 µg/mL with 0.12 mA, 0.33 mA and 0.54 mA current respectively. The C_{max} was found to be 46 times higher in L1 than L2. Passive delivery resulted in no detectable levels in the concentric probes, C1 and C2 while levels under iontophoresis were ~2 µg/mL in 6 hours in both C1 and C2.

Conclusion. An *in vitro* model has been developed for drug delivery into sutured surgical incisions and has been shown to be very promising for monitoring lidocaine levels as a function of applied current. The range of currents necessary to drive a sufficient amount of lidocaine into sutured skin has been successfully investigated. Based on drug levels delivered, an iontophoretic patch is likely to be useful for managing pain associated with incision

Introduction

Wound is an injury in which the skin or another external body surface is torn, pierced, or cut. Wound management primarily involves management of pain for prolonged period of time, preventing wound flora by antibiotics and application of wound healing agents (Liptak, 1997; McGrath and Breathnach, 2004).

Topical medications to relieve postoperative pain and prolong analgesia are used in many clinics (Bernards and Kopacz, 1999; Kenkel et al., 2004). Experimental studies have indicated that lidocaine does not alter wound healing process after surgical interventions such as liposuction (Kenkel et al., 2004). Recent findings reveal that topical application of therapeutic agents may not effectively benefit patients (Dixon et al., 2006). Iontophoresis, on the other hand, has been successfully used in clinical setting to deliver drugs across intact skin (Banga, 2006; Greenbaum, 2001). Iontophoretic delivery provides hydrophilic molecules a noninvasive route of administration through skin for controlled and programmed delivery and avoids first pass metabolism. Iontophoresis uses a small amount of current to push charged drugs deep into the skin

(Banga, 1998; Chien and Banga, 1989; Guy et al., 2000; Kalia et al., 2004).

Lidocaine is an amide-type local anesthetic agent and acts by inhibiting the ionic fluxes required for the initiation and conductance of impulses (Bernards and Kopacz, 1999).

This present investigation focuses on developing an *in vitro* wound healing model for drug delivery and tests it for iontophoretic delivery of lidocaine relative to drug delivery through intact skin. The present study used an *in vitro* setup in which human skin which has been excised and sutured is used as a model for drug delivery into sutured surgical incisions. Drug delivery as a function of current over time in agarose gel model was investigated, with the agarose gel serving to simulate the muscle. The delivery was monitored by four microdialysis probes, two in the skin and two in the gel. Probes placed in the skin measured penetration into excised area versus intact skin, while probes placed in the gel measured penetration as a function of depth.

Materials

Chemicals

4 % Lidocaine HCL Injection, USP (40 mg/mL), SeaKem Gold Agarose (Cambrex Bio Sciencs Rockland, Inc., ME), normal saline, methanol, and dibasic potassium phosphate.

Surgical Set Up

Human full thickness skin (NDRI, PA), surgical board, Nylon 4/0 sutures, Edge Ahead Stiletto / MVR knife, needle holder, skin mounting pins, tweezer, and forceps.

Microdialysis System

Microdialysis probes (Concentric and linear probes, 10 mm and 20 kDa membrane cut off), CMA/102 microdialysis pump, and 25 G guiding cannula.

Iontophoresis

Iontophoresis controller, Zinc and AgCl electrodes, electrical connections, cotton pads, and agarose mold to hold the skin.

Methods

Agarose Gel Mold

A beaker with saline was heated in a microwave. Then sufficient amount of agarose was added to obtain 0.9% w/v gel. The agarose was slowly added while stirring the solution. It was further reheated to boiling until all the

particles were dissolved. It was cooled to ~50°C prior to casting. The mold acts as a receiver chamber which is 6 cm in length. Two holes were made at 1 cm and 4 cm from the top. Provision was made to place AgCl electrode at the bottom of the mold (Fig. 4.1).

Iontophoresis

A one inch incision was made on full thickness human skin (5 x 6 cm) with a surgical blade and the edges were then mended with simple interrupted sutures. A simple linear probe (L1) was inserted into the incision after suturing. A second linear probe (L2) was placed intradermally next to the incision, into the intact skin (Figs. 4.1 & 4.2) using 25 G needle as guiding cannula.

The skin was placed over a mold having agarose gel and two concentric probes (C1 and C2) placed in the gel 1 cm and 4 cm below the surface of the skin. A cotton pad with Zn electrode was used as anode and was filled with 500 µL of test compound. A silver chloride (AgCl) electrode was placed at the bottom of the gel. A current of 0.12 mA, 0.33 mA, and 0.54 mA was applied over 6 hours, with each setting being used for 2 hours (Fig. 4.3). The current was applied in a similar way in case of 24 hour study except the 0.54 mA current was maintained for the remaining study period

immediately at the end of second current setting, 0.33 mA. The cotton pad was soaked with 500 µL of lidocaine solution for 6 hours study, while 2 mL of above solution was placed during 24 hours study period in order to minimize the depletion of donor solution.

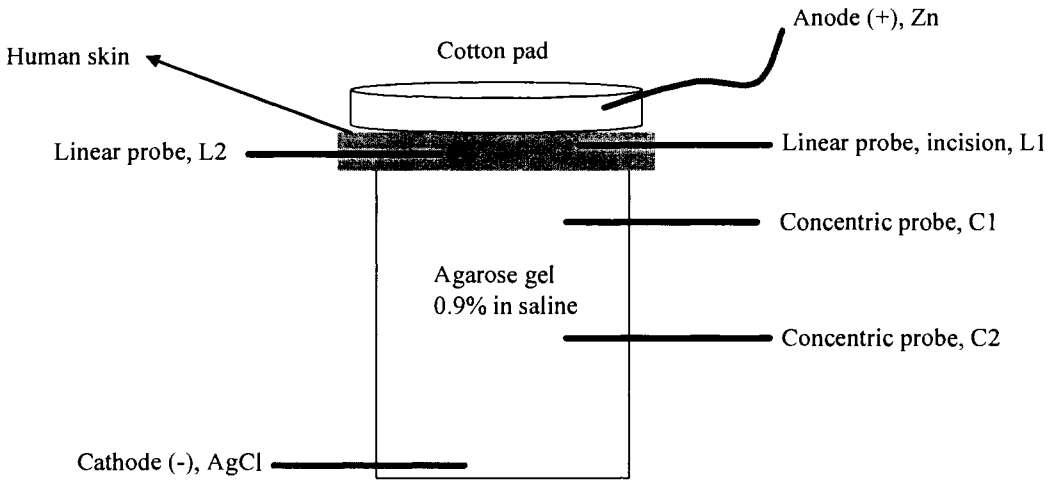

Figure 4.1 Schematic of wound healing model consisting of 60 mm mold made of 0.9% w/v agarose gel. Concentric probes (C1 & C2) were inserted 1 cm and 4 cm below the human skin. Linear probes (L1 & L2) were placed inside the skin and mounted on top of the gel. Cotton pad was soaked with lidocaine and Zn/AgCl electrodes were connected to an iontophoretic controller.

Microdialysis was performed at 2 µL/min flow rate. The dialysate samples were collected every 30 minutes and analyzed using HPLC immediately after collection. Initially, dialysate was collected every 30 min for four hours followed by 90 min sample collection interval during 24 hour iontophoretic delivery study.

HPLC Analysis

A stock solution of lidocaine was prepared by mixing 1 mg of lidocaine HCL in normal saline. The standard solutions were prepared by serial dilutions. The linear range was observed between 100 ng/mL and 100 µg/mL. Gemini C18 (4.6 x 150 mm, 5 µ) column was employed to measure lidocaine levels in the dialysate. Mobile phase consisted of 25 mM sodium phosphate (dibasic) and methanol with isocratic elution at 25% and 75% respectively with a flow rate of 1 mL/min. Dialysate samples were analyzed for lidocaine at 240 nm with 30 µL injection volume. The limit of detection was found to be 20 ng/mL.

Statistical Analysis

The data is shown as mean ± SE. Student's t - test (α = 0.05) was done for comparisons.

Results

Iontophoretic delivery (6 hours) increased the lidocaine levels in L1 probe three folds within one hour and five folds within six hours compared to passive delivery.

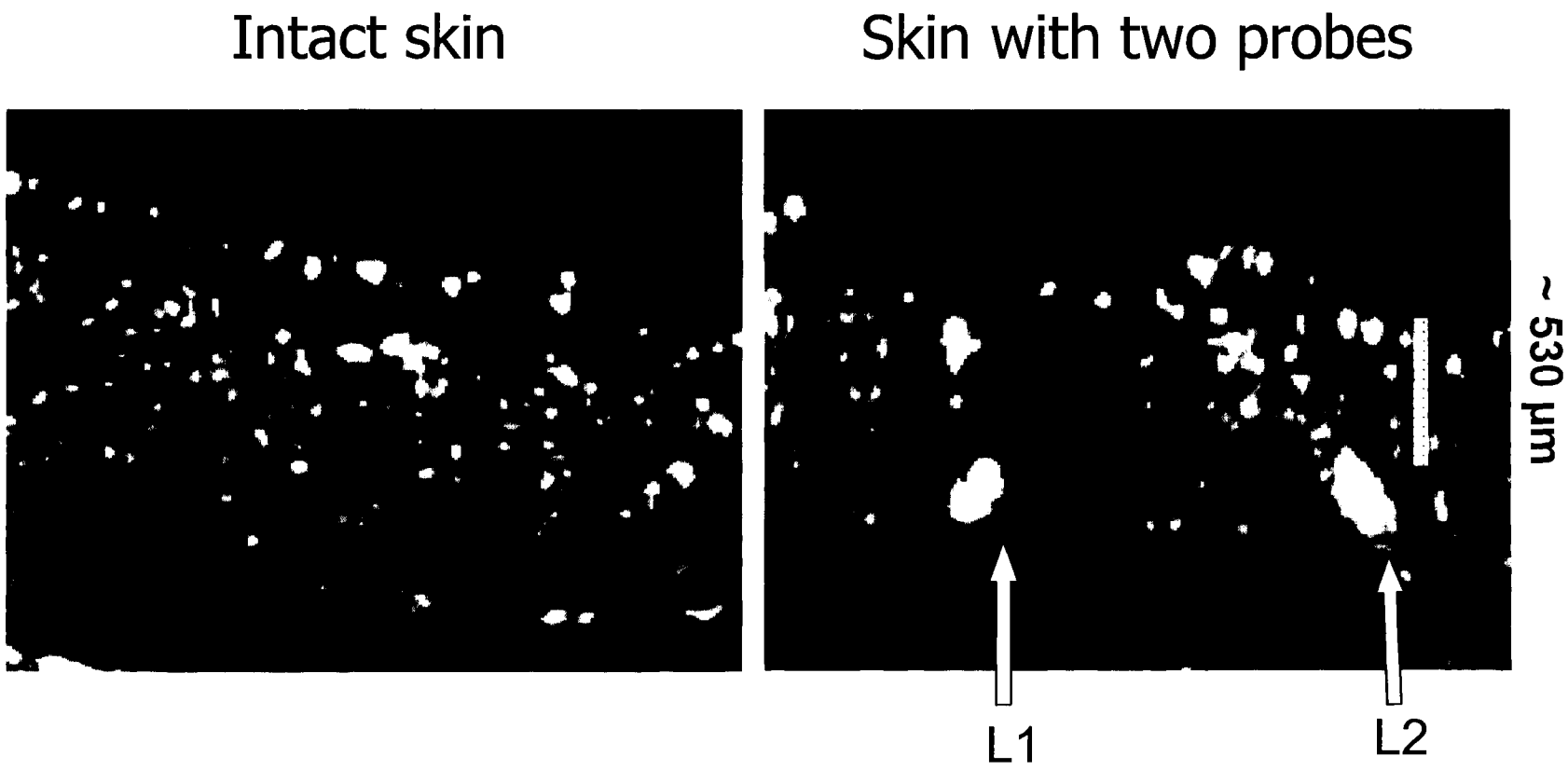

Figure 4.2 B-mode ultrasound scanning (Derma scan) depicting two linear probes in the skin (L1: Incision probe; L2: Intradermal probe).

The lidocaine levels inside the incision reached 114 µg/mL, 181 µg/mL and 196 µg/mL with 0.12 mA, 0.33 mA and 0.54 mA current respectively. The observed maximum concentration (C_{max}) was found to be 46 times higher in L1 than L2 (Fig. 4.3). The lidocaine levels were observed to be below the detection limit from concentric probes in iontophoresis and passive delivery from 6 hours study.

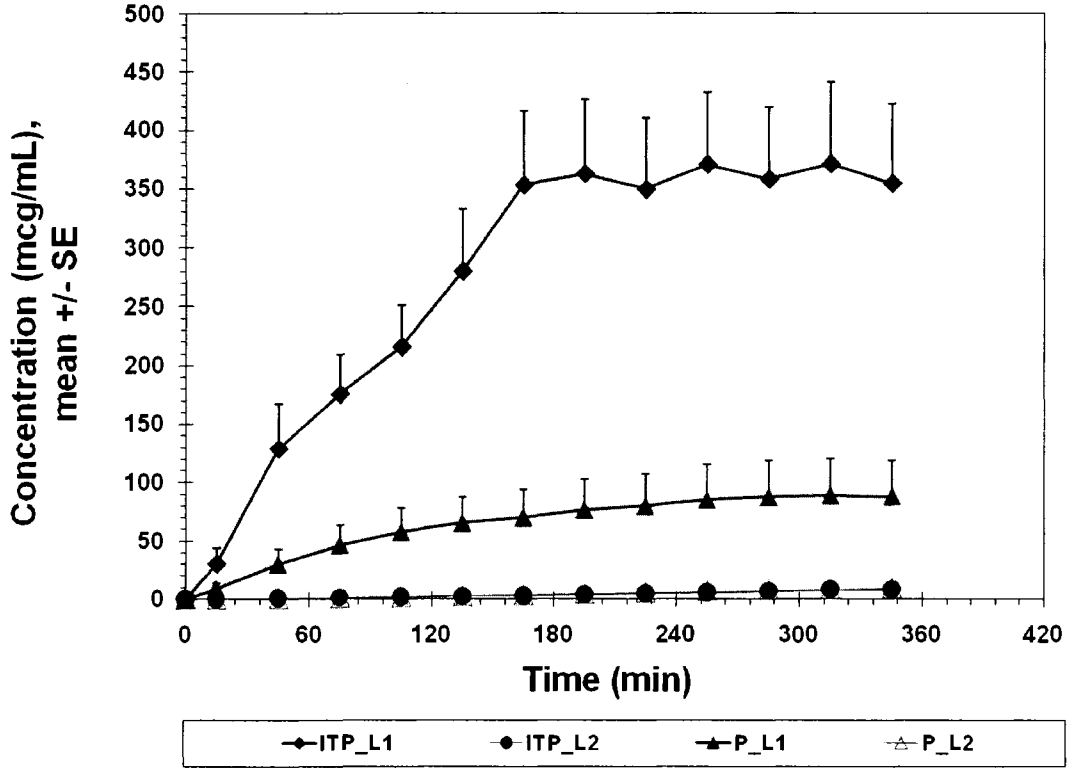

Figure 4.3 Linear probes: Iontophoresis Vs Passive delivery for 6 hours; Electric current of 0.12 mA, 0.33 mA, and 0.54 mA was applied for every two hours.

The lidocaine levels (24 hour delivery study) inside the incision reached 248 µg/mL, 783 µg/mL and 1048 µg/mL

with 0.12 mA, 0.33 mA and 0.54 mA current respectively. The C_{max} was noticed at 4.75 hours. The levels were dropped to 517 µg/mL in 24 hours. The C_{max} was found to be 26 times higher in L1 than L2 and the L1 levels were approximately 5 folds higher than L2 at the end of the iontophoresis. Though the levels in concentric probe, C1, were relatively minimal, the levels started to increase after 4 hours of iontophoresis and reached 43 µg/mL in 24 hours. No levels were detected in the probe, C2 (Fig. 4.4).

Discussion

Lidocaine has been used as an anesthetic agent for various procedures and it is widely used in dermatological procedures (Greenbaum, 2001). Lidocaine has been delivered by using ointments or injections. Injections are least preferred by many patients. Thus, iontophoresis offers an attractive method for delivering lidocaine to the deeper tissue (Wallace et al., 2001). Iontophoresis is gaining importance in clinical setting with the acceptance of Lidosite® to deliver lidocaine and Ionsys® to deliver fentanyl citrate (Ashburn et al., 1995; Kasha and Banga, 2008). Lidocaine acts as a vasodilator which increases its own clearance from the skin after topical administration, which has been believed to be one of the reasons for poor

patient compliance (Ashburn et al., 1997). Ashburn et al. have delivered lidocaine along with epinephrine to prolong anesthesia. Although epinephrine activates α2-adrenergic receptors, its mechanism for prolonging anesthesia results from minimizing the lidocaine clearance from the site of administration (Bernards and Kopacz, 1999). The effective dose for anesthetic effect during liposuction is very confusing as the FDA recommends 7 mg/kg which is quite different from reported values up to 55 mg/kg (Kenkel et al., 2004). Kenkel et al. have concluded from their study that tissue levels become subclinical within 4 - 8 hours of initial infiltration necessitating the need for patient controlled analgesia post operatively. Thus, the delivery of lidocaine as function of current was investigated using custom designed in vitro surgical incision model (Fig. 4.1). Microdialysis has been widely used for sampling drug levels in the interstitial fluid (Ault et al., 1992). This method offers simultaneous sampling with better temporal resolution for measuring the drug levels within the skin or subcutaneous tissue during topical/transdermal drug delivery (Ault et al., 1992; Benfeldt, 1999; Benfeldt et al., 1999; Benfeldt and Groth, 1998; Benfeldt and Serup, 1999; Kurosaki et al., 1998). Initially iontophoresis was

applied for 6 hours where the drug levels within the incision reached plateau after two hours (Fig. 4.3). This could be probably due to either depletion of the donor solution or the decreased iontophoretic efficiency. Iontophoretic delivery efficiency may be affected by the generation of competing ions, Zn^{2+}, from the anode (donor). This was further evidenced in another study where the patch was applied for 24 hours with enough lidocaine in the donor solution (Fig. 4.4) but still delivery decreased over time. However, incorporation of Zn^{2+} ions may be therapeutically beneficial as it helps in wound healing in the form of cofactor (Lansdown et al., 2007; Schwartz et al., 2005). The experimental results suggest that therapeutically effective levels can be delivered for prolonged period as a function of time to the deeper tissue and to the tissues surrounding the incision (Fig. 4.3 & 4.4). The concentration of lidocaine as such is much less important as the efficacy, and depth of penetration were not proportionally increased with increased concentration (Maloney et al., 1992). This further supported the idea of iontophoretic delivery of lidocaine to relieve post operative pain for prolonged period.

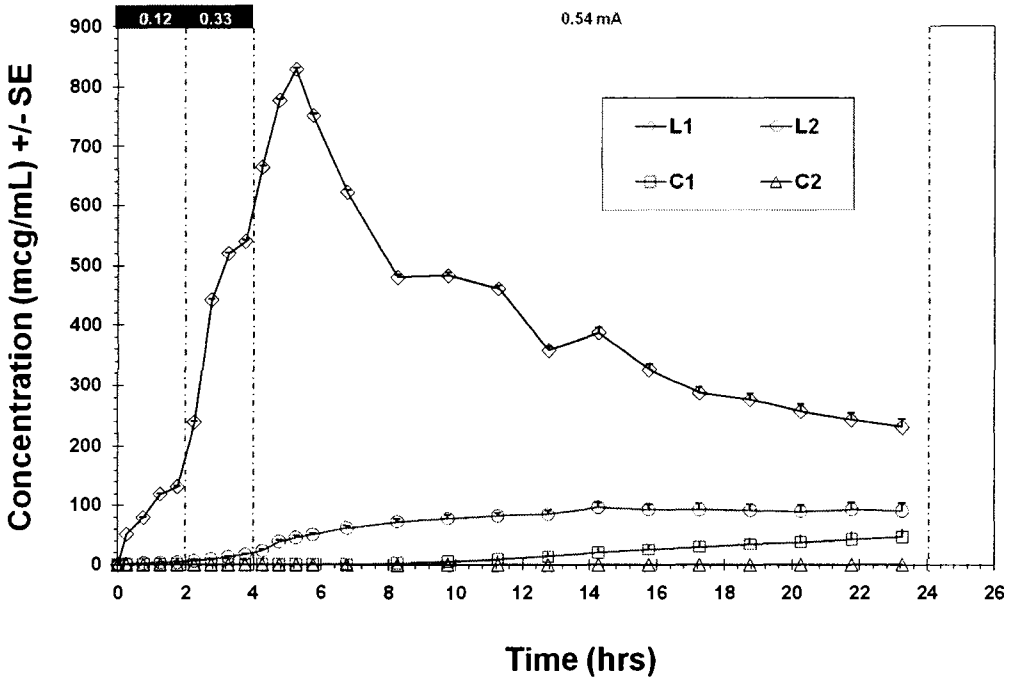

Figure 4.4 Iontophoretic delivery for 24 hours to demonstrate the effect of competing ions, Zn^{2+}, on the delivery efficiency.

In addition, iontophoresis per se had no influence on the wound healing process (Ernst et al., 1995). It has also been reported that lidocaine does not alter wound healing process (Drucker et al., 1998; Ernst et al., 1995).

Conclusion

An *in vitro* wound healing model has been developed for drug delivery into sutured surgical incisions and has been shown to be very promising for monitoring drug levels with respect to applied current. The range of currents necessary to drive a sufficient amount of lidocaine into sutured skin has been successfully investigated. An iontophoretic patch

is likely to be useful for managing pain associated with incision based on drug levels delivered for a prolonged period.

CHAPTER 5

SYSTEMIC DELIVERY OF SUMATRIPTAN BY IONTOPHORESIS

Abstract

Purpose. To determine the feasibility of transdermal delivery of sumatriptan by iontophoresis using hairless rat as animal model in the treatment of migraine.

Methods. Wearable Electronic Drug Delivery (WEDD®) patches were custom designed to fit the animal. Each patch was powered with a 7-volt system, consisting of a 1-volt Zn anode and Ag/AgCl cathode connected in series to two 3-volt lithium button cell batteries. Field effect transistor was placed between battery and cathode. CD hairless rats (250 - 400 g) were anesthetized with an intraperitonial injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Prior to placing the patch on the animal, an anode absorbent pad was loaded with 500 µL drug solution (4% w/v) while a cathode pad was loaded with 500 µL saline. Iontophoresis (0.1 mA/cm^2) was performed for three hours on abdominal area of the rat.

The current was monitored using multimeter. Skin irritation was measured using Transepidermal Water Loss (TEWL) before and after patch application. Blood samples were drawn at periodic intervals over 10 hrs. HPLC with fluorescence detection was employed for separation and detection of plasma samples after extraction.

Results. Plasma extraction and HPLC method was optimized. There was no significant evidence of irritation observed with iontophoretic delivery of sumatriptan in hairless rats. Mean clearance and mean half life were calculated as 701.98 ± 28.74 mL/hr and 1.69 ± 0.27 hr from intravenous administration. The F*Dose of ~5.1 mg was delivered iontophoretically while no apparent passive delivery was detected.

Conclusion. The results of this study suggest that WEDD® can be used to deliver sumatriptan in therapeutically relevant amounts and rates to other routes of delivery with improved therapeutic outcome.

Introduction

Migraine is a chronic condition that affects 12% of the population (Arulmozhi et al., 2005; Arulmozhi et al., 2006), occurring in all races, cultures and geographical locations. It is characterized by unilateral throbbing

head-ache with nausea, vomiting and sensitivity to light, sound and head movements. This episodic disorder may last from 2 to 4 days indirectly affecting the productivity at work. Triptans have been prescribed for treating migraine for many years. Current treatment strategies include oral, nasal, rectal delivery and subcutaneous injections. However, patients complain about limited efficacy, slow onset, and incomplete prevention of recurrence as well as unwillingness to take oral medication during migraine symptoms (Arulmozhi et al., 2005; Arulmozhi et al., 2006; Ferrari and Saxena, 1993). Though the subcutaneous injection of sumatriptan provides quick onset, it is least preferable due to irritation at the site of injection and low self compliance. Sumatriptan is proved to be very effective in the treatment of migraine (Femenia-Font et al., 2005b; Femenia-Font et al., 2005a). Therapeutic effect results from its selective stimulation of 5-HT_{1D} receptors present in the human basilar artery and in the vasculature of human duramater, which mediates the vasoconstriction.

The absolute bioavailability of sumatriptan is reported as 15, 14 and 96% after intranasal, oral and subcutaneous administration due to incomplete absorption or pre-systemic metabolism (Duquesnoy et al., 1998; Femenia-

Font et al., 2005b). Sumatriptan is a good candidate for iontohoresis due to its positive charge at pH 7.5. The cutaneous delivery of sumatriptan (pKa 9.6 & 12) would be an effective alternate approach for systemic delivery to improve the patient compliance. While the skin is typically not permeable to water-soluble ionized compounds, such delivery may be possible if the drug is pushed through the skin by iontophoresis (logP of -1.05 at pH 7.4)(Femenia-Font et al., 2006). Iontophoresis is a process which uses a small amount of electric current ($\leq$ 0.50 mA/cm^2) to push drug molecules into the skin. Previous in vitro studies indicate that passive delivery could not deliver enough sumatriptan to treat migraine (Femenia-Font et al., 2005b; Femenia-Font et al., 2005a). Increase in current density from 0.25 mA to 0.5 mA increased the cumulative amount delivered by anodal iontophoresis from 270 to 700 $\mu g cm^{-2}$. Femenia-Font et al. also reported enhanced cumulative amount delivered by lowering the NaCl (25 mM) in donor formulation (Femenia-Font et al., 2005a). In another study, Patel et al. have shown that the pH of the donor formulation has significant effect on cumulative amount delivered across porcine skin (Patel et al., 2006).

The objective of this study was to determine the feasibility of sumatriptan delivery using WEDD® technology (iontophoresis technology of Travanti Pharma) *in vivo* using hairless rat as an animal model.

Materials

Animals

Male CD-hairless rats (Charles River, Wilmington, MA) weighing 290-350 g were used. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). Food and water were provided *ad libitum*. The average number of replicates for each study was four.

Chemicals

Sumatriptan succinate was obtained from Travanti Pharma, MN. Terazosin and ethyl acetate (HPLC grade) were from Sigma (St. Louis, MO, USA). Male CD hairless rats were purchased from Charles River (CA), and were housed in the in-house animal facility at Mercer University until use. WEDD patches were obtained from Travanti Pharma (MN, USA). Centrifuge tubes, Centrifuge filters (Spin-X®, Corning, NY, USA), Acetonitrile (HPLC grade), Sodium hydroxide and monobasic sodium phosphate from Fisher Scientific (Pittsburgh, PA, USA). Gemini 5μ C_{18} column along with

Security guard cartridge system was purchased from Phenomenex (CA, USA).

Methods

In vivo Transdermal Drug Delivery of Sumatriptan

Transdermal studies were done using hairless rats as animal model as follows:

Iontophoretic patch - principle of operation

The WEDD® patches were custom designed to fit the animal. Each patch was powered by a 7-volt system, consisting of a 1-volt Zn anode and Ag/AgCl cathode connected in series to two 3-volt lithium button cell batteries. Field effect transistor (FET) and resistor were connected to cathode to regulate the flow of electric current inside the patch. Prior to placing the patch on the animal, an anode absorbent pad was loaded with 500 µL of 4% w/v of sumatriptan, and a cathode pad was loaded with 500 µL normal saline (Figure 1). Current flowing across electrodes was monitored during the delivery period to ensure proper electrical connections.

The electrochemistry involved at the electrode interfaces is shown below:

$$Zn \rightarrow Zn^{++} + 2e^{-}(\text{Anode})$$
$$AgCl + e^{-} \rightarrow Ag + Cl^{-}(\text{Cathode})$$

Male CD hairless rats (250-400 g) were anesthetized with an intraperitonial injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). The donor solution of sumatriptan was prepared in deionized water (4% w/v). The anode chamber was filled with 500 µL of donor solution while the cathode was filled with 500 µL of saline during iontophoresis. The current was monitored simultaneously using multimeter (Radio shack).

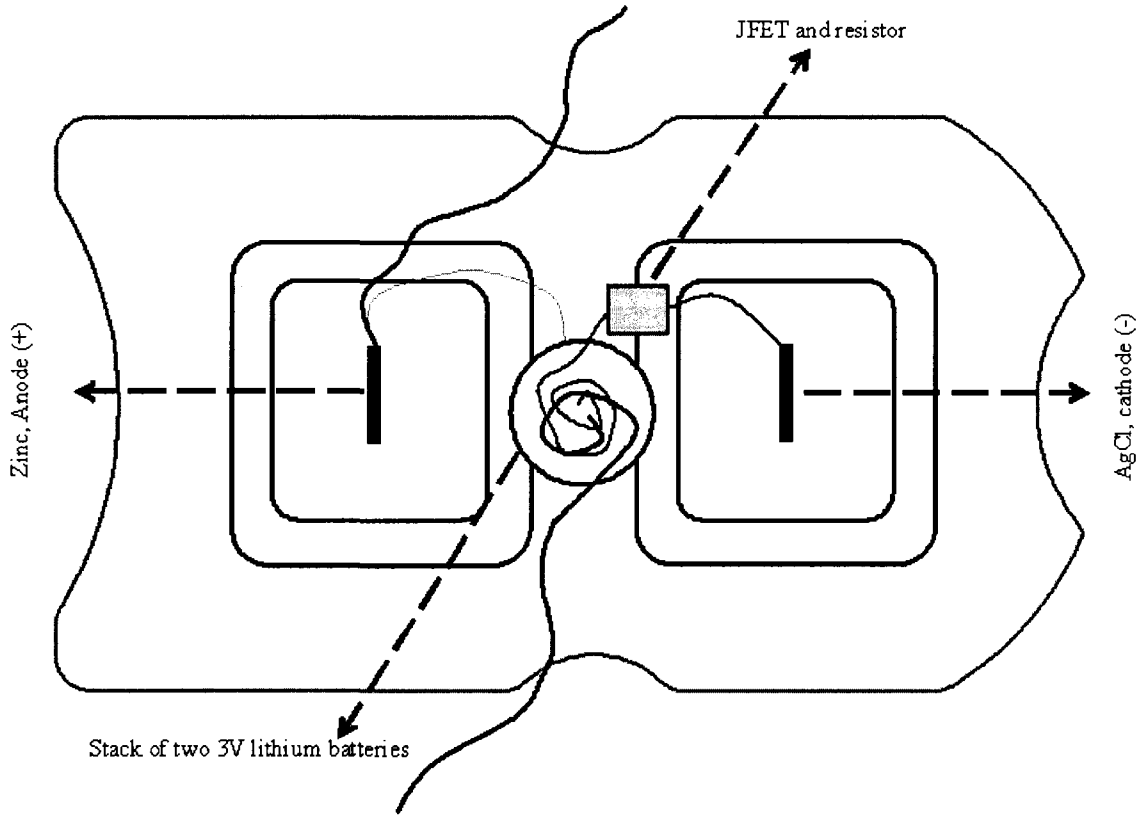

Figure 5.1 Schematic of WEDD employed in iontophoresis experiments. Zinc was used as an anode and AgCl as a cathode connected to the stack of two 3V lithium batteries. Field effector transistor and resistor were connected to the (-)ve pole of the lithium battery and to the cathode.

Skin irritation was measured using Transepidermal Water Loss (TEWL) before and after patch application. The patch was applied for three hours on abdominal area of the rat. Blood samples were drawn at 0, 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8 and 10 hrs for passive and iontophoretic delivery studies. The plasma was isolated by centrifugation at 10000 rpm for ten minutes.

Intravenous administration of sumatriptan

Male CD hairless rats (290-400 g) were used to administer sumatriptan (IV bolus dose: 1 mg/kg) by femoral vein injection using 25G needle (Dixon et al., 1993a). The stock solution of 1 mg/mL was prepared in sterile saline. The solution was administered accordingly to get a final dose of 1 mg/kg. The sample points were well dispersed to get plasma concentration-time profile. The blood was drawn at 0, 2, 5, 10, 15, 20, 30, 45min, 1 hr, 1.5, 2, 3, 4, 6, and 8 hrs after IV bolus administration. The data was analyzed by a noncompartmental analysis using WinNonlin (NCA Model 201, Win-Nonlin® version 5.0.1, Pharsight, CA) to obtain pharmacokinetic parameters, such as clearance and half life. The clearance value was used to calculate the dose delivered via passive or iontophoretic delivery studies by multiplying $AUC_{0-\alpha}$ and Cl_{IV}.

Extraction of plasma samples

Plasma collection tubes (MICROTAINER®), pre-coated with K_2EDTA, were used to collect the blood samples. The plasma was obtained by collecting the supernatant after centrifugation. Plasma samples were stored at -20°C prior to analysis. The supernatant (100 µL) was added with 100 µL of terazosin (20 ng/mL) and 100 µL of saline/standard solution of sumatriptan. This mixture was vortexed with 100 µL of 1 M NaOH. Then, plasma samples were extracted with 7 mL of ethyl acetate. The mixture was shaken on a horizontal shaker for 15 min at 250 rpm and centrifuged at 3000 rpm for 10 minutes at room temperature. The extract was transferred to a glass centrifuge tube and then evaporated to dryness under a nitrogen stream at 40°C. The residue was reconstituted with 300 µL mobile phase by vortex mixing for three times. The reconstituted solution was filtered though the membrane to remove any particulate matter before injecting into the HPLC. The general scheme is shown in Figure 5.2. The amount of sumatriptan succinate was expressed as base form for final quantification. Extraction efficiency was found to be 78% and 90% for sumatriptan and terazosin respectively.

HPLC Analysis

A stock solution of sumatriptan was prepared by mixing appropriate amount in methanol to a final concentration of 1 mg/mL. The solution was stored at -20^{0}C until further use. Terazosin stock solution was prepared in deionized water. The working solutions in saline were prepared freshly at the start of each analysis. Calibration curves prepared at a concentration range 125 - 5000 ng/mL. The chromatographic system consisted of Waters® alliance system with Waters® 2475 fluorescence detector. Empower® 2 software was employed for data acquisition and analysis. The separation was achieved using reverse phase HPLC column, C_{18} Gemini 5 µ (150 x 4.6 mm) attached to a guard column. The mobile phase consisted of potassium phosphate buffer (pH 7.5) and acetonitrile and gradient elution was used. The initial volume ratio (Potassium phosphate, 80: acetonitrile, 20) changed along a linear gradient to 75:25 over 4 min at a flow rate of 1 mL/min and to initial volume ratio following a linear gradient from 9 - 12 min at same flow rate with total run time of 12 min. The detection of sumatriptan was done at 220nm excitation and 350nm emission with simultaneous detection of internal standard, terazosin at

250 nm_{ex}, 370 nm_{em}. Peak area ratio was used for quantification.

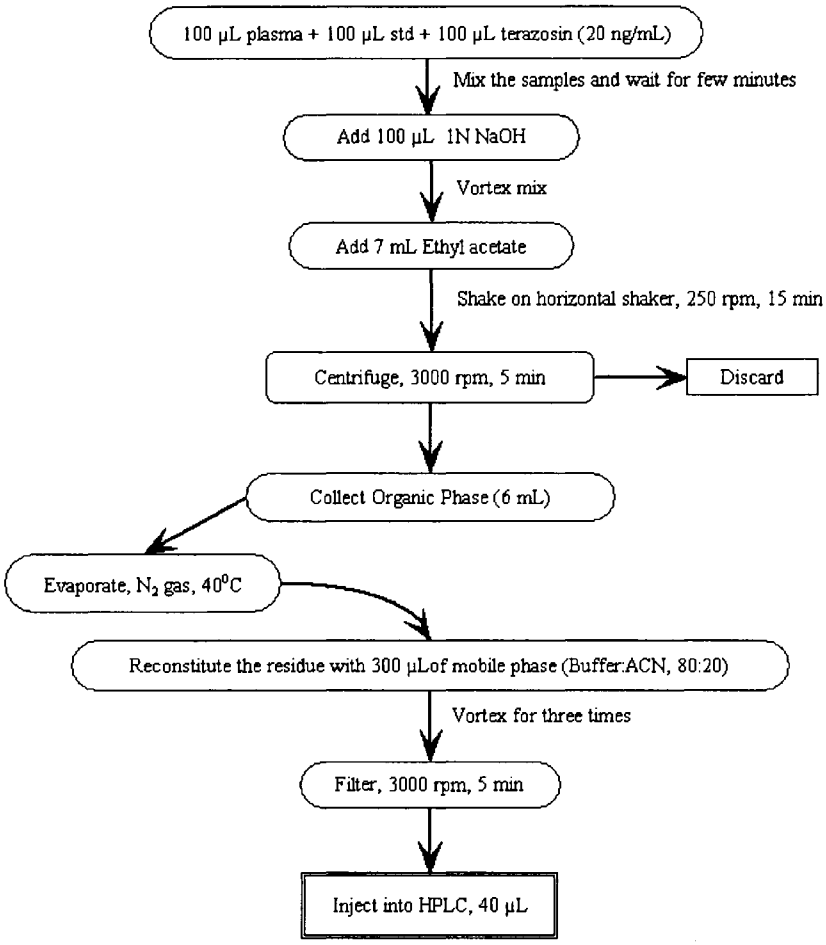

Figure 5.2 Schematic of plasma extractions.

Stability of Sumatriptan during Iontophoresis

Cyclic voltammetry is a unique technique for the electrochemical study of the redox systems (Huang et al., 2004). The stability of sumatriptan in an applied electric field was tested using cyclic voltammetry (Platinum disk working electrode/Ag counter electrode/Ag-AgCl reference

electrode). The voltage at working electrode, immersed in a test solution, is increased linearly and then decreased to its starting point, while current flow is monitored for an evidence of oxidation or reduction reactions. Ascorbic acid is known to undergo oxidative decomposition. It was chosen as a positive control in these experiments.

Pharmacokinetic Data Analysis

Plasma concentration versus time profile was analyzed using non-compartmental analysis (NCA) by WinNonlin® data analysis software. Clearance obtained from IV data was used to calculate the dose delivered during iontophoretic delivery based on the assumption that iontophoretic delivery provides a zero order infusion and follows linear kinetics (Chaturvedula et al., 2005). The following equation was used to calculate the F*dose delivered:

$$F^{*}\text{Dose delivered} = AUC_{0-\alpha} * \text{Clearance}_{IV} \quad (5.1)$$

Where, F represents the fraction of dose absorbed into the systemic circulation while clearance $_{IV}$ represents total body clearance obtained from IV bolus injection. Pharmacokinetic parameters such as maximum observed concentration (C_{max}), the time to reach maximum concentration (T_{max}), half-life ($T_{1/2}$), clearance from the

body (CL), area under the concentration-time profile ($AUC_{0-\alpha}$) were calculated.

Statistical Analysis

The data is shown as mean ± SE. Pharmacokinetic parameters were obtained for individual rat and then averaged. TEWL measurements were analyzed by using student's t-test at $\alpha = 0.05$.

Results and Discussion

There have been several analytical methods proposed to quantify sumatriptan (Dunne and Andrew, 1996; Duquesnoy et al., 1998; Femenia-Font et al., 2005c; Nozal et al., 2002b). Mostly recommended methods include HPLC with UV/Vis detector with different mobile phases (Nozal et al., 2002a). HPLC method with fluorescence detection is preferred to UV detection very often because of sensitivity and lack of interference peaks from plasma samples after extraction. Previously validated method (Ge et al., 2004) has been adopted and modified significantly to meet our need to analyze sumatriptan levels in plasma samples obtained from hairless rat. The assay was found to be reproducible throughout the experimental period.

Sumatriptan is known to cause serious reactions at the site of subcutaneous injection (Welch et al., 2000). It

could probably be due to the drug alone or vehicle in which it was injected. Transepidermal water loss is an indirect method of measuring barrier integrity where water evaporation from the skin is quantified before and after treatment. Thus, skin irritation was evaluated by using visual observation and TEWL measurements at the site of patch application. The site of patch application appeared to be normal. There is no significant evidence of irritation observed with passive and iontophoretic delivery of sumatriptan in hairless rats (t test, $\alpha = 0.05$) (Fig 5.3 & 5.4).

Pharmacokinetic data obtained after IV bolus administration of sumatriptan through femoral vein is found to be in accordance with previously reported data (Ayres et al., 1996; Dixon et al., 1993; Duquesnoy et al., 1998) (Fig. 5.5). The pharmacokinetic parameters are summarized in Table 5-1 & 5-2. The F*Dose was calculated as 5.1 mg in case of WEDD™ delivery for three hours at 0.1 mA/cm^2 current density while no apparent passive delivery was noticed (Fig. 5.6). The amount delivered by iontophoresis is comparable to subcutaneous injection of 6 mg/kg. The apparent half life calculated from iontophoretic data was found to be similar with IV bolus data.

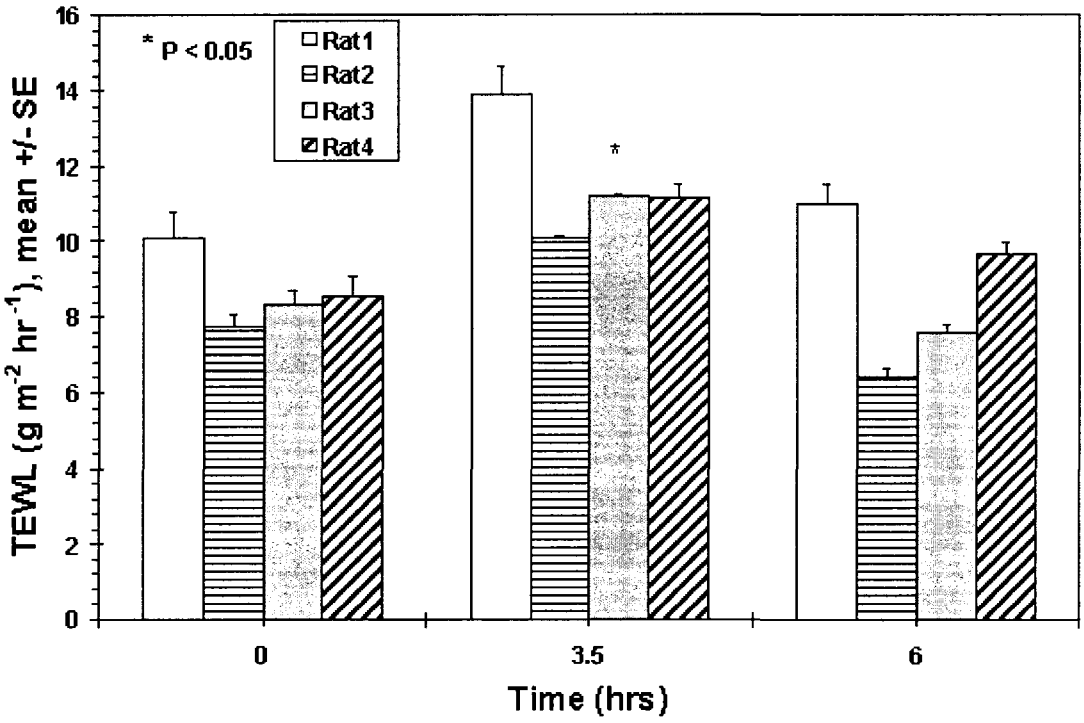

Figure 5.3 TEWL measurement obtained during iontophoretic delivery of Sumatriptan. Patch applied for 3 hrs (n=4). Student t-test was performed (α =0.05)

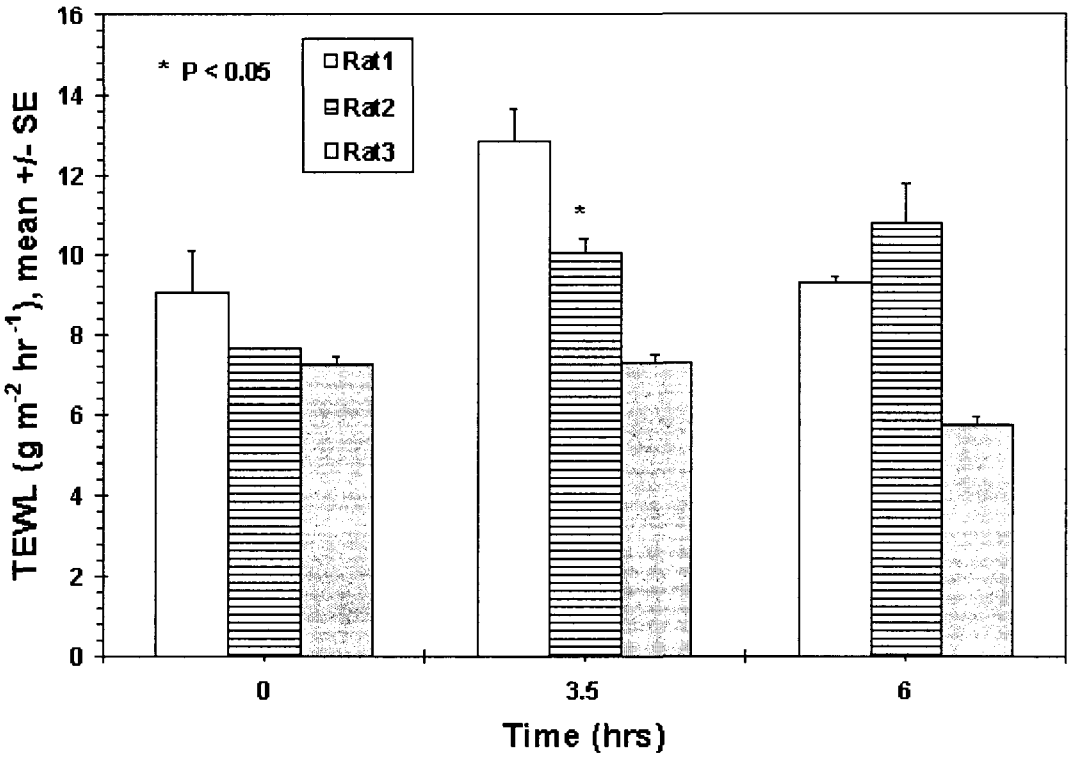

Figure 5.4 TEWL measurements of passive delivery of sumatriptan (n=3). Student t-test was performed (α =0.05)

It could be explained by no depot formation in the skin which would otherwise result in different release profile and more irritation at the site of patch application. Steady state levels of current (0.5 mA) were achieved throughout the patch application period indicating the integrity of the circuit system built into the patch. Field effector transistor (FET) and resistor were connected to negative pole of the lithium battery and to AgCl cathode in order to regulate the current flowing through the patch

(Fig. 5.1). Peak plasma concentration, C_{max} of 1344 (ng/mL), supersede other delivery modes observed in humans with 25 mg tablets (16.5), 20 mg nasal spray (12.9) and 25 mg suppository (22.9) (Patel et al., 2006)(Table 5-2). The stability of the compounds at electrode interface may be questionable due to possible decomposition by oxidation/reduction reactions at the working electrode. The decomposition products may not be resolved in HPLC as separate peaks unless an electrochemical detector is employed. However, cyclic voltammetry can be used to study the electro-active species employed in iontophoretic experiments. This method is further substantiated with the use of positive control which gets oxidized or reduced at certain applied potential. Ascorbic acid is known to degrade at positive applied potential as shown in Figure 5.7.

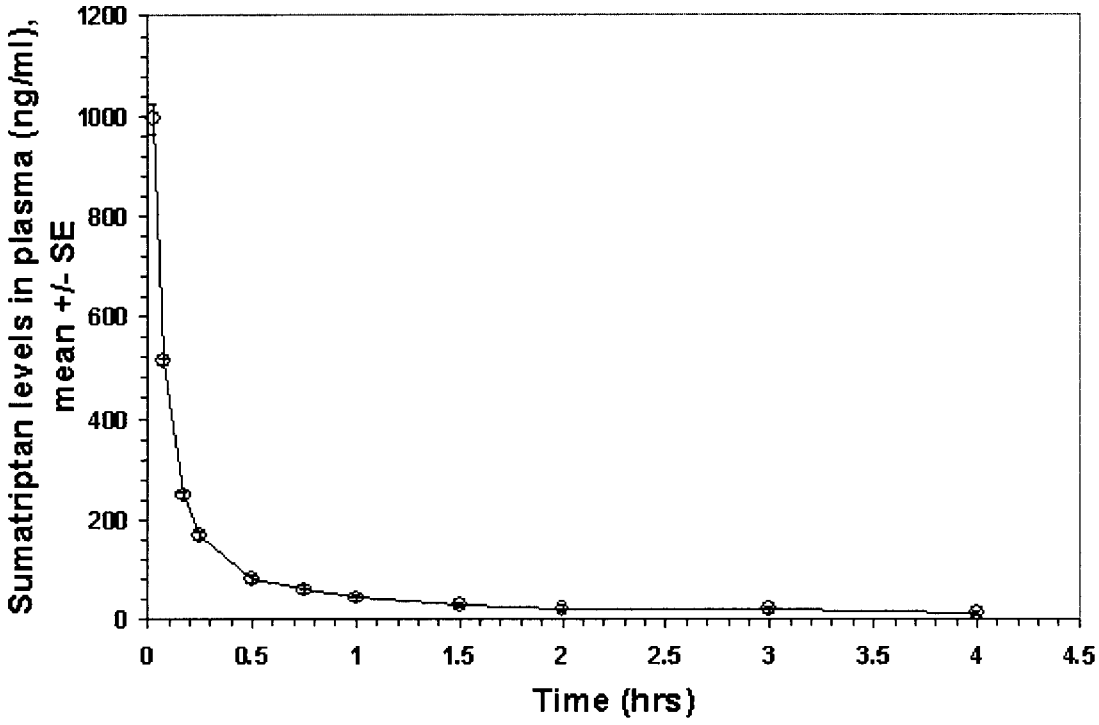

Figure 5.5 Plasma concentrations - time profile of sumatriptan, IV bolus administration (n=4).

Table 5-1 Pharmacokinetic parameters after IV bolus administration

Parameter	Units	Estimate ± SE
Terminal elimination rate constant (λz)	hr^{-1}	0.445 ± 0.10
Half life ($T_{1/2}$)	hr	1.69 ± 0.27
Clearance (CL)	mL/hr	701.98 ± 28.74
Volume of distribution (Vz)	mL	1677.99 ± 211.64
$AUC_{0-\alpha}$	hr*ng/mL	350.22 ± 33.23

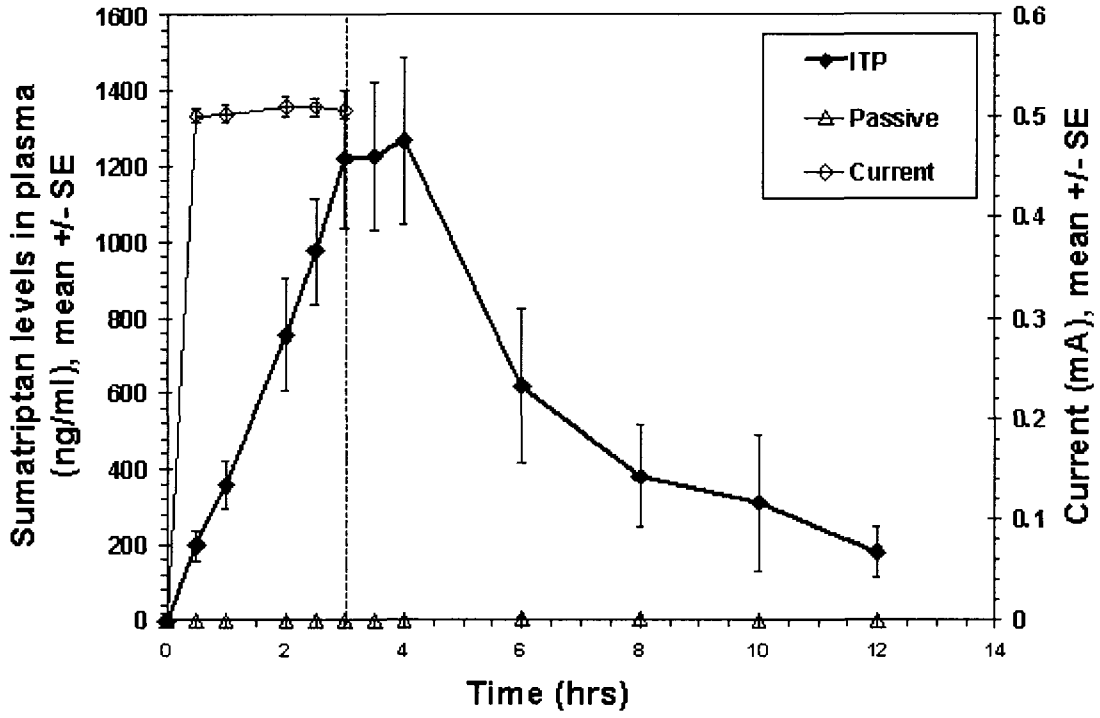

Figure 5.6 Comparison of iontophoretic and passive delivery of sumatriptan in hairless rats (n=4). Constant current was applied for three hours at 0.1 mA/cm^2 current density.

Table 5-2 Pharmacokinetic parameters of sumatriptan after transdermal iontophoretic delivery (n=4)

Parameter	Units	Estimate ± SE	
		Passive	Iontophoresis
$T_{1/2}$	Hrs	-	2.47 ± 0.36
*T_{max}	Hrs	-	3.00
C_{max}	µg/mL	0	1.34 ± 0.18
$AUC_{0-\alpha}$	hr*µg/mL	0	7.25 ± 1.57
F*Dose delivered	mg	0	5.10
Rate of delivery	mg/hr	0	1.70

*Median value was taken

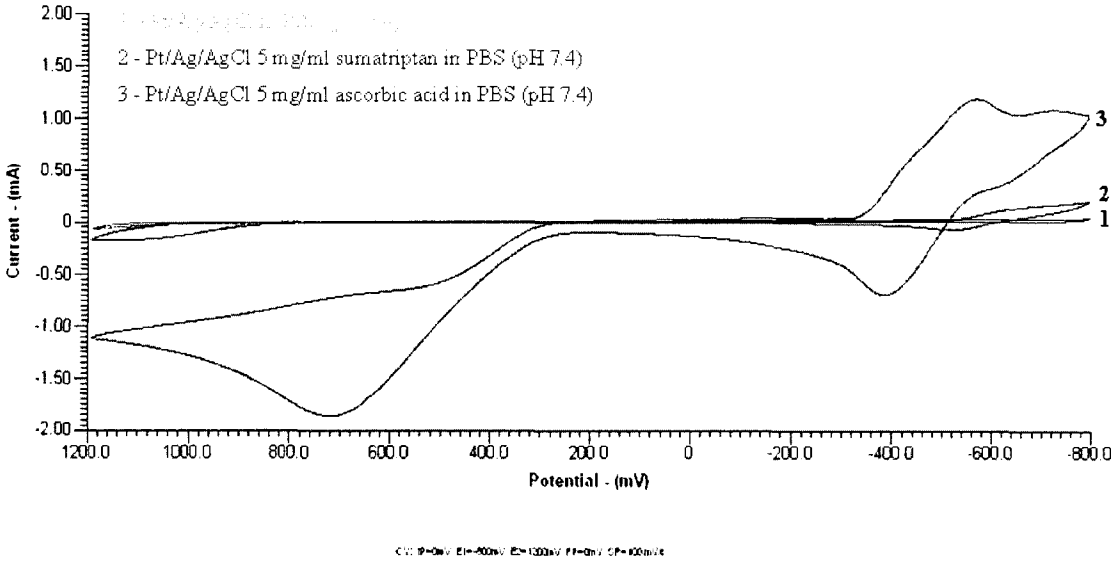

Figure 5.7 Cyclic voltammogram of sumatriptan (5 mg/mL in PBS). Platinum was used as working electrode, Silver as counter electrode and Ag/AgCl as reference electrode.

Conclusion

The results of this study suggest that Wearable Electronic Drug Delivery patch (WEDD™) can be used to deliver sumatriptan in amounts and rates similar to other routes of delivery with improved therapeutic outcome and patient compliance. This patch can be used for treating migraine attack. In addition, it minimizes the possibility of rebound head-ache by maintaining sumatriptan levels in plasma for a prolonged period of time.

CHAPTER 6

SINGLE - DOSE PHARMACOKINETIC STUDY OF TRANSDERMAL IONTOPHORETIC DELIVERY OF TIZANIDINE IN HAIRLESS RATS

Abstract

Purpose. To evaluate the single-dose pharmacokinetics of tizanidine when administered *via* iontophoretic patch to the hairless rat, targeting a total dose of 5, 10, and 15 mg/animal over 24 hours.

Methods. Wearable Electronic Drug Delivery (WEDD®) patches were custom designed to fit the animal. Each patch was powered with a capacity that exceeds charge dosage, consisting of anode pad and cathode pad. Donor electrodes, Zn and Ag, were evaluated for optimal delivery. The anode pad was formulated from 4% w/v tizanidine HCl, 2% w/v HPMC, 0.02% w/v propylparaben, and 0.18% w/v methylparaben; while cathode pad was formulated from 0.9% w/v NaCl, 2% w/v HPMC, 0.02% w/v propylparaben, and 0.18% w/v methylparaben. Male CD hairless rats (250 - 400 g) were anesthetized with an intraperitonial injection of ketamine (75 mg/kg) and xylazine (10 mg/kg).

Iontophoresis was performed for 24 hours with three different currents of 0.15, 0.30, and 0.45 mA for the 5, 10, and 15 mg/day target doses, respectively. The current was monitored using multimeter. Blood samples (~300 µL) were collected from the tail vein at 0, 2, 4, 6, 8, 12, 20, 24, 26, 30 and 48 hour post-patch activation and placed into K_2 EDTA tubes for plasma collection. Plasma samples were analyzed for tizanidine concentration by a LC/MS/MS method. Pharmacokinetic parameters were calculated using WinNonlin (5.0.1).

Results. Transdermal iontophoretic delivery of tizanidine was performed at three current settings to achieve the target doses (5, 10, and 15 mg per day). Iontophoretic delivery significantly increased the amounts delivered into the blood when compared to passive. Apparent half life from passive delivery was calculated to be 5.3 ± 4.1 hours with 77 CV% indicating high variability associated with terminal phase when compared to iontophoretic delivery. The F*dose delivered was calculated to be 0.45, 5.5, 10.9, 12.9 and 1.07 mg for passive, 0.3 mA (Zn), 0.3 mA (Ag), 0.45 mA (Ag), and 0.15 mA (Ag), respectively.

Conclusion. It is concluded that tizanidine HCl was successfully delivered iontophoretically in a hairless rat model. The bioavailability of tizanidine was not linear with the applied current. The Ag electrode was found to be more efficient compared to Zn as anode. The apparent half life was found to be similar amongst all groups tested ($p < 0.05$).

Introduction

Tizanidine (Tizanidine HCl)is a centrally acting α2-adrenoceptor agonist which diminishes spasticity by depressing polysynaptic reflexes and by increasing the presynaptic inhibition of motor neurons (Landau, 1995; Malanga et al., 2002; Wagstaff and Bryson, 1997). It has anti-nociceptive properties and has been used for the treatment of chronic headache (Arulmozhi et al., 2006; Granfors et al., 2004). The oral bioavailability is approximately 21% because of its extensive first-pass metabolism and it is metabolized mainly by CYP1A2 (Granfors et al., 2004; Wagstaff and Bryson, 1997). It follows linear pharmacokinetics in humans in the 0-20 mg dose range with elimination half life of ~2-4 hours (Shellenberger et al., 1999; Tse et al., 1987). Potential drug-drug interactions have been postulated due the involvement of CYP1A2

(Granfors et al., 2004). However, Shellenberger et al. have reported that there was no clinically relevant drug-drug interactions between tizanidine and baclofen (Shellenberger et al., 1999). Henney et al. have reported that food will significantly affect its pharmacokinetics (Henney and Shah, 2007). Tizanidine dose titration in the beginning of the treatment has been suggested due to high inter-patient variability and narrow therapeutic index (Henney and Runyan, 2008).

Iontophoresis is a delivery methodology which utilizes a low level DC current to propel charged ions non-invasively across the skin. Tizanidine, in its HCl salt form, is positively charged (Solubility of 29 mg/mL; pKa = 9) and therefore a candidate for iontophoretic delivery. It is believed that a constant transdermal delivery of tizanidine may avoid limitations associated with oral delivery. Thus, the objective of the present investigation was to study the single-dose pharmacokinetics of tizanidine when administered *via* iontophoretic patch to the hairless rat, targeting a total dose of 5, 10, and 15 mg/day.

Materials

Animals

Male CD-hairless rats (Charles River, Wilmington, MA) weighing 290-350 g were used. The research adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). Food and water were provided *ad libitum.*

Chemicals

WEDD® patches and formulations were obtained from Travanti Pharma (MN, USA). Anode pad was formulated from 4% w/v tizanidine HCl, 2% w/v HPMC, 0.02 % w/v propylparaben, and 0.18% w/v methylparaben while cathode pad was formulated from 0.9% w/v NaCl, 2% w/v HPMC, 0.02% w/v propylparaben, and 0.18% w/v methylparaben (cathode pad).

Miscellaneous

Dry absorbent pads (5 cm^2), 3 mL plastic syringes with a 1.5", 15 gauge flexible dispense tips. 8" stainless steel spatulas, analytical balance, petri dishes, plastic baggies, and tweezers

Methods

Gel Dispensing Procedure

The gel dispensing was done one day before the experiment so that the absorbent pads were properly imbibed

to ensure the uniformity of gel formulation. Following protocol was followed for gel dispensing:

1. Pulled up ~ 2 mL of gel using a 3 mL plastic syringe with a 1.5", 15 gauge flexible dispense tip.
2. Balance was tared (0.00 g) after placing dry absorbent pad into the petri dish (shiny side down).
3. The gel was dispensed (1.1 +/- 0.1 g gel) onto pad using 3 mL plastic syringe in spiral fashion.
4. Once gel has been uniformly spread onto pad, the gel was reweighed to ensure final weight of 1.1 +/- 0.1 g.
5. The pads were kept overnight to fully imbibe the gel prior to the iontophoresis experiment.

In vivo Iontophoretic Delivery

The *in vivo* experiments were designed to evaluate the feasibility of using the selected electric currents to achieve target dose level based on previous *in vitro* studies. The choice of Zinc or Ag as anode was also simultaneously evaluated for better efficiency (Table 6-1). The dosage range selected is approximately the range used for human dosing (Heazlewood et al., 1983; Wagstaff and Bryson, 1997).

Iontophoretic patch - principle of operation

The WEDD® patches were custom designed to fit the animal. Each patch was powered by a 7-volt system, consisting of a 1-volt Zn anode and Ag/AgCl cathode connected in series to two 3-volt lithium button cell batteries.

Table 6-1 Experimental design of single-dose pharmacokinetic study

Group No.	Treatment (current level)	Target Dose Level (mg/day)
1	IV	-
2	Passive	1
3	0.30 mA (Zn)	10
4	0.30 mA (Ag)	10
5	0.45 mA (tbd)	15
6	0.15 mA (tbd)	5

(tbd): To be decided from group 3 and 4

Field effect transistor (FET) and resistor were connected to cathode to regulate the flow of electric current inside the patch. The electrochemistry involved at the electrode interfaces is shown below:

$$Zn \rightarrow Zn^{++} + 2e^{-}(\text{Anode})$$
$$AgCl + e^{-} \rightarrow Ag + Cl^{-}(\text{Cathode})$$

Male CD hairless rats (250-400 g) were anesthetized with an intraperitonial injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Absorbent pads were loaded under respective electrode prior to the experiment. The patch was

applied for 24 hours, ensuring the proper placement of the patch. Electric current flowing across electrodes was monitored during the iontophoretic delivery period to ensure proper electrical connections. Iontophoresis was performed for 24 hours with three different current settings, 0.15, 0.30, and 0.45 mA, for the 5, 10, and 15 mg/day target doses respectively. Blood samples (~300 µL) were collected from the tail vein at 0, 2, 4, 6, 8, 12, 20, 24, 26, 30 and 48 hour post-patch activation and placed into K_2 EDTA tubes for plasma collection.

Intravenous administration of tizanidine

Male CD hairless rats were administered with tizanidine (IV bolus dose: 1 mg/kg) by femoral vein injection using 25 gauge needle. The stock solution of 1 mg/mL was prepared in sterile saline. The solution was administered accordingly to get a final dose of 1 mg/kg. The sample points were well dispersed to get plasma concentration-time profile. The blood was drawn at 0, 5 min, 30 min, 1 hr, 2, 4, 6, 8, and 24 hours after IV bolus administration. The data was analyzed by non-compartmental analysis using WinNonlin (NCA Model 201, Win-Nonlin® version 5.0.1, Pharsight, CA) to obtain pharmacokinetic parameters, such as clearance and half life. The clearance

value was used to calculate the dose delivered *via* passive or iontophoretic delivery studies by multiplying $AUC_{0-\alpha}$ and Cl_{IV}.

HPLC Analysis

Immediately after blood collection, the tube was gently inverted several times to mix the anticoagulant (K_2EDTA) with the blood sample. Samples were placed in wet ice until centrifugation. The plasma fraction was separated by centrifugation in refrigerated centrifuge (4°C to 8°C). All samples were clearly labeled in a fashion that identifies the animal ID#, dose group, time point, and the collection date and placed into a freezer at approximately -70°C within approximately 2 hours after collection. Plasma samples were analyzed for tizanidine concentration using a validated HPLC/MS/MS method (Keystone Laboratories, Inc., Northwales, PA).

Pharmacokinetic Data Analysis

Plasma concentration versus time profile was analyzed using non-compartmental analysis (NCA) by WinNonlin® data analysis software. Clearance obtained from IV data was used to calculate the dose delivered during iontophoretic delivery based on linear and similar pharmacokinetics from both routes (Chaturvedula et al., 2005; Siegel et al.,

2007; Stagni and Shukla, 2003). The following equation was used to calculate the F*dose delivered:

$$F^*\text{Dose delivered} = AUC_{0-\alpha} * \text{Clearance}_{IV} \quad (6.1)$$

Where, F represents the fraction of dose absorbed into the systemic circulation while clearance $_{IV}$ represents total body clearance obtained from IV bolus injection. Plasma concentration - time profiles of passive, iontophoretic and IV delivery were plotted. PK parameters such as AUC_{last}, AUC_{inf}, T_{max}, C_{max}, $t_{1/2}$, absolute bioavailability, and delivery rate (mg/hr) from 0-6, 0-12 and 0-24 hr) were calculated using WinNonlin (5.0.1) software.

Statistical Analysis

The average number of replicates for each study was four. The data is shown as mean ± SE. Pharmacokinetic parameters were obtained for individual rat and then averaged. The unpaired t-test was performed with Welch's correction at 95% confidence interval (α =0.05).

Results and Discussion

Transdermal iontophoretic delivery of tizanidine was performed at three current settings to achieve the target doses (5, 10, and 15 mg per day). Iontophoretic delivery has significantly increased the amounts delivered into the

blood when compared to passive (Figs. 6.1 – 6.5). Apparent half life from passive delivery was calculated to be 5.3 ± 4.1 hours with 77 CV% indicating high variability associated with terminal phase when compared to iontophoretic delivery. The elimination half life was similar in all groups. Relatively higher intersubject variability of PK parameters was observed from the passive delivery when compared to iontophoresis (Figs. 6.1 – 6.5; Table 6-1). Tizanidine PK was not affected when administered in the form of tablets and capsule during fasted conditions but not under fed conditions possibly due to drug - food interactions (Henney and Shah, 2007). Plasma concentration – time profiles of transdermal delivery were shown to produce less variability associated with initial absorption phase. AUC and C_{max} increased proportionally with applied current (Table 6-2).

Initially, middle level current was chosen and evaluated for better anode (Zn/Ag). The iontophoretic delivery efficiency was found to be better with Ag as anticipated. It is probably because of the electrochemistry as Ag gets precipitated upon oxidation to AgCl. In contrast, Zn gets oxidized to Zn^{2+} which competes with drug ions. Though the calculated F*Dose delivered was found to

be relatively higher in case of silver, pharmacokinetic parameters were found to be variable with Ag anode. This could be due to precipitation of AgCl at the skin surface by outward flow of chloride ions from the skin. Thus, precipitation might have affected the iontophoretic permeation pathways resulting in relatively variable profile (Fig. 6-2 & 6-3), though the patch was built in with FET transistor which regulates the electric current flowing in the patch.

Table 6-2 Pharmacokinetic parameters of IV bolus administration

Parameter	Units	Estimate ± SE
Elim. rate const. (λz)	1/hr	0.78 ± 0.17
Half life	Hrs	0.93 ± 0.21
Clearance	mL/hr/kg	3935 ± 518
Vol. of dist.(Vz)	mL/kg	5434 ± 1871
$AUC_{0-\alpha}$	hr*ng/mL	226 ± 30

The partial areas such as AUC_{0-6}, AUC_{0-12}, and AUC_{0-24} increased proportionally in each group. The delivery rates calculated from partial areas (dose delivered/24 hours) were found to be approximately constant in contrast to the levels declining much before the patch removal (Figs. 6.1 -

6.4). It is plausible that the variability associated with transdermal iontophoretic delivery must be from elimination phase. There was no indication of any irritation at the site of iontophoresis as the patch was applied for extended period of time.

Table 6-3 Pharmacokinetic parameters from different groups

Group No. (Target Dose)	Rat	Tmax (hr)	Cmax (ng/mL)	AUClast (hr*ng/mL)	AUCinf (hr*ng/mL)	Half_Life (hr)
2 (1 mg/day, passive)	Mean	5.5	30.5	584.1	733.7	5.3
	SD	1.9	25.2	440.3	432.0	4.1
	Median	5.0	23.8	472.8	650.3	4.1
	CV%	34.8	82.6	75.4	58.9	77.1
3 (10 mg/day, Zn anode)	Mean	11.5	548.0	8774.2	8896.0	6.8
	SD	12.4	454.4	7246.1	7380.9	1.8
	Median	6.0	347.2	5574.8	5648.0	6.9
	CV%	107.6	82.9	82.6	83.0	26.5
4 (10 mg/day, Ag anode)	Mean	16.0	995.4	17520.1	17539.7	3.3
	SD	7.3	475.3	5133.1	5130.1	0.7
	Median	16.0	919.1	17090.5	17099.5	3.2
	CV%	45.6	47.7	29.3	29.2	20.2
5 (15 mg/day, Ag anode)	Mean	11.0	1277.3	24622.7	20736.0	3.7
	SD	6.6	564.0	13247.9	13087.5	0.8
	Median	9.0	1183.2	25058.3	14431.6	3.3
	CV%	60.3	44.2	53.8	63.1	21.2
6 (5 mg/day, Ag anode)	Mean	5.5	87.9	1707.9	1726.3	5.0
	SD	1.0	10.6	149.5	155.1	2.3
	Median	6.0	88.5	1701.0	1723.1	5.7
	CV%	18.2	12.0	8.8	9.0	45.4

Table 6-4 Calculated dose delivered from different groups

Group No.	Treatment (current level)	Target Dose Level (mg/day)	F*Dose delivered (mg/day)
1	IV	1	-
2	passive	1	0.45
3	0.30 mA (Zn)	10	5.5
4	0.30 mA (Ag)	10	10.9
5	0.45 mA (Ag)	15	12.9
6	0.15 mA (Ag)	5	1.07

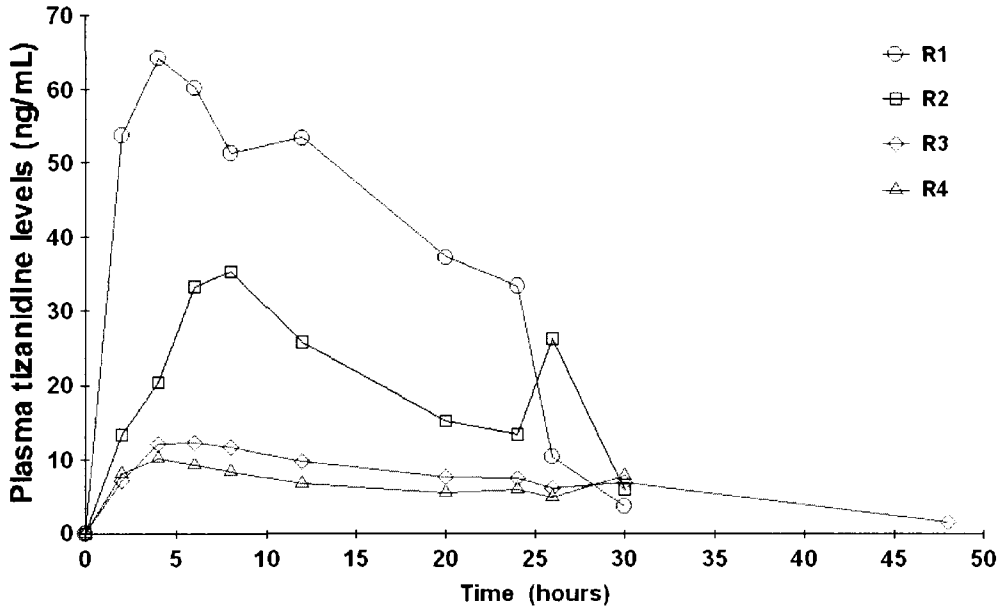

Figure 6.1 Plasma concentrations - time profile of passive delivery of tizanidine (passive). The patch was applied for 24 hours (n=4). Data is displayed on linear scale.

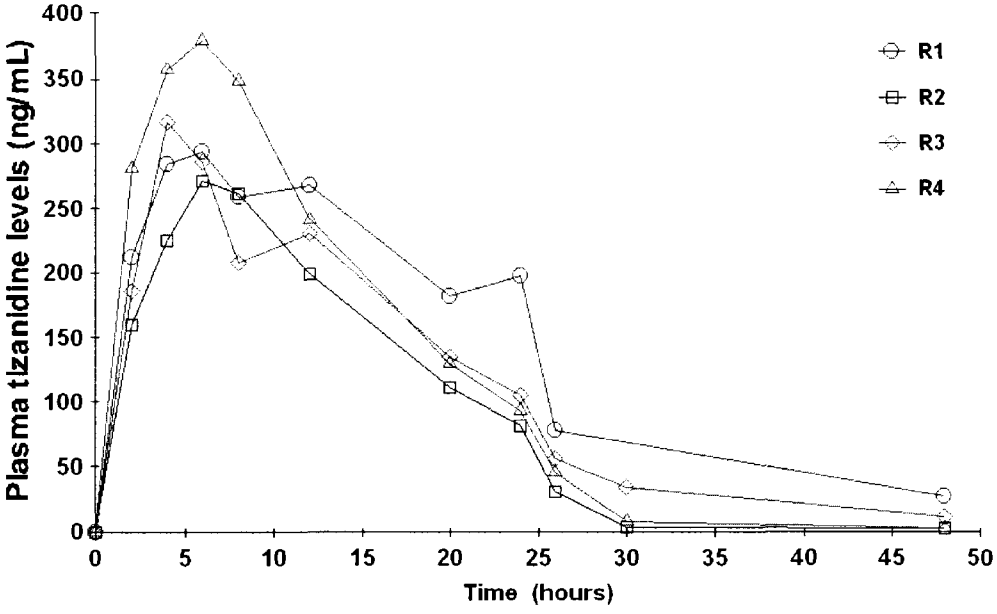

Figure 6.2 Plasma concentrations - time profile of iontophoretic delivery of tizanidine (0.3 mA, Zn anode). The patch was applied for 24 hours (n=4). Data is displayed on linear scale.

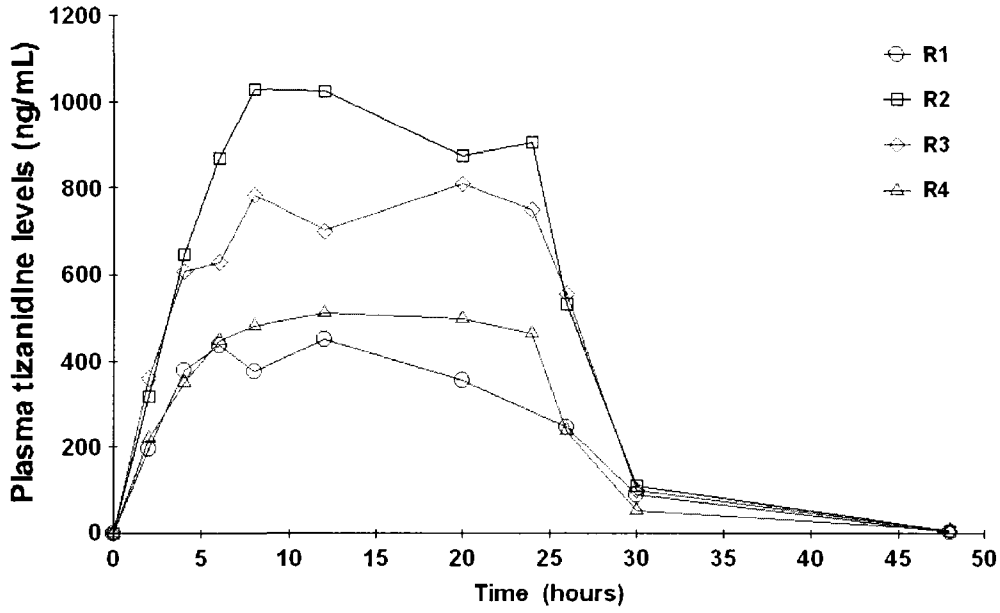

Figure 6.3 Plasma concentrations - time profile of iontophoretic delivery of tizanidine (0.3 mA, Ag anode). The patch was applied for 24 hours (n=4). Data is displayed on linear scale.

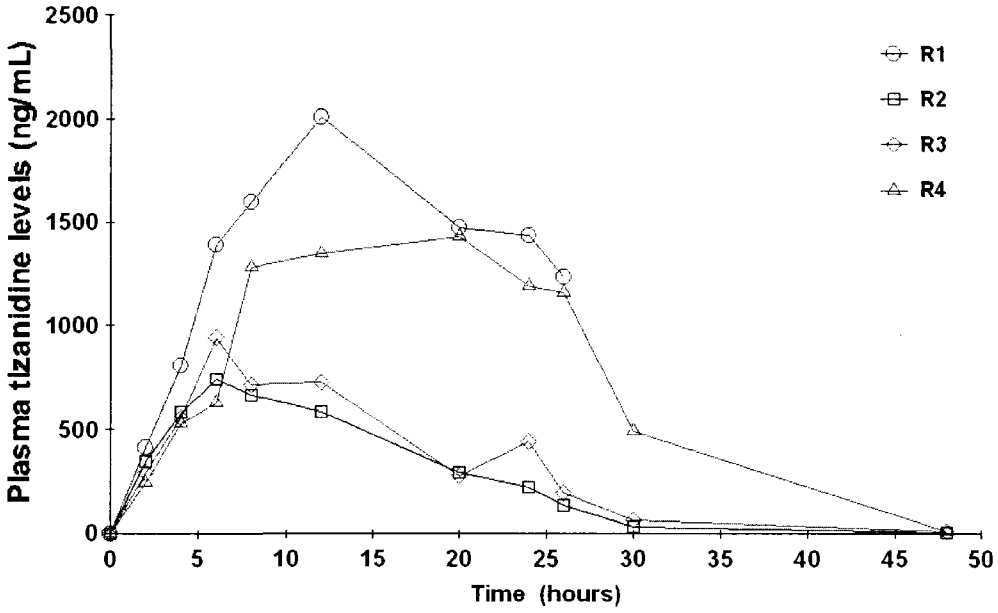

Figure 6.4 Plasma concentrations - time profile of iontophoretic delivery of tizanidine (0.45 mA, Ag anode). The patch was applied for 24 hours (n=4). Data is displayed on linear scale.

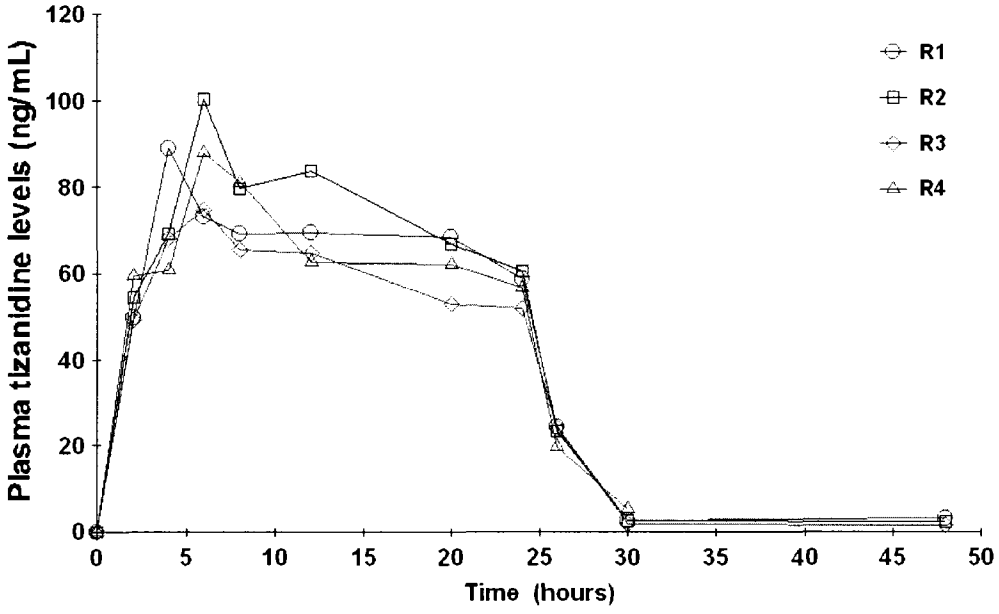

Figure 6.5 Plasma concentrations - time profile of iontophoretic delivery of tizanidine (0.15 mA, Ag anode). The patch was applied for 24 hours (n=4). Data is displayed on linear scale.

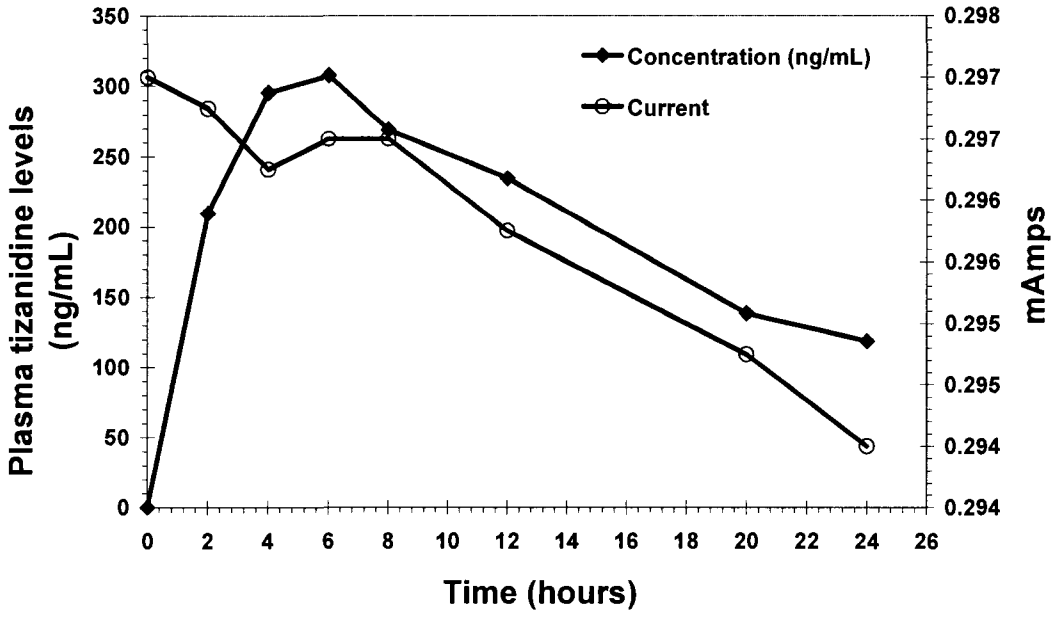

Figure 6.6 Relationship between tizanidine levels and electric current flowing across electrodes -Group 3 (0.3 mA, Zn anode).

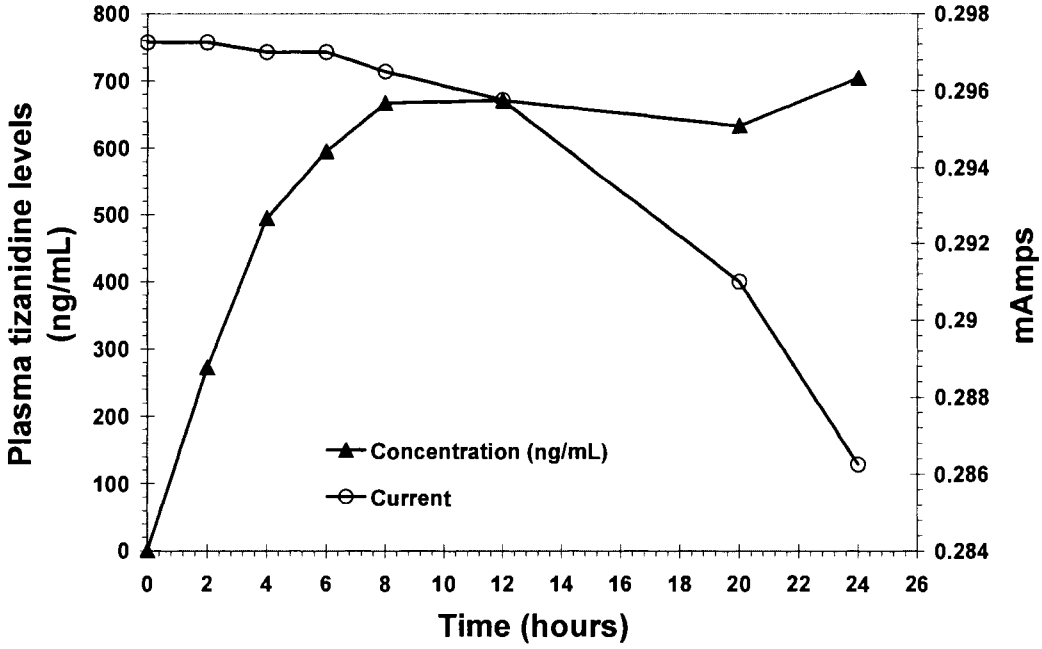

Figure 6.7 Relationship between tizanidine levels and electric current flowing across electrodes -Group 4 (0.3 mA, Ag anode).

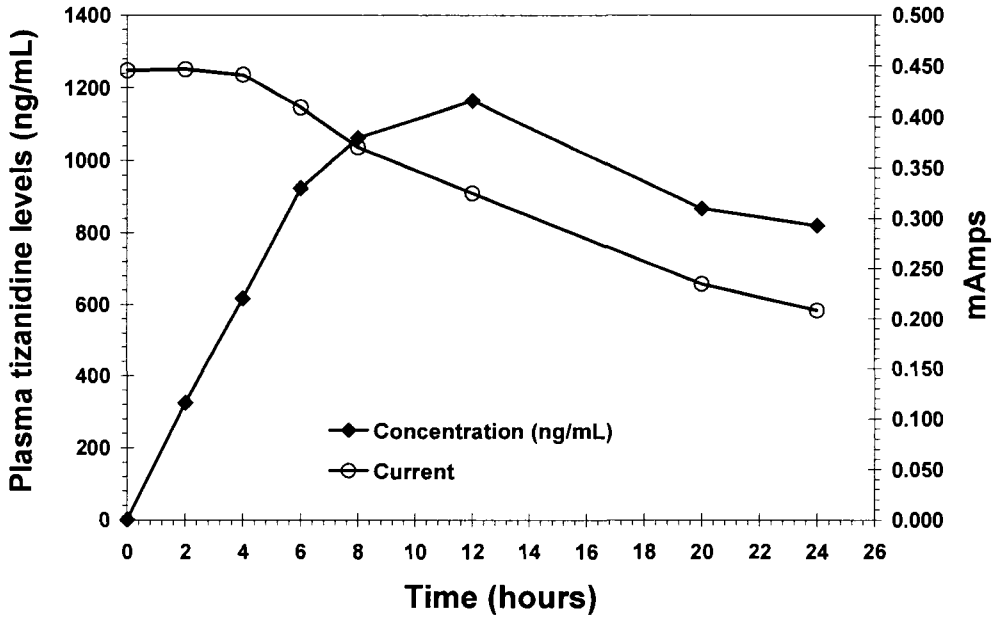

Figure 6.8 Relationship between tizanidine levels and electric current flowing across electrodes -Group 5 (0.45 mA, Ag anode).

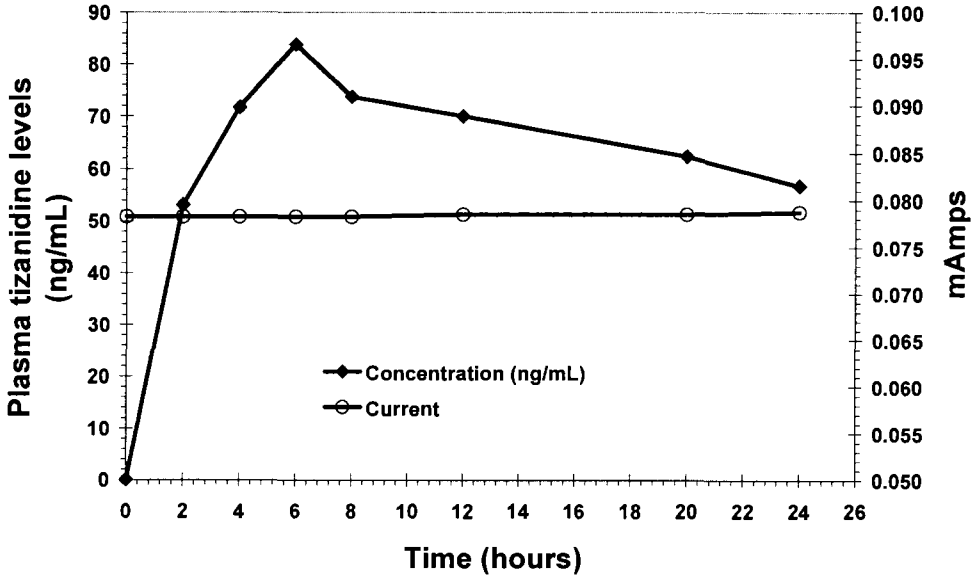

Figure 6.9 Relationship between tizanidine levels and electric current flowing across electrodes -Group 6 (0.15 mA, Ag anode).

Conclusion

It is concluded that tizanidine HCl was successfully delivered iontophoretically in a hairless rat model. The bioavailability of tizanidine was not linear with the applied current. The Ag electrode was found to be more efficient compared to Zn as anode. The apparent half life was found to be similar amongst all groups tested ($p < 0.05$). Thus, the WEDD patches can be used to deliver tizanidine in clinical setting with better patient compliance.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Competitive landscape among the pharmaceutical companies exists with patent expiration of block buster drugs to sustain competition from generic makers. Large companies have started acquiring small scale specialty pharmaceutical companies to develop novel drug delivery products and extend their pipeline as part of lifecycle management, for example, Pfizer has recently acquired PowderMed in 2006; and ALZA was previously acquired by Johnson & Johnson. Complex innovations often lead to high cost, which may not be covered by the insurance. Cost effective delivery technology with patient friendly devices need to be developed, for example, IontoPatch™ and Companion 80™ which cost about eight dollars per patch. Iontophoresis seems to be one of the promising novel drug delivery systems in terms of price and insurance coverage in near future.

The iontophoretic devices approved by FDA (Iontocaine, 1996; LidoSite, 2004), have already been well accepted in clinical setting and IONSYS (2006) will be introduced into the US hospital market. The transdermal iontophoretic delivery of therapeutic agents will continue to be an area of high growth in addressing the unmet need of drug delivery methods, particularly in the areas of chronic CNS disorders (Parkinson's, Depression, and ADHD), pain management, and women's health.

The current research was focused on the evaluation of a unique patch, Wearable Electronic Disposable Drug Delivery (WEDD®), for topical as well as for transdermal delivery. The novelty of iontophoretic patch comes from its ability to integrate electronics along with button cell batteries compared to the conventional table-top iontophoretic controllers. The WEDD® patches were custom designed to fit the animal. Each patch was powered by a 7-volt system, consisting of a 1-volt Zn anode and Ag/AgCl cathode connected in series to 3-volt lithium button cell batteries. Field effect transistor (FET) and resistor were connected to cathode to regulate the flow of electric current inside the patch.

Diclofenac is widely prescribed as an oral medication, but due to its gastrointestinal (GI) side effects such as gastric ulcers and GI bleeding, extensive first pass metabolism (~ 50%) and short biological half life, an alternative delivery approach is desirable. One such approach, iontophoretic delivery, can potentially provide deeper tissue penetration without compromising target tissue concentrations. The objective of this present investigation was to use custom-designed wearable disposable drug delivery (WEDD®) patches to deliver therapeutic levels of diclofenac to the subcutaneous tissue. Microdialysis (MD) was used as a sampling technique to measure the effectiveness of delivery.

Topical medications to relieve postoperative pain and prolong analgesia are used in many clinics. Recent findings reveal that topical application of therapeutic agents may not effectively benefit patients for prolonged period of time, for example, post surgical pain. An *in vitro* wound healing model has been developed for drug delivery into sutured surgical incisions and has been shown to be very promising for monitoring lidocaine levels with respect to applied current. The range of currents necessary to drive a

sufficient amount of lidocaine into sutured skin has been successfully investigated.

Migraine is a chronic condition that affects 12% of the population, occurring in all races, cultures and geographical locations. It is characterized by unilateral throbbing head-ache with nausea, vomiting and sensitivity to light, sound and head movements. This episodic disorder may last from 2 to 4 days indirectly affecting the productivity at work. The absolute bioavailability of sumatriptan is reported as 15, 14 and 96% after intranasal, oral and subcutaneous administration due to incomplete absorption or pre-systemic metabolism. In addition, patients complain about rebound headaches after its administration by conventional routes, probably due to the rapid elimination from the body. WEDD® patches were used to deliver sumatriptan in amounts and rates similar to other routes of delivery with improved therapeutic outcome and patient compliance. This patch can be used for treating migraine attack. In addition, it minimizes the possibility of rebound head-ache by maintaining sumatriptan levels in plasma for a prolonged period of time.

Tizanidine HCl is a centrally acting α2-adrenoceptor agonist which diminishes spasticity by depressing

polysynaptic reflexes and by increasing the presynaptic inhibition of motor neurons. The oral bioavailability is approximately 21% because of its extensive first-pass metabolism. It is known for its drug-drug and food-drug interactions. Thus, iontophoresis offers a better alternative to deliver tizanidine in therapeutic amounts. Tizanidine HCl was successfully delivered in a hairless rat model using WEDD® patches, though the bioavailability of tizanidine was not linear with the applied current. The Ag electrode was found to be more efficient compared to Zn as anode.

It can be concluded from these studies that the WEDD® patches are effective in delivering therapeutic agents for localized delivery or transdermal delivery. The uniqueness of the patch with built-in electronics provides reliability as well as better patient compliance. It can also be used for relieving post surgical pain.

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