PREDICTION OF PLASMA CONCENTRATION PROFILE DURING SINGLE AND REPEATED SKIN APPLICATIONS OF INDOMETHACIN AND ITS CALCIUM SALT OINTMENTS

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To determine the plasma concentrations and to clarify pharmacokinetic behavior from a pharmaceutical application viewpoint, indomethacin (IND) and its calcium salt (IND-Ca) in the form of gel ointments were applied singly and repeatedly to rat abdominal skin under occlusions. In order to describe the plasma IND concentration profile during repeated percutaneous administration, a highly simple pharmacokinetic model including a first-order absorption process with a lag time was applied. IND and IND-Ca were readily absorbed with a short lag time from IND-Ca ointment (1% as IND, 0.14 g/cm², 3.0 × 1.2 cm area) containing Azone®. Even when the applied dose of IND-Ca ointment was decreased to one-fourth (1.0 × 1.0 cm area), the plasma IND concentrations were maintained above the effective levels for 24h. The use of the pharmacokinetic model was justified by good agreement with observed data obtained after repeated percutaneous application of ointments. Thus, the plasma IND concentration-time courses during repeated percutaneous administration can be predicted by using the model.

Keywords — percutaneous absorption; repeated application; indomethacin; indomethacin calcium; kinetic model; ointment; Azone; rat; plasma concentration prediction

INTRODUCTION

Indomethacin (IND) is one of the most extensively used nonsteroidal anti-inflammatory agents for oral or rectal administration in the treatment of rheumatoid arthritis, gout, ankylosing spondylitis and osteoarthritis of large joints but the gastro-intestinal side effects of IND restrict its use in the treatment of certain other diseases.¹⁾ Recently, an IND gel ointment has been developed and used for topical application.²⁾ The IND gel ointment is shown to be very effective for exterior diseases such as arthritis deformans, arthralgia, muscle ache, tenositis and tenosynovitis but to be less effective for interior diseases, *e.g.* the inflammation of lumbar vertebra and hip joint.³⁾

Our previous studies with IND-calcium (IND-Ca) gel ointment containing Azone® (AZ) indicated that the salt of IND was significantly absorbed into the rat skin and that therapeutically effective concentrations were maintained for 48 h.⁴⁾ Thus, the IND-Ca gel ointment is useful in a topical dosage form aimed at systemic effect of the anti-inflammatory drug.

Many reports regarding topical dosage form aimed at systemic effect have been published.⁵⁾ However, the pharmacokinetics of IND after

topical application has not been fully clarified, even though a pharmacokinetic model which can account for the percutaneous absorption of IND after a single topical administration was presented by Naito *et al.*⁶⁾

The primary aim of the present study was to propose a simple pharmacokinetic model that describes the percutaneous absorption and to clarify pharmacokinetic behavior during repeated topical applications. The second objective was to obtain therapeutically effective concentrations at small doses of the ointment for practical application.

MATERIALS AND METHODS

Materials — IND (J. P. grade) and AZ were generous gifts of Sumitomo Seiyaku Co., Ltd. and Nelson Research and Development Co., respectively. Penfluridol, an internal standard for gas-liquid chromatography, was purchased from Janssen Pharmaceutica. Trisilildiazomethane as a methylating agent and Hiviswako 104® were purchased from Tokyo Kasei Kogyo Co., Ltd. and Wako Junyaku Co., Ltd. respectively. Calcium salt of IND was prepared according to a previous method.⁷⁾ All other chemicals used were of reagent grade.

TABLE I. Compositions of IND and IND-Ca Ointments and Application Area

Exp.	Content (% w/w)				Application area (cm × cm)
	Drug ^{a)}	DMSO	EtOH	AZ ^{b)}	
1 IND	1	10	20	6	3.0 × 1.2
2 IND-Ca	1	10	20	6	3.0 × 1.2
3 IND-Ca	1	10	20	6	1.0 × 1.0

a) Drug content as IND. b) AZ (1-dodecyl-azacycloheptane-2-on). Sorbitan monooleate (6%) was added as an emulsifier.

Preparation of Ointments — IND or IND-Ca was dissolved in dimethylsulfoxide and mixed with an ointment base (Hiviswako 104[®]) containing water, ethanol, diisopropyl adipate, diisopropanolamine and/or absorption enhancers (AZ and sorbitan monooleate). Details of the ointment composition are listed in Table I.

Intravenous (i.v.) Administration — IND (4 mg/kg) was administered intravenously as a solution in 0.1 M phosphate buffer (pH 7.0) after dissolving in a small amount of 0.1 N NaOH.

In Vivo Percutaneous (p.c.) Absorption Experiments — A) A Single Administration: Male Wistar rats weighing 210–230 g were used in the *in vivo* experiment. On the day before the experiment, the rat jugular vein was cannulated with a polyethylene tubing and the hair of the abdominal area was carefully removed according to the method as described in previous papers.^{4,8)} On the next day, 0.14 g/cm² of ointment was uniformly spread over the shaved abdominal skin (3.0 × 1.2 or 1.0 × 1.0 cm area) under pentobarbital anesthesia. The ointment remained in contact with the skin for 8 h under occlusions, and then the unabsorbed ointment was wiped off with absorbent cotton soaked in warm water, as described in a previous paper.⁴⁾ Blood samples (0.25 ml) were collected periodically after dosing through the heparinized polyethylene tubing. The plasma was separated immediately by centrifugation and stored frozen until assay.

B) Repeated Administration: The ointment was applied for 8 h by the same method as the single administration and the application was repeated for 3 d. The application area is described in Table I. For Exps. 1 and 2, the first and third application of ointment was done on the same site, but for Exp. 3 each application site was different.

Analytical Method — IND in plasma samples

was determined by the method⁹⁾ described previously with a slight modification: trisilildiazomethane was used as methylating agent instead of diazomethane.

Pharmacokinetic and Statistical Analyses — To analyze the plasma IND concentration during multiple p.c. dosing, the data, after a single dosing, were fitted to the 2-compartment model including a first-order absorption process with a lag time (t_{lag}) which is the time to reach the steady-state absorption rate and was determined by simulation, in which the parameters, α , β , V_1 and k_{21} were fixed at values obtained from i.v. data:

$$C_t = \frac{F \cdot X_0 \cdot k_a}{V_1} \left\{ \frac{(k_{21} - k_a)}{(\alpha - k_a)(\beta - k_a)} e^{-k_a(t - t_{lag})} + \frac{(k_{21} - \alpha)}{(k_a - \alpha)(\beta - \alpha)} e^{-\alpha(t - t_{lag})} + \frac{(k_{21} - \beta)}{(k_a - \beta)(\alpha - \beta)} e^{-\beta(t - t_{lag})} \right\} \quad (1)$$

where k_a is the absorption rate constant and F the fraction of the drug absorbed. All plasma data were analyzed by the non-linear iterative least squares regression analysis, MULTI.¹⁰⁾

The area under the plasma drug concentration-time curve (AUC) after i.v. and p.c. administrations was calculated by using all the experimental points in the trapezoidal method. The absolute bioavailability was calculated by the following equation:

$$\text{bioavailability (\%)} = \frac{AUC_{p.c.} \cdot \text{Dose}_{i.v.}}{AUC_{i.v.} \cdot \text{Dose}_{p.c.}} \times 100$$

where $AUC_{p.c.}$ and $AUC_{i.v.}$ are AUC after p.c. and i.v. administrations, respectively. In a single

i.v. dosing, the plasma IND concentration data for individual animals were fitted to the equation:

$$C_t = A e^{-\alpha t} + B e^{-\beta t} \quad (2)$$

where C_t is the drug concentration at time t , A , α , B and β are the biexponential equation constants.¹¹⁾ The means of all data are presented with their standard deviations (S.D.). Statistical analysis was performed using the non-paired Student's t -test, and a p -value of 0.05 or less was considered to be significant.

RESULTS

A small amount of drugs (1%) and small areas of application sites were used in this experiment, for practical purposes, compared with amounts used (5% ointment, 1 or 0.3 g, 3.0×3.0 or 1.7×1.7 cm area) in a previous paper.⁴⁾

Single p.c. Administration and a Pharmacokinetic Model

The plasma IND concentrations after a single p.c. application (0.14 g/cm^2 , 3.0×1.2 or 1.0×1.0 cm area) of ointments are shown in Fig. 1. IND and IND-Ca were readily absorbed through the skin with a short lag time, and the peak plasma concentration ($C_{\max}$) occurred about 8 h after treatment. To describe the plasma concentration pattern of IND after p.c. administration, a simple pharmacokinetic model including a first-order absorption process with a lag time, as shown in Fig. 2, was used. The time course of plasma concentration after p.c. administration was simulated using the model. The parameters in Eq. 1, α , β , V_1 and k_{21} , were fixed at the values obtained from i.v. data shown in Table II. The simulation curve and the best-fit

FIG. 1. Plasma Concentration of IND and Fitting Curve after Single p.c. Administration

Solid line represents a fitting curve calculated by means of the 2-compartment model in Fig. 2 and each point represents the mean $\pm$ S.D. of 4–6 rats. ●, IND ointment with AZ (Exp. 1); ■, IND-Ca ointment with AZ (Exp. 2); ▲, IND-Ca ointment with AZ (Exp. 3).

FIG. 2. Two-Compartment Model Including a First-Order Absorption Process with Lag Time

Where D is the drug administered, k_a is the absorption rate constant, k_{12} and k_{21} are distribution rate constants and k_{el} is the elimination rate constant.

TABLE II. Kinetic Parameters after i.v. and Single p.c. Administrations

Parameter	i.v.	Parameter	p.c.		
			Exp. 1	Exp. 2	Exp. 3
A ($\mu\text{g/ml}$)	24.91 ± 4.83	F	0.184 ± 0.019	$0.364 \pm 0.035^a)$	$0.315 \pm 0.052^b)$
α (h^{-1})	0.623 ± 0.072	k_a (h^{-1})	0.122 ± 0.026	0.124 ± 0.022	0.163 ± 0.052
B ($\mu\text{g/ml}$)	2.371 ± 0.162	t_{lag} (h)	2.95 ± 0.44	2.84 ± 0.44	3.43 ± 0.04
β (h^{-1})	0.122 ± 0.004				
k_{21} (h^{-1})	0.166 ± 0.021				
V_1 (l/kg)	0.144 ± 0.021				

Each value represents the mean $\pm$ S.D. of 4–6 rats. a) $p < 0.01$, b) $p < 0.05$ compared with Exp. 1.

FIG. 3. Plasma Concentration of IND during Repeated p.c. Administration

Solid line represents the simulation curve and each point represents the mean $\pm$ S.D. of 4–6 rats. The dose of p.c. administration was 0.14 g/cm². Symbols are the same as in Fig. 1.

parameters, F , k_a , t_{lag} , are shown in Fig. 1 and Table II, respectively. The absorption rate constant, k_a and t_{lag} were not significantly different among the ointments. However, the fraction of drug absorbed (F) from IND-Ca ointment (Exp. 2) was significantly higher than that from IND ointment (Exp. 1) ($p < 0.01$).

Repeated p.c. Administration and Prediction of Plasma Drug Concentration Time Courses

The plasma IND concentrations and kinetic parameters during repeated p.c. application of ointments are shown in Fig. 3 and Table III, respectively.

The third dosing of Exp. 1 (IND) or 2 (IND-Ca) was applied to the same site as the first dosing. Drastic increases in the C_{max} and AUC after the third dosing were observed in comparison with those after the first dosing, suggesting that the enhancing effect of AZ was retained for a long time. For Exp. 3 (1.0 $\times$ 1.0 cm area), in which application sites were changed daily for 3 d, no additional effect was seen. Each AUC of IND-Ca (Exp. 2) during repeated p.c. administration was 3 times higher than that of IND (Exp. 1).

The solid line in Fig. 3 shows the simulation

TABLE III. Kinetic Parameters after Repeated Percutaneous Administration

Day	Parameter	Exp.		
		1	2	3
1	$AUC(\mu\text{g} \cdot \text{h/ml})$	50.07 $\pm$ 10.01	168.27 $\pm$ 38.64	33.84 $\pm$ 5.94
	$C_{max}(\mu\text{g/ml})$	4.143 $\pm$ 0.864	10.373 $\pm$ 3.421	2.757 $\pm$ 0.917
	$C_{min}(\mu\text{g/ml})$	0.915 $\pm$ 0.142	2.621 $\pm$ 1.490	0.454 $\pm$ 0.212
2	$AUC(\mu\text{g} \cdot \text{h/ml})$	61.01 $\pm$ 11.01	151.49 $\pm$ 52.64	38.81 $\pm$ 11.43
	$C_{max}(\mu\text{g/ml})$	3.900 $\pm$ 1.101	9.666 $\pm$ 5.946	2.449 $\pm$ 0.433
	$C_{min}(\mu\text{g/ml})$	0.857 $\pm$ 0.310	1.706 $\pm$ 1.416	0.505 $\pm$ 0.181
3	$AUC(\mu\text{g} \cdot \text{h/ml})$	71.62 $\pm$ 17.75 ^{b)}	207.87 $\pm$ 41.34	38.75 $\pm$ 12.45
	$C_{max}(\mu\text{g/ml})$	5.897 $\pm$ 0.619 ^{a,c)}	16.754 $\pm$ 3.182 ^{a,c)}	2.673 $\pm$ 0.592
	$C_{min}(\mu\text{g/ml})$	0.730 $\pm$ 0.215	1.886 $\pm$ 0.899	0.323 $\pm$ 0.108

Each value represents the mean $\pm$ S.D. of 4–6 rats. a) $p < 0.02$, b) $p < 0.05$ compared with day 1. c) $p < 0.02$ compared with day 2.

curve of multiple p.c. dosing, calculated by using the parameters obtained from the single dosing (Table II) and i.v. data. For Exp. 3, a good fit was obtained for the calculated line compared with the experimental data. However, for Exps. 1 and 2, in which the third application was done at the same site as the first application, the plasma concentrations after the third dosing were higher than the fitting curve, and the simulation curve calculated did not agree wholly with the observed values. The pharmacokinetic model could describe the plasma IND concentration profile during repeated p.c. administration. Consequently, it is possible to predict the plasma concentrations after repeated p.c. applications by using the model.

DISCUSSION

It is reported that when the commercial product of IND ointment (10 g) is applied to human skin, the plasma peak concentration is approximately 20 ng/ml and only 1% of the applied dose is excreted into the urine during 3 d.¹²⁾ Kanno has reported that repeated administration of commercial IND ointment significantly increased the drug levels at the application site and its surroundings, but not in the blood.³⁾ After a single p.c. administration of IND-Ca ointment (2%, 1.5×1.2 cm area, data not shown) the bioavailability was about 70% for 36 h. In spite of the small dose compared with that in a previous paper,⁴⁾ this value was similar to that after oral or rectal administration of IND (80–100%).¹³⁾ In this study, a higher plasma concentration was experimentally observed for Exp. 2 (IND-Ca ointment), suggesting the rapid penetration of drug through the skin and therapeutically effective levels (0.5 – 3.0 $\mu\text{g/ml}$)¹⁴⁾ were maintained over 24 h after Exp. 2 administration.

Watton *et al.* describe that in *in vitro* experiments, the enhancing effect of AZ remains in rat skin after removing the drug solution containing AZ.¹⁵⁾ Similar results were obtained after repeated dosing of ointments (Exps. 1 and 2), suggesting that the effect of AZ remained for about 48 h after the first dosing. This also means that the changes in stratum corneum induced by AZ are not restored for 48 h after the dosing. Therefore, it should be emphasized that the alteration of application sites is necessary for multiple administration of ointments, in both animal and human

experiments, to obtain the appropriate pharmacokinetic response. This approach of treatment was strengthened by the data of Exp. 3 in which the ointment was applied to the different sites. The effect on drug penetration by dimethylsulfoxide (DMSO), a penetration enhancer, and sorbitan monooleate must also be considered in this study. However, it was already demonstrated in our previous paper that these reagents did not induce a significant enhancing effect in the gel ointment under the occlusion.⁴⁾ In addition, all of these ointments, which contained AZ, DMSO, sorbitan monooleate and drug together, produced no apparent skin irritation after an 8-h application. Thus, the enhancing effect based on skin irritation was not involved in the result obtained.

Naito and Tsai presented a pharmacokinetic model which can account for the p.c. absorption of IND after topical administration.⁶⁾ Their model describes the plasma concentration profile at the early time stage, however it does not include the elimination phase. In order to explain the plasma concentration–time data of IND after p.c. administration, we have applied a simple pharmacokinetic model including a first-order absorption process with a lag time. The time course of plasma IND levels obtained during the repeated administration of ointments (Exps. 1, 2 and 3) was similar to that of repeated oral dosing. Therefore, it may be possible to adapt the pharmacokinetic model for oral administration to the p.c. dosing with slight modifications. The stratum corneum has a reservoir function for many drugs. The first step for p.c. penetration of drugs is drug saturation of the stratum corneum, so that drugs can penetrate the more interior layers of the skin. Thus, it is reasonable to step up the saturation time of drugs (t_{lag}) in the stratum corneum before the systemic circulation in the skin permeation kinetics. Since the time for drug release from ointments was exceedingly short compared with the t_{lag} , the release time was not considered in the present model. The model shown in Fig. 2, including the t_{lag} , was adapted to the observed data during repeated dosing. A good fit was obtained between the observed and calculated plasma IND levels for Exps. 1, 2 and 3, except for the levels on the third day of Exps. 1 and 2. Thus, the seemingly complicated absorption process in skin was simplified by the lag time (t_{lag}).

In clinical therapeutics, the pharmacokinetic analyses must be performed by a simple method using limited data. In this study the proposed approach might be useful for developing insight into the prediction of plasma concentration-time course during multiple p.c. dosing of IND and IND-Ca ointments, by using limited data.

In conclusion, our proposed model in the present paper finds support in good agreement with observed data obtained during repeated p.c. administration of the ointments. Therefore, the model appears to be useful for the prediction of plasma concentration-time courses after p.c. administration of IND.

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