In Vivo Bioavailability and Metabolism of Topical Diclofenac Lotion in Human Volunteers

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Purpose. The primary objective of this study was to determine the rate and extent of transdermal absorption for systemic delivery of diclofenac from Pennsaid (Dimethaid Research, Inc.) topical lotion into the systemic circulation after the lotion was applied to human volunteers, in an open treatment, non-blinded, non-vehicle controlled study. In addition, the *in vivo* metabolism of this topical diclofenac lotion has also been studied.

Methods. Human volunteers were dosed with topical [^{14}C]-diclofenac sodium 1.5% lotion on the knee for 24 h. Sequential time blood and urine samples were taken to determine pharmacokinetics, bioavailability and metabolism.

Results. Topical absorption was 6.6% of applied dose. Peak plasma ^{14}C occurred at 30 h after dosing, and peak urinary ^{14}C excretion was at 24–48 h. The urinary ^{14}C excretion pattern exhibits more elimination towards 24 h and beyond, as opposed to early urinary ^{14}C excretion. This suggests a continuous delivery of [^{14}C]-diclofenac sodium from the lotion into and through skin which only ceased when the dosing site was washed. Skin surface residue at 24 h was 26 ± 9.5% dose (remainder assumed lost to clothing and bedding). Extraction of metabolites from urine amounted to 7.4–22.7% in untreated urine, suggesting substantial diclofenac metabolism to more water soluble metabolites, probably conjugates, which could not be extracted by the method employed. Two Dimensional TLC analysis of untreated urine showed minimal or no diclofenac, again emphasizing the extensive *in vivo* metabolism of this drug. Treatment of the same urine samples with the enzymes sulfatase and β-glucuronidase showed a substantial increase in the extractable material. Three spots were consistently present in each sample run, namely diclofenac, 3'hydroxy diclofenac and an intermediate polar metabolite (probably a hydroxylated metabolite). Therefore, there was significant sulfation and glucuronidation of both diclofenac and numerous hydroxy metabolites of diclofenac, but many of the metabolites/conjugates remain unidentified.

Conclusions. There was a continuous delivery of diclofenac sodium from the lotion into and through the skin, which ceased after the dosing site was washed. The majority of the material excreted in the urine were conjugates of hydroxylated metabolites, and not the parent chemical, although further identification is required.

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INTRODUCTION

Diclofenac (2-[(2,6-dichlorophenyl)amino]phenylacetate)), a non-steroidal anti-inflammatory drug, is indicated for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. This phenylacetic acid reduces production of prostaglandins, thromboxane, and leukotrienes and inhibits the release of arachidonic acid, the precursor of the prostaglandins. The common route of administration of diclofenac is oral. 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). The most common side effects of oral therapy are gastric or duodenal ulceration and bleeding which occurs in 1% of patients at three to six months. Occasionally, diarrhea, fluid retention and renal dysfunction are noted. 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 extensively 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 containing 1.5% w/w diclofenac sodium 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. *In vitro* percutaneous absorption studies have proven this to be the case, after mutliple doses of this lotion to human skin (8).

The primary objective of this study was to determine the rate and extent of transdermal absorption for systemic delivery of diclofenac from Pennsaid (Dimethaid Research, Inc.) topical lotion into the systemic circulation after the lotion is applied to the knee of human volunteers, in an open treatment, non-blinded, non-vehicle controlled study. Based on the analyses of a pilot study, an appropriate blood sampling regimen was established for the main study. As already stated, oral diclofenac is rapidly metabolised to a number of hydroxylated metabolites. These metabolites, as well as unchanged diclofenac, are excreted primarily in the urine and bile as glucuronide and sulfate conjugates. Therefore, the *in vivo* metabolism of this topical diclofenac lotion has also been studied.

MATERIALS AND METHODS

Bioavailability Study

Test Article and Dose Formulation

Diclofenac (Pennsaid) topical lotion (Lot #4063), a colorless liquid, was supplied by Dimethaid Inc. The lotion contains 1.5% (w/w) diclofenac sodium and 48.1% (w/w) dimethyl sulfoxide. [^{14}C]-Diclofenac sodium (phenylacetic acid ring-U-^{14}C-diclofenac sodium was purchased from Amersham International Place (Little Chalfont, Buckinghamshire England) and purified by Wizard Laboratories Inc., (West Sacramento, CA). The specific activity was 34 μCi/mg and radiochemical purity was 99.0% analyzed by 2D-TLC. Appropriate quantities of [^{14}C]-diclofenac sodium were added to the topical lotion to yield the

desired concentration and specific radioactivity. The formulation was stirred 30 min before sampling and dosing. Sponsor data show the diclofenac in this formulation to be stable for the time course of this study. Multiple sampling of formulation before and during the study show the formulation to be uniformly mixed.

Human Volunteers

Human volunteers were recruited from the University of California, San Francisco (UCSF). Four normal, healthy, men and women, aged from 45 to 76 years, were selected. The average height was 175 ± 10 cm and the average weight was 82 ± 14 kg. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, gastrointestinal, neurological, hematological and dermatologic disease and without hypersensitivity or contraindications to diclofenac, ASA or any other NSAID, alcohol, glycerin, propylene glycol, and dimethyl sulfoxide as determined by medical history. They did not receive any medication before or during the study and had not been involved in any other clinical trial within the previous 60 days. The study was approved by the UCSF Committee on Human Research and each volunteer signed a consent form. Volunteers were selected on a random basis.

Dosing Procedure

A 200 cm^2 area was marked on the left knee. The marked area received a single topical application of [^{14}C]-diclofenac topical lotion (1.0 ml containing 15 mg diclofenac and 50 μCi ^{14}C). After topical application, the dosed area was kept clear of clothing for the first 10 h, then clothing was allowed to touch the knee.

Skin Washing and Analysis

Twenty-four hours after dosing, the dosed site was washed using gauze pads (Sherwood Medical, St. Louis, MO), IVORY liquid soap (Proctor & Gamble, Cincinnati, Ohio) and water. The washing procedure involved one wash with 50% soap solution (v/v) followed by one deionized water wash. A second 50% soap solution (v/v) was followed by two water washes. Individual gauze pads were placed in a borosilicate glass vial containing 10 ml of methanol, sonicated and shaken for 2 hours, to extract ^{14}C material (9). To 1 ml aliquots of these extracts 10 ml scintillation cocktail was added (UniverSol, ICN, Biomedicals, Costa Mesa, CA) and radioactivity was quantified by liquid scintillation spectroscopy (LSS).

Blood Sample Collection and Analysis

A 20 gauge sterilized Landmark Midline Catheter (Menlo Care, Inc., Menlo Park, CA) was placed in the ventral forearm, near the elbow flexure, before dosing. Blood (10 ml) was sampled every hour for 10 hours, using the catheter and thereafter by venopuncture at 24 hourly intervals. The blood samples were immediately placed in a EDTA Vacutainer (Becton Dickinson VACUTAINER Systems, Rutherford, New Jersey) and then centrifuged using an Eppendorf Micro-Centrifuge at 10,000 ×g for 1 min to separate plasma and packed blood cells. The time points of blood collection were adjusted at 1, 2, 4, 6, 8, 10,

24, 48, 72, 96, and 120 h after the application of the lotion. 1 ml Aliquots of plasma were used to determine the radioactivity.

Urine Sample Collection and Analysis

Urine samples were collected prior to dose application (time 0), 0–2, 2–4, 4–6, 6–8, 8–10, 10–24, 24–48, 48–72, 72–96, and 96–120 h after application. Triplicate aliquots of urine (1 ml) were used to determine radioactivity. Time 0 values were used as background and subtracted from sample values. The remainder of samples was kept in high density polyethylene containers and stored in a freezer (-20°C) for further determination of diclofenac and its metabolites by TLC.

Scintillation Counting

All radioactivity measurements were conducted using a Model 1500 Liquid Scintillation Counter (Packard Instruments). Weighed aliquots of urine, skin wash, and cover wash samples were mixed directly with UniverSol Scintillation Cocktail and analyzed for radioactivity.

Data Analysis

Noncompartment pharmacokinetic parameters from plasma levels of each volunteer versus time data were determined by using the program PCNONLIN (Scientific Consulting Inc., North Carolina). C_{max} is the peak drug concentration. T_{max} is the time at which C_{max} occurred. $T^{1}/_{2}$ is the half life of terminal rate constant. $AUC_{(0-last)}$ is determined as area under the plasma concentration-time curve up to the last measured time point.

Metabolism Study

Sulfatase (Type V; EC 3.1.6.1, pure (β-glucuronidase activity <2 Sigma units/mg)) and β-glucuronidase (Type L-II; EC 3.2.6.1) were obtained from Sigma Chemicals Inc., St. Louis, MO. Other chemicals were of analytical grade, purchased from standard suppliers.

Collection and Storage of Urine Samples

The samples were frozen at -20°C until required for metabolite analysis. Only those samples with significant amounts of radioactivity were used.

Subjects and Urine Samples Chosen for Metabolic Determination Were

Volunteer number 01, 24–48 h urine sample, volunteer number 02, 10–24 h urine sample, volunteer number 04, 10–24 h urine sample. Other samples did not contain sufficient quantities of radioactive material to determine metabolites.

Extraction of Urine

40 ml of urine sample was placed into a large round bottom flask and 80 ml 5 N phosphoric acid added. After mixing, 120 ml hexane: isopropyl alcohol (9:1) was added and shaken vigorously for at least 5 min. The organic (top) layer was separated by freezing the aqueous phase over a mixture of dry ice and isopropyl alcohol. The organic phase was transferred

to a second, smaller round bottom flask and rotary evaporated to remove all of the solvent (10,11). This extraction procedure was repeated twice with the same urine sample. The resulting dry extract was reconstituted in 2 ml hexane:isopropyl alcohol (9:1). This was again blown to dryness under a constant stream of nitrogen gas. This dry sample was then kept at $-20°C$ until required for TLC analysis.

Thin Layer Chromatography (TLC)

Two dimensional TLC was used to determine the metabolic profile of the organic phase extracts from the urine samples. Selecto Scientific flexible, 200 μm silica gel 60, F-254 plates (20 × 20 cm) were used. Metabolite standards and radiolabelled diclofenac standards were spotted in two reference lanes, in order that one lane ran for each solvent system employed. Two solvent systems were run: System 1, Hexane:Ethyl Acetate: Acetic Acid (50:50:2)—First dimension; System 2, Chloroform: Ethyl Acetate:Acetic Acid (6:3:1)—Second dimension. Just prior to running TLC plates the extracted samples were reconstituted in 40 μl hexane:isopropyl alcohol (9:1). One sample was spotted in the lower left-hand corner of the plate approximately 2 cm from the lower and left edges of the plate. The first dimension was run with solvent system 1 until the solvent front had travelled 12 cm and the plate was allowed to dry thoroughly. The plate was rotated through 90° and run in the second dimension using solvent system 2, again for 12 cm. Plates were run in sealed double-well glass tanks. Non-radioactive metabolite standards were visualized under ultraviolet light and radioactive areas (both standard diclofenac and sample) visualized using a Molecular Dynamics Phosphorimager (Oakland, CA).

Metabolite Standards

The following metabolite standards were used for TLC:

1. 3'-hydroxy diclofenac;
2. 4'-hydroxy diclofenac;
3. 5-hydroxy diclofenac;

4. 3-hydroxy-4-methoxy diclofenac;
5. 4',5-dihydroxy diclofenac.

Sulfatase and β-Glucuronidase Treatment (10)

The same urine samples were used as previously described. Two aliquots of 40 ml urine were taken, one for sulfatase treatment and one for β-glucuronidase treatment.

Sulfatase Treatment. The urine samples were acidified to a pH of 5.0, by the addition of 5 N phosphoric acid (1.5 ml). 21.1 mg sulfatase was added to the urine sample, giving a total activity of 160 units. After vigorous mixing, the urine/sulfatase was then incubated for 18 h at 37°C. After treatment the extraction procedure was the same as already described.

β-Glucuronidase Treatment. The urine samples were acidified to a pH of 6.8, by the addition of 5 N phosphoric acid (0.5 ml). 57.8 mg β-glucuronidase was added to the urine sample, giving a total activity of 900,000 units. After vigorous mixing, the urine/β-glucuronidase was then incubated for 18 h at 37°C. After treatment the extraction procedure was the same as already described.

Data Analysis

Analysis of TLC plates was performed by comparing RF values (the distance each spot travelled from the origin divided by the total distance the solvent front travelled) of each standard and sample. To aid in comparisons between different TLC plates, run on separate occasions, each RF value was divided by the RF value of the radioactive diclofenac standard run on the same plate. Only the second dimension RF values were used for comparison. The second solvent run showed no such RF variability, as the metabolites and diclofenac had already been separated during the first dimension.

Table 1. Summary of Diclofenac Recovered from Urine and Plasma Samples After *In Vivo* Topical Application of the Lotion[a]

| Collection period (h) | Urine | | | | | Plasma | |
| | Percent applied dose excreted | | Diclofenac equivalent (μm) | | | | |
	Mean ±	SD	Mean ±	SD		Mean ±	SD
0–1	—	—	—	—		0.60	0.28
1–2	0.004	0.007	0.670	1.160		0.82	0.51
2–4	0.008	0.008	1.410	1.347		1.88	1.77
4–6	0.016	0.013	2.728	2.184		1.90	1.14
6–8	0.071	0.078	12.086	13.411		4.54	3.32
8–10	0.062	0.059	10.697	10.060		7.31	3.43
10–24	0.954	0.595	163.337	102.155		11.78	4.27
24–48	1.550	0.752	265.229	129.580		7.11	0.35
48–72	0.691	0.249	118.052	42.410		5.19	1.42
72–96	0.431	0.151	73.764	26.058		3.67	0.94
96–120	0.243	0.047	41.590	8.011		2.81	1.13
Cumulative	4.031	1.808	689.563	311.031			

[a] Mean ± SD, n = 4.

Table 2. Pharmacokinetic Parameters of Diclofenac After Topical Application of the Lotion to Human Skin *In Vivo*

Parameter	Units	Volunteer number				Mean	SD
		01	02	03	04		
T_{max}	hr	24	24	48	24	30	12
C_{max}	ng/ml	10.6	17.4	7.3	12.0	11.8	4.2
$T^{1}/_{2}$	hr	117.4	73.1	74.7	88.4	88.4	20.5
$AUC_{(1-120)}$	ng*hr/ml	621.5	897.9	655.3	695.9	717.6	124.0
$AUMC_{(1-120)}$	ng*h/ml	31945.9	41800.6	37633.9	31519.3	35724.9	4916.9
$MRT_{(1-120)}$	hr	51.4	46.6	57.4	45.3	50.2	5.5

Note: T_{max}, time to maximum concentration (t); C_{max}, maximum plasma concentration (amt/vol); $T^{1}/_{2}$, half-life (t); $AUC_{(1-120)}$, area under the curve (amt/vol*t); $AUMC_{(1-120)}$, area under the first moment curve (amt/vol*t^{2}); $MRT_{(1-120)}$, mean residence time (t).

RESULTS

Bioavailability

Urine

Summarized data are shown in Table 1. The earliest urinary radioactivity appeared at 0–2 h after topically dosing. The peak urinary excretion was reached at 24–48 h period for all volunteers, at 1.55 ± 0.75% of applied dose. The mean value of total urinary radioactivity recovery was 4.03 ± 1.81% of the applied dose by 120 h.

Plasma

Summarized data are shown in Table 1 and pharmacokinetics parameters in Table 2. Detectable [^{14}C]-diclofenac radioactivity in plasma appeared at 0–1 h after topical dosing. The mean value of time to achieve peak level in plasma, T_{max}, was 30 ± 12 h and C_{max} was 11.8 ± 4.2 ng/ml for all volunteers.

Dermal application of diclofenac appears to produce a prolonged delivery of drug into the systemic circulation, compared to oral dosing (where T_{max} is generally 2–5 h, even for sustained-release formulations) (12–13).

Residual Skin Surface Material

Summarized data are shown in Table 3. After the 24 h exposure period, the mean recoverable radioactivity was 26.1 ± 9.5% of the applied dose.

Metabolism Study

Extraction Efficiences

The extraction efficiency for diclofenac spiked urine was 92.6 ± 0.3% (mean ± SD, n = 6). Table 4 shows the recovery of radioactivity after urine extraction, for all three volunteers. For untreated urine there was a low, but variable, recovery of radioactivity in the solvent extract. The percentage of the total radioactivity recovered was 7.4 ± 1.8%, 7.7 ± 2.2% and 12.7 ± 1.6%, for subjects 01, 02 and 04, respectively. The remainder of the radioactivity was unextractable, remaining in the aqueous phase, presumably as conjugated diclofenac and/or other polar metabolites. After treatment of the same urine samples with either sulfatase or β-glucuronidase the amount of extractable radioactivity increased in all of the subjects (Table 4). The extractable radioactivity increased to 20.6 ± 5.5%, 16.6 ± 6.1% and 34.5 ± 8.9% (sulfatase) and 23.1 ± 3.8%, 23.4 ± 7.4% and 41.9 ± 11.6% (β-glucuronidase), for subjects 01, 02 and 04, respectively.

Thin Layer Chromatography

Untreated Urine. TLC analysis of the organic extracted samples showed the presence of the parent compound, diclofenac, in two of the three subjects (01 and 04), but not in subject 02. Tables 5–7 shows that two identical metabolites were extracted from the urine of all three subjects. One with a relative RF value of 0.92–0.94, which corresponds to the 3'-hydroxy diclofenac metabolite standard. This metabolite was the most abundant moeity present in all three urine samples.

Table 3. Recovery of Residual Radioactivity from Skin Surface Washes After *In Vivo* Topical Application of the Lotion

Wash Number	Percent applied dose recovered. Mean±	SD	Diclofenac equivalent. Mean±	SD
1	5.1	2.1	869.4	347.2
2	6.9	2.4	1154.1	417.5
3	5.2	2.2	889.3	374.0
4	4.6	1.9	782.1	332.9
5	4.3	3.0	739.4	516.2
Total	26.1	9.5	4426.7	1650.6

Table 4. Effect of Sulfatase and β-Glucuronidase Treatment on the Extractable Material in Human Urine After Topical Application of the Lotion

	Percent extracted from urine (Mean ± SD, n = 3)		
	Subject #01	Subject #02	Subject #04
Untreated	7.4 ± 1.8	7.7 ± 2.2	12.7 ± 1.6
Sulfatase treatment	20.6 ± 5.5	16.6 ± 6.1	34.5 ± 8.9
β-Glucuronidase treatment	23.1 ± 3.8	23.4 ± 7.4	41.9 ± 11.6

Table 5. Effect of Sulfatase and β-Glucuronidase Treatment on the Extractable Material in Human Urine After Topical Application of Diclofenac Sodium. TLC Analysis. Subject #01

	Spot ID	Relative RF[a]	Designation	Abundance[b]
Untreated	i	1.00	Diclofenac standard	—
	ii	0.83	Intermediate polar metabolite	0.7
	iii	0.94	3'-OH Diclofenac	2.2
	iv	1.02	Diclofenac	1.0
				Total: 3.9
Sulfatase treated	i	1.00	Diclofenac standard	—
	ii	0.83	Intermediate polar metabolite	5.2
	iii	0.89	3'-OH Diclofenac	36.0
	iv	1.01	Diclofenac	9.0
				Total: 50.2
β-Glucuronidase treated	i	1.00	Diclofenac standard	—
	ii		Intermediate polar metabolite	9.9
	iii	0.91	3'-OH Diclofenac	29.5
	iv	1.03	Diclofenac	13.0
	v	0.70	Unknown polar metabolite	2.5
	vi	0.80	Unknown polar metabolite	5.0
				Total: 52.9

[a] RF value of sample spot/RF value of radioactive diclofenac standard.
[b] Average spot density from phosphorimage analysis (= Volume/Area).

The second, with a relative RF value of 0.83–0.85, did not match the known metabolites and is probably another intermediate polar metabolite (i.e., another hydroxylated moeity). No other radioactive metabolites were detected in the extract.

Sulfatase/β-Glucuronidase Treated Urine. Treatment of the same urine samples with sulfatase resulted in a substantial increase in the abundance of radioactive material on the TLC plate (by phosphorimage analysis) from each sample (Tables 5–7). This increase ranged from a 10-fold increase in subject 04 to a 17-fold increase in subject 02. The same three major spots (relative RF values: 0.83–0.85, 0.89–0.92 and 1.01–1.04 (diclofenac)) were observed as in untreated urine, with the 3'-hydroxy diclofenac being the major metabolite present. The two metabolites and the parent drug, diclofenac, all increased in abundance, suggesting that all were sulfated *in vivo*. The

Table 6. Effect of Sulfatase and β-Glucuronidase Treatment on the Extractable Material in Human Urine After Topical Application of Diclofenac Sodium. TLC Analysis. Subject #02

	Spot ID	Relative RF[a]	Designation	Abundance[b]
Untreated	i	1.00	Diclofenac standard	—
	ii	0.83	Intermediate polar metabolite	1.3
	iii	0.92	3'-OH Diclofenac	1.8
				Total: 3.1
Sulfatase treated	i	1.00	Diclofenac standard	—
	ii	0.83	Intermediate polar metabolite	15.1
	iii	0.92	3'-OH Diclofenac	18.5
	iv	1.04	Diclofenac	13.1
	v	0.70	Unknown polar metabolite	1.8
	vi	1.08	Unknown non-polar metabolite	5.4
				Total: 53.9
β-Glucuronidase treated	i	1.00	Diclofenac standard	—
	ii	0.82	Intermediate polar metabolite	32.1
	iii	0.90	3'-OH Diclofenac	36.0
	iv	1.00	Diclofenac	23.1
	v	0.72	Unknown polar metabolite	5.8
	vi	0.85	Intermediate polar metabolite	9.7
	vii	0.99	Diclofenac	23.0
	viii	1.07	Unknown non-polar metabolite	6.2
				Total: 135.9

[a] RF value of sample spot/RF value of radioactive diclofenac standard.
[b] Average spot density from phosphorimage analysis (= Volume/Area).

Table 7. Effect of Sulfatase and β-Glucuronidase Treatment on the Extractable Material in Human Urine After Topical Application of Diclofenac Sodium. TLC Analysis. Subject #04

	Spot ID	Relative RF[a]	Designation	Abundance[b]
Untreated	i	1.00	Diclofenac standard	—
	ii	0.85	Intermediate polar metabolite	6.8
	iii	0.92	3'-OH Diclofenac	10.7
	iv	1.05	Diclofenac	8.8
				Total: 26.3
Sulfatase treated	i	1.00	Diclofenac standard	—
	ii	0.85	Intermediate polar metabolite	17.3
	iii	0.89	3'-OH Diclofenac	97.7
	iv	1.03	Diclofenac	72.2
	v	0.98	Diclofenac	69.2
				Total: 256.4
β-Glucuronidase treated	i	1.00	Diclofenac standard	—
	iii	0.92	3'-OH Diclofenac	227.1
	iv	1.07	Diclofenac	42.5
				Total: 269.6

[a] RF value of sample spot/RF value of radioactive diclofenac standard.
[b] Average spot density from phosphorimage analysis (= Volume/Area).

extracted urine of subject 02 also showed the presence of two other unidentified metabolites, one more polar and another less polar than diclofenac. Both were of low abundance, and not present in the untreated urine extract.

β-Glucuronidase treatment again showed an increased abundance of radioactivity, increasing by 14-fold, 44-fold and 10-fold, for subjects 01, 02 and 04, respectively. The increase in subject 02 was much greater than that observed for the sulfatase treatment, suggesting that much more of the diclofenac and/or metabolites were glucuronidated rather than sulfated. The other two subjects showed similar levels to that observed after sulfatase treatment, indicating equal amounts of both sulfation and glucuronidation. As with sulfatase treatment, the major spot was that corresponding to the 3'-hydroxy diclofenac metabolite. Other unknown spots were identified in the extracts from subjects 01 and 02.

DISCUSSION

The study determined the topical bioavailability of [14C]-diclofenac sodium in a lotion formulation applied to the knee of human volunteers for 24 h. Total urinary diclofenac excretion was 4.0 ± 1.8% applied dose. Percent dosed absorbed was calculated as follows:

$$\text{Percent Dose Absorbed} = \frac{(^{14}\text{C urinary excretion}_{topical})}{(^{14}\text{C urinary excretion}_{iv})} \times 100$$

The intravenous dose is assumed to be 100% bioavailability due to injection, and the above equation accounts for ^{14}C excretion by other body routes (feces, CO_2) or retained in the body. 61% of diclofenac sodium administered i.v. to man is excreted in urine (14). Given this, the percent dose absorbed is 4.03/61 × 100 = 6.61% dose absorbed. Peak plasma ^{14}C occurred at 30 ± 12 hours after dosing, and peak urinary diclofenac excretion was at 24–48 h. The urinary diclofenac excretion pattern exhibits more excretion towards 24 h and beyond, as opposed to early urinary diclofenac excretion. This would suggest a continuous delivery of [14C]-diclofenac sodium from the

lotion into and through skin which only ceased when the dosing site was washed. The AUC values are comparable to those published in the literature after oral dosing. For example, Willis and Kendall (15) reported AUC values of 1.43–1.47 μg*h/ml, compared to 0.7247 μg*h/ml from these studies after topical application. This again indicates good delivery of diclofenac sodium from the lotion.

Since the topical application is non-occlusive and without protection for 14 hours after dosing, it is assumed that the remainder of the dose was lost to clothing and bedding (sheets, blankets) during the 24 h dosing period. However, since 26% was recovered from the skin surface after 24 h, the dose was assumed to be in contact with skin for the total 24 hour dosing period.

From the data presented it is obvious that the majority of the recovered material in human urine after topical application of the lotion is not the parent drug. In untreated urine between 73% and 92% of the total radioactivity was unextractable, i.e., water soluble. These metabolites of diclofenac remain largely unidentified. A proportion of this unextractable material was sulfate and glucuronide conjugates, of both diclofenac and its metabolites. However, even after sulfatase and β-glucuronidase treatment the majority of the radioactivity remained unextractable. Hydroxylated products and conjugates (mainly sulfates and glucuronides) will be formed either during passage through the skin or, more likely, in the liver after absorption, before being excreted in the urine. TLC analysis showed the presence of many metabolites, some having similar relative RF values as known standards (such as 3'-hydroxy diclofenac), while others were unknowns. From *in vitro* dermal metabolism studies performed with the same topical lotion (unpublished data), primarily diclofenac was recovered in the receptor fluid. Therefore, it may be assumed that the majority of the diclofenac metabolism observed *in vivo* was due to liver hydroxylation/conjugation. After oral or i.v. dosing of diclofenac in man very little drug is excreted unchanged. Diclofenac undergoes hydroxylation (cytochrome P450) and subsequent conjugation with both sulfate and glucuronide (16). The major hydroxylated

product of diclofenac metabolism in man is 4'-dehydroxydiclo-fenac (17), which was not definitively identified in these studies. However, there are conflicting reports within the literature regarding diclofenac metabolism. This has been suggested to be due to instability and spontaneous hydrolysis of glucuronide conjugates, which can isomerize to form a isoglucuronide (16).

In conclusion, there was a continuous delivery of diclo-fenac sodium from Pennsaid lotion into and through the skin, which ceased after the dosing site was washed. Biotransforma-tion of diclofenac was extensive, with little unchanged diclo-fenac being excreted in the urine. There was a certain degree of sulfate and glucuronide conjugation, but some of the metabolites/conjugates remain unidentified.

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REFERENCES

1. B. L. Seth. Comparative pharmacokinetics and bioavailability study of percutaneous absorption of diclofenac from two topical formulations containing drug as a solution gel and as an emulsion gel. *Arzneim-Forsch.* **42**:120–122 (1992).
2. H-O. Ho, F-C. Huang, T. D. Sokoloski, and M-T. Shue. The influence of cosolvents on the *in vitro* percutaneous penetration of diclofenac sodium from a gel system. *J. Pharm. Pharmacol.* **46**:636–642 (1994).
3. A. Sioufi, F. Pommier, F. Boschet, J. Godbillion, D. Lauoignat, and D. Salliere. Percutaneous absorption of diclofenac in healthy volunteers after single and repeated topical application of diclo-fenac emulgel. *Biopharmaceut. Drug Disp.* **15**:441–449 (1992).
4. S. P. Vyas, R. Singh, and R. K. Asati. Liposomally encapsulated diclofenac for sonophoresis induced systemic delivery. *J. Micro-encapsulation.* **12**:149–154 (1995).
5. T. Nishihata, K. Kotera, Y. Nakano, and M. Yamamazaki. Rat percutaneous transport of diclofenac and influence of hydroge-nated soya lecitin. *Chem. Pharm. Bull.* **35**:3807–3812 (1987).
6. Y. Obata, K. Takayama, H. Okabe, and T. Nagai. Effect of cyclic monoterpenes on percutaneous absorption in the case of a water-soluble drug (diclofenac sodium). *Drug Des. Del.* **6**:319–328 (1991).
7. T. Nishihata, A. Kamada, K. Takahashi, K. Matsumoto, K. Shino-zaki, Y. Tabata, M. Keigami, T. Miyagi, and N. Tastumi. Percuta-neous absorption of diclofenac in rats and humans: Aqueous gel formulation. *Int. J. Pharm.* **46**:1–7 (1988).
8. P. G. Hewitt, N. Poblete, R. C. Wester, J. Z. Shainhouse, and H. I. Maibach. *In vitro* cutaneous disposition of a topical diclo-fenac lotion in human skin: effect of a multi-dose regimen. *Pharm. Res.* **15**:988–992.
9. R. C. Wester, J. Melendres, S. Serranzana, and H. I. Maibach. Time-response necessary in validation for extraction of pesticides from cloth patches used in field exposure studies. *Arch. Environ. Contam. Toxicol.* **27**:276–280 (1994).
10. K. K. H. Chan, K. H. Vyas, and K. Knuck. A rapid and sensitive method for the determination of diclofenac sodium in plasma by high-performance liquid chromatography. *Analytical Let.* **15**:1649–1663 (1982).
11. H. Stierlin and J. W. Faigle. Biotransformation of diclofenac sodium in animals and man. II. Quantitative determination of the unchanged drug and principal phenolic metabolites, in urine and bile. *Xenobiotica* **9**:611–621 (1979).
12. M. M. Hasan, N. M. Najib, and H. Muti. A comparative bioavail-ability study on two sustained-release formulations of diclofenac sodium following a single dose administration. *Int. J. Clin. Phar-macol. Ther. Toxicol.* **31**:387–391 (1993).
13. H. Mascher. The pharmacokinetics of a new sustained-release form of diclofenac sodium in humans. *Drug Des. Del.* **4**:303–311 (1989).
14. V. A. John. The pharmacokinetics and metabolism of diclofenac sodium (voltarol[R]) in animals and man. *Rheumatol. Rehabili.* **17**(Suppl):22–35 (1979).
15. J. V. Willis and M. J. Kendall. Pharmacokinetic studies on diclo-fenac sodium in young and old volunteers. *Scand. J. Rheumatol-ogy* **22**(suppl):36–41 (1978).
16. N. M. Davis and K. E. Anderson. Clinical pharmacokinetics of diclofenac. *Drug Dis.* **33**:184–213 (1997).
17. P. D. Fowler. Voltarol: Diclofenac sodium. *Clin. Rheum. Dis.* **5**:427–464 (1979).