Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(7):2701-2713

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Can diclofenac ester prodrug promote direct penetration across the skin?

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ABSTRACT

This research project was to determine the extents of direct penetration across live rat skin from topical application of diclofenac and its ester prodrug. Diclofenac and its prodrugs were formulated into patches with different pressure sensitive adhesives. In vitro flux studies across the human epidermis and across hairless rat skin were conducted. Direct penetration across live rat skin from topical application of either a diclofenac acid patch or a glycerol diclofenac ester patch were evaluated with a dual agar gel in situ rat model. Diclofenac ester prodrugs showed higher in vitro fluxes from patches with polyisobutylene adhesives, while diclofenac acid showed higher fluxes in patches with polyacrylate adhesives. Direct penetration from diclofenac acid patch accounted for 0.12% of the drug absorbed into the body and 78% of the drug collected in the agar gel, and the numbers from glycerol diclofenac ester patch were 0.083% and 77%, respectively. Topical application of either diclofenac acid patch or its ester prodrug patch demonstrated some direct penetration across the live rat skin, but the extent of direct penetration across the live rat skin from the glycerol diclofenac ester patch was not advantageous over that from the diclofenac acid patch.

Key words: Diclofenac; Prodrug; Transdermal; Skin; Penetration.

INTRODUCTION

Oral nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in relief of pain from musculoskeletal diseases such as osteoarthritis and rheumatoid arthritis. However, oral NSAIDs usually cause significant adverse effects such as gastric intestinal bleeding and ulceration. Topical NSAIDs, by directly applying the medication to the skin of the affected area, usually showed much less systemic drug exposure [1], and thus much less systemic adverse effects. Topical diclofenac products (i.e., Voltaren gel and Pennsaid solution) are commonly prescribed for osteoarthritis. Based on a recent review on the clinical studies of topical diclofenac products for pain relief in knee arthritis, the pain relief effect from topical diclofenac products was only 10% better than that from their placebo in patients over a 12-week period (60% of the patients felt better from the use of topical diclofenac *vs.* 50% from the use of the placebo) [2]. As the systemic drug concentration from topical diclofenac is very low, in order for topical diclofenac to be effective, sufficient direct penetration of diclofenac into the local tissues is necessary for its efficacy.

Mixed experimental results were reported on the drug concentrations in local tissues from topical application of NSAIDs. In one study, diclofenac gel was applied on one knee and there was no difference in diclofenac concentrations in synovial fluid between the drug-applied knee and the contralateral knee [3]. In a different study, compared to oral administration, topical ketoprofen plaster provided lower plasma, synovial tissue, and synovial fluid concentrations, but higher cartilage and meniscus concentrations [4]. In another study, similar plasma concentrations in human subjects were observed from topical diclofenac and oral diclofenac, but topical diclofenac led to significant higher muscle concentration at the application site [5]. Such mixed results indicated big variations in direct penetration from topical NSAIDs.

It is of great interest to patients with arthritis that topical products are capable of providing direct penetration to deeper tissue such as the subcutaneous, muscle or joint tissues underneath the skin at the application site. However, development of such topical products is challenging. One reason is that only limited amount of drug can be delivered into intact skin due to the skin barrier effect. But more importantly, it is because of the skin blood flow acting as a sink that clears away most of the drug entering into the skin before it is available for penetration into deep tissue [6]. It was shown in several studies that topical drug direct penetration into deep tissues could be enhanced by lowering the skin blood flow either with co-delivery of vasoconstrictors [7, 8] or applying a cold water jacket on the local skin [9]. There is not yet a device that can conveniently apply cold temperature to skin for a sustained period of time. The co-delivery of vasoconstrictors for lowering the skin blood flow was only achieved under iontophoresis conditions or passive diffusion on compromised skin such as stratum corneum stripped skin.
The direct penetration of topical drug into deep tissue is also dependent on the physicochemical properties of the drug molecules. Roberts's group investigated the direct penetration from dermal application of a range of drug molecules including NSAIDs [10], lidocaine [11], some bases and steroids [12] with different physicochemical properties. They discovered that the direct penetration of lidocaine, some bases and steroids could reach as deep as 1 cm into tissues, while the direct penetration of some NSAIDs could not exceed beyond 3 mm deep. They suggested that lidocaine and the unionized bases or steroids were more lipophilic at physiological pH and with lower plasma protein binding, and thus, were to a less extent being cleared away by dermal blood circulation. On the other hand, the NSAIDs were ionized at physiological pH and with very high plasma protein binding, and thus were at a higher extent being cleared away by the dermal blood circulation. The less extent of NSAID direct penetration into deep tissues was also reported by other researchers [13, 14].

A majority of NSAIDs have a carboxyl group, are ionized at physiological pH (hydrophilic), and have very high plasma protein binding. With such physicochemical properties, topical NSAIDs would have very limited direction penetration into deep tissues. Such physicochemical properties could be modified with the formation of NSAID prodrugs, *i.e.*, forming of an ester prodrug between diclofenac acid and an alcohol. It is expected that the ester prodrug would be unionized and more hydrophobic at physiological pH, and have lower plasma protein binding. Indeed, it was demonstrated that methyl salicylate, a prodrug, was better than its parent drug salicylic acid in direct penetration into deep tissues [15, 16]. Would some diclofenac ester prodrug also show better direct penetration into deep tissue than its parent drug? If it is true, the topical product of such prodrug could improve the treatment efficacy for millions of arthritic patients. However, this important question has not been addressed by other researchers before. We previously synthesized several diclofenac ester prodrugs and discovered that some of the ester prodrugs showed better *in vitro* flux across the skin than that of the parent drug [17]. However, it was unknown whether the diclofenac ester prodrugs would also show better direct penetration into local tissue, which was one of the objectives to be investigated in this study. It is well known that skin blood flow clears most of the drug molecules penetrated into the skin and thus hinders topical drug direct penetration into deeper tissues. A dual agar gels *in situ* rat model has been demonstrated its validity in the evaluation of topical drug direct penetration into local tissues across the skin [18, 19]. Thus this model was adapted in this study to evaluate the direct penetration of diclofenac and its prodrugs. In addition, saturated aqueous solutions of the diclofenac ester prodrugs were used in the evaluation of their flux across the skin in our previous study [17]. Since those ester prodrugs are fairly unstable in aqueous solutions, a non-aqueous formulation system would be more suitable for the delivery of those prodrugs into the body. Pressure sensitive adhesives such as polyacrylate and polyisobutylene are widely used in transdermal patches and such formulation system offers a non-aqueous environment. Thus another purpose of this study was to screen the pressure sensitive adhesives to obtain suitable patch formulations in the delivery of the diclofenac ester prodrugs into the skin.

EXPERIMENTAL SECTION

2.1. Materials

Diclofenac sodium was obtained from Gallipot, Inc. Pressure sensitive adhesives Durotak 387-2287, Durotak 87-901A, and Durotak 87-608A were gifts from Henkel, Inc. Human cadaver skin was purchased from New York Firefighter skin bank. Transdermal backing film (CoTran 9722) and 1022 release liner were gifts from 3M. Female hairless rats were purchased from Charles River laboratory. The diclofenac prodrugs: glycerol diclofenac ester (GD), ethylene glycol diclofenac ester (ED), methanol diclofenac ester (MD) were synthesized as described in our previous paper [17]. Fresh human plasma was purchased from Zen-Bio, Inc.

2.2. Preparations of the diclofenac and its prodrugs patches

Three pressure sensitive adhesives were investigated in the formulation of transdermal patches namely: Durotak 387-2287 (polyacrylate adhesive with hydroxyl group), Durotak 87-901A (polyacrylate adhesive with no functional group), Durotak 87-608A (polyisobutylene adhesive). The drug or prodrug was added to obtain a final concentration of 10% w/w with respect of the drug to the final dry adhesive layer weight. 3% oleic acid was added as a permeation

enhancer and 3% silicon dioxide was added as a suspending agent. The drug adhesive mixture was cast at a thickness of 0.254 mm on a release liner.

2.3. Evaluation of the in vitro flux across human skin

Human Epidermis (HE) was separated from human cadaver skin by a heat separation method. The HE with a size around 2 cm x 2 cm square was mounted on Franz diffusion cell with stratum corneum side facing the donor chamber. A circular patch with a diameter of 1.2 cm was then applied to the HE surface. The Franz cell diffusion surface area was 0.65 cm^2. The receiver chamber was filled with 5 ml of 0.15 M phosphate buffer saline solution at pH 7.4 and maintained under stirring condition at 37 °C. At time 2, 4, 6, 8, 12, 24, 30, 36, and 48 hours, the whole 5 ml was taken and replaced with 5 ml fresh media. The drug or prodrug concentration in the receiver media was analyzed with a HPLC method. Triplicated experiments were conducted for each patch.

2.4. Evaluation of the in vitro flux across hairless rat skin

Female hairless rats were euthanized and then the abdominal skin was separated out. The fat tissue under the skin was scraped off with scissors. A 2 cm x 2 cm squares was mounted on Franz diffusion cells with stratum corneum side facing the donor chamber, a circular patch with a diameter of 1.2 cm was then applied to the skin surface. The Franz cell diffusion surface was 0.65 cm^2. The receiver chamber was filled with 5 ml of phosphate buffer saline solution. At time 2, 4, 6, 8, 12, 24, 30, 36, and 48 hours, the whole 5 ml was taken and replaced with 5 ml fresh media. The drug or prodrug concentration in the receiver media was analyzed with a HPLC method. In addition, the flux of diclofenac from a commercial available product (Voltaren gel) across the hairless rat skin was also determined here. This was to serve as a reference point for the flux results we obtained from our patch formulations. Triplicated experiments were conducted for each condition. The animal protocols used in this study were fully approved by the Institutional Animal Care and Use Committee (IACUC) at the Idaho State University.

2.5. Determination of plasma protein binding and bioconversion rate

The diclofenac prodrugs (GD, ED, MD) or diclofenac acid (DA) was dissolved in DMSO solution at suitable concentrations, then a small amount of the drug solution (20 µl) was spiked into 2 ml of 30 mg/ml human serum albumin in phosphate buffer solution at pH 7.4 in a dialysis tube (MWCO = 5000) for dialysis against the same phosphate buffer for 6 hours at room temperature. The concentration of the drug in the dialysis tube ($C_{total} = C_{free} + C_{bound}$) and drug concentration outside the dialysis tube (C_{free}) was determined by HPLC. The drug plasma protein binding was determined by the ratio of C_{bound}/C_{total}.

The bioconversion rate of the diclofenac ester prodrugs in fresh rat plasma was determined in a previous study [17]. The bioconversion rate of the diclofenac ester prodrugs in human plasma was determined here with the same method. Briefly, diclofenac prodrugs in DMSO solutions with appropriate concentrations was spiked into human plasma and then incubated in 37°C water bath shaker. Aliquots were taken from the plasma periodically into a methanol solution to stop the bioconversion and also to precipitate out the plasma protein. Then the samples were centrifuged and supernatants were analyzed with an HPLC method.

2.6. Determination of the pharmacokinetic parameters from IV injection.

Diclofenac sodium in phosphate buffer saline solution was prepared at 500 µg/ml. 200 µl of the solution was injected into the hairless rat via the saphenous vein. 0.5 ml of blood sample was taken with the tail bleeding method at 10, 30, 60, 120, 180, and 240 min after the injection. The blood sample was centrifuged at 5000 rpm for 15 min to collect the plasma. The drug concentration in the plasma was analyzed with an HPLC method. The plasma concentrations at different time points were simulated with WinNolin 5.02 software, a bolus IV injection with two-compartmental model was used, the volume of distribution of the central compartment (V_d) and the total clearance (CL) from the central compartment were obtained from the software simulation.

2.7. Evaluation of direction penetration with a dual agar gel in situ rat model.

This study followed the method developed by Hasegawa *et al.* [18] with some modification (see Figure 1A). Specifically, hairless rats were anesthetized with isoflurane and maintained a body temperature of 36 to 37 °C. An incision (5 cm wide) was cut (horizontal) along the lower abdominal skin, the abdominal skin was carefully separated from the abdominal muscles to ensure no subcutaneous bleeding, then two pieces of agar gel (1.0% agarose in 0.15M phosphate buffer solution at pH 7.4), each filled in a polyethylene dish of 2.5 cm wide, 3.8 cm long, and 0.5 cm thick, were inserted into the space between the hypodermis and the abdominal muscles with the open surface facing dermis, one in the left side and one in the right side. A patch of the same size of the gel dish was applied exactly on the right abdominal skin where the gel was located, and at 2, 4, 6, 8, 10, 12 hrs time points, the two pieces of gel were taken out from the body, and at 2, 4, 6, 8, 10 hrs time points two fresh pieces of gel were inserted into the specified place, and then the incision was closed with Michel's clamps. The gel underneath the application site was served as a receptor to collect the drug penetrated through the skin from the patch. The other

piece of gel on the contralateral site was served as a control for drug penetration into the gel through systemic blood circulation (assuming systemic circulation contributes to the same amount of drug to both gels). At the same time points, 0.5 ml of blood was taken from the rat tail. The amount of drug in the gel dish and the plasma drug concentration were analyzed by HPLC. Four rats were used for each patch formulation in the *in situ* rat study.

More schematic illustration of the dual agar gel model is shown in Figure 1B with modification from Hasegawa et al [18]. Drug from the patch permeates into the skin with part of the drug goes into systemic circulation and part of the drug penetrates directly to deep tissue (here this amount of the drug is collected by the agar gel at the application site: $X_{gdirect}$). In addition, some drug from systemic circulation is redistributed to deep tissue (here the amount of the drug is collected by the gel dish at the contralateral site: X_{gc}). The gel dish at the application site also has similar contribution from systemic circulation, so the total amount of the drug in the gel dish at the application site can be expressed as: $X_{gtotal} = X_{gdirect} + X_{gc}$. In addition, based on CL and V_d determined from IV injection, the plasma drug concentration (C_b) after patch application and the amounts of drug in each of two gel dishes, the total amount of the drug absorbed into systemic circulation from time 0 to time t can be expressed:

$$\sum X_{b0-t} = CL \cdot AUC_{0-t} + C_b \cdot V_d \quad (1)$$

where AUC_{0-t} is the area under the plasma concentration time curve from time 0 to time t calculated by trapezoidal rule. The total amount of the drug directly penetrated into the gel dish:

$$\sum X_{gdirect} = \sum X_{gtotal} - \sum X_{gc} \quad (2)$$

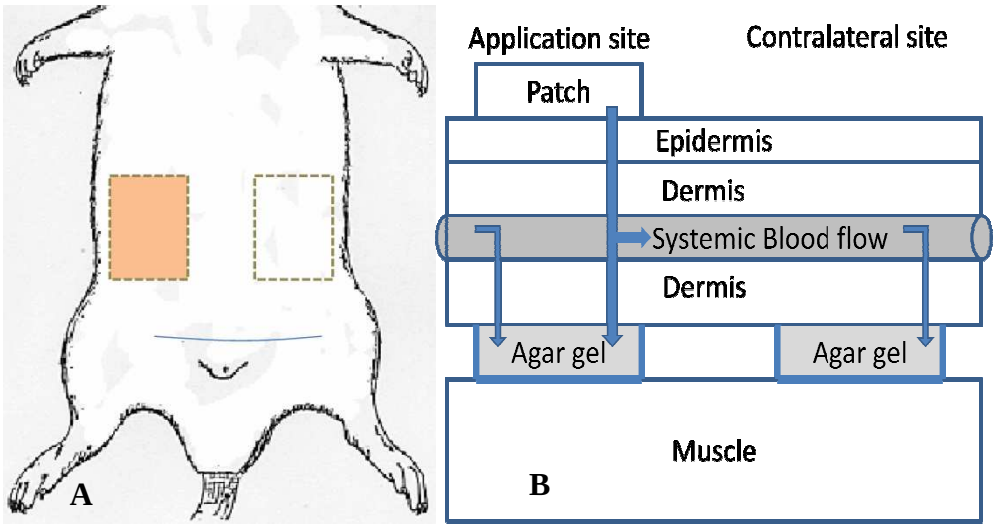
The percentage of the drug in the gel dish that was due to direct penetration:

$$\text{Direct\%} = \sum X_{gdirect} / \sum X_{gtotal} \quad (3)$$

In addition, the fraction (**f**) of the drug accounted for the direct penetration compared to the total amount of drug delivered into the body by the patch:

$$f = \sum X_{gdirect} / \sum X_{b0-t} \quad (4)$$

Figure 1. (A) Schematic diagram of a hairless rat being applied with a patch on the abdominal skin, an incision at the low abdominal skin, and two agar gels (dash line) were inserted under the abdominal skin. (B) Schematic diagram of the dual agar gel rat model where the topical patch provides penetration into the skin, direct penetration into the agar gel, and redistribution of drug into agar gel from blood circulation.



2.8. Sample analysis

For the plasma samples, 200 µl of plasma was taken and added 50 µl of 100 µg/ml ibuprofen as internal standard. 5 ml of Hexane : Tetrahydrofuran (90:10) was added to each sample, and then the sample was shaken in orbital shaker at 400 rpm for 1 hr, and then centrifuged to collect the upper organic layer. The organic layer was evaporated to dry in a vacuum oven and then redissolved in 200 µl of a solution (25/75 acetonitrile/0.02 M phosphate buffer at pH 7.4). 50 µl of sample was injected for HPLC analysis. For agar gel samples, The samples were mixed with 10 ml mixture of hexane : tetrahydrofuran (95:5), and then homogenized for 1 min, and then shake in orbital shaker for 1 hours, then centrifuged to collect the organic phase, the organic phase was evaporated to dry in vacuum oven, and

then redissolved with 200 µl of the solution (25/75 acetonitrile/0.02 M phosphate buffer at pH 7.4) for HPLC analysis.

The HPLC assay method was the same as our previous study [17]. The DA and its prodrugs were analyzed with a HP 1050 HPLC with quaternary pump and DAD detector. The column used was a *Zorbax* XDB-C18 4.6 x 150 mm 5.0 micron *HPLC column*. The mobile phases were acetonitrile and 0.02 M citrate buffer at pH 6.0 with a gradient method. The flow rate was 1 ml/min, and the detection UV wavelength was 280 nm.

RESULTS AND DISCUSSION

3.1. In vitro flux across human epidermis

We fabricated the patches for diclofenac acid (DA), glycerol diclofenac ester (GD), ethylene glycol diclofenac ester (ED), and methanol diclofenac ester (MD) with the three different adhesives: Durotak 387-2287, Durotak 87-901A, and Durotak 87-608A. The drug or prodrugs were uniformly dispersed in the adhesive layer of the final patches with no visible crystal formation over a 6-month period and all the patches showed sufficient tackiness (tested by finger).

In vitro flux study across human epidermis was conducted on those patches. The prodrugs maintained their ester form in the samples from the receiver chambers, especially in the first 12 hrs with no parent drug detected in the samples. However, some small amount of DA was detected in the samples in later time points, especially at the 24 hr and 48 hr time points with a span of 12 hrs in sampling time. Since the human epidermis was prepared with a heat separation method, most esterase present in the skin was probably destroyed, and thus little prodrug was converted to the parent drug in the skin and most the prodrug permeated through the skin in their ester form. However, the prodrugs are not very stable in aqueous solution with half lives from 25 to 50 hrs [17], thus some of the prodrugs could be possibly hydrolyzed to the parent drug in the receiver chamber between the sampling points. With this in consideration, we combined the amounts of prodrug and parent drug in the samples for plotting the cumulative amount of drug permeated across the skin and in the calculation of the flux across the skin.

For the patches made with the polyacrylate adhesive Durotak 387-2287, the cumulative amount of the prodrugs permeated through the human epidermis over the experiment period was much lower from all diclofenac prodrug patches than that from the diclofenac patch (Figure 2A). In addition, among the diclofenac prodrugs, GD showed the highest amount permeated across the skin, and MD showed the lowest amount permeated across the skin. We also observed similar results from the patches made with another polyacrylate adhesive Durotak 87-901A, with DA showing the highest amount permeated across the epidermis, and MD showing the lowest penetration (Figure 2B). In the patches made with a polyisobutylene based adhesive Durotak 87-608A, the GD and ED patches showed similar amount of prodrugs permeated across the skin, and they were higher than that from the DA patch (more than double), but MD still had the lowest permeation across the skin (Figure 2C).

The fluxes and lag times for all the patches determined from Figure 2 were summarized in Table 1. DA showed the highest flux in Durotak 87-901A, while GD and ED showed the best fluxes in Durotak 87-608A. The inferiority in the fluxes of the prodrugs from the patches with polyacrylate adhesives was probably due to a strong interaction between the ester prodrugs and the ester type adhesives. On the other hand, the hydrophobic parent drug DA probably had stronger interaction with the polyisobutylene adhesive and thus showed much lower fluxes.

Table 1. A summary of the *in vitro* fluxes across human epidermis.

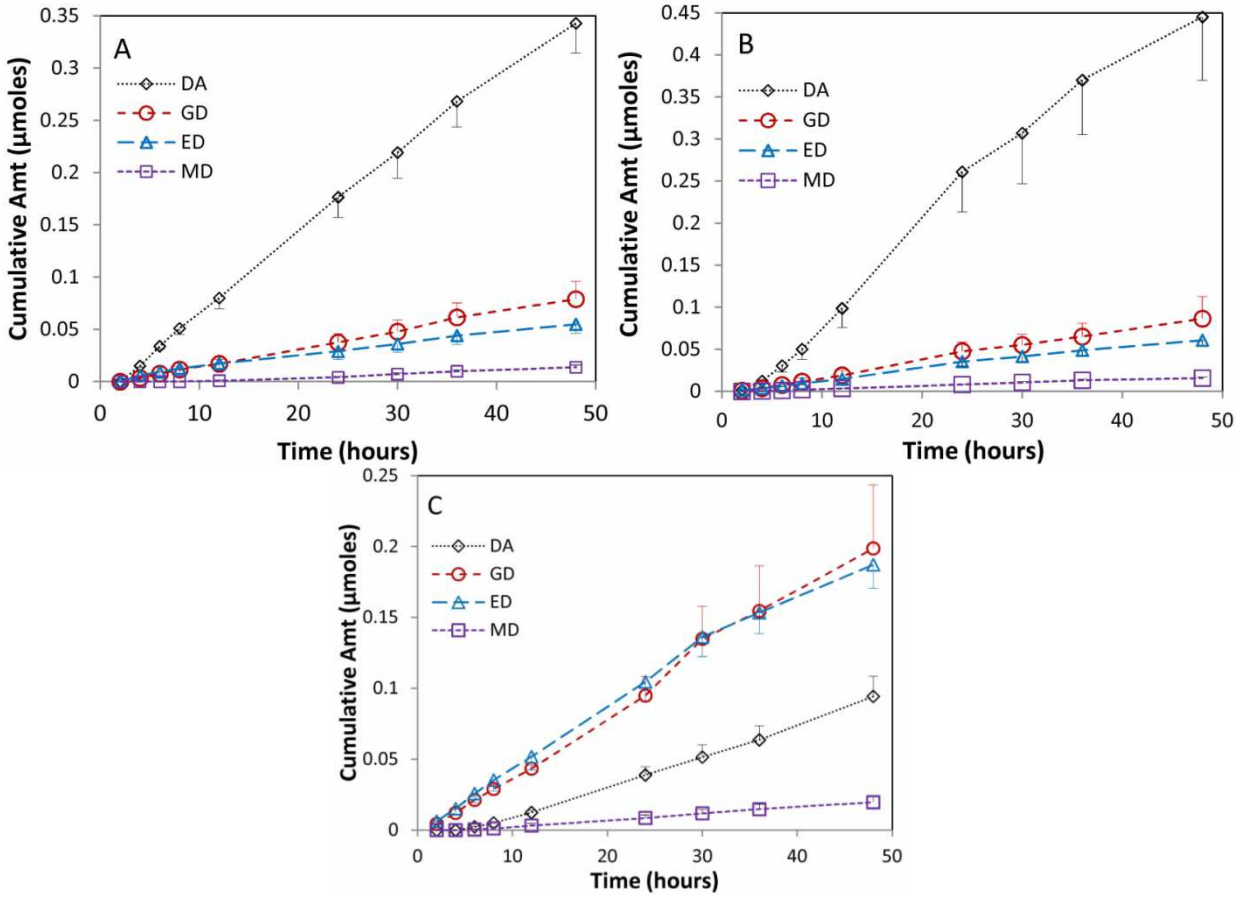
Drug/Prodrug	Adhesive	Flux (nmol/cm^2/hr)	Lag time (hr)
DA	DuroTak-2287	11.54 ± 0.80	0.39 ± 1.01
GD		2.75 ± 0.59	2.14 ± 0.94
ED		1.67 ± 0.14	- 4.06 ± 5.75
MD		0.57 ± 0.07	10.60 ± 1.49
DA	DuroTak-901A	15.06 ± 2.41	- 0.91 ± 1.38
GD		2.89 ± 0.86	- 0.13 ± 1.26
ED		1.99 ± 0.23	- 1.72 ± 0.85
MD		0.55 ± 0.08	0.36 ± 1.83
DA	DuroTak-608	3.55 ± 0.55	6.84 ± 0.29
GD		6.89 ± 1.93	1.99 ± 1.77
ED		5.97 ± 0.34	- 3.33 ± 2.10
MD		0.73 ± 0.14	4.94 ± 1.89

The number after ± is the standard deviation.

The purpose of this study was to screen the diclofenac and its prodrugs patch formulations with different adhesives, and to identify the ones with high in vitro fluxes for further studies. Human cadaver skin can be readily obtained from skin bank, and with that flux screening on large number of patch formulations can be done on the skin from the same donor. The using of skin from the same donor can potentially lower the variation of the flux study. We

discovered that the diclofenac acid in Durotak 87-901A patch and the Prodrugs (GD and ED) in Durotak 87-608A provided the best flux results, and thus they were chosen for further study.

Figure 2. Cumulative amount of diclofenac or its ester prodrugs penetrated across the human epidermis from the *in vitro* flux study. (A) Diclofenac or its ester prodrugs in patches with adhesive Durotak 387-2287. (B) Diclofenac or its ester prodrugs in patches with Durotak 87-901A. (C) Diclofenac or its ester prodrugs in patches with Durotak 87-608A.



3.2. In vitro flux across hairless rat skin

We further determined the flux behavior across hairless rat skin of the DA Durotak 87-901A patch and the ED and GD Durotak 87-608A patches. Unlike the flux study with human epidermis, the hairless rat skin had the full thickness dermis layer. This can significantly increase the lag time for the penetration to reach steady state. In addition, the skin was fresh excised from rats; some enzyme activity was retained in the skin, especially in the initial experiment period, which could help us understand how fast the prodrug converting back to the parent drug during the penetration across the skin. In addition, we also conducted an in situ study with rats and obtained the amount of drug absorbed in the body, the in vitro study with rat skin would also enable us to conduct an in vitro in vivo correlation check.

It is shown in Figure 3A that the DA flux across hairless rat skin from the DA Durotak 87-901A patch was almost two times of that from the commercial Voltaren gel (Lot#10071758), which indicated that the flux provided by the patch formulation was clinically significant. The flux across hairless rat skin was similar to the flux across human epidermis for the DA Durotack-901 patch, but the lag time was much bigger for the permeation across the hairless rat skin, due to the full thickness of the rat skin (see Table 1 and Table 2). The amount of Voltaren gel loaded in the Franz diffusion cells was 50 µl, and with a diffusion area of 0.64 cm^2, it was probably much more than the amount of gel used per cm^2 skin area for patient use. With this approach, the Voltaren gel also provided a relative stable steady flux over a long period of time. Unlike the patches, in the case of applying such gel on patient skin, because of thinner application and fast drying of the gel material, a sustained steady state flux may not occur. The permeation across hairless rat skin from the GD Durotak 87-608A patch is shown in Figure 3B. Large percentage of the drug collected in the receive chamber was in the parent drug form, especially in the first 12 hours. This is contrary to the permeation across human cadaver epidermis skin, in which most of the drug in the receiver chamber

was in the prodrug ester form. This was probably due to the hairless rat skin was freshly excised, which may retain some enzyme activity that converted the GD to its acid drug form either in the skin or in the receiver chamber. It would be very likely that the stratum corneum was the main permeation barrier and GD had to penetrate across the stratum corneum layer of the hairless rat skin before being converted, thus we calculated the flux of GD across the hairless rat skin by combining both the diclofenac acid form and the ester form in the receiver chamber. With that, the flux of GD delivered by the patch across the hairless rat skin was similar or even a little higher than that across the human skin (Table 1 and Table 2), and a larger lag time was also observed in the flux across the rats skin.

The permeation across hairless rat skin from the ED Durotak 87-608A patch is shown in Figure 3C. Most of the drug in the receiver chamber was also in the parent drug form in the first 12 hours, which indicated high bioconversion in the skin. Comparing to flux results from the GD patch, the fraction of the prodrug converted to the parent drug in the receiver chamber was even larger, which indicated the faster bioconversion of ED than GD in the skin. The total flux across the hairless skin for ED delivered by the patch was less than that across human epidermis (Table 1 and Table 2), and it was also less than half of that from the GD patch shown in Figure 3B. ED was more hydrophobic than GD and less soluble in aqueous solution (almost 10 times less soluble than GD) [17], thus the dermis layer of the rat skin may pose a significant barrier for its permeation.

3.3. Plasma protein binding and bioconversion rate

The fraction of diclofenac or its prodrugs binding to plasma protein are listed in Table 3. The diclofenac ester prodrugs have significantly lower fraction of plasma protein binding than that of the parent drug, which means that the prodrugs may have almost 10 times higher unbounded free prodrug than that of the parent drug. The bioconversion rates of the prodrugs in human plasma and in rat plasma were also listed in Table 3. In rat plasma, GD showed the slowest bioconversion rate. This is also consistent with the slower bioconversion of GD than that of ED we observed during penetration across the fresh excised rat skin in the flux study. But in human plasma, GD showed the fastest bioconversion rate. The bioconversion rates in human plasma of the ester prodrugs were much slower than those in the rat plasma. Our intention was to choose one diclofenac prodrug to compare with diclofenac acid for their direct penetration across live rat skin in the in situ rat study. Since GD showed higher flux across the rat skin and also slower bioconversion in rat plasma than those of ED, the GD Durotak 87-608A patch and DA Durotack 87-901A patch were selected for further *in situ* rat study.

Table 2. A summary of the *in vitro* fluxes across hairless rat skin.

Drug/Prodrug	Flux (nmol/cm^2/hr) at 4 - 12 hr	Flux (nmol/cm^2/hr) at 12-48 hr	Lag time (hr)
DA in Durotak-901A	8.33 ± 0.94	12.84 ± 1.27	7.09 ± 0.73
GD in Durotak-608	5.97 ± 1.03	9.12 ± 0.42	6.08 ± 1.85
ED in DuroTak-608	2.38 ± 0.30	3.92 ± 0.34	7.90 ± 0.24
Voltaren gel	4.90 ± 0.93	6.69 ± 0.74	5.43 ± 1.03

The number after ± is the standard deviation.

Table 3. A list of the plasma protein binding and bioconversion results.

	DA	GD	ED	MD
Fraction Binding to Plasma Protein (%)	99.2%	91.0%	88.3%	--[a]
Bioconversion in Human Plasma (half life in min)	--	50.8	122	604
Bioconversion in rat plasma (half life in min)	--	11.3[b]	1.66[b]	1.62[b]

[a] *Experiment was not conducted because of extremely low aqueous solubility of MD.*
[b] *Experiment results from previous study[17].*

3.4. Pharmacokinetic analysis of IV injection of diclofenac sodium

The rat plasma concentrations from the IV injection are shown in Figure 4. The data points were fit with a two-compartment IV injection model and the obtained pharmacokinetic parameters from the fitting are listed in Table 4. The volume of distribution (Vd) of the central compartment and the total clearance (CL) were similar to the values reported in a study on a subcutaneous injection of diclofenac to rats [20]. Those parameters are used for the calculation of drug absorbed into the body from the transdermal patches in the *in situ* rat study. This is a part of the information needed in order to determine the in vitro in vivo correlation. In addition, this information is also needed to determine the fraction of drug went to direct penetration compared to the amount of absorbed in the body.

Figure 3. Cumulative amount of diclofenac or its ester prodrugs penetrated across the hairless rat skin from the *in vitro* flux study.

(A) Diclofenac acid in Durotak 87-901A or Voltaren gel. **(B)** Glycerol diclofenac ester in Durotak 87-608A patch. **(C)** Ethylene glycol diclofenac ester in Durotak 87-608A patch.

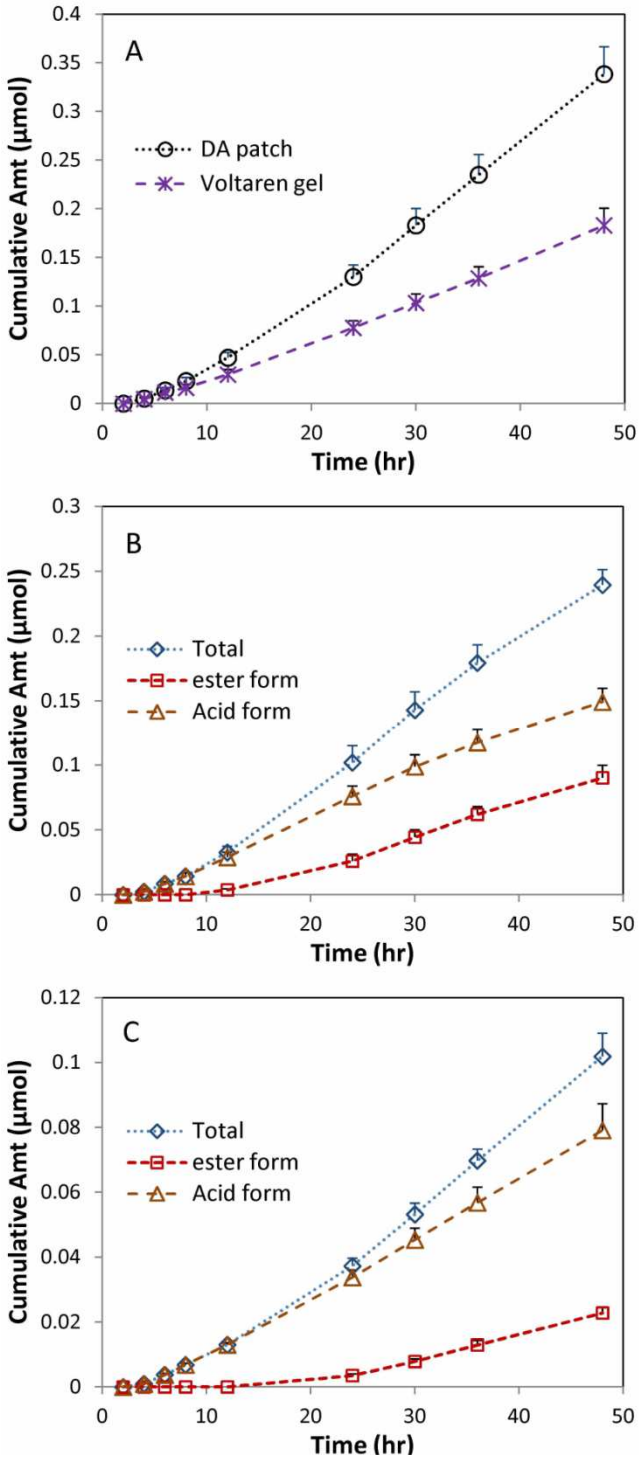


Figure 4. The diclofenac plasma concentrations over the time from I.V. injection of 100 µg of diclofenac sodium into hairless rats

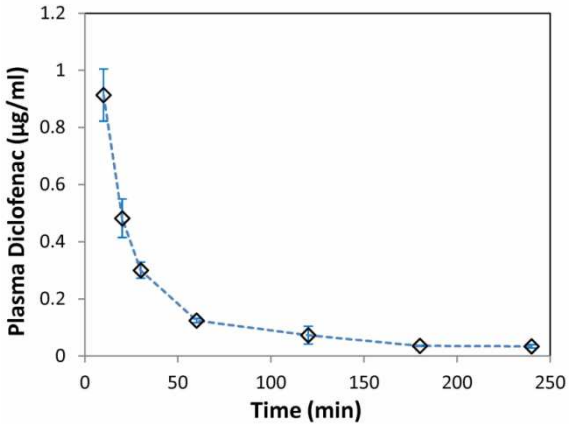


Table 4. The pharmacokinetics parameters from IV injection based on the WinNonlin analysis.

V_d (ml)	52.3 ± 1.4
K10 (min^{-1})	0.045 ± 0.005
K12 (min^{-1})	0.034 ± 0.005
K21 (min^{-1})	0.018 ± 0.006
CL (ml/min)	2.34 ± 0.31

The number after ± is the standard deviation.

3.5. Direct penetration from in situ rat study

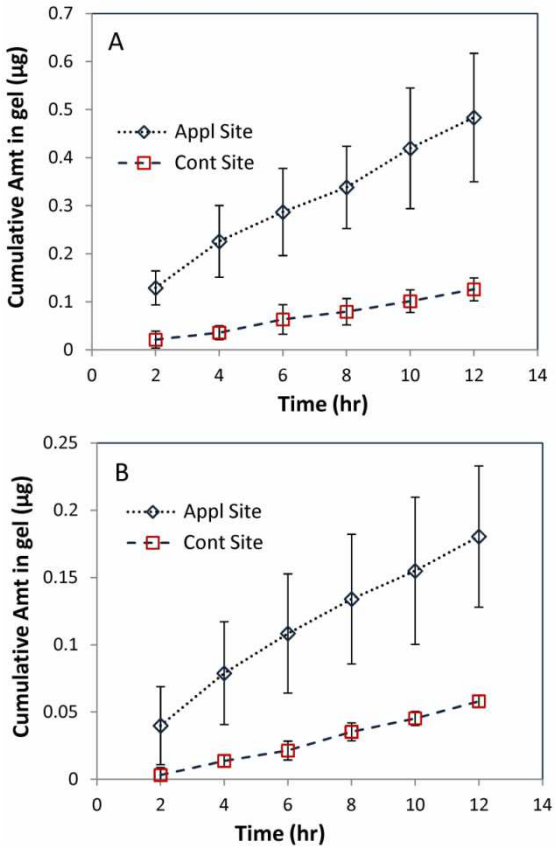
The cumulative amount of diclofenac collected in the agar gel dish under the skin applied with the DA Durotak 87-901A patch and also the amount of diclofenac in the agar gel disk under the contralateral skin site are shown in Figure 5A. Presumably, the amount of drug collected in the agar dish at the contralateral site (X_{gc}) was contributed from the redistribution of diclofenac from the systemic blood circulation. And the amount of drug collected in the agar dish at the patch application site (X_{gtotal}) was contributed from both the direct penetration across the skin from the patch and also the drug redistributed from the systemic circulation. The amount of the drug contributed from the direct penetration would be the difference of the drug collected between the two agar gel dishes. Based on the data in Figure 5A and Equation 3, roughly 78% (Direct%) of the drug in the gel dish at the application site was due to direct penetration from the DA patch.

The cumulative amount of diclofenac collected in the agar gel dish right under the skin applied with the GD Durotak 87-608A patch and also the amount of diclofenac in the agar gel dish under the contralateral skin site are shown in Figure 5B. No prodrug was detected in the agar gel dishes, which means all prodrug penetrated into the skin was converted back to the parent drug. In addition, the less amount of the drug collected by the agar gels from the GD patch than that from the DA patch was probably due to the less amount of drug delivered into the skin by the GD patch, as demonstrated from the *in vitro* flux study (Figure 3A and 3B). Based on the data in Figure 5B, roughly 77% (Direct%) of the drug in the gel dish was due to direct penetration from the GD patch. The percentages of drug contributed from direction penetration were similar between the GD patch and the DA patch.

The diclofenac concentrations in rat plasma over the time from the DA patch or the GD patch applied on the skin during the *in situ* rat study are shown in Figure 6A. No GD was detected in the plasma samples. The DA patch provided higher plasma diclofenac level, which was almost double of that from the GD patch. Based on the diclofenac plasma concentrations and also the pharmacokinetic parameters from the IV injection study, the amount of drug absorbed into the systemic blood circulation was calculated from Equation 1 and plotted in Figure 6B for both patches. From the plot, the amount of drug delivered into the systemic blood circulation from the GD patch was only half of that delivered from the DA patch.

Based on the amount of drug absorbed into the body from Figure 6B for both patches and also the amount of drug penetrated through the hairless rat skin in the first 12 hours from the *in vitro* flux study (Figure 3A and 3B), we observed a very strong *in vitro in vivo* correlation as shown in Figure 7 for both the DA patch and the GD patch. This again supported that the amount of drug absorbed into the body we calculated in Figure 6B was valid. We also calculated the fractions of direction penetration to the agar gels compared to the total amount of drug absorbed into the body based on Equation 4 for both patches, which are 0.12% for the DA patch and 0.083% for the GD patch.

Figure 5. (A) The cumulative amount of diclofenac collected in the agar gels underneath the skin of the application site and the contralateral site from topical application of the diclofenac patch. (B) The cumulative amount of diclofenac collected in the agar gels underneath the skin of the application site and the contralateral site from topical application of the glycerol diclofenac ester patch.



We showed in this study that both glycerol diclofenac ester (GD) and ethyl glycol diclofenac ester (ED) had lower plasma protein binding than the parent drug, and therefore, they would be cleared away in a less extent by the blood circulation in the skin as compared to the parent drug. In addition, the direct penetration of drug molecules across a tissue layer is dependent on the concentration gradient of the free drug (unbounded molecules). Even though the fraction of unbounded drug in tissues and the fraction of unbounded drug in plasma protein are different, Roberts and Cross observed there was a direct correlation between the two fractions [21]. Based on that, we could probably assume that the fraction of unbounded diclofenac ester prodrugs was also higher than the fraction of unbounded diclofenac in the tissues, and thus topical application of diclofenac ester prodrugs could be advantageous in direct penetration into deep tissue.

We showed in a previous study that some diclofenac ester prodrugs can provide higher flux across the skin in a aqueous media [17]. Due to the diclofenac ester prodrugs being prone to hydrolysis in aqueous media, we here investigated the feasibility of formulating these prodrugs into non-aqueous pressure sensitive adhesive patch system. We showed that the polyacrylate based adhesives such as Durotak 387-2287 and Durotak 87-901A were suitable for the parent drug DA to provide high fluxes across the skin, but not for those ester prodrugs, possibly due to strong prodrug adhesive interactions. On the other hand, the polyisobutylene based adhesive (Durotak 87-608A) patches offered better fluxes for the diclofenac ester prodrugs, with the fluxes across the skin being similar or slightly better than those from their saturated aqueous solution as we observed previously [17].

Figure 6. (A) Diclofenac plasma concentrations in hairless rats over the time from topical application of the diclofenac acid patch or the glycerol diclofenac ester patch. **(B)** The amount of diclofenac absorbed into the systemic blood circulation from the topical application of the diclofenac acid patch or the glycerol diclofenac ester patch.

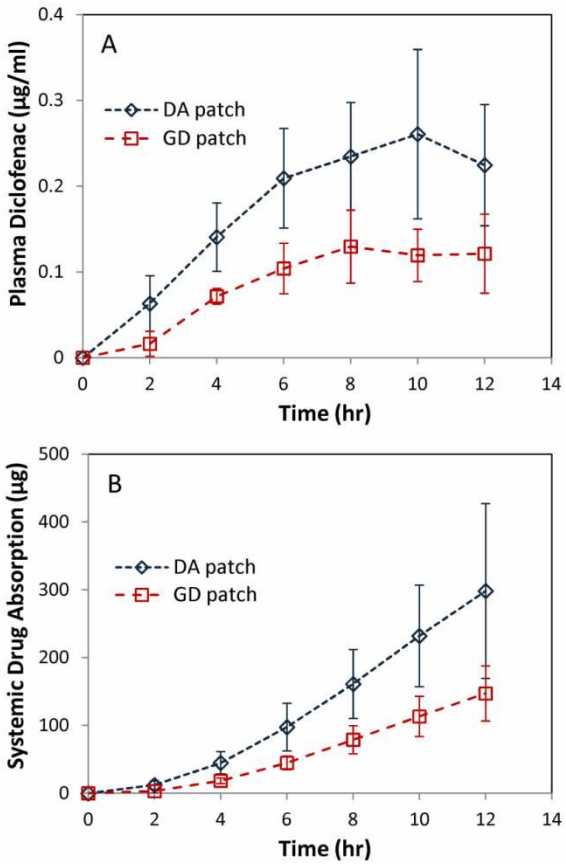
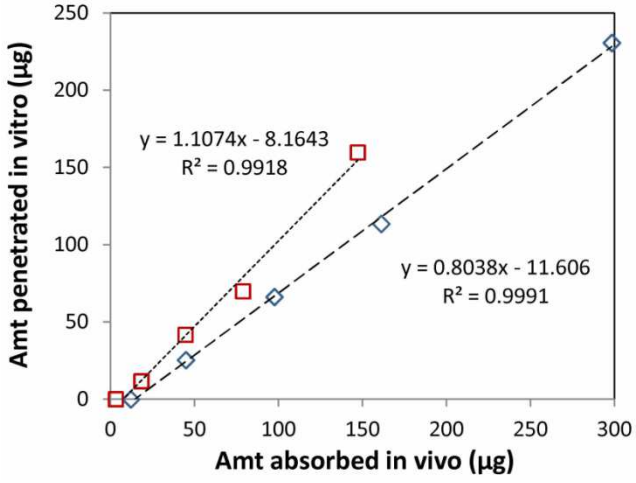


Figure 7. The correlation between the amount of drug penetrated across the skin from *in vitro* flux study demonstrated in Figure 3 and the amount of drug absorbed into the systemic blood circulation form the *in situ* study demonstrated in Figure 6. (◊) for diclofenac acid patch; (□) for the glycerol diclofenac ester patch.



An in-situ rat model was previously proposed by Sugibayashi group in investigation of topical drug direct penetration across the skin [14, 18, 19]. This model retained an intact blood circulation in the skin and provided a mean to determine the contributions from direct penetration and also from systemic circulation. We adapted the dual agar gel *in situ* rat model in this study to investigate the topical application of diclofenac or its ester prodrug in direct penetration across the skin. We observed that the amount of drug collected in the agar gel underneath the skin at the application site was consistently higher than the amount of drug in the agar gel at the contralateral site for both the diclofenac and its prodrug patches, with around 77% contributed from the direct penetration. However, the amount of the drug collected in the agar gel underneath the skin at the application site was very small compared to the total amount of drug absorbed into the circulation system, with only 0.12% contributed from the direct penetration from

the diclofenac patch and 0.083% from the GD patch. These results indicate the skin blood flow cleared most of the absorbed drug with little drug available for direct penetration into deep tissues.

Contrary to our initial expectation, the topical delivery of glycerol diclofenac ester did not show advantage over the topical delivery of the parent drug diclofenac in terms of the direct penetration from our *in situ* rat study. This was probably due to the fast bioconversion of GD back to the parent drug diclofenac in the rat skin. The bioconversion of GD in rat plasma is fast with a half life only 11.3 min, even though it is the slowest one among the three diclofenac prodrugs (GD, ED, MD). The bioconversion of GD in the rat skin could also be very fast, as we showed from the *in vitro* flux study across fresh hairless rat skin, with a significant amount of drug in the receiver chamber in the parent drug form, especially in the initial 12 hours when the enzymes in the skin may be still active. Such fast bioconversion of GD in the skin could render it to lose its prodrug form in the skin and thus lose its advantage over the parent drug in direct penetration into deep tissues.

The bioconversion rates of the diclofenac prodrugs in human plasma are much slower, compared to those in the rat plasma (Table 4), which probably indicates a slower bioconversion in the human skin also. Because of such big differences in bioconversion rates for the ester prodrugs between the human and the rats, it is possible that the prodrug may still be advantageous over diclofenac in human application. To verify that, future study on human subjects with the topical application of diclofenac ester prodrugs may be needed.

As we discussed in the introduction section, the efficacy from current topical diclofenac products was only marginal because of insufficient direct penetration. In this paper, we confirmed that topical application of diclofenac provided very limited amount of drug (0.12%) for direct penetration, and the majority of the drug went to systemic circulation. We adapted a dual agar gel model proposed by Sugibayashi's group [14, 18, 19] for the direct penetration study. We showed that the direct penetration results from our diclofenac patch were similar to the results they obtained for flurbiprofen [18]. These results further supported the validity of the dual agar gel *in situ* rat model for the direct penetration study. However, we discovered that the direct penetration from the diclofenac ester prodrug was not better than that from diclofenac, mainly due to the fast bioconversion of the prodrug in the rat, while such bioconversion was much slower in human. This indicates that the *in situ* rat model may not be suitable for investigation of direct penetration in drugs that have large difference in metabolic rates between that in the human skin and that in the rat skin.

CONCLUSION

Diclofenac ester prodrugs were more suitable to be formulated in patches with polyisobutylene adhesive, while the parent drug diclofenac was more suitable to be formulated in patches with polyacrylate adhesives. Diclofenac ester prodrugs showed lower plasma protein binding than diclofenac. The bioconversion of diclofenac ester prodrugs to diclofenac in human plasma was much slower than that in rat plasma. Topical application of diclofenac patch and its prodrug glycerol diclofenac ester patch showed direct penetration across rat skin, but the direct penetration only accounted a very small fraction of the drug absorbed into the body. Glycerol diclofenac ester did not show advantage over diclofenac in the direct penetration across live rat skin.

Acknowledgments
This project was supported partly by the INBRE Program, NIH Grant Nos. P20 RR016454 (National Center for Research Resources) and P20 GM103408 (National Institute of General Medical Sciences).

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