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Subcutaneous concentrations following topical iontophoretic delivery of diclofenac

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ABSTRACT: A self-contained Wearable Electronic Disposable Drug Delivery (WEDD®) patch was used to demonstrate that diclofenac levels delivered by iontophoresis are greater than estimated minimal effective concentrations in local subcutaneous tissue and are also greater than either passive transdermal or intravenous delivery using hairless rats. *In vitro* iontophoretic delivery was evaluated to optimize donor cell formulation using Franz diffusion cells and 1000 NMWL Millipore ultrafiltration membrane. *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. Blood and microdialysis samples were collected at different time points after patch application. Current levels increased to 1.0 mA at 30 min, then fell to a steady state of ~ 0.4 mA. Both WEDD® and passive patches produced measurable levels of diclofenac in the subcutaneous tissue below the application site (C_{max} ± SE = 113.3 ± 61.7 ng/mL and 36.3 ± 15.9 ng/mL, respectively). The dose delivered in six hours 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 intravenous administration of 1.5 mg/kg diclofenac solution. The trend indicates that WEDD® can be used to successfully deliver diclofenac to subcutaneous tissue to concentrations higher when compared to either passive delivery or intravenous dosing of 1.5 mg/kg.

Keywords: NSAID, self-contained patches, WEDD®, cutaneous drug delivery, microdialysis, cyclic voltammetry

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1. 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 (*1*). 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 (*2,3*). Diclofenac is widely prescribed as an oral medication, but due to its gastrointestinal side effects such as gastric ulcers and gastrointestinal 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 (*4*). Diclofenac potassium (p*K*a 4.0) has partition coefficient (*5*) 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 (*6*). 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 when compared to passive delivery.

Iontophoresis is a technique where ions are transferred into the body using an applied electric field (*7*). This technique offers an advantage of delivering larger quantities of a given drug when compared to passive delivery (*8*). 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 (*8*). 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. In addition, measurements were indirect, using radiolabeled 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 (*9*) 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 (*10-12*). 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 microdialyisis (*13*). With dermal microdialysis, probe insertion and control over insertion depth are difficult, and the probe itself can increase skin thickness (*14,15*).

The present study had several objectives. In a first *in vitro* portion of the study, formulation of the donor electrode-pad was investigated as a means to improve delivery efficiency with an objective of optimizing the total delivery at lower and more acceptable current level. The *in vitro* portion of the study also served to evaluate the effect of potential formulation additives (such as hydrogels and anti-microbial compounds) on the iontophoretic delivery efficiency. Yet another objective of our *in vitro* studies was to evaluate the electrochemical stability of diclofenac under conditions of applied voltage, using cyclic voltammetry. In a second *in vivo* portion of this study, custom-designed Wearable Electronic Drug Delivery (WEDD®) patches were used to deliver diclofenac. Subcutaneous microdialysis (MD) sampling and blood sampling were performed simultaneously to measure the effectiveness of delivery. The *in vivo* portion of this study also served to evaluate passive delivery and intravenous (IV) injection administered drug, to compare both localized and systemic levels measured to those obtained by iontophoresis.

2. Materials and Methods

2.1. *Animals*

Male CD-hairless rats (Charles River, Wilmington, MA, USA) 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.

2.2. *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% sodium chloride USP was purchased from Baxter Healthcare Corporation (Deerfield, IL, USA). Water, acetonitrile, glacial acetic acid, and sodium acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). All solvents used were of HPLC grade.

2.3. *Cyclic voltammetry system*

A BASi Epsilon (Bioanalytical Systems, Inc., West Layfayette, IN, USA) electrochemical analyzer using a platinum working electrode, silver-silver chloride reference electrode, and titanium-wire counter electrode was used for cyclic voltammetry. Figure legends (Figures 2 and 3) contain experimental sweep conditions.

2.4. *Microdialysis system*

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.

2.5. *In vitro iontophoretic delivery of diclofenac*

Side-by-side cells (n = 4) were used in this study with a 1 mL donor volume, a 3 mL receiver chamber, and a 0.64 cm^2 connection area. The donor cell was filled with a diclofenac solution of varying formulation, with an immersed silver chloride cathode. The receiver cell was filled with 0.9% saline, with an immersed silver anode. As a barrier to Passive flux, a 1000 NMWL Millipore ultrafiltration membrane was used to separate donor and receiver chambers. For power, a voltage/resistance circuit was connected in series to provide approximately 1 mA of current flow. Applied charge dosage was monitored as an integration of current over time. As a control, passive studies were conducted (to measure delivery without any applied current) and tested at the same time-points as corresponding active cells. Samples were extracted from the receiver chamber, then diluted and analyzed using UV spectrophotometer at 277 nm against a standard curve.

2.6. *In vivo iontophoretic delivery of diclofenac potassium*

2.6.1. *Iontophoretic patch – principle of operation*

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. The anode absorbent pad was loaded with 500 µL normal saline solution and the cathode pad was loaded with 500 µL of 20 mg/mL diclofenac potassium. Electric current was monitored during the delivery period to ensure proper electrical connections immediately after patch application. The electrochemistry involved at the electrode interfaces:

$$Zn \rightarrow Zn^{++} + 2e^{-} \text{ (Anode)};$$
$$AgCl + e^{-} \rightarrow Ag + Cl^{-} \text{ (Cathode)} \quad (1)$$

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, 10 × 0.5 mm, 20 kDa molecular weight cut-off) was inserted subcutaneously into the abdominal area of the rat using a guide cannula, and sutured into the skin (*16*). Drug containing reservoir of the patch was aligned exactly above the site where microdialysis probe was inserted. Microdialysis probe was 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 h. For iontophoretic and passive patch testing, serum samples were collected at 0, 1, 2, 4, 6, 8, and 10 h. 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.

2.6.2. *Microdialysis probe recovery*

Probe implantation is followed by 1 h recovery period prior to microdialysis probe calibration. During this time, probes were perfused with 0.9% w/v NaCl solution at 2 µL/min flow rate. Microdialysis probe recovery *in vivo* was determined by perfusing 500 ng/mL of diclofenac solution at 2 µL/min flow rate. The samples (dialysate) were collected simultaneously every 30 min. In parallel, collected dialysate was analyzed immediately for the drug content using HPLC until three steady values were obtained. Then the recovery factor (RF) was calculated by the following formula.

$$RF = (C_p - C_d)/C_p \quad (2)$$

Where, C_p = concentration of perfusate; C_d = concentration of microdialysate.

2.6.3. *Intravenous administration of diclofenac potassium*

Animals were anesthetized as described before. Diclofenac solution was prepared using water for injection, which 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 min). Serum was collected after clotting and stored at −20°C until HPLC analysis.

2.6.4. *Analytical method*

A Waters® Alliance HPLC system with Empower® software and a Waters® 2475 fluorescence detector was used. Several methods have been reported to analyze diclofenac levels in the biological fluids (*17-25*). The operating parameters were adopted from literature and the simplified HPLC assay was validated for intra-day and inter-day variations. All *in vivo* samples were analyzed using C18, Varian microsorb-MV (250 × 4.6 mm, 5 µm) column with fluorescence detection at 282 nm and 365 nm as excitation and emission wavelengths, respectively. The elution was performed using mobile phase, sodium acetate (0.075 M; pH 5) and acetonitrile (55:45). Serum samples were extracted by adding 50 µL of 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 was mixed by pipetting up and down. After cooling for two minutes, 50 µL of 560 ng/mL of internal standard, naproxen, was added and vortexed for few seconds. Supernatant was obtained after centrifugation at 3,500 rpm for three minutes and injected into HPLC for analysis.

2.7. *Cyclic voltammetry*

Cyclic voltammetry is a unique technique for the electrochemical study of the redox systems (*26*). The stability of diclofenac in an applied electric field was tested using cyclic voltammetry (Pt disk electrode/Ag-AgCl reference/Ti 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 the evidence of oxidation or reduction reactions.

2.8. *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 $AUC_{0\text{-inf}}$, terminal elimination rate constant (λz), clearance/F, and C_{max} were calculated. 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:

$$F^*\text{Dose delivered} = AUC_{\text{Iontophoretic}} \times \text{Clearance}_{IV} \quad (3)$$

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

$$R_0 = F^*\text{Dose delivered/Duration of patch application} \quad (4)$$

Where, 'F' represents the fraction of dose absorbed into systemic circulation. F*Dose delivered was calculated as a single function from Equation 3.

2.9. *Statistical analysis*

The data is presented as mean ± SE. Pharmacokinetic parameters were calculated for individual rat, and then mean was calculated. Student's paired *t*-test with two tailed distribution was performed for comparisons at $p = 0.05$ set a priori as significant.

3. Results and Discussion

3.1. *In vitro iontophoretic delivery of diclofenac*

In the first portion of our *in vitro* testing, diclofenac concentration was varied in the donor cell and measured the efficiency at which diclofenac was delivered by iontophoresis. It was hypothesized that as donor concentration is increased, improved delivery efficiency would be seen owing to a more favorable ratio of drug ions relative to competing ions present (Table 1). It was concluded 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 (Table 1). Therefore, by employing the potassium salt form, the total dosage of the Hui *et al*. study (*8*) was possible using much less current. In the second part of the *in vitro* study, the effect of excipients added to a formulation containing 20 mg/mL diclofenac potassium was investigated. The total diclofenac delivered, following a 3 mA•hr (180 mA•min) applied charge dosage, was measured with different donor cell formulations (Table 2). In general, we did not find significant variation in efficiency as a function of the additives tested (Figure 1). We hypothesize that the lower efficiency noted with the PVA formulation may be due to ionic impurities.

The stability of diclofenac in an applied electric field was assessed using cyclic voltammetry (Figures 2 and 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 (Figures 2 and 3).

Table 1. Delivery efficiency of donor cell formulation

Donor cell formulation*	Delivery efficiency ± SD**
4 mg/mL (12.6 mM); sodium	7.1 ± 1.5
8 mg/mL (25.1 mM); sodium	10.3 ± 0.7
18 mg/mL (56.6 mM); sodium	9.2 ± 1.6
20 mg/mL (59.8 mM); potassium	17.6 ± 1.0
30 mg/mL (89.8 mM); potassium	17.5 ± 1.9
40 mg/mL (125.7 mM); sodium	9.5 ± 2.1

* Total diclofenac concentration and salt form. ** µg per mA•min applied charge, to a charge dosage between 150 and 180 mA•min.

3.2. *In vivo iontophoretic delivery of diclofenac potassium*

The linear range of the HPLC assay for serum extractions was obtained between 10-1,000 ng/mL with extraction efficiency of greater than 90%. The mean recovery factor from microdialysis probe calibration was calculated to be 0.75. Recovery factor was later used to calculate actual concentrations surrounding the microdialysis probe.

Table 2. List of donor cell formulations

Formulation	Content
Control	1 mL of 20 mg/mL diclofenac potassium in distilled water
A	+ 2% HPMC
B	+ 10% polyvinylalcohol
C	+ 0.2 mg/mL benzalkonium chloride
D	+ 1% butyl alcohol
E	+ 1.5 mg/mL methylparaben, 0.2 mg/mL butylparaben at pH 7.45
F	+ 1.5 mg/mL methylparaben, 0.2 mg/mL butylparaben at pH 7.68
G	+ 1.5 mg/mL methylparaben, 0.2 mg/mL butylparaben at pH 8.06
H	Control formulation delivered from flex printed electrode

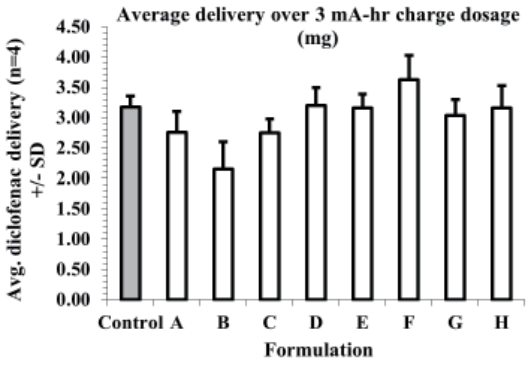

Figure 1. Effect of excipients added to 20 mg/mL diclofenac potassium donor cell formulation (*n* = 4).

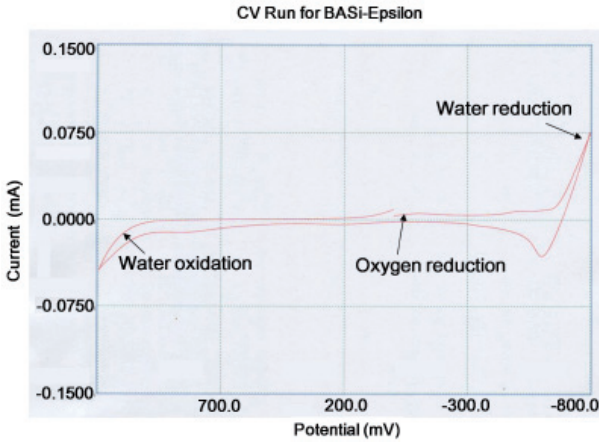

Figure 2. Cyclic voltammogram of 0.1 M TES buffer solution, pH 7.4; number of data points: 4000, current full scale: 1 mA, switching potential 1: −800 mV, switching potential 2: 1,200 mV, initial and final potential: 0 mV, scan rate: 100 mV/sec, filter: 10 Hz, sample interval: 1 mV, number of segments: 3, quiet time: 2 sec.

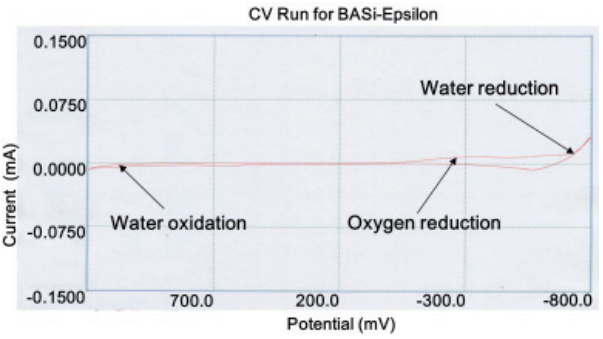

Figure 3. Cyclic voltammogram of 0.1 M TES buffer (pH 7.4) with 5 mg/mL diclofenac potassium; number of data points: 4000, current full scale: 1 mA, switching potential 1: −800 mV, switching potential 2: 1,200 mV, initial and final potential: 0 mV, scan rate: 100 mV/sec, filter: 10 Hz, sample interval: 1 mV, number of segments: 3, quiet time: 2 sec.

The current measurements for the Active WEDD® patches are shown in Figure 4. Current levels peaked at approximately 1.0 mA at 30 min, and then fell to a steady state of approximately 0.4 mA. Both passive and WEDD® patches produced measurable levels of diclofenac in the subcutaneous region below the application site (Figure 5). 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 (Figure 5). However, minimal levels in the subcutaneous tissue may be surprising when compared to similar studies that we have conducted with Granisetron (*16*) and another study with diclofenac (*8*). The anticipated subcutaneous concentrations of diclofenac were about 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 (Figure 5). Diclofenac is known to bind with proteins; as binding is approximately 99.7 % in plasma, and approximately 99.5 % in synovial fluid (*27*). The protein-bound forms of diclofenac will not diffuse

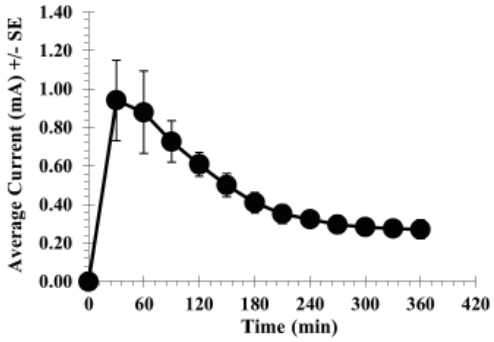

Figure 4. Average iontophoretic current during six hours of WEDD® patch application (*n* = 4).

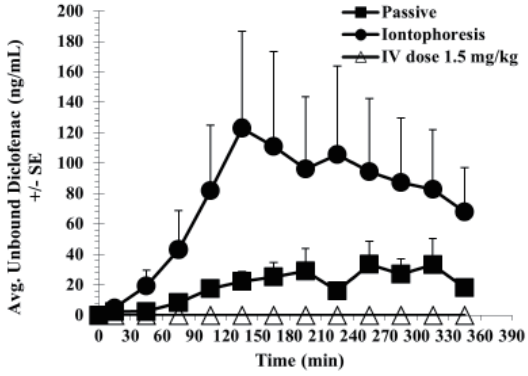

Figure 5. Average microdialysate levels in the subcutaneous tissue (*n* = 4).

through the dialysis membrane of the microdialysis probe. Since significant protein levels are found in the interstitial fluid of rats (*28*) and humans (*29*), 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.

It was noticed that an increase in the number of HPLC peaks over time at elution times slightly different from the diclofenac peak. These peaks were found in active, passive and *in vivo* calibration data sets; and therefore, it is not indicative of diclofenac degradation from an electric field. However, these peaks may be related to diclofenac that can be attributed to percentage of protein bound diclofenac, capable of entering the microdialysis probe. The stability of diclofenac in an applied electric field was further confirmed by investigation using cyclic voltammetry (Figures 2 and 3).

A high degree of variability is also noted; with approximately 100% coefficient of variation seen in both WEDD® 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 (*1*). 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 (*1*).

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

Parameter	Units	Estimate ± SE
Elim. rate const. (λz)	min^{-1}	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

Table 4. Pharmacokinetic parameters of diclofenac

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

The pharmacokinetic parameter, clearance, from IV bolus study was used to calculate F*Dose delivered as a single function from equation 3 (Table 3). Both passive and active WEDD® patches produced measurable levels of diclofenac in the serum (Table 4). In comparing WEDD® active delivery with passive, a trend very similar to our microdialysis results indicates that active delivery shows serum concentrations two-fold higher, and faster than passive delivery (Figure 6 and Table 4). 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.

In a first animal administered with a 1.5 mg/kg IV dosage, a microdialysis probe was inserted into the same subcutaneous space as in the passive and active patch testing. This was performed to measure an amount reached subcutaneously *via* IV compared to the amounts reached *via* transdermal patches. The MD results after IV dosing for the animal were below the detection limits at all time points (Figure 5). 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.

The minimal effective concentration (MEC) of unbound diclofenac was estimated to be in the range of 0.5 to 2.5 ng/mL based on the following assumptions: estimated MEC of total diclofenac in synovial fluid is 100-500 ng/mL (*27*). Protein binding of diclofenac was measured to be 99.5% in synovial fluid (*27*). Therefore, 0.5% unbound fraction of total diclofenac (100-500 ng/mL) is 0.5-2.5 ng/mL. Thus, unbound concentration from subcutaneous microdialysis was multiplied by a factor, 200, to compare with our serum result. This factor is supported as our estimated total subcutaneous

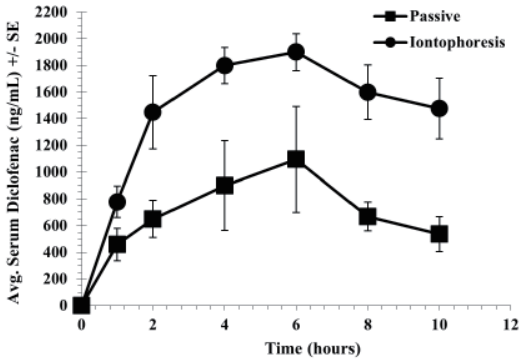

Figure 6. Average serum diclofenac levels after passive and iontophoretic delivery (n = 4).

concentration of 18,840 ng/mL is comparable to a previous study with direct measurement of ~11,000 ng/mL subcutaneous diclofenac after iontophoresis (*8*).

In spite of their potential use as anti-inflammatory agents, NSAIDs exhibit undesirable side effects when administered by oral route. Topical application provides an attractive choice to target deeper tissue beneath the skin for various clinical conditions that calls for NSAIDS. However, conventional formulations are not able to target deeper tissue while minimizing the systemic exposure. Passive delivery of diclofenac to the deeper tissue was shown to be highly variable (*1,8*). There have been contradictory findings reported in the literature related to direct deeper tissue penetration of diclofenac (*8*). In our study, subcutaneous levels by iontophoresis have also shown variability possibly due to the variability associated with microdialysis probe recovery of higher tissue concentrations (iontophoresis) compared to lower tissue concentrations (passive). In contrast, pharmacokinetic parameters of serum samples from passive delivery shown to be highly variable when compared to WEDD® application in hairless rats (Table 4). This can be attributed partly to different diclofenac transport pathways in the skin during iontophoresis and passive delivery.

4. Conclusion

The results of this study suggest that a user-friendly, disposable WEDD® 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.

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Conflict of interest

There is no conflict of interest but Dr. Banga has served as a consultant to Travanti in the past.

References

1. Dehghanyar P, Mayer BX, Namiranian K, Mascher H, Muller M, Brunner M. Topical skin penetration of diclofenac after single- and multiple-dose application. Int J Clin Pharmacol Ther. 2004; 42:353-359.
2. Peters DCFoster RH. Diclofenac/hyaluronic acid. Drugs Aging. 1999; 14:313-319.
3. Merk HF. Topical diclofenac in the treatment of actinic keratoses. Int J Dermatol. 2007; 46:12-18.
4. Boinpally RR, Zhou SL, Poondru S, Devraj G, Jasti BR. Lecithin vesicles for topical delivery of diclofenac. Eur J Pharm Biopharm. 2003; 56:389-392.
5. Raz I, Hussein Z, Samara E, Ben David J. Comparative pharmacokinetic analysis of a novel sustained-release dosage form of diclofenac sodium in healthy subjects. Int J Clin Pharmacol Ther Toxicol. 1988; 26:246-248.
6. Fini A, Fazio G, Gonzalez-Rodriguez M, Cavallari C, Passerini N, Rodriguez L. Formation of ion-pairs in aqueous solutions of diclofenac salts. Int J Pharm. 1999; 187:163-173.
7. Ajay K.Banga. Therapeutic Peptides and Proteins: Formulation, Processing and Delivery Systems. CRC Press/Taylor & Francis, Boca Raton, FL, USA, 2006.
8. Hui X, Anigbogu A, Singh P, Xiong G, Poblete N, Liu P, Maibach HI. Pharmacokinetic and local tissue disposition of [14C]sodium diclofenac following iontophoresis and systemic administration in rabbits. J Pharm Sci. 2001; 90:1269-1276.
9. Stagni GShukla C. Pharmacokinetics of methotrexate in rabbit skin and plasma after iv-bolus and iontophoretic administrations. J Control Release. 2003; 93:283-292.
10. Stagni G, Ali ME, Weng D. Pharmacokinetics of acyclovir in rabbit skin after i.v.-bolus, ointment, and iontophoretic administrations. Int J Pharm. 2004; 274:201-211.
11. Fang JY, Sung KC, Lin HH, Fang CL. Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: *In vitro* and *in vivo* studies. Int J Pharm. 1999; 178:83-92.
12. Fang JY, Hsu LR, Huang YB, Tsai YH. Evaluation of transdermal iontophoresis of enoxacin from polymer formulations: *In vitro* skin permeation and *in vivo* microdialysis using Wistar rat as an animal model. Int J Pharm. 1999; 180:137-149.
13. Graumlich JF, McLaughlin RG, Birkhahn D, Shah N, Burk A, Jobe PC, Dailey JW. Subcutaneous microdialysis in rats correlates with carbamazepine concentrations in plasma and brain. Epilepsy Res. 2000; 40:25-32.
14. Groth L, Jorgensen A, Serup J. Cutaneous microdialysis in the rat: Insertion trauma studied by ultrasound imaging. Acta Derm Venereol. 1998; 78:10-14.
15. Groth LSerup J. Cutaneous microdialysis in man: Effects of needle insertion trauma and anaesthesia on skin perfusion, erythema and skin thickness. Acta Derm Venereol. 1998; 78:5-9.
16. Chaturvedula A, Joshi DP, Anderson C, Morris R, Sembrowich WL, Banga AK. Dermal, subdermal, and systemic concentrations of granisetron by iontophoretic delivery. Pharm Res. 2005; 22:1313-1319.
17. Avgerinos A, Karidas T, Malamataris S. Extractionless high-performance liquid chromatographic method for the determination of diclofenac in human plasma and urine. J Chromatogr. 1993; 619:324-329.
18. Blagbrough IS, Daykin MM, Doherty M, Pattrick M, Shaw PN. High-performance liquid chromatographic determination of naproxen, ibuprofen and diclofenac in plasma and synovial fluid in man. J Chromatogr. 1992; 578:251-257.
19. Godbillon J, Gauron S, Metayer JP. High-performance liquid chromatographic determination of diclofenac and its monohydroxylated metabolites in biological fluids. J Chromatogr. 1985; 338:151-159.
20. Lee HS, Kim EJ, Zee OP, Lee YJ. High performance liquid chromatographic determination of diclofenac sodium in plasma using column-switching technique for sample clean-up. Arch Pharm (Weinheim). 1989; 322:801-806.
21. Millership JS, Hare LG, Farry M, Collier PS, McElnay JC, Shields MD, Carson DJ. The use of hydrophilic lipophilic balanced (HLB) copolymer SPE cartridges for the extraction of diclofenac from small volume paediatric plasma samples. J Pharm Biomed Anal. 2001; 25:871-879.
22. Moncrieff J. Extractionless determination of diclofenac sodium in serum using reversed-phase high-performance liquid chromatography with fluorimetric detection. J Chromatogr. 1992; 577:185-189.
23. Rbeida O, Christiaens B, Hubert P, Lubda D, Boos KS, Crommen J, Chiap P. Evaluation of a novel anion-exchange restricted-access sorbent for on-line sample clean-up prior to the determination of acidic compounds in plasma by liquid chromatography. J Chromatogr A. 2004; 1030:95-102.
24. Riegel MEllis PP. High-performance liquid chromatographic assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids. J Chromatogr B Biomed Appl. 1994; 654:140-145.
25. Roskar RKmetec V. Liquid chromatographic determination of diclofenac in human synovial fluid. J Chromatogr B Analyt Technol Biomed Life Sci. 2003; 788:57-64.
26. Huang T, Gao P, Hageman MJ. Rapid screening of antioxidants in pharmaceutical formulation development using cyclic voltammetry--potential and limitations. Curr Drug Discov Technol. 2004; 1:173-179.
27. Radermacher J, Jentsch D, Scholl MA, Lustinetz T, Frolich JC. Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. Br J Clin Pharmacol. 1991; 31:537-541.
28. Wiig H, Reed RK, Tenstad O. Interstitial fluid pressure, composition of interstitium, and interstitial exclusion of albumin in hypothyroid rats. Am J Physiol Heart Circ Physiol. 2000; 278:H1627-H1639.
29. Fogh-Andersen N, Altura BM, Altura BT, Siggaard-Andersen O. Composition of interstitial fluid. Clin Chem. 1995; 41:1522-1525.

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