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Skin permeation of indomethacin from gel formed by fatty-acid ester and phospholipid

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Abstract

We selected 20 fatty-acid esters from cosmetic ingredients and investigated their suitability as a vehicle for absorption-type ointments using indomethacin (IM) as a model drug. Five fatty-acid ester groups, octanoate, isononanoate, myristate, palmitate and stearate, were selected. The total number of carbon atoms in the fatty-acid esters ranged from 17 to 34, and all of these esters are liquid at room temperature except stearyl caprylate. The solubility of IM was higher in fatty-acid esters containing fewer carbon atoms. The permeation rate of IM from a fatty-acid ester suspension through excised hairless rat skin was proportional to its solubility in the suspension, i.e. about 1–3 $\mu g/cm^2/h$. Gels were then formed from esters by the addition of a hydrogenated phospholipid. The permeation rate of IM from gels were 4–20 $\mu g/cm^2/h$ which were higher than those from suspensions because IM was present in amorphous state with phospholipid. High permeation rates from gels of fatty-acid esters in which side chains were present on both fatty-acid and alcohol moieties were observed.

Keywords: Skin permeation; In vitro; Fatty-acid ester; Phospholipid; Indomethacin; Hairless rat skin

1. Introduction

In the formulation of a percutaneous absorption-type ointment, the selection of an appropriate vehicle is important because the skin permeation rate of a drug is strongly affected by the type of vehicle used. In general, aqueous bases composed of aqueous polymers and hydrophilic solvents are used. However, these vehicles cannot be applied to the skin when the solvent irritates the skin. Thus, the selection of a vehicle is important also from the viewpoint of patient benefit. However, oil bases are unsuitable as percutaneous absorption-type vehicles because of poor solubility and permeability of the drugs (Shima et al., 1981), in spite of their safety.

We previously reported that gels with a consistency appropriate for use as ointments were formed by addition of a hydrogenated soybean phospholipid (HSL) to hydrocarbon oils or fatty-acid esters (Fujii et al., 1986a; Fujii et al., 1986b). We have examined the usefulness of the gel of

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Table 1
Fatty acid esters used in this experiment

Name	Abbreviation	Number of Carbon Atoms			Structure	
		R1	R2	Total	R1	R2
Isocetyl Octanoate	ICIO	8	16	24	iso	iso
Cetyl Octanoate	CIO	8	16	24	iso	nor
Cetyl Caprylate	CC	8	16	24	nor	nor
*Isostearyl Octanoate	ISIO	8	18	26	iso	iso
Stearyl Octanoate	SIO	8	18	26	iso	nor
Stearyl Caprylate	SC	8	18	26	nor	nor
Octyl Isononanoate	IOIN	9	8	17	iso	iso
Isononyl Isononanoate	ININ	9	9	18	iso	iso
*Isodecyl Isononanoate	IDIN	9	10	19	iso	iso
*Isotridecyl Isononanoate	ITIN	9	13	22	iso	iso
Isopropyl Myristate	IPM	14	3	17	nor	iso
*Isotridecyl Myristate	ITM	14	13	27	nor	iso
Isocetyl Myristate	ICM	14	16	30	nor	iso
Isopropyl Palmitate	IPP	16	3	19	nor	iso
Octyl Isopalmitate	IOIP	16	8	24	iso	iso
Octyl Palmitate	IOP	16	8	24	nor	iso
Isocetyl Palmitate	ICP	16	16	32	nor	iso
* Isopropyl Isostearate	IPIS	18	3	21	iso	iso
* Isocetyl Isostearate	ICIS	18	16	34	iso	iso
Isocetyl Stearate	ICS	18	16	34	nor	iso

R1, fatty-acid moiety; R2, alcohol moiety; iso, with side chains; nor, without side chains.
*: mixture of esters of which side chain number and position differ from each other.

liquid paraffin (LP) as a vehicle for several anti-inflammatory drugs (Henmi et al., 1994). The rate of permeation of those drugs was improved by the solubilizing effect of HSL; however, the permeation rate is lower than that of commercial hydrogel-type ointments. To make the same permeation rate as that from commercial hydrogel-type ointments, a skin-permeation enhancer such as menthol was necessary. Moreover, the feeling after application of gel with LP is very oily.

Therefore, we selected 20 fatty-acid esters (Esters) from cosmetic ingredients and tried to use them instead of LP. They are commonly used as the oil phases of creams and do not have as oily a feeling after application as LP. There have been some studies in which short-chain Esters were used as skin-permeation enhancers (Catz and Friend, 1989), or Esters were mixed with aqueous vehicles (Ozawa et al., 1988, Sato et al., 1988, Sugibayashi et al., 1988). However, there is no report as yet on drug permeation using a series of medium-to-long-chain Esters. Thus, the permeation rate of a drug from suspension was initially determined and then gels were prepared and the permeation rate from the gels was investigated. Indomethacin (IM), of which solubility and permeation rate are low, was used as a model drug.

2. Materials and methods

2.1. Materials

IM (JP XII grade) was purchased from Nippon Bulk Yakuhin Co., Ltd. (Osaka). Esters used in this experiment are shown in Table 1. They are divided into 5 groups: octanoate, isononanoate, myristate, palmitate and stearate. The total number of carbon atoms in these Esters ranged from

17 to 34. They are liquid at room temperature except SC. They have side chains on both or either of the fatty acid or the alcohol moiety except SC and CC. Those marked with * are a mixture of analogues. IPM, ICIS and IPP were provided by Nikko Chemicals Co., Ltd. (Tokyo). ITM was a gift from Kurary Co., Ltd. (Tokyo). Other Esters were gifts from Kokyu Alcohol Kogyo Co., Ltd. (Chiba). Esters except SC were of Japanese Standards of Cosmetic Ingredients grade or Japanese Cosmetic Ingredients Codex grade. Liquid paraffin (LP) was used as standard oil. Hydrogenated soybean phospholipid (HSL) was provided by Nikko Chemicals Co., Ltd. (Lecinol S-10 lot 2951, phospholipid content > 80%).

2.2. Solubility of IM in Esters

An excess amount of IM was suspended in Esters and incubated at 37°C overnight. After centrifugation, the supernatant was extracted with pH 7.1 phosphate buffer solution (PBS). The concentration of IM in PBS was determined by high-performance liquid chromatography (HPLC). Solubilities of IM in Esters were calculated taking into account the Ester/PBS partition coefficients.

A fixed amount of IM (0.1% interval) was added to Esters with or without HSL and the mixture was heated at 95°C. The maximum IM concentration at which no drug particles were detected by the naked eye was determined as its solubility at 95°C.

2.3. Preparation of gels

HSL, the water content of which was controlled to 0.7–0.9%, and IM were mixed well, added to Esters contained in a flask, capped tightly, and then heated at 95°C in a water bath with stirring until a homogeneous solution was obtained. The solution was packed in metal ointment tubes and cooled to 20°C in water for 30 min. Then the tubes were maintained at 40°C in an air incubator for 3 days, followed by storage at room temperature. The final HSL concentration was fixed at 15%.

2.4. Permeation and release studies

Skin permeation rate was measured in a modified Franz-type diffusion cell apparatus. The effective area available for permeation was 1.1 cm^2. The receptor compartment was filled with 15 ml of isotonic PBS (pH 7.1), maintained at 37°C, and mixed with a star-head magnet at 600 rpm. A piece of suitably sized abdominal skin from hairless male rats (5–6 weeks old, Saitama Laboratory Animals, Saitama) was excised immediately before the skin permeation experiments. In the case of suspension formulation, a piece of skin was mounted on the cell and approximately 0.1 g of suspension was poured onto it. In the case of gel formulation, approximately 0.1 g of gel was spread on the cornual layer side of the excised skin, and was mounted on the cell. At appropriate times, 200 μl aliquots were withdrawn from the receptor compartment. The same volume of PBS was added to the receptor compartment after withdrawal to keep the volume constant. Each experiment was carried out for 10 h.

After the skin permeation studies, the IM concentration in the skin was determined 24 h after application. Sample-applied skin was cut off from the margin to set to the cell, and wiped with paper moistened with LP. Then the skin was homogenized with a methanol-water mixture and centrifuged at 3000 rpm. The IM concentration in the supernatant was measured by HPLC.

The release rate of IM from the gel was measured in the same cell apparatus as that used for the permeation test. Approximately 0.1 g of gel was spread on a membrane filter (cellulose nitrate, pore size 0.45 μm, Advantec Toyo, Tokyo), which was then mounted on the cell. Each experiment was carried out for 4 h using the same procedure as that of the permeation test.

2.5. Analysis of IM concentration

IM concentrations were determined using HPLC. The analytical system consisted of a pump (LC 6A, Shimadzu, Kyoto), a UV detector (SPD 6A, Shimadzu) operated at 264 nm and an integrator (CPR 4A, Shimadzu). The sample withdrawn from the receptor compartment was

injected by an autoinjector equipped with a system controller (SIL 9A, SCL 6B, Shimadzu). The column (TSK-GEL ODS-120T, 150 mm × 4.6 mm i.d., Toso Co., Ltd., Tokyo) was eluted at ambient temperature with a mobile phase of 0.1% phosphoric acid solution:methanol, (25:75), at a flow rate of 1 ml/min.

3. Results

3.1. Solubility of IM in Esters

The solubility of a drug in an oily vehicle is generally low. For example, the IM solubility in LP, a typical oily vehicle, is 9 μg/ml. The IM solubilities in the Esters under various conditions are shown in Table 2. The IM solubilities at 37°C varied from 0.55 mg/ml (ICIS) to 2.30 mg/ml (IPM). These values are markedly higher than the IM solubility in LP, i.e. by 60- and 250-fold, respectively. Fig. 1 shows the relationship between total number of carbon atoms and solubility of IM at 37°C. The solubility of IM increases as the total number of carbon atoms decreases.

Table 2
Solubility of IM in esters under various conditions

Name	Solubility of IM (mg/ml)		
	37°C	95°C	
		Without HSL	With HSL
ICIO	0.79	7	25
CIO	0.89	6	26
CC	1.12	11	
ISIO	0.71	5	25
SIO	0.73	9	24
SC	1.16	10	
IOIN	2.03	17	40
ININ	1.68	16	38
IDIN	1.53	14	37
ITIN	1.14	10	26
IPM	2.30	18	40
ITM	0.72	7	25
ICM	0.65	6	26
IPP	1.81	16	38
IOIP	1.09	10	27
IOP	1.04	10	28
ICP	0.59	6	26
IPIS	1.48	15	36
ICIS	0.55	7	27
ICS	0.57	6	26

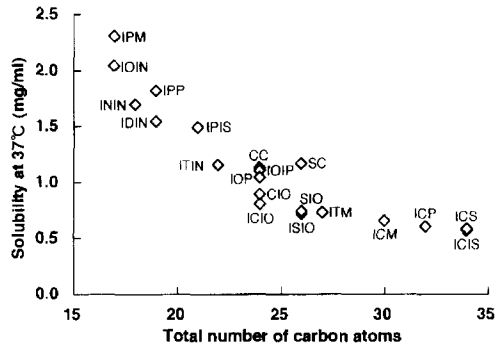

Fig. 1. Relationship between total number of carbon atoms and solubility of IM in Esters at 37°C.

The IM solubility at 95°C was about 10-fold that at 37°C. Moreover, at 95°C, the IM solubility in the presence of 15% HSL ranged from 24 to 40 mg/ml, and was about 20 mg/ml higher than that in the absence of HSL. We found in the solid dispersion study (Fujii et al., 1988) that IM interacts weakly with phosphatidylcholine, which is one of the principal components of HSL, and exists in an amorphous state. Therefore, the IM solubility became high when HSL was added. In contrast, the solubility of IM with HSL addition at 37°C could not be determined, because the soluble and insoluble version of IM could not be separated in gel.

3.2. Permeation from suspension

The cumulative amount of IM permeated per unit area from its suspension in various Esters was determined. Each Ester preparation was saturated with excess solid IM to ensure an equal and constant driving force. Permeation profiles of IM from IOIN suspension and ICIS suspension, which showed the highest and lowest permeation rates, respectively, are shown in Fig. 2. IM showed no permeation from LP suspension even after 10 h, but 3.8 $\mu g/cm^2$ of IM had permeated from ICIS suspension after 10 h. In all cases studied, linear relationships between cumulative

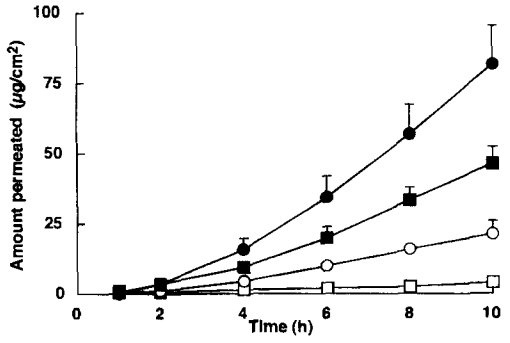

Fig. 2. Permeation profiles of IM from suspension and gel through excised hairless rat skin. ●, IOIN gel (1% IM); ■, ICIS gel (1% IM); ○, IOIN suspension; □, ICIS suspension. Each value represents the mean ± S.D. ($n \geq 3$).

amount of IM permeated and time from 4 to 10 h was observed; thus, the steady state permeation rate was determined from the slope.

Fig. 3a shows the relationship between IM solubility at 37°C and permeation rate from suspension. They showed good correlation (permeation rate = 1.29 × solubility, $r = 0.996$). The permeation coefficient of IM was 3.7×10^{-7} cm/s independent of the Esters used. This indicated that none of the Esters have a special skin permeation-enhancing effect.

3.3. Permeation from gel formed by hydrogenated phospholipid

The permeation rates of IM from Esters suspensions were markedly higher than that from LP suspension. The highest permeation rate was 2.85 $\mu g/cm^2/h$; however, this was not as high as that from a commercial one, 8–10 $\mu g/cm^2/h$. We previously reported that HSL changed an oil into a gel and that the permeation rate of IM from a gel consisting of LP and HSL was markedly higher than that from an LP suspension. Therefore, gels were formed using Esters instead of LP. SC and CC were not included in the gel studies, because their consistency was so high that they could not be squeezed out from the ointment tubes.

The permeation rates of IM from ICIS gel and IOIN gel were 10- and 4-fold higher than those from ICIS suspension and IOIN suspension, respectively (Fig. 2). For all Esters studied, the permeation rates of IM from gel containing 1% IM ranged from 5–10 $\mu g/cm^2/h$, and were higher than those from suspension. The relationship between solubility of IM in Esters at 37°C and permeation rate from gel containing 1% IM is shown in Fig. 3b. The permeation rate from the gels increased with increasing solubility of IM in the Esters used to form the gels at 37°C.

There was also a possibility that more than 1% IM dissolved in gel due to the solubilizing effect of HSL. Thus, the concentration of IM in the gel was increased to 1.5% and 2%. Fig. 4 shows some examples of the change in permeation rate when the IM concentration in the gel was 1, 1.5 and 2%. Maximum permeation rates were observed at an IM concentration of 2% in the case of IPM and at an IM concentration of 1.5% in the case of high-solubility Esters such as IOIN, ININ and

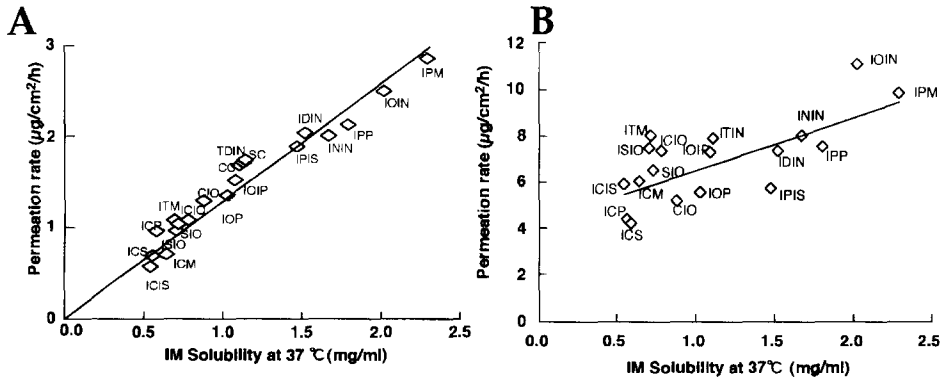

Fig. 3. Relationship between IM solubility at 37°C and permeation rate from suspension (a) and from gel containing 1% IM (b).

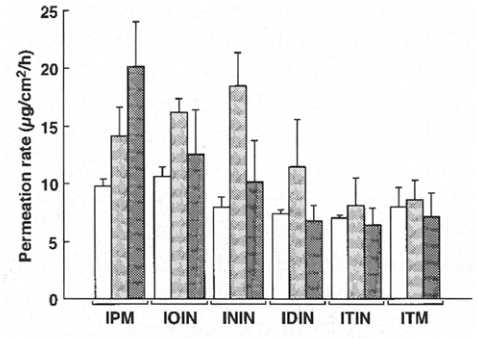

Fig. 4. Effect of IM concentration in gel on permeation rate of IM through excised hairless rat skin. IM concentration: □ 1%; ▨ 1.5%; ▩ 2%. Each value represents the mean ± S.D. ($n \geq 3$).

IDIN. In the case of low-solubility Esters, the permeation rate was independent of the IM concentration.

Fig. 5 shows the relationship between solubility of IM at 37°C and maximum permeation rate from gel. The results suggest that a maximum permeation rate is achieved when Esters in which IM is highly soluble are used in the gel formulation. However, the permeation rates from gels of Esters of which solubilities were almost the same differed from each other. These results indicate that factors other than solubility in Esters at 37°C influence the permeation rate in the case of gel. To determine whether these factors are related to properties of the skin or of the vehicle, release rates of IM from gels were determined. The amount of IM released was correlated with the square root of time; thus the release rate was determined from the slope. Fig. 6 shows the

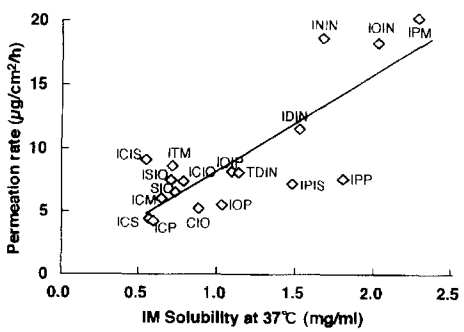

Fig. 5. Relationship between IM solubility at 37°C and maximum permeation rate from gel.

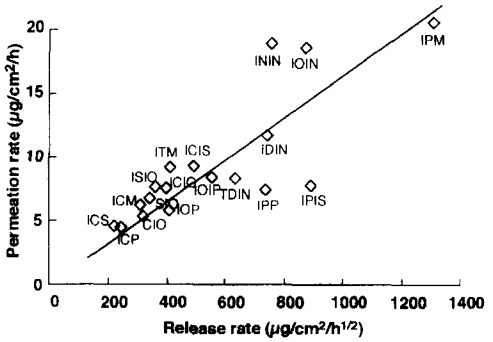

Fig. 6. Relationship between release rate and maximum permeation rate from gel.

strong correlation between release rate and maximum permeation rate of IM from gel. A factor influencing the permeation rate also influenced the release rate; thus, it is probably related to properties not of the skin but of the vehicle used and might be solubility of IM in the vehicle. It is considered that the supersaturation state is more stable in gels of which the permeation and the release rate are high.

To identify the above factor, the permeation rates from gels containing Esters with the same total numbers of carbon atoms but different structures were compared. The permeation rates from gels containing Esters which have side chains on both the fatty acid and the alcohol moieties were higher than those from gels containing Esters which have side chains on either the fatty acid or the alcohol moiety, despite the fact that they have similar IM solubilities in Esters at 37°C (Fig. 7). The permeation rate from gel was not affected when the chain length of the fatty acid moiety was switched with that of the alcohol moiety, i.e. (ICIO, IOIP) and (CIO, IOP).

4. Discussion

Esters are often used as the oil phases of cosmetic creams, because the feel of the skin after application is better than that of hydrocarbon oils. It is said that the higher their molecular weight the safer Esters are to the skin (Suzuki and Ogo, 1973). IPM was reported not to induce

erythema in humans (Ozawa et al., 1988); however, another report indicated that IPM irritated the skin (Tomita, 1976). Thus, we used Esters with a total number of carbon atoms equal to or higher than that of IPM. As the total number of carbon atoms increased, the freezing point became high. The presence of unsaturated bonds lowered the freezing point; however, these bonds are easily oxidized. Therefore, Esters of which both or either of the fatty acid or the alcohol moiety has side chains were selected for study. CC and SC, which are liquid at 37°C, were also selected.

Higuchi (1960) demonstrated that the permeation rate from suspension is maximum and constant regardless of the vehicle used because the thermodynamic activity of the drug is equal to that of the solid drug in suspension. However, the permeation rate from Esters suspension was not equal to each other but proportional to IM solubility in Esters at 37°C in our study. This indicated that Esters affected the properties of the skin and that the effective activity coefficient of IM in the skin barrier might be changed. Catz and Friend (1989) reported that there was no direct relationship between IM solubility and permeation rate from suspensions of short-chain Esters such as ethyl acetate and methyl valerate. They also mentioned that there was a specific enhancer/skin interaction. However, in our experiment, the permeation rate was directly proportional to IM solubility in the Esters at 37°C. Therefore the IM concentration in the skin was measured. As shown in Fig. 8, the IM concentration in the skin was also proportional to IM solubility in the Esters at 37°C. It was considered that the Esters themselves partitioned to the skin, and the amount of IM that partitioned to the skin became high proportional to the IM solubility in the Esters. The high IM concentration in the skin resulted in a high permeation rate in the case of the high-IM-solubility Esters suspension. This was the reason that the permeation coefficient apparently remained the same among the Esters in our studies.

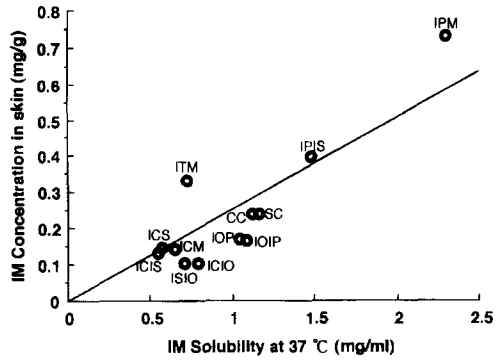

Fig. 8. Relationship between IM solubility at 37°C and IM concentration in skin.

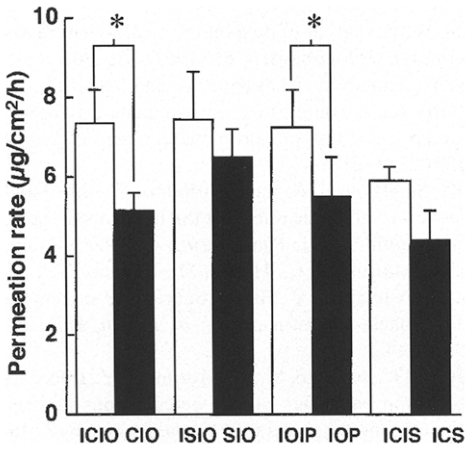

Fig. 7. Effect of structure of Esters on permeation rate of gel containing 1%; IM □, both R1 and R2 have side chains; ■, either R1 or R2 has side chains. Each value represents the mean ± S.D. ($n \geq 3$). Statistically significant difference: $^*P < 0.05$

The permeation rates of IM from gels were considerably higher than those from IM suspensions in Esters. As mentioned above, the permeation rate is maximum from suspension because thermodynamic activity of the drug is maximum. When HSL was added, the solubility became high, due to interaction between HSL and IM. Some of the IM in the gel might be present as an amorphous state and formed a supersaturation state, so the activity of IM might be higher than that in suspension. The transport rate from a supersaturated solution is proportional to the degree of supersaturation (Davis and Hadgraft,

1991). The solubility of IM in gels cannot be determined because the soluble and insoluble IM cannot be separated in the gel. Therefore, the permeation rate from gels with various IM concentration were determined. As the concentration of IM increased, the permeation rate from the gel increased in the case of Esters with high IM solubility. This indicates that the IM in the gel was solubilized by HSL and that the solubilities of IM in the gels containing Esters with low and high IM solubilities might be near 1% and 1.5%, respectively, and be higher than that in LP gel, 0.5%. A high IM concentration sometimes resulted in a low permeation rate. This was attributed to unstable supersaturation and occurrence of recrystallization at high IM concentration.

In the case of the gel formulation, the permeation rate also seemed to depend on the solubility of IM in the Esters at 37°C. This indicated that the degrees of supersaturation in the gels were almost the same for all Esters, so that the permeation rates from the gels were about 7-fold those from suspensions of Esters used for the gels, though release and permeation rates of IM from the gels containing ICIO, ISIO, IOIP and ICIS were higher than those from the gels containing CIO, SIO, IOP and ICS, respectively, in spite of the similar solubilities in Esters at 37°C and permeation rates from suspension. The release from solution type ointment was proportional to the drug concentration in vehicle (Higuchi, 1962). Also, it was considered that IM solubility in the gel of Esters with side chains on both the fatty acid and the alcohol moieties kept higher, so that the degree of supersaturation was higher than that in gel of Esters with side chains on either the fatty acid or the alcohol moiety.

As a consequence, the permeation rate of IM from Esters was found to depend on solubility of IM in Esters at 37°C, because the partition of IM in the skin is proportional to the solubility. Also, the supersaturation degree in gel affected on the permeation from gel. To obtain a high permeation rate of IM, gels with shorter chain length and side chains Esters must be selected.

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