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Research Papers

Percutaneous absorption of diclofenac in rats and humans: aqueous gel formulation

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Summary

The rat dorsal percutaneous absorption of sodium diclofenac increased with an increase in sodium diclofenac concentration in an applied aqueous solution, but the bioavailability was poor. The addition of 10% w/w ethanol in the aqueous solution containing sodium diclofenac apparently increased the percutaneous absorption of diclofenac, which was performed by the increase in the sodium diclofenac concentration in the applied solution rather than by an increase in dorsal skin permeability to diclofenac. The administration of sodium diclofenac in the aqueous gel formulation, which was prepared with hydrogenated soya phospholipid, increased the plasma diclofenac concentration in rat significantly. The formulation causing high diclofenac concentration in rat plasma also resulted in a large accumulation of diclofenac in the dorsal subcutaneous tissue. The dorsal percutaneous administration of sodium diclofenac in the aqueous gel form to healthy human subjects caused a gradual increase of plasma diclofenac concentration during the experimental period of 8 h. The relative bioavailability of sodium diclofenac in the gel form in one subject was about 7% of that after rectal administration of a commercial suppository of sodium diclofenac.

Introduction

As described by Ostrenga et al. (1971), in the formulation of vehicle for topical drugs, the efficacy of such dosage forms is often dependent on the composition of the vehicle. The ability of a drug in a topical formulation to penetrate the skin and to exert its effect is dependent on two consecutive events. The drug must first diffuse out of vehicle to the skin surface and then it must penetrate this natural barrier en route to the site of action. These two processes are intimately related, and both are dependent upon the physical properties of the drug, vehicle, and barrier. It has been proposed (Scheuplein and Blank, 1973) that

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the major permeability barrier in stratum corneum results from an ordered intracellular lipid matrix and has a low water content. It has been reported that surfactants disorganize the lipid matrix (Walter et al., 1982, 1984) and that organic solvents extract lipid to increase the percutaneous permeability (Scheuplein and Blank, 1973).

We have recently reported (Nishihata et al., 1987) that the aqueous gel formulation, which was prepared with hydrogenated soya phospholipid alone, increased the in vitro permeability of rat skin to diclofenac. Since phospholipids are surfactants, a mechanism behind the effect of hydrogenated soya phospholipids might be dependent on the disorder of the lipid matrix of the stratum corneum. Another problem of a topical formulation of diclofenac in an aqueous system is the low solubility of diclofenac. To develop a practical topical formulation of diclofenac, it seems to be required to increase the solubility of diclofenac in the formulation.

In the present study, we investigated the effect of various aqueous gels, prepared with hydrogenated soya phospholipid, on percutaneous absorption of diclofenac in rats and humans.

Materials and Methods

Materials

Sodium diclofenac was supplied by Ciba Geigy Japan (Takarazuka, Japan). Hydrogenated soya phospholipid (phospholipid) was supplied by Nihon Surfactant Co. (Tokyo, Japan). Triglyceride (melting point, 33–35 °C) was supplied from Nippon Oil and Fat Co. (Tokyo, Japan). Other reagents used were of analytical grade.

Preparation of aqueous gels of diclofenac

The codes for and constituents of aqueous gel are listed in Table 1. Sodium diclofenac was dissolved in 0.1 M sodium phosphate buffer (pH 7.2) at 50 °C or in the mixture of the phosphate buffer and ethanol at room temperature. (The solubility of sodium diclofenac in the buffer is 13.4 mM at room temperature and 45.7 mM at 50 °C, and the solubility in the buffer containing 10% w/w ethanol is 51.4 mM at room temperature.) Other ingredients were then added to the above solution which was agitated at 80 °C for 10 min, and then homogenized for 10 min. With agitation, the mixture was cooled to room temperature to obtain the desired aqueous gel formulations. The codes and constituents of other test solutions, which were prepared at room temperature, are listed in Table 2.

In vivo rat percutaneous absorption of diclofenac

Dorsal hair of Wistar male rats weighing 160–180 g was shaved with an electric clipper (Sanyo, Tokyo). After anesthetizing a rat with sodium pentobarbital (30 mg/kg, i.p. for the first injection, and then 15 mg/kg was administered at

TABLE 1

The codes for and constituents of aqueous gels

	Code			
	A	B	C	D
Constituents				
sodium diclofenac	1.0	1.0	1.0	2.25
phospholipid	6.8	6.8	6.8	6.8
triglyceride	0	6.8	6.8	6.8
buffer	92.20	86.40	76.40	74.15
ethanol	0	0	10.0	10.0
Total	100	100	100	100
Dose of gel, g				
for rat	0.5	0.5	0.5	0.22
for man	–	–	–	2.67

All values in g. Buffer: 0.1 M sodium phosphate buffer, pH 7.0.

TABLE 2

The codes for and constituents of the solutions

Code	Constituents				Dose of solution (g)
	Sodium diclofenac (mg)	Buffer (mg)	Ethanol (mg)	Total (g)	
1	10	4990	0	5	2.5
2	10	2990	0	3	1.5
3 (in suspension)	10	990	0	1	0.5
4	10	4490	500	5	2.5
5	10	2690	300	3	1.5
6	10	890	100	1	0.5

Buffer: 0.1 M sodium phosphate buffer. Solution or suspension was prepared at room temperature. The solubility of sodium diclofenac in the buffer is 13.4 mM at room temperature and 45.7 mM at 50 °C, and the solubility in the buffer containing 10% w/w ethanol is 51.4 mM at room temperature. Thus, about 60% of sodium diclofenac in Code 3 is present in suspension.

3 h intervals during experimentation), rats were placed on a hot surface at 38 °C. The test solutions and gels were administered with a columnar cylinder (20 mm inner diameter and 50 mm height), which was fixed on the dorsal skin surface with glue (Aron Alpha, obtained from Toa Chemicals Co., Tokyo) at the edge. After the administration, 200 μl of blood was collected from the right femoral vein via a cannula at designated time intervals for 12 h and centrifuged to obtain plasma. After 12 h, the skin surface was rinsed gently with 5 ml of ethanol, and the skin tissue including subcutaneous tissue was excised by cutting with scissors. The tissue without the surface layer, which was removed by stripping, was homogenized in saline to measure the diclofenac content in the tissue. Diclofenac solution (at a dose of 0.5 mg which was dissolved in 0.5 ml saline) was administered i.v. via the jugular vein. Area under the curve (AUC) of diclofenac concentration in plasma was determined by the trapezoidal rule.

In vivo percutaneous absorption of diclofenac in man

Two grams of the gel of Code D in Table 1 were spread on 50 cm^2 of aluminum foil and attached to the dorsal skin surface of 4 healthy male subjects (56–75 kg, and 35–38 years old). After the administration, blood samples were collected at 2 h intervals for 8 h and centrifuged to obtain plasma.

Assays

Diclofenac was assayed by HPLC (Yaginuma et al., 1981). 100 μl of rat plasma or the tissue homogenate were deproteinized with 400 μl of acetonitrile. After centrifugation, the collected supernatant was dried under nitrogen. The assay limitation for diclofenac in the rat samples was 40 ng/ml. The assay for diclofenac in human plasma was performed after the following extraction procedure. One ml of 6% w/v trichloroacetic acid solution was added to one ml of plasma, and the 10 ml of chloroform was added to the solution, followed by shaking for 10 min. After centrifugation, the chloroform phase was collected. This chloroform extraction was repeated 3 times. The combined chloroform was evaporated under nitrogen and the residue was dissolved in 100 μl of the mobile phase. The limitation of assay in human plasma was 5 ng/ml, and the recovery of diclofenac from human plasma was $96.2 \pm 2.7\%$ ($n = 16$).

Statistical analyses

Statistical analyses were performed by Student's *t*-test.

Results and Discussion

Rat percutaneous absorption of diclofenac in aqueous solution

After an application of aqueous diclofenac solution of Code 1 of Table 2 (buffer alone) on rat skin surface at 5 mg dose of drug, the plasma diclofenac concentration increased up to 2 h and then remained at constant levels from 4 h to 12 h (Fig. 1A). The addition of ethanol to the solution

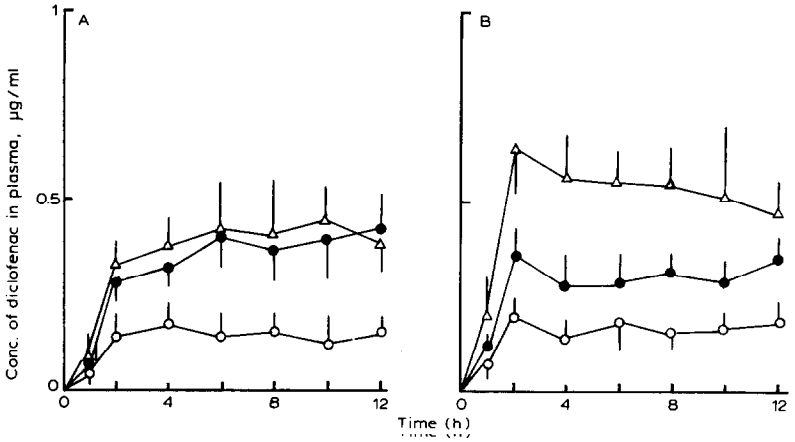

Fig. 1. Plasma diclofenac concentration in rats as a function of time after the percutaneous administration of the diclofenac solution at a dose of 5 mg sodium diclofenac. (A): ○, 2.5 g Code 1; ●, 1.5 g Code 2; △, 0.5 g Code 3. B: ○, 2.5 g Code 4; ●, 1.5 g Code 5; △, 0.5 g Code 6. Each value represents the mean ± S.D. ($n = 4$).

(10% w/w) (Code 4 of Table 2 and Fig. 1B)) resulted in similar plasma concentration profiles of diclofenac to those obtained without ethanol. An increased diclofenac concentration from a decreased volume of the administered solution at constant dorsal skin surface area resulted in increased plasma diclofenac independent of whether the solution contained ethanol (Code 5) or not (Code 2). When the weight of the administered solution was decreased to 0.5 g, some sodium diclofenac was present as a suspension in the buffer alone (Code 3, see note in Table 2), but diclofenac completely dissolved in the solution containing 10% ethanol (Code 6). The plasma concentration of diclofenac was observed to be greater for formulation Code 6 than that obtained for Code 3 (Fig. 1). The AUCs of plasma diclofenac for 12 h after percutaneous administra-

TABLE 3

AVC of plasma diclofenac concentrations for 12 h after percutaneous administration of sodium diclofenac and accumulation of diclofenac in the subcutaneous tissue under the applied skin

Code	Dose (mg)	AUC (μg/h/ml)	AUC for Code X / AUC for Code 1	Skin permeation (μg/min/cm^2)	Accumulation (μg/g tissue)
I.V.	0.5	6.01 ± 0.27			undetected
P.C. 1	5.0	1.26 ± 0.47	1	4.6×10^{-2}	15.7 ± 6.9
2	5.0	2.91 ± 0.92	2.3	10.2×10^{-2}	26.4 ± 4.2
3	5.0	3.27 ± 1.40	2.6	11.6×10^{-2}	30.1 ± 9.2
4	5.0	1.47 ± 0.54	1.2	5.7×10^{-2}	12.5 ± 4.1
5	5.0	2.63 ± 1.17	2.1	9.1×10^{-2}	29.1 ± 13.3
6	5.0	4.18 ± 1.12 *	3.3	12.9×10^{-2}	36.4 ± 9.2
A	5.0	13.11 ± 3.94	10.4	54.5×10^{-2}	58.1 ± 20.6
B	5.0	11.64 ± 4.12	9.2	47.6×10^{-2}	69.5 ± 26.2
C	5.0	11.15 ± 3.26	8.8	45.2×10^{-2}	65.9 ± 16.0
D	5.0	19.17 ± 5.64 **	15.2	72.2×10^{-2}	91.3 ± 39.2

The ratio of AUC was determined by comparison of AUC after percutaneous administration (P.C.) of various formulations to that after Code 1. Skin permeation of diclofenac was calculated by the equation in the text. Accumulation of diclofenac in the tissue was determined at 12 h after the administration. Each value represents the mean ± S.D. ($n = 4$).

*, $P < 0.1$ vs Code 3; **, $P < 0.05$ vs Codes B and C; $P < 0.1$ vs Code A.

tion to rats are summarized in Table 3. The AUCs increased with the diclofenac concentration in the administered solution independent of the presence of ethanol. The AUC was not affected by the presence of ethanol when the following solutions were applied: Code 1 as compared with Code 4 and Code 2 with Code 5. However, an increase of AUC after the application of Code 6 (solution) was observed in comparison to Code 3 (suspension). Thus, it is presumed that the 10% ethanol in the administered solution affects the skin penetration of diclofenac by increasing the solubility of diclofenac in the administered solution rather than by altering the skin's barrier properties.

The value of AUC/dose for percutaneous administration of sodium diclofenac was very small in comparison to that for i.v. administration (Table 3). The low AUC for percutaneous administration may be due to low skin permeability. The authors have reported (Nishihata et al., 1987) that penetration of diclofenac through whole rat skin in vitro correlates with tissue accumulation of diclofenac. Therefore, accumulation of diclofenac in the subcutaneous tissue was also measured after in vivo study (Table 3). The accumulation of diclofenac increased with the diclofenac concentration in the solution, in spite of the constant dose of diclofenac. Thus, it is again concluded that the accumulation of diclofenac in the tissue caused the high concentration of diclofenac in plasma. This accumulation of diclofenac in the subcutaneous tissue may be estimated from the plasma diclofenac concentration.

Rat percutaneous absorption of diclofenac from an aqueous gel formulation

The percutaneous application of a 5 mg sodium diclofenac aqueous gel (Table 1) as compared to the solution (Fig. 1) caused a significant increase of plasma diclofenac concentration (Fig. 2). At constant diclofenac concentration, the presence of ethanol in the gel did not influence the plasma diclofenac concentration; among Codes A, B, and C in Table 1, no differences were observed in the plasma diclofenac concentration profiles (Fig. 2) or AUC for 12 h (Table 3). The AUCs obtained for Code A, B, and C were 3 times larger than that for Code 6, which exhibited the largest AUC

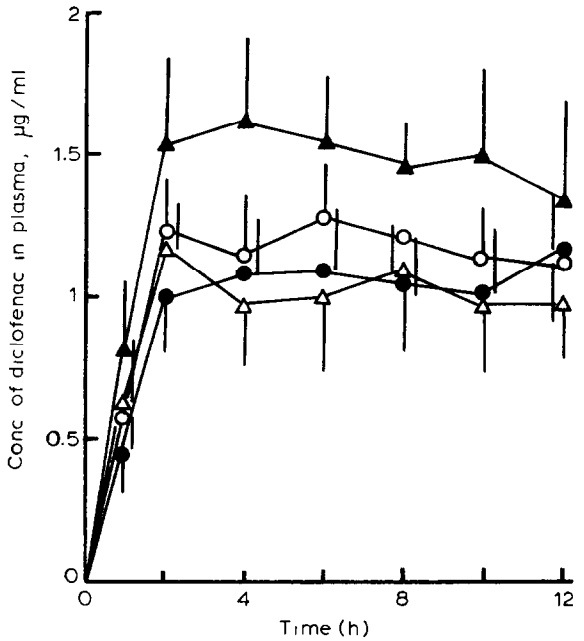

Fig. 2. Plasma diclofenac concentration in rats as a function of time after the percutaneous administration of the gel formulation at a dose of 5 mg of sodium diclofenac. ○, Code A; ●, Code B; △, Code C; ▲, Code D. Each value represents the mean ± S.D. ($n = 4$).

among the solution treatments (Table 3). The application of Code D gel, which contained the highest diclofenac concentration, increased the plasma diclofenac concentration markedly (Fig. 2). The accumulation of diclofenac in the tissue increased with the plasma diclofenac concentration (Table 3).

The authors have reported (Nishihata et al., 1987) that the phospholipid in the buffer increased the penetration and accumulation of diclofenac in the in vitro penetration study using rat skin. Although the effect of phospholipid in increasing penetration of diclofenac was presumed due to the surfactant properties of phospholipids, interpretation of the results is ambiguous. However, since it was observed in our previous report that phospholipids did not affect the in vitro tissue content of diclofenac when subcutaneous tissue was directly exposed to the test solution, the effect of phospholipid in increasing the percutaneous absorption of diclofenac seems likely to

result from an alteration of the barrier properties of stratum corneum.

Apparent skin permeation of diclofenac in the in vivo study after application of gel formulations was calculated by the following equation.

$$\text{Clearance} = \text{Dose}_{iv}/\text{AUC}_{iv}$$

Skin permeation

= (Clearance)(Steady-state plasma levels from each gel formulation)/(Surface area)

Where steady-state plasma levels of diclofenac was determined by the mean of the plasma concentration of diclofenac from 4 h to 12 h for each study in Figs. 1 and 2. As shown in Table 3, the skin permeation of diclofenac after application of gel formulations was greater than that after application of aqueous solution.

Percutaneous absorption of diclofenac in human subjects

To examine the practical formulation of diclofenac, the gel of Code D was investigated in the human study. As shown in Fig. 3, plasma diclofenac concentrations were detected during the experimental period after an administration of Code D. The increase in plasma diclofenac concentration occurred slowly in comparison with the rat experiment, but the concentration was quite constant from 4 h to 8 h (Fig. 3). The histology of human skin differs from that of rat skin which has many well-developed hair follicles and this difference may relate to functional barrier differences. To estimate the bioavailability of percutaneous absorption of diclofenac in the gel form of Code D, AUC for 8 h after percutaneous administration was compared to that after rectal administration of a commercial suppository of sodium diclofenac in one subject. As shown in Table 4, the relative bioavailability of diclofenac Code D was approximately about 7%.

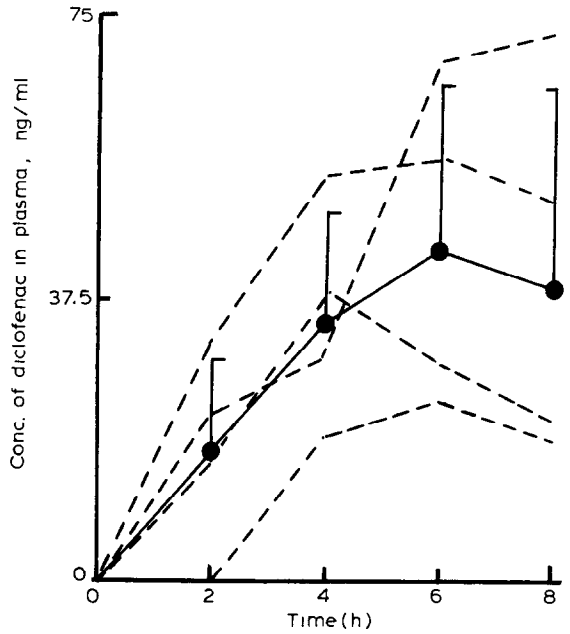

Fig. 3. Plasma diclofenac concentrations in human subjects after the percutaneous administration of Code D at a dose of 60 mg of sodium diclofenac. Each value represents the mean ± S.D. ($n = 4$). Dashed lines represent the plasma diclofenac concentration profiles for each subject after the administration.

TABLE 4

AVC of plasma diclofenac concentrations for 8 h after percutaneous administration of the gel of Code D in healthy human subjects

	Dose mg	AUC ng/h/ml	AUC/dose $\times 10^6$ h/ml	AUC for Code D / AUC for supp.
Mean of 4 subjects Code D	60	235.6 ± 107.4 *	3.9	
One subject of 37 years old and 75 kg				
Suppository	25	1027	41.1	
Code D	60	187.3	3.1	0.076

AUC ratio of diclofenac was determined by comparison of AUC after the percutaneous administration of Code D to that after the rectal administration of a commercial suppository. * The value represents the mean ± S.D. ($n = 4$).

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