International Journal of Pharmaceutics, 24 (1985) 115–124
Elsevier

IJP 00811

Percutaneous absorption of diclofenac sodium ointment

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(Received September 24th, 1984)
(Modified version received November 26th, 1984)
(Accepted December 4th, 1984)

Summary

In order to allow a high concentration of the drug to reach the lesion, preventing disorders in the digestive tract as a result of the oral administration of an anti-inflammatory steroid-type agent and to attain a sufficient plasma concentration to obtain an effective systemic effect, we prepared ointments and studied the percutaneous absorption of the drug in rabbits. Diclofenac sodium was rarely absorbed precutaneously when given as a simple ointment, hydrophilic ointment, absorption ointment, macrogols ointment described in the Japanese Pharmacopoeia, and carboxyvinylpolymer as gel ointment were used as the ointment bases. Therefore, we used a gel ointment made with methylcellulose as the ointment base and added isopropyl myristate to it as a sorbefacient, obtaining a good plasma concentration. Using the values obtained by the intravenous injection of diclofenac sodium, the parmacokinetics of the plasma concentration obtained by the percutaneous absorption was studied by two-compartment models containing two first-order rate constants. In addition, the retention of this drug in skin and the test for the loss of water and ethanol from the ointment were studied.

Introduction

Diclofenac sodium (mol. wt. 318.13) is a non-steroid-type of anti-inflammatory agent and is widely used clinically because of its strong analgesic, antipyretic and anti-inflammatory effect. It is known that this drug inhibits biosynthesis of the prostaglandin in vivo and in vitro and the drug is considered to have only a slightly

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adverse effect on the stomach and intestines (Manasse et al., 1978).

The in vivo metabolism of diclofenac sodium in humans were reported by Willis et al. (1980, 1981a and b) and others and the metabolic products obtained when it was administered to animals were reported by Stierlin et al. (1979a and b), Tsuchiya et al. (1980) and others.

Studies of the pharmacokinetics of diclofenac sodium were carried out by Willis and others (1978, 1979), Said and Sharaf (1981) and others. Furthermore, Riess and Stierlin (1978) mentioned that diclofenac circulates in the intestine and liver and binds with plasma protein at a high concentration level. At the same time, they stated that this binding with protein is not strong enough to inhibit the binding of other drugs including tolubutamide and warfarin with protein. Yaginuma et al. (1981, 1982) carried out a study on the rectal absorption of diclofenac sodium for the purpose of increasing its bioavailability. We prepared ointments and studied the percutaneous absorption of diclofenac sodium in rabbits to allow the drug to reach the inflammatory lesion directly at a high concentration level and prevent disorders in the digestive tract as a result of the administration of the drug. The results are reported herein.

Materials and Methods

Materials

The following reagents were used in this study: diclofenac sodium[1], methylcellulose (metolose SM-4000), hydroxypropylmethyl cellulose (metolose 65SH-4000)[2], benzene, ethyl alcohol, ethyl ether, methyl alcohol, 2.5 N phosphoric acid, 0.1 N sodium hydroxide, sulfuric acid, D-(+)-glucose (anhydrous), isopropyl myristate [3], dieldrin, propylene glycol [4] and *n*-hexane [5].

Animals

Rabbits weighing 2.0–2.3 kg (Keari, Japan) were used. The hair was removed with electric hair clippers from a 10×15 cm area of the skin of the addominal region 20 h before the experiment on percutaneous absorption. Each group consisted of 5 rabbits.

Intravenous injection and blood collection

To prevent hemolysis, diclofenac sodium, 2.5 mg/kg, was dissolved in 2 ml of a 5.8% glucose solution and the mixture was injected into the auricular artery of the rabbits. A T-shaped catheter (Okasan Kagaku, Kyoto, Japan) was inserted into the carotid artery and blood samples (about 0.5 ml) were collected sequentially (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 h).

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[1] Fujisawa Yakuhin Kogyo, Osaka, Japan.
[2] Shin-etsu Kagaku Kogyo, Tokyo, Japan.
[3] Nakarai Chemicals, Kyoto, Japan.
[4] Wako Pure Chemicals Industries, Osaka, Japan.
[5] Kanto Chemicals, Tokyo, Japan.

The blood samples were transferred to heparin-treated test tubes and centrifuged for 5 min at 3000 rpm to obtain 0.1 ml of plasma.

Preparation of ointment

We placed 3 g of methylcellulose (MC) or 3 g of hydroxypropylmethylcellulose (HPMC) into a beaker, added 20 ml of boiled distilled water, and mixed the suspension thoroughly. Then, 31.57 ml of cold water was rapidly added to the suspension and the mixture was thoroughly stirred in ice. In another beaker 12.00 g of propylene glycol, 30.00 g of ethanol and 2.00 g of isopropyl myristate, and 1.43 g of diclofenac sodium was added and dissolved completely. The mixture was gradually added to MC or HPMC which was dissolved in water and was stirred until it became a gel. If the amount of isopropyl myristate or diclofenac sodium was changed, the volume of distilled water was adjusted to give a total amount of 100 g. Table 1 shows the formula of the diclofenac sodium ointment in the experiment.

Percutaneous absorption

On the hair-free abdominal region of the rabbits, 7.0 g of the diclofenac sodium gel ointment which had been spread uniformly over a sheet of flannel cloth, 6×10 cm^2, was applied. Then, Saran Wrap (Asahi-Dow, Japan) was placed over this cloth and all the four edges were sealed with plastic tape (Nichiban). Blood samples were collected sequentially (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 h) and the plasma obtained was analyzed.

Homogenate of skin sections

The ointment was removed with a spatula and the surface to which the ointment had been applied was cleaned 100 times with cotton made wet with water, and then wiped 100 times with dry cotton.

Skin sections were cut from the epidermis to the dermis (2.0×10 cm^2) with a razor and weighed. Then they were sliced into pieces with a razor and homogenized for 5 min at about 1000 rpm with a glass homogenizer by using 10 ml of 0.001 N NaOH as the solvent. We designated 0.1 ml of the suspension as the test drug.

TABLE 1

FORMULA OF DICLOFENAC SODIUM OINTMENT

Ingredient	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Methyl cellulose (%)	3.00	–	3.00	3.00	3.00	3.00
Hydroxypropylmethyl cellulose (%)	–	3.00	–	–	–	–
Propylene glycol (%)	12.00	12.00	12.00	12.00	12.00	12.00
Ethanol (%)	30.00	30.00	30.00	30.00	30.00	30.00
Iso-propyl myristate (%)	2.00	2.00	2.00	2.00	1.00	5.00
Diclofenac sodium (%)	1.43	1.43	0.72	2.86	1.43	1.43
Distilled water (%)	51.57	51.57	52.28	50.14	52.57	48.57

Assay of diclofenac

Diclofenac was extracted from the plasma according to the method of Geiger et al. (1975). The extract was converted to the methylester derivative with the same method described by Ikeda et al. (1980), which was injected into a gas chromatograph equipped with ECD. That is, 4.0 ml of 2.5 N phosphoric acid and 6.0 ml of benzene were added to 0.1 ml of plasma and the mixture was shaken for 45 min, at 120 strokes/min, then centrifuged for 5 min at 3000 rpm. After that, 5.0 ml of the benzene phase was transferred to another test tube, and 3.5 ml of 0.1 N NaOH was added to it. The mixture was shaken for 5 min at 240 strokes/min and centrifuged for 5 min at 3000 rpm, and the supernatant was removed with an aspirator. Subsequently, 3.0 ml of the NaOH phase was removed, and 1.0 ml of 2.5 N phosphoric acid and 3.5 ml of the benzene phase was added to it. The mixture was centrifuged for 15 min at 240 strokes/min and was centrifuged for 5 min at 3000 rpm. Then 3.0 ml of the supernatant was pipetted into a test tube equipped with a screw cap and evaporated to dryness in a water bath at 40°C with a mild N_2 gas flow. To the residue, 0.15 ml of methanol containing 0.5% H_2SO_4 was added and the mixture was incubated for 1 h at 60°C. After it was cooled, 0.3 ml of purified water and 1.3 ml of *n*-hexane were added, and the mixture was stirred for 20 s in a mixer, then centrifuged for 5 min at 3000 rpm. Following this procedure, 1.0 ml of the organic phase was removed and evaporated to dryness in a water bath at 40°C with a mild N_2 gas flow. A 0.1 ml of *n*-hexane solution containing dieldrin (20 ng/ml internal standard) was added to the residue and the mixture was thoroughly stirred in a mixer and 2 μl of the mixture was injected into a gas chromatograph.

Gas chromatography

Diclofenac was assayed by gas chromatography under the following conditions:
gas chromatograph: Shimazu GC-6AMPFE
detector: electron capture (^{63}Ni 10 m Ci)
pulse rate: 10 kHz
column: 1% OV-17 chromosorb W; AW-DMCS 80-100 mesh 1.5 m × 3 mm
carrier gas: nitrogen
column temperature: 220°C
injection temperature: 280°C
detector temperature: 280°C.

Results and Discussion

In order to study the percutaneous absorption of an ointment pharmacokinetically, diclofane sodium was given intravenously to rabbits and the plasma concentration was measured sequentially (Fig. 1A). The pharmacokinetic analysis was performed and the observed values were obtained which complied well with the two-compartment model. On the other hand, Yamaoka et al. (1978) reported about statistical estimation of pharmacokinetic equation using the Akaike's information criterion (AIC) in which minimum value is regarded as the best representation of

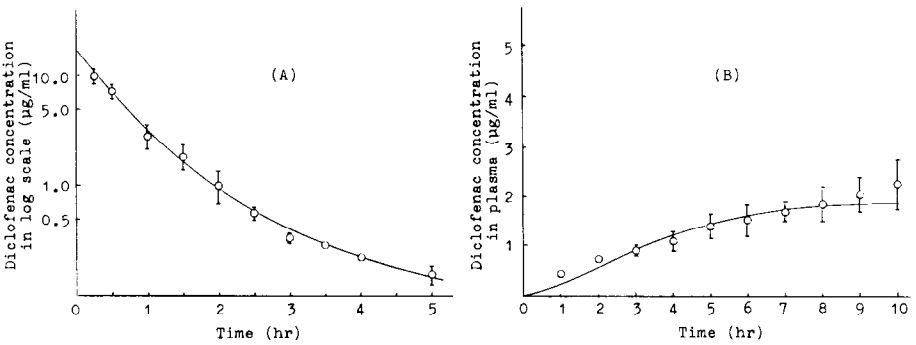


Fig. 1. Diclofenac concentration in plasma. A: after intravenous administration of diclofenac sodium (2.5 mg/kg) to rabbits. B: after application of the ointment (Sample B *). Each point represents the mean obtained from 5 rabbits with the standard error. Solid line (A) represents the curve calculated by using parameters in Table 2 and solid line (B) is the curve calculated from Eqn. 1 in Scheme 1.

TABLE 2

PHARMACOKINETIC PARAMETERS FOR INTRAVENOUS ADMINISTRATION OF DICLOFENAC SODIUM (2.5 mg/kg)

Parameter	Value
α	1.83 h^{-1}
β	0.44 h^{-1}
k_e	1.46 h^{-1}
k_{12}	0.26 h^{-1}
k_{21}	0.55 h^{-1}
V_c	302 ml

α and β are hybrid first-order rate constants; k_e = elimination rate constant from the central compartment; k_{12} = rate constant from the central to tissue compartment; k_{21} = rate constant from the tissue to central compartment; V_c = distribution volume of central compartment.

experimental data. In the present work, the equation of the two-compartment model yielded the lowest AIC among the various models, supporting its suitability to the model. The pharmacokinetic values obtained are shown in Table 2. As the ointment bases, a simple ointment as oleaginous base, hydrophilic ointment (O/W type) and absorption ointment (W/O type) as emulsion base, macrogols as water-soluble base described in the Japanese Pharmacopoeia, and carboxyvinylpolymer as gel base were used. Each ointment was adjusted to contain 1.43% diclofenac sodium. These ointments were applied to the abdominal region of the rabbits and the plasma concentration was measured, only to obtain unfavorable results. Therefore, we studied percutaneous absorption by using MC and HPMC as the gel ointments with and without additives. First, Sample B was prepared by using HPMC as the base and was applied to the skin (Table 1). Fig. 1B shows the plasma concentration when Sample B was used. In this case, the ointment was adjusted to contain 2.0%

* See Table 1.

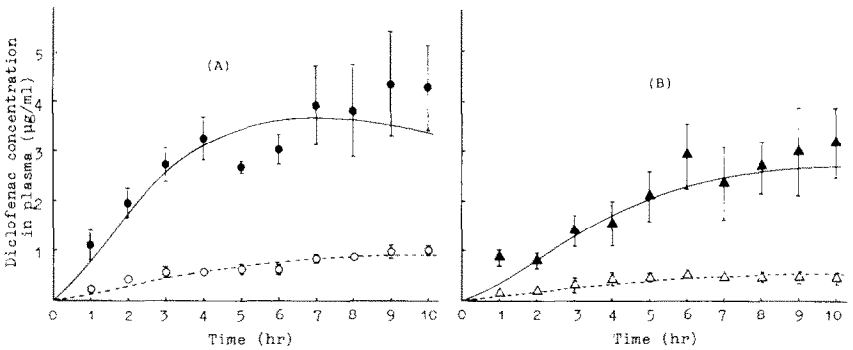


Fig. 2. Diclofenac concentration in plasma after application of ointments. Key: ○———○, Sample E *; ●------●, Sample F *; △———△, Sample C *; ▲------▲, Sample D *. Each point represents the mean obtained from 5 rabbits with the standard error. Solid and broken lines were calculated from Eqn. 1 in Scheme 1.

TABLE 3

PHARMACOKINETIC PARAMETERS FOR PERCUTANEOUS ABSORPTION

Ointment type	f [a]	k_r (h^{-1})	k_a (h^{-1})	Ratio of $AUC_{0\to10}$ [b]
Sample A	0.26	0.12	0.10	1.00 (15.16 ± 2.63 μg·h·ml^{-1})
Sample B	0.21	0.12	0.10	0.86
Sample C	0.12	0.12	0.10	0.25 [c]
Sample D	0.15	0.12	0.10	1.27
Sample E	0.12	0.09	0.10	0.41 [c]
Sample F	0.30	0.27	0.10	1.92 [c]

[a] Fraction of drug absorbed to the drug in the ointment base.
[b] Each ratio was calculated value when $AUC_{0\to10}$ of diclofenac after application of Sample A was 1.00.
[c] Significantly different from the Sample A, $P > 0.05$.

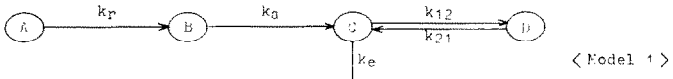
isopropyl myristate as the sorbefacient. When $AUC_{0\to10}$ was compared between Sample A and Sample B, it was found that Sample A yielded a much larger $AUC_{0\to10}$ than Sample B. Therefore, in the present work, we used MC as the base and measured the plasma concentration sequentially in ointments prepared under various conditions. With regard to changes in the concentration of isopropyl myristate in the ointment, the $AUC_{0\to10}$ of Sample A (2.0% isopropyl myristate was added to the ointment) was greater than that of Sample E (1.0% isopropyl myristate was added (Fig. 2A) and it was found to increase further if 5% isopropyl myristate was added (Fig. 2A) Sample F). However, regarding the degree of increase in $AUC_{0\to10}$, it was greater if $AUC_{0\to10}$ was compared with regard to the addition of 1% or 2% isopropyl myristate than if it was compared with regard to the addition of 2% or 5% isopropyl myristate. Therefore, we decided to add 2.0% isopropyl myristate. Fig. 2B shows the plasma concentrations of the ointments containing the main

* See Table 1.

drug, i.e. 0.72 or 2.86% diclofenac sodium. Table 3 shows that $AUC_{0\to10}$ obtained from the gel ointments using MC as the base and containing 0.72, 1.43 or 2.86% diclofenac sodium. As indicated in Table 3, the ratios of AUC were 0.25, 1.00 and 1.27, respectively. Considering the degree of increase for $AUC_{0\to10}$, we designated the concentration of diclofenac sodium as 1.43%. Then, a pharmacokinetic study of the percutaneous absorption of the ointments was carried out. Recently, Guy et al. (1982) reported about a new pharmacokinetic model for percutaneous absorption and first-order kinetics are assumed for the reserving process. However, no experimental evidence was shown in the reserving process. In the present study, the reserving rate constant could not be determined, and therefore percutaneous absorption of diclofenac was presumed to follow Model 1 in Scheme 1. The k_r is not

Scheme 1

Drug in ointment Drug in skin Drug in plasma Drug in tissue



〈Model 1〉

$$C_C = \frac{k_r\, k_a\, f\, A_0}{Vc}\left[\frac{k_{21}-\alpha}{(\beta-\alpha)(k_r-\alpha)(k_a-\alpha)}e^{-\alpha t} + \frac{k_{21}-\beta}{(\alpha-\beta)(k_r-\beta)(k_a-\beta)}e^{-\beta t} + \frac{k_{21}-k_r}{(\alpha-k_r)(\beta-k_r)(k_a-k_r)}e^{-k_r t} + \frac{k_{21}-k_a}{(\alpha-k_a)(\beta-k_a)(k_r-k_a)}e^{-k_a t}\right]$$

〈Kan. 1〉

C_C : Diclofenac concentration in central compartment

f : Fraction absorbed

A_0 : Dose

regarded as a simple rate constant but as an apparent release rate constant including the diffusion rate constant and other unknown factors. Arbitrary values were substituted in f, k_r and k_a and a combination of these data corresponding to the actual measurement value was obtained. As a result, 0.10 h^{-1} was obtained as a k_a for the average of the respective ointments. Therefore, it is considered that once diclofenac sodium is released from the ointment base, it is absorbed according to a regular absorption rate constant. In this case, if k_a is presumed to be constant, 0.10 h^{-1}, f and k_r are fixed as certain values. The values are shown in Table 3. The solid line and broken line in Figs. 1B, 2A, 2B and 3A are theoretical curves obtained from these constants. The increase and decrease in the concentration of isopropyl myristate was found to affect f and k_r and changes in the concentration of diclofenac sodium in the base were found to influence f. In order to study the retention in the skin, MC gel ointment (Sample A, Table 1) containing 2% isopropyl myristate and 1.43% diclofenac sodium was used. At the desired time after it was applied to the abdominal region of the rabbits, the animals were sacrificed and 2.0 cm^2 of skin was removed from the area of application. This skin was homogenized and the amount of diclofenac in the skin from the corneum to the dermis was determined. The results are shown in Fig. 3A. Some degree of retention of the diclofenac in the skin was observed if this base was used. Also, 6 h after the application, the concentration of

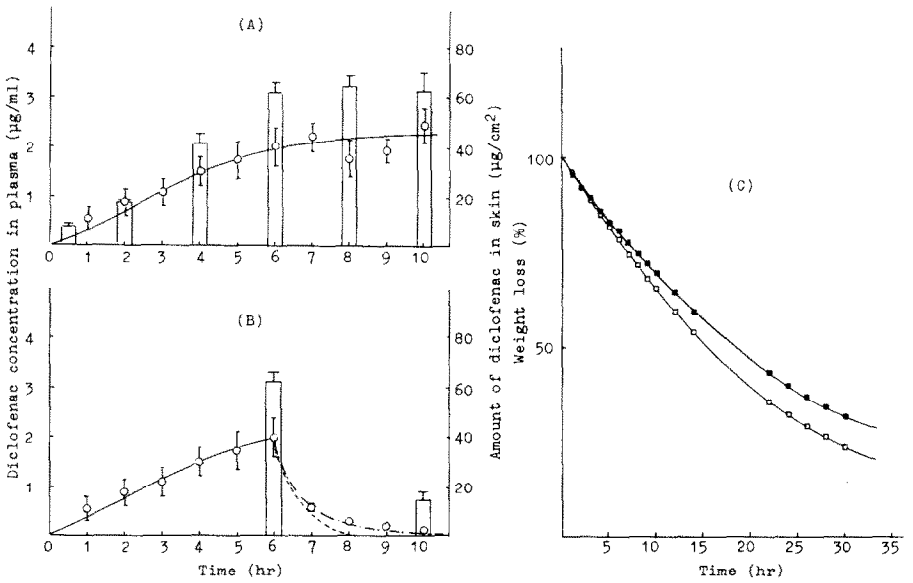


Fig. 3. Diclofenac in plasma and skin. A: After application of the ointment (Sample A *). B: when ointment (Sample A *) was removed 6 h after the application. Each point or column exhibits the mean obtained from 5 rabbits with the standard error in plasma or in skin. Solid line, dash-and-dot line and broken line were derived from Eqn. 1 in Scheme 1, Eqn. 2 in Scheme 2 and Table 2, respectively. Loss of water and ethanol from ointment at 37 ± 1°C (C) Key: ■———■, Sample A *; □———□, an ointment which is commercially available.

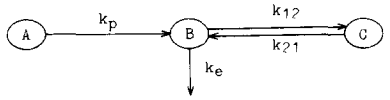
diclofenac in the skin sections was measured in a region 1.0 cm and 3.0 cm away from the area of application, and the respective ratios to the concentration obtained from the site of application at the same time were found to be 2.8 and 0.3%, respectively. Therefore, the infiltration of diclofenac into the skin was demonstrated although the degree was slight. The plasma concentration was measured sequentially after the ointment (Sample A) was applied to the abdominal region of rabbits for 6 h and then the region was cleaned. The theoretical curve of disappearance of diclofenac from the blood 6 h later was obtained from each kind of parameter in Table 2, which is represented as a broken line in Fig. 3B. However, the actual curve did not correspond to this, and diclofenac was present in the plasma even 10 h later as shown by the dash-and-dot line although the amount was small. If this difference in the plasma concentration is considered to be due to the release of the diclofenac sodium retained in the skin into the plasma and this is presumed to follow the two-compartment model containing one first-order release process (Model 2 in Scheme 2), its release rate k_p became 1.10 h^{-1} when obtained by the fitting technique. The drug was found to remain in the skin, as shown in Fig. 3B, although the plasma concentration was low 10 h later.

The results obtained from the experiment on the loss of water and ethanol from

* See Table 1.

Scheme 2

Drug in skin Drug in plasma Drug in tissue



〈Model 2〉

$$C_B = \frac{k_p \cdot f \cdot A_0}{V_c}\left[\frac{k_{21} - k_p}{(\alpha - k_p)(\beta - k_p)} \cdot e^{-k_p \cdot t} + \frac{k_{21} - \alpha}{(k_p - \alpha)(\beta - \alpha)} \cdot e^{-\alpha \cdot t} + \frac{k_{21} - \beta}{(k_p - \beta)(\alpha - \beta)} \cdot e^{-\beta \cdot t}\right] \quad \langle \text{Eqn. 2} \rangle$$

C_B : Diclofenac concentration in central compartment

f : Fraction absorbed

A_0 : Dose

the ointment (Sample A) used in the retention experiment are shown in Fig. 3C. When analgesic and anti-inflammatory gel ointments (Inteban, Sumitomo Chemicals) which are sold on the market were compared with this ointment, there was not so great a difference in the changes in the weight of the ointment due to the loss of water and ethanol between the two. Therefore, it is almost certain that this ointment has a stability parallel to that of drugs sold on the market. It has been reported that the C_{max} of the plasma concentration is within 1–2 μg/ml in most people if a routine dose of this ointment is administered once to humans (Willis and Kendall, 1978). Sample A ointment attained a plasma concentration of 1–2.5 μg/ml 2–10 h later when it was applied to rabbits.

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