In Vitro Cutaneous Disposition of a Topical Diclofenac Lotion in Human Skin: Effect of a Multi-Dose Regimen

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Purpose. This study determines comparative bioavailability of diclofenac sodium lotion compared to an aqueous solution after topical application to viable human skin *in vitro*. In addition, the difference between a single dose and multiple doses (8 times) was also determined.
Methods. An *in vitro* flow-through diffusion cell system was employed, using radiolabelled diclofenac sodium.
Results. Multiple doses of lotion (2 μl/cm^2 and 5 μl/cm^2) delivered a total of 40.1 ± 17.6 μg and 85.6 ± 41.4 μg diclofenac, respectively, at 48 h, compared to only 9.4 ± 2.9 μg and 35.7 ± 19.0 μg absorbed after topical application of diclofenac as an aqueous solution ($P < 0.05$). A single dose study showed no statistical difference between diclofenac delivered in lotion or an aqueous solution. Over 48 h the total absorption from lotion was 10.2 ± 6.7 μg and 26.2 ± 17.6 μg (2 μl/cm^2 and 5 μl/cm^2, respectively), compared to 8.3 ± 1.5 μg and 12.5 ± 5.7 μg from an aqueous solution. Both single doses of lotion and aqueous diclofenac showed decreased diclofenac absorption into the receptor fluid between 12 and 24 h. However, when applied multiple times, absorption from lotion was continually increasing up to 48 h. The total dose accountability ranged from 76.8 ± 8.2% to 110.6 ± 15.1% of the applied dose.
Conclusions. Diclofenac lotion exhibited enhanced diclofenac percutaneous absorption rate through human skin (mass, flux and partition coefficient) when applied a multiple number of times and this enhanced absorption was maintained over 48 h. This suggests that a constituent of the lotion (DMSO) will enhance human skin absorption of diclofenac when used in a multi-dose regimen, but not after a single dose.

KEY WORDS: percutaneous absorption; diclofenac; multi-dose; human skin.

INTRODUCTION

Diclofenac, a non-steroidal anti-inflammatory drug, has been widely used in the treatment of rheumatoid arthritis and osteoarthritis. However, oral delivery of this drug poses certain disadvantages, such as, fast first-pass metabolism and adverse side-effects (including, gastrointestinal reactions and idiosynchratic drug reactions). Therefore, alternative routes of administration have been sought. The skin has become increasingly important in this regard, and many drugs have been formulated in transdermal delivery systems, including diclofenac itself (1–4). However diclofenac sodium is not easily absorbed through the skin due to its hydrophilic nature (5). Much work has concentrated on using percutaneous absorption enhancers or co-solvents to increase penetration (6–7). A new diclofenac sodium lotion has been developed for topical application, which includes the absorption enhancer dimethyl sulfoxide (DMSO). It is expected that the addition of DMSO may increase the *in vivo* permeation rate of diclofenac through the skin into the deeper target tissues beneath the skin.

This study determines comparative bioavailability of diclofenac sodium lotion compared to an aqueous solution of diclofenac sodium after topical application to viable human skin *in vitro*. In addition, the difference between a single dose and multiple doses (8 times over 48 h) was also determined.

MATERIALS

[^{14}C]-Diclofenac sodium was purchased from Amersham Life Sciences and further purified by Wizard Laboratories (Sacramento, CA). Specific activity was 9.76 mCi/mmol and radiochemical purity was 99.0%. The Pennsaid™ lotion (lot number 977132), was provided by Dimethaid Research Inc. (Markham, Ontario, Canada), formulated without diclofenac. Lactate diagnostic kit was obtained from Sigma Chemical Co (St Louis, MO). All other chemicals were of analytical grade from standard suppliers.

METHODS

Dose Preparation

i) Lotion: 6 mg of [^{14}C]-Diclofenac sodium was added directly to 400μl lotion, giving a final concentration in the formulation of 1.5%.

ii) Aqueous solution: 6 mg of [^{14}C]-Diclofenac sodium was added directly to 360 μl HPLC grade water (40 μl ethanol was required to dissolve the solid diclofenac) giving a final concentration in the formulation of 1.5%.

Test System

Human cadaver skin from five different donors was dermatomed prior to delivery, using a Padgett Electrodermatome, to a target thickness of 500 μm. Each skin sample was placed in Eagles Minimum Essential Media (MEM) and stored refrigerated at 1 ± 4°C, until use within 5 days after death. This preservation/use regimen follows that used by the human skin transplant bank (8) and the work of Bronaugh *et al.* (9). Wester *et al.* (10) has shown skin to be viable using this storage regimen.

Dose Application

The formulations are identified as A, B, C, D, E, F, G, H and I. Distinctions among these are as follows:

A. 2 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium lotion.[5] Single dose to 1 cm^2 skin area.

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[5] The lotion contains: 45% DMSO, 11% glycerin, 11% ethanol, 11% propylene glycol and water.

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B. 5 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium lotion. Single dose to 1 cm^2 skin area.

C. 2 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium in HPLC grade water (+10% ethanol). Single dose to 1 cm^2 skin area.

D. 5 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium in HPLC grade water (+10% ethanol). Single dose to 1 cm^2 skin area.

E. 2 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium lotion. Multiple doses to 1 cm^2 skin area, at times 0 h, 4 h, 8 h, 12 h, 24 h, 28 h, 32 h and 36 h.

F. 5 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium lotion. Multiple doses to 1 cm^2 skin area, at times 0 h, 4 h, 8 h, 12 h, 24 h, 28 h, 32 h and 36 h.

G. 2 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium in HPLC grade water (+ 10% ethanol). Multiple doses to 1 cm^2 skin area, at times 0 h, 4 h, 8 h, 12 h, 24 h, 28 h, 32 h and 36 h.

H. 5 μl 1.5% (1000 ppm) [^{14}C]-Diclofenac sodium in HPLC grade water (+ 10% ethanol). Multiple doses to 1 cm^2 skin area, at times 0 h, 4 h, 8 h, 12 h, 24 h, 28 h, 32 h, and 36 h.

I. Viability control (glucose metabolism to lactate).

Diffusion Study

The human skin was clamped into a continuous flow-through diffusion cell system (11). The glass cells had a 1 cm^2 surface area of exposed skin, which was maintained at approximately 32°C using a Lauda Heating Circulator (Lauda, West Germany). The receptor fluid, MEM, with 50 μg/ml gentamicin sulfate added to ensure sterility during the running of the experiment, was pumped at a rate of 3 ml/h. Receptor fluid samples were collected every 4 h up to 48 h.

Forty eight hours after dose application, skin surface wash was performed on all skin samples (washed once with 50% Ivory™ liquid soap/Nanopure water, and then rinsed twice with Nanopure water). Wash and rinse samples for individual skin surfaces were combined.

After skin surface washing, ten consecutive cellophane tape strips were performed on all skin samples (except for the lactate viability control skin), and added to a separate vial. The remaining skin was placed in separate scintillation vials and digested by adding 4 ml of Packard Soluene-350 for 7–10 days at room temperature.

Duplicate aliquots (500–1000 μl) of the receptor fluid fractions were taken and assayed for radioactivity by liquid scintillation spectrometry (Packard 1500 counter), after addition of 15 ml scintillation cocktail (UniverSol, ICN). All other samples (tape strips, skin digest solutions and skin surface washes) were also counted for radioactivity after addition of 10–15 ml UniverSol.

Lactate Assay

An assay for the energy metabolism, by the donor skin, of glucose to lactate in the receptor fluid perfusate (which contains glucose) was used to verify that individual skin sources were viable. One undosed, viability control, penetration cell was run concurrently, specifically for this purpose. Receptor fluid samples from this cell were collected every 4 h up to 48 h and were frozen immediately to be analyzed. Samples were measured for lactate using a Sigma Diagnostic kit. A lactate calibration standard curve was constructed using the kit and MEM, and measured at 340 nm using a Hitachi Spectrophotometer.

Statistical Analysis

Statistical analysis was performed using Students t-test (both unpaired and paired). The limited number of skin samples precluded the use of more sophisticated tests, such as covariance.

RESULTS

Percutaneous Absorption of Diclofenac Sodium

The *in vitro* percutaneous absorption (receptor fluid + stratum corneum + skin residue) of diclofenac as mass (μg) absorbed for single and multiple doses are given in Table 1, as well as Figures 1 and 2, and the statistical summary for total absorption is given in Table 2. For single doses (2 μl *vs* 5 μl) there was no statistical difference for lotion (A *vs* B) and aqueous solution (C *vs* D), nor was there any statistical difference between Pennsaid lotion and aqueous solution for the single dose (A *vs* C; B *vs* D). Multiple dosing was superior to single dosing (A *vs* E; B *vs* F; D *vs* H) except for 2 μl aqueous solution (C *vs* G). The lotion was statistically greater than aqueous solution ($P = 0.005$; $P = 0.04$) for 2 μl/cm^2 and 5 μl/cm^2 multiple dosing, respectively, showing that a constituent of the lotion enhanced human skin absorption of diclofenac when used in a multi-dose regimen.

Dose Accountability

The human skin surface was washed at the end of the experiment (48 h) to remove any residual radioactivity which did not penetrate the stratum corneum. Table 3 gives the data as percent of applied dose, including dermal reservoir, skin surface wash and dose accountability. The recovery from the skin surface ranged from 57.8 ± 26.3% to 94.2 ± 19.1%. Total recovery of radioactivity was good for the single dose study, ranging from 92.8 ± 18.6% to 110.6 ± 15.1%. For the multi-dose study recovery from lotion was also good (100.7 ± 7.1% and 90.6 ± 3.0%), however, only 82.0 ± 9.7% and 76.8 ± 8.2% was recovered when aqueous diclofenac was applied.

Steady State Flux and Kp Values

Flux values are calculated during steady state absorption into the receptor fluid, and are expressed a mass transfer/unit area of skin/unit time (namely, μg/cm^2/h). The apparent permeability coefficient (Kp), of particular value since it is independent of the applied dose, is calculated from the following equation: Kp = absorption (μg)/ (surface area × concentration (μg/cm^3) × time). Table 4 shows the data calculated for all formulations. As observed previously, applying a single dose of diclofenac (in lotion or as an aqueous solution) had no effect on either the flux or partition coefficient. However, multiple doses of lotion significantly increased the penetration of diclofenac compared to application in an aqueous vehicle.

Viability Control/Lactate Production

The calibration curve obtained for lactate showed a correlation coefficient of 1.00, and data for each skin source are represented in Figure 3. Lactate was produced for each skin source (and hence viable) through until approximately 32 h.

Table 1. *In vitro* Percutaneous Absorption of Diclofenac from Pennsaid Lotion and an Aqueous Solution

Pennsaid Lotion[a]:				
Parameter measured	A (30μg[b], single)	B (75μg[b], single)	E (240μg[b], multiple)	F (600μg[b], multiple)
Receptor fluid	3.4 ± 3.5	9.8 ± 12.4	4.9 ± 3.4	11.5 ± 11.3
Skin residue	3.3 ± 2.4	7.6 ± 5.5	10.5 ± 6.2	38.0 ± 24.0
Stratum corneum	3.5 ± 2.8	8.9 ± 4.9	24.8 ± 12.0	36.0 ± 30.5
Total absorption	10.2 ± 6.7	26.2 ± 17.6	40.1 ± 17.6	85.6 ± 41.4
Aqueous Solution[c]:				
Parameter measured	C (30μg[b], single)	D (75μg[b], single)	G (240μg[b], multiple)	H (600μg[b], multiple)
Receptor fluid	2.3 ± 2.4	3.0 ± 2.4	0.8 ± 0.7	5.8 ± 4.1
Skin residue	4.3 ± 0.8	3.8 ± 3.0	2.6 ± 1.5	8.5 ± 5.5
Stratum corneum	1.8 ± 0.9	5.5 ± 2.4	6.1 ± 4.3	21.4 ± 18.7
Total absorption	8.3 ± 1.5	12.3 ± 5.7	9.4 ± 2.9	35.7 ± 19.0

Note: Multiple doses *vs* single dose.
[a] Mass (μg) recovered. Mean ± SD, n = 5.
[b] Total dose applied to skin.
[c] Mass (μg) recovered. Mean ± SD, n = 4 or 5.

DISCUSSION

The *in vitro* percutaneous absorption of diclofenac sodium, applied as two different formulations (i.e., lotion and aqueous) and as a single or multi-dose regimen, has been studied in viable human skin. Penetration of diclofenac sodium from the lotion vehicle through and into human skin was extensive after a single dose, reaching approximately 35% of the applied dose (either 2 μl or 5 μl). A high proportion of this actually penetrated through the skin, demonstrating good transdermal delivery of diclofenac to the deeper layers of skin *in vivo*. After a single dose of the lotion, absorption kinetics were relatively rapid over the first 12 h, after which time the rate slowed up to 48 h. This was in contrast to the absorption profile observed when the lotion was applied a multiple number of times. In this case the percentage dose absorbed was lower, but appeared to be linear over the time course of the experiment, and may even have still been increasing at 48 h. The linear kinetics after multiple dosing may be due to the presence of absorption enhancers in the lotion, namely, DMSO. Repeated application

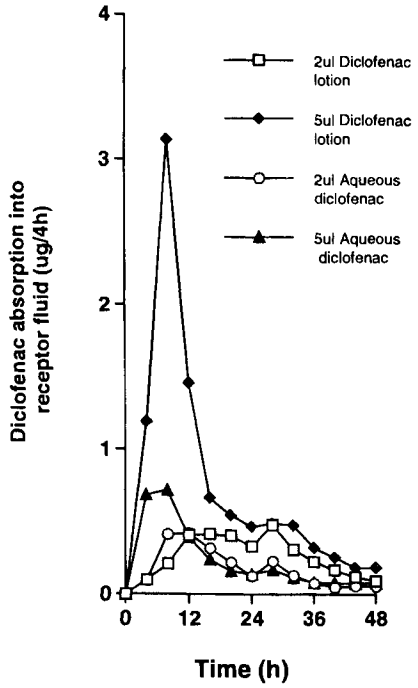

Fig. 1. Absorption (μg/4 h) of Diclofenac sodium through human skin *in vitro* after single dose of diclofenac lotion and aqueous diclofenac. Mean, n = 4 or 5.

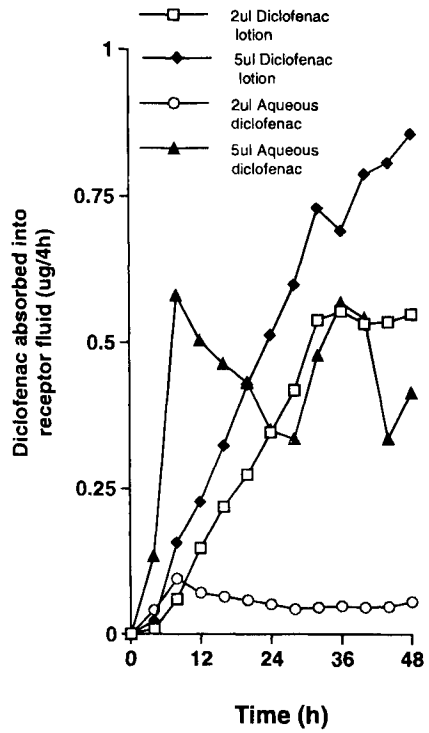

Fig. 2. Absorption (μg/4 h) of Diclofenac sodium through human skin *in vitro* after mutlipe doses of diclofenac lotion and aqueous diclofenac. Mean, n = 4 or 5.

Table 2. Statistical Summary

Treatment	Statistic[a] (P =)
Diclofenac lotion	
A vs B - single dose	0.09[b]
E vs F - multiple doses	0.05[c]
A vs E - single vs multiple	0.007[c]
B vs F - single vs multiple	0.02[c]
Diclofenac aqueous solution	
C vs D - single dose	0.16[b]
G vs H - multiple doses	0.02[c]
C vs G - single vs multiple	0.42[b]
D vs H - single vs multiple	0.03[c]
Diclofenac lotion vs aqueous	
A vs C - single dose	0.57[b]
B vs D - Single dose	0.13[b]
E vs G - multiple doses	0.005[c]
F vs H - multiple doses	0.04[c]

[a] Students t-test.
[b] Non-significant.
[c] Statistically significant.

Table 4. Steady State Flux and Apparent Kp Values[a]

Formulation dosed	Flux (ng/cm^2/h)	Kp (cm/h)
A	71.2 ± 72.7	$4.7 \times 10^{-6} \pm 4.8 \times 10^{-6}$
B	252.2 ± 271.8	$16.8 \times 10^{-6} \pm 18.1 \times 10^{-6}$
C	47.7 ± 49.0	$3.1 \times 10^{-6} \pm 3.2 \times 10^{-6}$
D	63.0 ± 50.7	$4.1 \times 10^{-6} \pm 3.3 \times 10^{-6}$
E	101.8 ± 70.0	$6.7 \times 10^{-6} \pm 4.6 \times 10^{-6}$
F	240.9 ± 235.6	$16.0 \times 10^{-6} \pm 15.7 \times 10^{-6}$
G	16.3 ± 17.7	$1.0 \times 10^{-6} \pm 1.1 \times 10^{-6}$
H	130.9 ± 94.7	$8.7 \times 10^{-6} \pm 6.3 \times 10^{-6}$

[a] Mean ± SD, n = 4 or 5.

of DMSO may keep increasing the transfer of diclofenac through the skin in to the receptor fluid. It may also aid in "dragging" through diclofenac already within the skin from previous applications. Therefore, a multiple dose regimen appears to produce a more sustained delivery of diclofenac, over 48 h, than that of a single dose. This increasing penetration rate of diclofenac sodium has been observed previously *in vivo* after multiple dosing (3). After multiple applications of diclofenac in a gel to human volunteers Sioufi *et. al.*, (3) showed a prolonged absorption, with an increasing rate up to 24 h in 7 out of 10 subjects. They also reported great variability in plasma concentration levels, which is what was observed in our studies. This could be due to many factors of the individuals skin, namely skin permeability, which will be dependent upon stratum corneum thickness, age, skin hydration, cutaneous metabolism during penetration and the general condition of the donor skin (12). In addition, Wester *et. al.*, (13) reported a similar affect of azone, which could enhance its own absorption after repeated application to human volunteers, and could potentially accumulate within the skin. There are conflicting reports in the literature on transdermal diclofenac penetration. A study by Radermacher *et al.* (14) showed only minimal direct transport of diclofenac into the ipsilateral knee joint after repeated cutaneous application.

Few reports have been published relating to multi-dose topical drug administration, despite the clinical and occupational relevance. Several workers showed no significant differences between a single dose and multiple doses in man, for compounds such as malathion, hydrocortisone, testosterone and estradiol (15). Wester *et al.* (16) showed an increased absorption after long-term administration of hydrocortisone to the rhesus monkey. This has been suggested to be due to the chemical nature of hydrocortisone, which may have caused thinning of the skin during the 14 days of the experiment. Muller *et al.* (17) recently reported human *in vivo* studies with topical diclofenac.

Table 3. *In Vitro* Percutaneous Absorption from Pennsaid Lotion and an Aqueous Solution

Pennsaid Lotion[a]				
Parameter measured	A (2μl, single)	B (5μl, single)	E (2μl, multiple)	F (5μl, multiple)
Receptor fluid	11.4 ± 11.6	13.1 ± 16.6	2.0 ± 1.4	1.9 ± 1.9
Skin residue	11.0 ± 7.8	10.1 ± 7.4	4.4 ± 2.6	6.3 ± 4.0
Stratum corneum	11.6 ± 9.3	11.8 ± 6.5	10.3 ± 5.0	6.0 ± 5.1
Total absorption	33.9 ± 22.3	35.0 ± 23.3	16.7 ± 7.3	14. ± 6.9
Skin surface recovery	62.5 ± 28.1	57.8 ± 26.3	84.0 ± 8.5	76.3 ± 9.7
Total recovery	96.4 ± 6.7	92.8 ± 18.6	100.7 ± 7.1	90.6 ± 3.0
Aqueous Solution[a]				
Parameter measured	C (2μl, single)	D (5μl, single)	G (2μl, multiple)	H (5μl, multiple)
Receptor fluid	7.6 ± 7.9	4.0 ± 3.3	0.3 ± 0.3	1.0 ± 0.7
Skin residue	14.3 ± 2.8	5.0 ± 4.0	1.1 ± 0.6	1.4 ± 0.9
Stratum corneum	5.8 ± 2.8	7.3 ± 3.2	2.5 ± 1.8	3.6 ± 3.1
Total absorption	27.8 ± 5.0	16.3 ± 7.5	3.9 ± 1.2	5.9 ± 3.2
Skin surface recovery	79.5 ± 19.9	94.2 ± 19.1	78.1 ± 9.9	70.8 ± 8.2
Total recovery	107.2 ± 22.7	110.6 ± 15.1	82.0 ± 9.7	76.8 ± 8.2

Note: Multiple doses *vs* single dose.
[a] Percent of the applied dose recovered. Mean ± SD, n = 4 or 5.

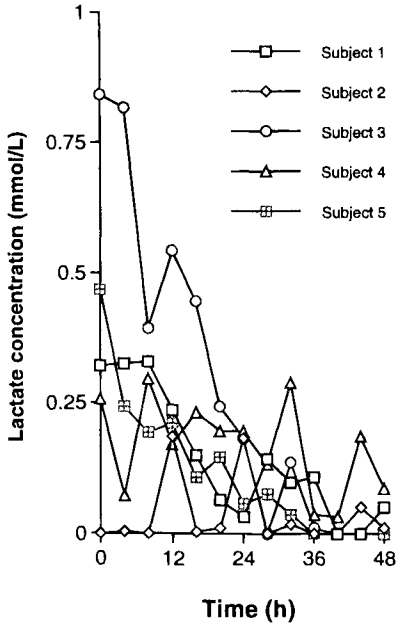

Fig. 3. Lactate production, and release into the receptor fluid during absorption through human skin *in vitro*.

They concluded that transdermal penetration of diclofenac after single doses is unpredictable, as well as being strongly influenced by individual skin properties.

CONCLUSIONS

When diclofenac sodium was applied in an aqueous vehicle a similar picture was observed as for the lotion used here. The percent dose absorbed after mutiple doses was lower than after a single dose. Again, after a single dose the absorption kinetics showed a slowing in the penetration rate between 12 and 24 h. When applied multiple times the rate of absorption was linear over 48 h. However, there was a much lower absorption into the receptor fluid, skin and stratum corneum after multi-dosing compared to that from the lotion. This suggests that the increased absorption of diclofenac in the lotion multi-dose study was due to a constituent of the formulation (presumably DMSO), and not entirely the dosing regimen. DMSO is an absorption enhancer for compounds such as hydrocortisone (18) and salicylic acid (19) and is thought to function by solvation of the polar head groups of the stratum corneum lipids.

These *in vitro* data suggest a sustained level of diclofenac penetrating the skin. However, the biological efficacy of this lotion must be determined clinically. If the absorbed diclofenac is taken in to the systemic circulation too quickly then concentrations in the deeper target tissues (i.e., muscle and joints) may be inefficient.

In conclusion, the lotion exhibited enhanced diclofenac percutaneous absorption through human skin when used in a multi-dose regimen. This higher penetration was also constant over the time period studied, suggesting that under normal use conditions therapeutic levels may be maintained. It should be noted that the clinical regimen for the lotion is the effective multi-dose regimen shown in this study.

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