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Biopharmaceutical Characteristics of Indomethacin Gel Ointment

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The characteristics of indomethacin (ID) gel ointment were compared with those of conventional types of ointments.

1) The percutaneous absorption of ID from a 50% alcoholic solution occurred by a simple diffusion process, since the percentage of absorbed ID did not depend on the concentration of ID.

2) The *in vitro* release of ID from the gel ointment was greater than that from conventional ointments.

3) The gel ointment had greater volatility and the concentration of ID in the gel base doubled in 4 hours under the conditions of 75% relative humidity at 25°C.

4) Reflecting the results of *in vitro* studies, the absorption of ID from the gel ointment into rat skin and muscle was greater than that from conventional types of ointments.

5) From the time course of muscle ID levels, little lag time of ID transfer through the skin was apparent.

Keywords——indomethacin; gel ointment; percutaneous absorption; rat; *in vitro* release; volatility

Indomethacin (ID) is a potent nonsteroidal anti-inflammatory agent, which has been limited to oral and rectal administration.[1,2]

An ID gel ointment has recently been developed for topical application. It was reported that the gel ointment of ID exerted substantial anti-inflammatory effect[3] and the presence of ID in the muscle and the skin was demonstrated through percutaneous absorption experiments.[4,5]

Percutaneous absorption of a drug from an ointment involves two consecutive processes, that is, drug release from the ointment base and subsequent drug absorption through the skin barrier. In the cases of conventional types of ointments, for example, oily, emulsified and water-soluble ointments, many studies have been accumulated on the *in vitro* drug release profiles from the ointment base and *in vivo* drug absorption.[6] It has been pointed out that drug release from the ointment base is one of indices of bioavailability for the ointment.[7] However, extensive biopharmaceutical studies on the gel ointment have not yet been performed, since this type of ointment has been introduced to the market quite recently.

In this study, the basic absorption process of ID and the characteristics of the gel ointment were compared with those of conventional ointments.

Experimental

Materials and Preparation of Ointments——Indomethacin was synthesized and micronized to 1—2 μ by Sumitomo Chemical Co. Plastibase was obtained from Japan Squibb Co. Other ointment bases were as described in J.P.IX. The ointments containing 1% ID were prepared by the methods of J.P.IX. Both white petrolatum and plastibase contained 1% liquid paraffin. As the gel ointment, commercially available Inteban Ointment® (Sumitomo Chemical Co.), prepared according to the reported method,[8] was employed. A list of the ointments used is shown in Table I. Precoated Kieselgel 60 F_{254} and cellulose F_{254} plates were obtained from E. Merck.

Percutaneous Absorption from Solution——The procedure of the percutaneous absorption experiment with a recirculation apparatus followed the method of Washitake *et al.*[9] The temperature of the solution and the recirculation flow rate were controlled at 36±1°C, 15±2 ml/min, respectively. The recirculation

Table I. Classification of the Ointments used in the Experiments

Classification	Ointment
Oily base	White petrolatum ointment Plastibase ointment
Emulsified base	Absorption ointment (w/o) Hydrophilic ointment (o/w)
Water-soluble base	Macrogol ointment
Gel base	Gel ointment

experiments were carried out with ethanol: pH 6.0 phosphate buffer (1: 1) solution of ID at the following concentrations; 5, 50, 200 and 500 μg/ml. Twenty ml of the 50% alcoholic solution was initially placed in the recirculation cell. In this experiment, the net water flux was found to be negligible using phenol red as an indicator. An aliquot of 1 ml was taken up from the cell periodically after initiation. Indomethacin was extracted with dichloroethane and was assayed spectrophotometrically at 320 nm.

Release of ID from the Ointments——The release of ID from the ointment base was determined using the apparatus shown in Fig. 1 (Toyama Sangyo Co., TMS 103). A 500 ml aliquot of 0.01 M, pH 6.0 phosphate buffer solution was warmed to 37°C in the flask and about 3 g of the ointment was applied to Toyo filter paper No. 2 at the bottom of the cylinder. The temperature of the solution was held at 37°C, with stirring at 150 rpm. An aliquot of 5 ml was taken up through a sampling hole at 15, 30 and 60 min after initiation.

The Volatility of Various Ointments——About 3 g of each of the ointments shown in Table I was spread on filter paper and allowed to stand at 25°C, 75% relative humidity. The weight of the ointment was measured periodically.

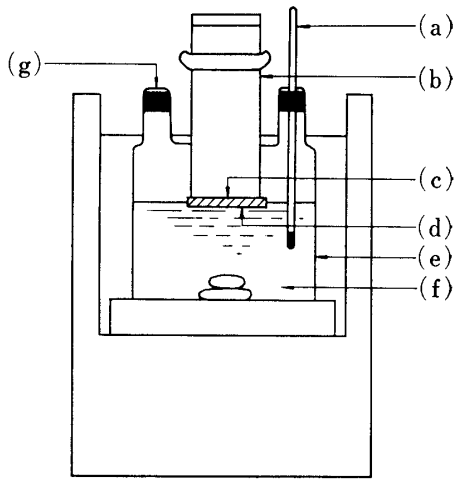

Fig. 1. *In Vitro* Release Apparatus

(a) thermometer.
(b) cylinder.
(c) ointment.
(d) filter paper.
(e) flask.
(f) phosphate buffer (pH 6.0).
(g) sampling hole.

Thin-Layer Chromatography (TLC)——About 10 mg of each of the ointments was spotted on TLC plates of silica gel and cellulose. The plates were developed with *n*-octyl alcohol and distilled water, respectively. The pattern of drug release from the ointment was examined under ultraviolet (UV) light.

Percutaneous Absorption from the Ointments——Male Wistar albino rats weighing 200—250 g were used. The procedure for determining percutaneous absorption from the ointment was the same as described in the report of Ishihama *et al.*[5] The hair of the abdominal skin was cut with an electric hair clipper and a razor on the day prior to application of the ointments. On the next day, the rats were anesthetized with 2 ml of a 10% (w/v) urethane aqueous solution. After they had been fixed on their backs, about 100 mg of ointment (Table I) was spread on the skin (7.07 cm^2). After 6 h, unabsorbed ointment was wiped off with ethanol: water (1: 1) solution. The skin and the muscle of the application site were removed.

The skin was finely cut up with scissors and extracted with ethanol: water (1: 1) solution for 15 h at room temperature with continuous shaking. The muscle was homogenized with ethanol: 0.1 M citric acid, pH 5.0 (1: 1) solution.

Assay of ID——In the *in vitro* and *in vivo* experiments, ID was extracted with dichloroethane, methylated with diazomethane and assayed by electron capture gas chromatography based on the method of Jensen.[10] Before methylation, 2,4,6-triphenyl-nitrobenzene was added as an internal standard. A Shimadzu GC-5A gas chromatography (Kyoto, Japan) equipped with a ^{63}Ni-electron capture detector was used under the following conditions: column, 0.3 cm × 1.5 m glass tube packed with 3% OV-1 on Gas Chrom Q 60/80; column temperature, 250°C; injection port temperature, 280°C; detector block temperature, 300°C, carrier gas, 99.999% N_2 (38 ml/min).

Results and Discussion

The Basic Kinetics of the Percutaneous Absorption of ID

It has been widely accepted that there is a rapid transient diffusion followed by a steady state diffusion in the process of percutaneous drug absorption.[11] Thus, to clarify the basic

kinetics of the percutaneous absorption of ID through the rat skin, a 50% alcoholic solution of ID was used as the experimental system, so that the interaction between ID and the ointment base was eliminated. The results of *in situ* recirculation experiments are shown in Fig. 2. The concentration of ID in the alcoholic solution decreased linearly after the rapid decrease at the initial stage. The rapid transient diffusion might be attributed speculatively to the adsorption rate to the skin surface and the diffusion rate into the unstirred water layer in the skin both being rapid compared with the overall diffusion rate of the drug through the skin tissue.[12] Similar experiments were performed with various concentrations of ID. The results are shown in Fig. 3. It is evident that the percentage of absorbed ID was constant for every tested concentration.

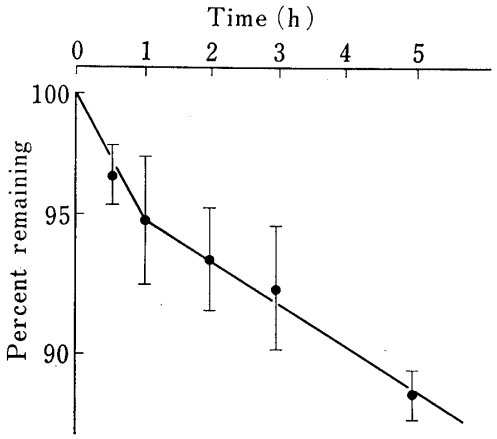

Fig. 2. Logarithmic Plot of Remaining ID in the Recirculating Solution

Initial concentration of ID in the solution was 200 μg/ml. Each point represents the mean ± S.E. of 4 rats.

These results suggest that the absorption of ID occurs by a simple diffusion process, since the percentage of absorbed ID does not depend on the concentration of ID.

In Vitro Release of ID from Various Ointments

It has been suggested by Washitake *et al.* that there are two processes in percutaneous absorption, that is, direct absorption into the skin and indirect absorption of drug *via* the aqueous phase of the *stratum corneum*, and that the latter is the main process of drug absorption from an ointment.[7] In the latter case, the release of ID from an ointment base into the aqueous phase must be considered as the major factor in the percutaneous absorption of ID.

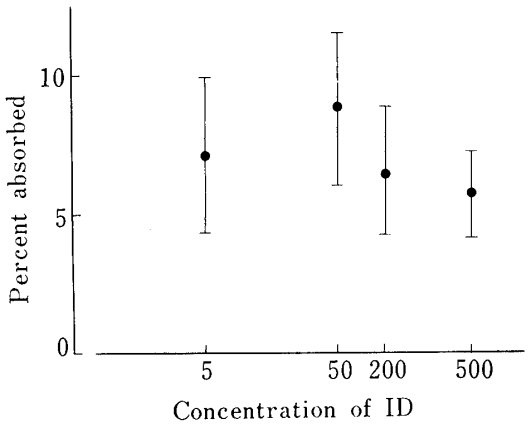

Fig. 3. Effect of ID Concentration on Percutaneous Absorption from the Reciruclating Solution during 3 Hours

Each point represents the mean ± S.E. of 4 rats.

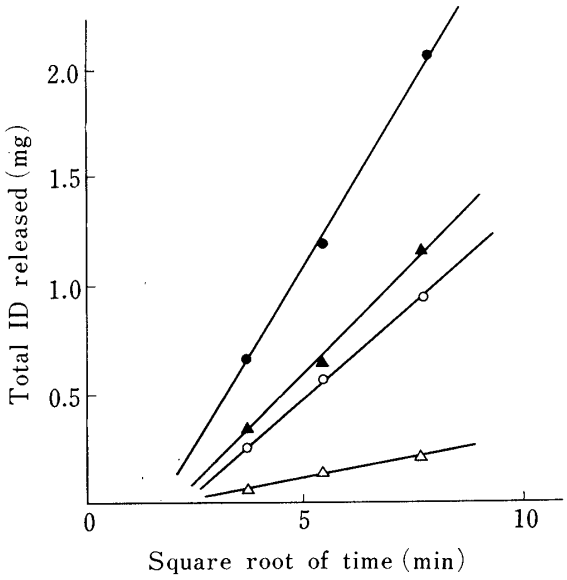

Fig. 4. Effect of Ointment Base on the Release of ID

●; gel ointment, ○; hydrophilic ointment, ▲; absorptive ointment, △; white petrolatum ointment, plastibase ointment.

The amount of each ointment used in the release test was about 3 g.

Each point represents the mean of 3 replicates.

Although various methods have been reported to examine drug release from ointments,[13–16] a unified method has not been established so far. Thus, the release of ID from various ointments into a pH 6.0 phosphate buffer was examined using the release apparatus shown in Fig. 1.

Figure 4 illustrates the variation of ID release from various ointments containing the same amount of ID. The experimental data were fairly well approximated by a straight line when the amount of ID released was plotted *versus* t,$^{1/2}$ where t is the time. Similar phenomena have been reported for the release of corticoids and benzocaine from various ointments. The rate of ID release was found to be in the order gel ointment>absorptive ointment> hydrophilic ointment > white petrolatum ointment≒plastibase ointment.

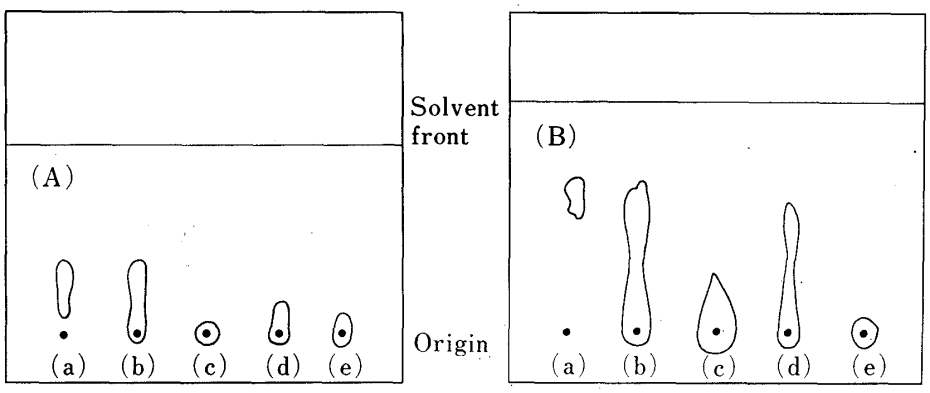

Fig. 5. Thin–Layer Chromatography of ID Ointments

(a) indomethacin (standard), (b) gel ointment, (c) macrogol ointment, (d) hydrophilic ointment, (e) white petrolatum ointment, plastibase ointment.
(A) developed with *n*-octyl alcohol, (B) developed with distilled water. Indomethacin was detected under a UV lamp.

In this experiment, dissolution of the base into the aqueous phase is inevitable. However, it is not a serious problem within one hour, except in the case of the macrogol ointment. The macrogol base is readily soluble in water and the release rate could not be determined by this method.

Thin-Layer Chromatography

In order to evaluate the ID release characteristics of both water-soluble ointments and oily ointments, thin–layer chromatography (TLC) was employed. The release of ID from the ointment base was examined with distilled water or *n*-octyl alcohol as the developing solvent. As shown in Fig. 5, the release of ID from the gel ointment was better than those from the conventional ointments, including macrogol ointment. The extent of ID release from the ointments decreased in the following order: gel ointment>hydrophilic ointment>white petrolatum ointment>macrogol ointment.

The above results are consistent with those of the *in vitro* study using the release apparatus shown in Fig. 1. Therefore, this method is considered to be a convenient one for the study of *in vitro* drug release profiles from various ointments.

The Volatility of the Various Ointments

As the gel ointment contains many volatile components such as ethanol and water, the effect of volatility might be more marked than in the cases of other ointments when applied on the skin. Thus, the volatility properties of the gel and conventional ointments were examined under the conditions of 75% relative humidity at 25 °C. Figure 6 shows that the 4-hour volatility of the gel ointment is greater than those of the other ointments.

It has been pointed out that a linear relationship exists between release rate and drug

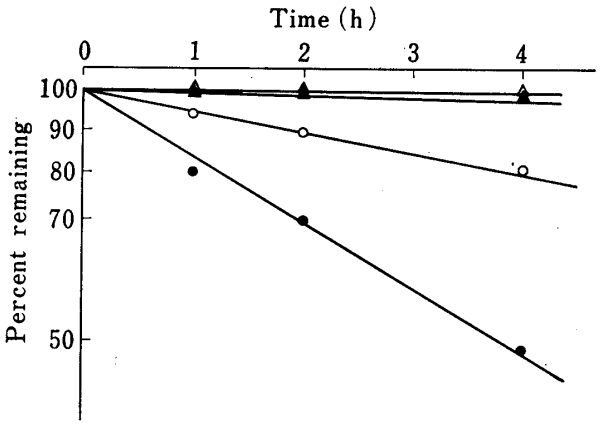

Fig. 6. The Volatility of the Various Ointments under 75% Relative Humidity at 25 °C

●; gel ointment, ○; hydrophilic ointment, ▲; absorptive ointment, △; white petrolatum ointment, plastibase ointment.

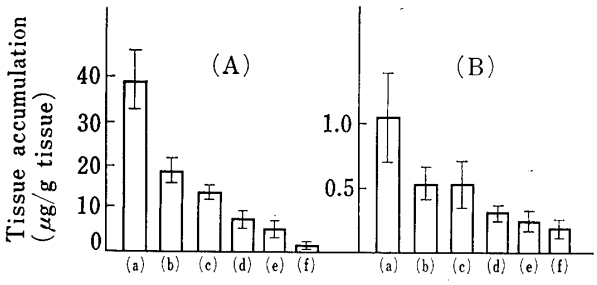

Fig. 7. Absorption of ID into the Skin and the Muscle of Rats at 6 Hours after Topical Application

(A) in the skin, (B) in the muscle.
(a) gel ointment, (b) absorptive ointment, (c) hydrophilic ointment (d) white petrolatum ointment, (e) plastibase ointment, (f) macrogol ointment.
Concentration of ID in the ointment was 1%.
Results are the means±S.E. of 5 rats.

TABLE II. The Relase Rate of ID from the Concentrated Gel Ointment

% concentrated	Relase rate (μg/min)
0	31.7
20	42.0
50	56.9

Results are the means of 3 replicates.

concentration in the base when the drug is completely dissolved in the base.[13] Therefore, it is considered that the concentration of ID in an ointment causes a corresponding increase in the amount of ID released from an ointment. The weight of the gel ointment decreased to a half in 4 hours. Thus, the release rate of ID from gel ointment previously concentrated to 50 or 80% of the initial weight was examined using the *in vitro* release apparatus. As shown in Table II, the release rate of the former was 56.9 μg/min, and that of the latter was 42.0 μg/min. The amount of ID released from the ointment increased in proportion to the concentration of ID in the ointment.

This result is consistent with the characteristics of the gel ointment suggested by the *in vitro* release profile.

Percutaneous Absorption of ID from the Various Ointments

Since the release of ID from the gel ointment was found to be better than that from other ointments using the *in vitro* release apparatus and the TLC method, the transfer of ID into the tissues from the gel ointment is expected to be improved.

Thus, comparative percutaneous absorption studies of ID from the gel and from conventional ointments were carried out. The results are presented in Fig. 7. The concentration of ID absorbed from the gel ointment was the highest, that is, 38.5 μg/g skin, and 1.1 μg/g muscle. On the other hand, the extent of absorption of ID from other ointments decreased in the following order: absorptive ointment≒hydrophilic ointment>white petrolatum ointment≒plastibase ointment>macrogol ointment.

The relationship between the *in vitro* release rate of ID and the skin accumulation is shown in Fig. 8. A good correlation was observed between the *in vitro* and *in vivo* studies. Similar tendencies were also found between the data obtained by the TLC method and the data of the *in vivo* study.

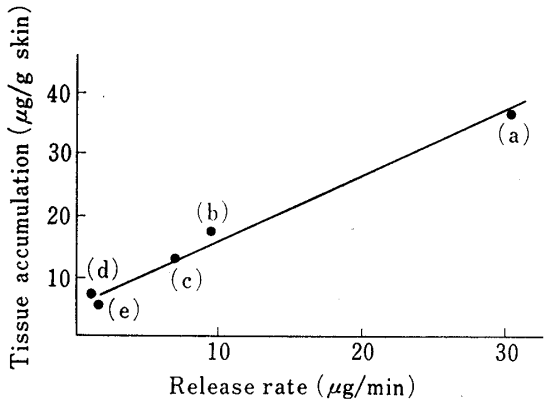

Fig. 8. The Relationship between *in Vitro* Release Rate and *in Vivo* Skin Accumulation

(a) gel ointment, (b) absorptive ointment, (c) hydrophilic ointment, (d) white petrolatum ointment, (e) plastibase ointment.

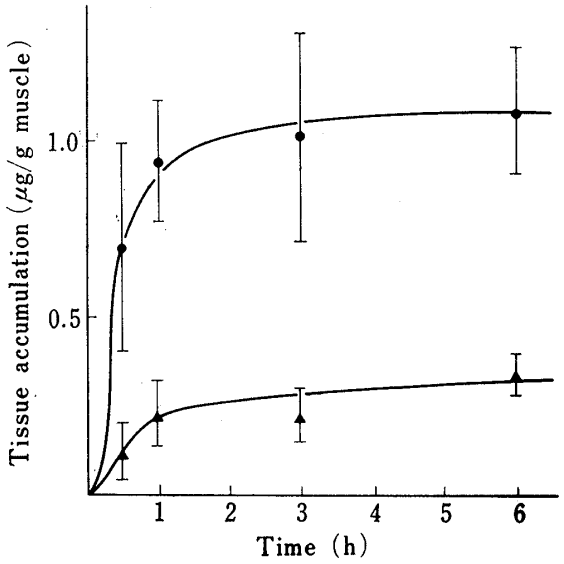

Fig. 9. Time Course of ID Absorption into the Muscle

●; gel ointment ▲; white petrolatum ointment. Each point represents the mean ± S.E. of 5 rats.

It has also been noted that the *stratum corneum* constitutes the main barrier for drug penetration,[12,17–19] and that a drug can not pass easily through the skin at an early stage.[20,21] Since the gel base contains much water, drug penetration may be accelerated in the case of the gel ointment due to the saturation of the *stratum corneum* with water.[22] Therefore, it was considered that the lag time of ID penetration into the muscle would be shorter for the gel ointment compared with the conventional types of ointment.

The concentration of ID in the muscle was therefore determined periodically after application of the gel ointment and white pertrolatum ointment. The results are shown in Fig. 9. Although the ID level in the muscle was markedly greater for the gel ointment, the pattern of ID transfer into the muscle was essentially similar for both ointments and little lag time was observed in ID absorption from both ointments into the muscle.

Thus, it was confirmed by the results of various *in vitro* and *in vivo* experiments that, as regards drug absorption, the gel ointment shows excellent drug release properties.

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