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Effect of phosphatidylcholine on skin permeation of indomethacin from gel prepared with liquid paraffin and hydrogenated phospholipid

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Abstract

The effects of hydrogenated and unhydrogenated phosphatidylcholine (HPC, PC) on the permeation of indomethacin (IM) through hairless rat skin were investigated using liquid paraffin (LP) and a gel prepared with LP and hydrogenated soybean phospholipid (HSL). IM solubility at 95°C increased in proportion to the concentration of HPC or PC, whereas solubility at 37°C did not increase with HPC. IM showed no permeation until 10 h from LP without HPC/PC, but permeated at rates of ≈ 5 and 10 μg/cm^2 within 10 h from LP with HPC and PC, respectively. The permeation from the gel with various formulations (HSL, 15%; PC/HPC, 0–5%; IM, 0.5–2%) was determined. Permeation rates were 1.7–4.8 μg/cm^2 per h and were proportional to the skin concentration. Skin concentration was correlated to the release rate from the gel. We concluded that IM was solubilized by phospholipids, high activity in the vehicle led to high partition of IM in skin, and permeation increased due to a high skin concentration. © 2001 Published by Elsevier Science B.V.

Keywords: Skin permeation; Phosphatidylcholine; Indomethacin; Oily gel; Hairless rat skin

1. Introduction

The addition of phospholipids to various types of vehicles, such as liposomes and microemulsions, enhances the permeation of drugs through the skin (Bonina et al., 1995; Yokomizo and Sagitani, 1996; Dreher et al., 1997). The above researchers used natural (unhydrogenated) high-purity phosphatidylcholine (PC) and suggested that PC interacted to some degree with the skin.

We previously reported that the use of an oily gel (Gel), prepared using hydrogenated soybean phospholipid (HSL) and liquid paraffin (LP), enhanced skin permeation of several anti-inflammatory drugs (Henmi et al., 1994). This effect might be a result of the solubilizing effect of HSL. A high concentration of HSL in the gel resulted in a high level of solubilization of the drugs, but the consistency of the Gel was inappropriate for application on the skin. Furthermore, we deter-

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mined that the permeation rate of indomethacin (IM) using the Gel was influenced by the composition of HSL: permeation rates for Gels prepared with HSL containing 25 and 20% PC were 3.0 and 1.7 μg/cm^2 per h, respectively. These results suggest that PC plays an important role in the solubilization of IM.

The present study investigates whether an improved formulation of gel, as well as improved application, results in a greater permeation rate of IM by adding hydrogenated and unhydrogenated PC (abbreviated HPC and PC, respectively) to the gel. Furthermore, the effects of HPC or PC on skin permeation of IM were compared to that of LP alone.

2. Materials and methods

2.1. Materials

IM (JP XII grade) was purchased from Nippon Bulk Yakuhin (Osaka, Japan). LP (P-70) was provided by Nikko Chemicals (Tokyo, Japan). HSL (Lecinol S-10 lot 2951, phospholipid content above 80%, and PC content $\approx$20%), HPC (Lecinol S-10EX, PC content 95%) and PC (Epikuron 200, Lukas Meyer, PC content above 95%) were also provided by Nikko Chemicals. Other reagents were of analytical or HPLC grade.

2.2. Solubility of indomethacin at 37°C

An excess amount of IM and a fixed amount of phospholipid were added to LP and the mixture was maintained at 37°C over 2 days with shaking. Following centrifugation, $\approx$0.1 g of supernatant was diluted with ethanol to an appropriate concentration, and the concentration of IM was then determined by UV absorption at 318 nm.

2.3. Solubility of indomethacin at 95°C

A fixed amount of IM (0.1% interval) was added to LP with or without phospholipid, and the mixture was heated to 95°C. Solubility of IM was defined at 95°C as the maximum IM concentration at which no drug particles were detected by the naked eye.

2.4. Preparation of formulations

A fixed final concentration of 1% IM, was mixed with LP, and PC or HPC and heated at 95°C for 30 min. The mixture was then cooled to room temperature and allowed to stand for 1 day.

Gels were prepared by mixing HSL (water content controlled at 0.7–0.9%) and IM, and adding this to LP in a flask which was then capped tightly, and 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. The tubes were then maintained at 40°C in an air incubator for 3 days, followed by storage at room temperature (Fujii et al., 1986). The final HSL concentration was 15%. PC or HPC was added to LP with HSL and IM, using the same procedure as above, except incubation occurred at 30°C when PC was added rather than at 40°C.

2.5. Measurement of consistency

Consistency was determined by load weights spreading 0.40 g of Gel at a 0.50 mm thickness using a pressure tension meter (Tensiprsser® Taketomo Electric, Tokyo, Japan). The resultant consistency of white petrolatum was $\approx$1.5 kgf.

2.6. Permeation studies

Skin permeation was measured by a modified Franz-type diffusion cell apparatus. The effective area available for permeation was 1.1 cm^2. Isotonic phosphate buffer solution (16 ml, pH 7.1, maintained at 37°C) was placed in the receptor compartment and mixed with a star-head magnet at 600 rpm. A sample of abdominal skin from a 5- to 6-week-old, hairless male rat (Saitama Laboratory Animals, Saitama, Japan) was excised immediately prior to the skin permeation experiments. The skin sample was then mounted on the cell and $\approx$0.1 g of LP with IM and PC/HPC was poured over it to formulate the suspension. Approximately 0.1 g of Gel was applied to the stratum corneum side of the excised skin to formulate the Gel, which was then mounted on the cell. At appropriate time intervals, 200-μl aliquots

were taken from the receptor compartment. To maintain a constant volume, an identical volume of fresh solution was added to the receptor compartment.

2.7. Indomethacin concentration in skin

Following the skin permeation studies, IM concentration in the skin was determined 24 h after application. Sample applied skin was excised from the margin of the cell, and wiped with paper moistened with LP. The skin was then homogenized in a methanol–water mixture and centrifuged at 3000 rpm. The IM concentration in the supernatant was measured by HPLC.

2.8. Release studies

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 applied to a membrane filter (cellulose nitrate, pore size 0.45 μm; Advantec Toyo) which was then mounted on the cell. Each experiment was conducted for 8 h using the same procedure as for the permeation test.

2.9. Analysis of indomethacin concentration

IM concentrations were determined using HPLC. Analysis occurred using a pump (LC 6A, Shimadzu, Kyoto, Japan), UV detector (SPD 6A, Shimadzu) operated at 264 nm and an integrator (CPR 4A, Shimadzu). The sample extracted from the receptor compartment was injected using 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., Tosoh, Tokyo, Japan) 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 and discussion

3.1. Effect of PC and HPC on the solubilities of IM using LP

IM dissolved only slightly in LP, ≈0.05% at 95°C and 0.001% at 37°C. Table 1 shows the solubilities of IM in LP at 95 and 37°C in the presence of a phospholipid. As in previous reports (Henmi et al., 1994), IM solubility in LP at 95°C increased in proportion to the concentration of HSL. IM solubility also increased in proportion to the concentration of PC or HPC, increasing 5-fold when compared to that of HSL. PC content in HSL was about 20%. This suggests that PC interacted with IM, and IM dissolved in LP. The solubilizing effects of PC and HPC were almost identical, and hydrogenation had no effect on IM solubility at 95°C.

Table 1
Solubilities of IM in LP at 37 and 95°C in the presence of various concentrations of phospholipid

Concentration of phospholipid in LP (%)	Solubility of IM at (w/w%)				
	37°C		95°C		
	PC	HPC	PC	HPC	HSL
0	0.001	0.001	<0.05	<0.05	<0.05
1	0.3	0.004	0.6	0.6	–
2	0.6	0.004	1.1	1.2	–
5	1.2	0.004	3.0	3.4	–
10	–	–	–	–	1.2
15	–	–	–	–	1.8
20	–	–	–	–	2.7

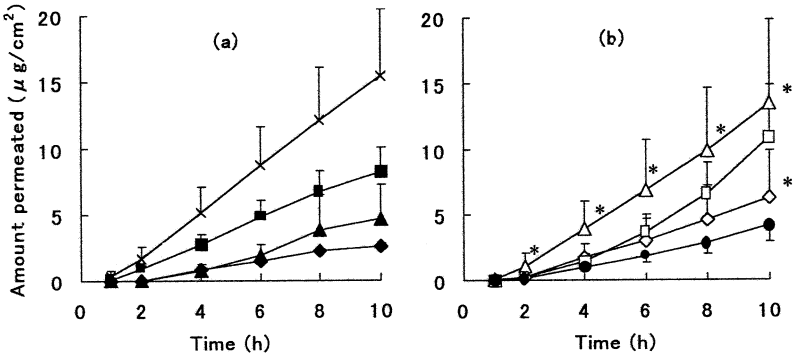

Fig. 1. Permeation profiles of IM from LP suspensions in the presence of HPC or PC. (a) HPC 1% (◆), 2% (▲), 5% (■), Gel (×); and (b) PC l% (◇), 2% (△), 5% (□), water suspension (●). Each point represents the mean ± S.D. of at least three experiments. (*) Significant difference between identical concentrations of PC and HPC.

In contrast, the solubilizing effects of PC and HPC at 37°C differed. Only PC was effective on IM solubility. This was, in part, due to dissolution of PC in LP at 37°C, which is in contrast to HPC. Furthermore, the difference in phase transition temperature (Tc) of PC and HPC affected the interaction with IM. We found that IM dissolved in PC and resulted in a solid dispersion system when they were heated above Tc (Fujii et al., 1993). The Tc of PC is below room temperature, whereas the Tc of HPC is 85°C. Thus, HPC was effective on IM solubility only when heated to a high temperature.

3.2. Permeation of IM from LP using PC or HPC

When HPC or PC concentrations were greater than 2%, 1% IM was soluble at 95°C. The formulations with HPC or PC were yellow in color due to solubilization of IM, even when maintained at room temperature. A large number of precipitates were observed in LP with HPC, due to the low solubility of HPC in LP. No obvious precipitate was detected for PC, however, the formulations were viscous. The solubility of IM could not be measured at room temperature because of the high viscosity of the formulations.

Fig. 1 shows the permeation profiles of IM from LP suspension with PC or HPC, from Gel and from water suspension. After 10 h of water suspension, IM had permeated 4 μg/cm^2, whereas IM was not detected even after 10 h during LP suspension using neither PC nor HPC. Permeation from suspension must be the same because drug activity is same as a solid (Higuchi, 1960). The low solubility of IM in LP suggests that the dissolution rate of IM into LP might be a rate-limiting step.

The permeation of IM increased to 0.9 μg/cm^2 per h with a 5% increase in the concentration of HPC. In contrast, IM was not detected even after 10 h when IM and HPC suspended in LP at room temperature was applied to the skin. These results suggest the importance of the heating process to enable permeation of IM. The effect of PC on the permeation rate of IM was about 2-fold greater than that of HPC. When 2% PC was added, the permeation rate was 1.6 μg/cm^2 per h and was almost the same as that from Gel. When 5% PC was added, the permeation profile changed, showing a profile similar to that of water suspension and a longer lag time than those of other formulations, except that of water suspension, was observed. The reason remains unclear, but sufficient PC to solubilize 1% of IM existed only in this formulation, whereas other formulations appeared to change character within 10 h probably because of water from skin.

Fig. 2 shows the IM concentrations in skin 24 h after the application of each formulation. Maximum drug concentration in the skin is reached independent of the vehicle when drug suspension is applied. However, skin concentrations of IM

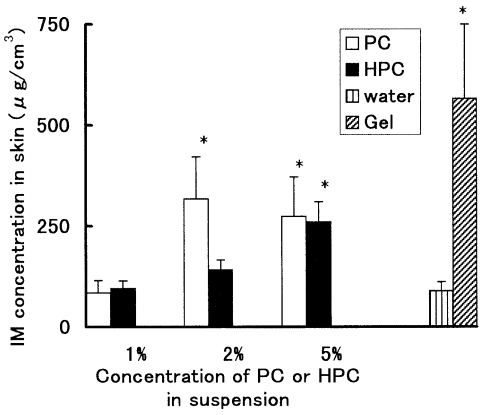

Fig. 2. IM concentration in skin 24 h after application of LP suspension with HPC or PC, Gel and water suspension. Each column and bar represents the mean and S.D. of at least three experiments. (*) Significant difference from water suspension ($P < 0.05$).

after the application of some formulations were higher than that of water suspension. It was considered that IM interacted with PC or HPC, solubilized, i.e., supersaturated in LP, and partitioned to the skin higher concentration.

3.3. Consistency of the Gel following the addition of HPC or PC

LP containing HPC or PC were effective vehicles for permeation. However, these were not suitable dosage forms because their condition changed with time. Therefore, we attempted to prepare a more effective and convenient formulation by adding HPC or PC to Gel. The consistency of Gel was 1.3 kgf and it increased with the concentration of the phospholipid added: 1% of PC or HPC showed 1.8 and 1.6 kgf and 2% of PC or HPC showed 2.1 and 2.0 kgf, respectively. Gel containing 5% HPC showed a consistency of 3.1 kgf, although with 5% PC added, no further increase in consistency was observed. Consistency was suitable for application in all formulations.

3.4. The release of IM from Gel following the addition of HPC or PC

Release rates of IM from an ointment base differ between solution- and suspension-type ointments (Higuchi, 1960). The release rate from a solution-type ointment is proportional to the initial concentration and the square root of the diffusion coefficient (Higuchi, 1962), whereas that from a suspension-type ointment is not directly proportional to the initial drug concentration. It was unclear in Gels whether all of the IM interacted with the phospholipid and dissolved or whether some of the IM existed as crystals. Thus, the release rate of IM from Gel was calculated from the linear slope of the profile of the square root of time versus amount released.

Fig. 3(a) shows the effect of HPC and PC on the release from Gel containing 1% IM, which improved the release rate slightly. However, the release rates decreased slightly from gel contain-

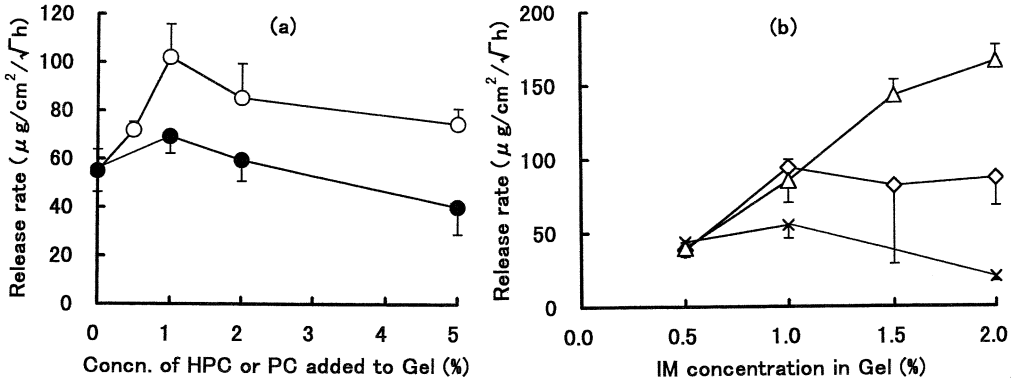

Fig. 3. Effect of formulation on the release rate of IM from Gel with 15% HSL. (a) PC (○), HPC (●); IM concentration was fixed at 1%; and (b) without PC (×), PC 1% (◇), PC 2% (△). Each point represents the mean ± S.D. of at least three experiments.

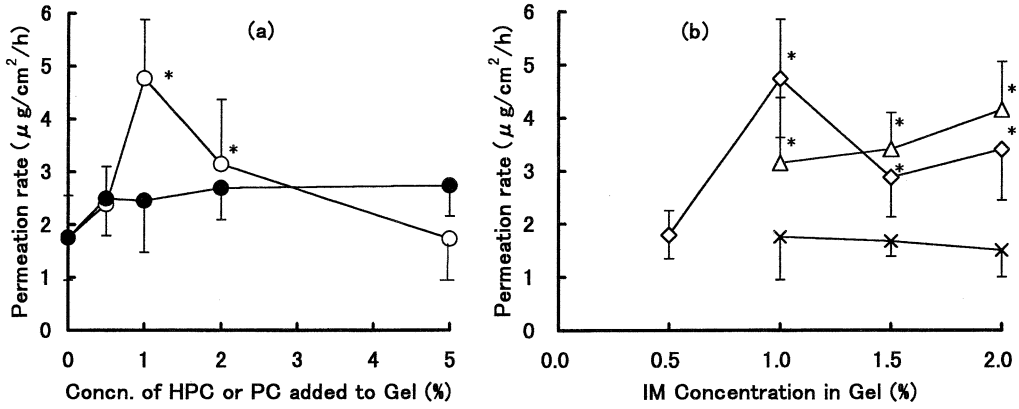

Fig. 4. Effect of formulation on the permeation rate of IM from Gel with 15% HSL. (a) PC (○), HPC (●); IM concentration was fixed at 1%. (b) Without PC (×), PC 1% (◇), PC 2% (△). Each point represents the mean ± S.D. of at least three experiments. (*) Significant difference between Gel containing 1% IM and with no extra phospholipid ($P < 0.05$).

ing 5% HPC. The release rate from Gel containing 1% PC was two times that from Gel without extra phospholipid, but it also decreased at higher concentrations of PC.

Fig. 3(b) shows the effect of IM concentration on the release rates from Gel with or without PC. When 0.5% of IM was added to Gel, no difference in release rates was observed between the three formulations. Without PC, the release rates from Gel containing 0.5 and 1% IM were almost identical and that from Gel containing 2% IM was lower than that from Gel containing 0.5 or 1% IM. Using 1% PC, the release rate from Gel containing 1% IM was two times that from Gel containing 0.5% IM. However, the release rates did not change with a higher concentration of IM in Gel. When 2% of PC was added, the release rate was in proportion to the IM concentration added to Gel up to 1.5% of IM. These results suggested that 0.5% of IM was solubilized in Gel without PC, 1% with 1% PC, and 1.5–2% with 2% PC. Gels containing a higher concentration of IM may be suspension-type ointments. The release rate from a suspension-type ointment was not proportional to the drug concentration but should be higher with higher drug concentration, which was not in the cases of these formulations. Overdosing IM, in a crystalline form, would produce negligible changes in the release rate, because IM solubility in LP is very low (9 μg/ml) and requires heating to interact with PC. Using Gel with 2% IM and no PC, IM did not dissolve completely and crystals remained even at high temperatures. Remaining crystals formed cores of recrystallization of IM once dissolved in the interaction with phospholipid and the release rate was lower than those from 0.5 or 1% IM formulation.

Greater solubility was expected at higher PC concentrations using 1% IM. Thus, the release rate was expected to be the same as for PC concentrations over 1%. However, the observed release rates were lower using 2 or 5% of PC. We believe that the diffusion coefficient decreased due to high viscosity of the vehicle. Upon the addition of HPC to Gel, the release rate did not improve as much as for PC, likely due to the fact that the solubilization effect may have been lower than that of PC. As the HPC concentration increased, viscosity increased and release rates decreased to a similar extent as PC.

3.5. The permeation of IM from Gel following addition of HPC or PC

Fig. 4(a) shows the IM permeation rates from Gels containing 1% IM and various concentrations of HPC or PC. When HPC was added, permeation rates were slightly higher than those from the formulation without HPC/PC. However, the increase was not significant, showing a 1.5-

fold maximum increase regardless of the additional concentration. The addition of 1% PC improved the permeation rate 2.8 times compared to the formulation without PC. However, when 5% PC was added, no difference was observed in the permeation rate when compared to the formulation without PC. Fig. 4(b) shows the effect of IM concentration on the permeation rate. The permeation rate decreased using 1% PC when IM concentration was 1.5 or 2%. Permeation rate remained unchanged by IM concentration using 2% PC.

Permeation rate is expressed as CKD/L (C, concentration in vehicle; K, partition coefficient between skin and vehicle; D, diffusion coefficient in skin; L, diffusion length), i.e. permeation rate depends on the drug concentration and diffusion coefficient in the skin. Fig. 5 shows the relationship between permeation rate and IM concentration in the skin. A significant correlation ($r = 0.830$) was seen between these two factors. This relationship exists not only for Gel formulation but also for LP suspension with phospholipid and water suspension.

Fig. 6 shows the relationship between IM concentration in the skin and release rate, which also shows a significant correlation ($r = 0.817$). As mentioned above, the release rate correlated with the solubilized concentration of IM in Gel. Thus, a high skin concentration of IM is also dependent upon solubilization of IM in Gel.

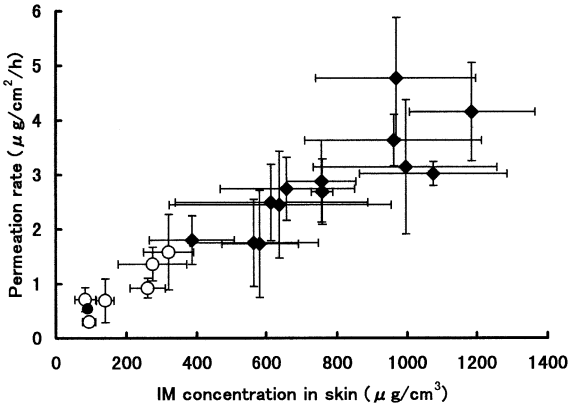

Fig. 5. Relationship between IM concentration in skin and permeation rate. Gel with various formulations (◆), LP suspension (○), water suspension (●). Each point represents the mean ± S.D. of at least three experiments.

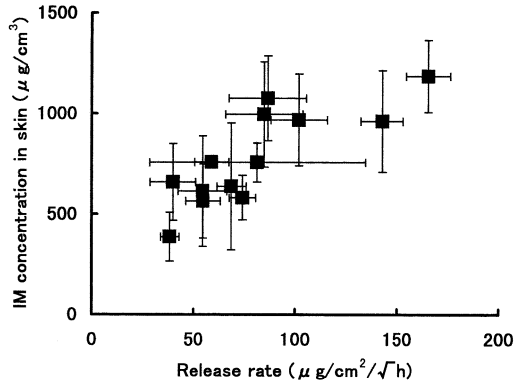

Fig. 6. Relationship between release rate and IM concentration in skin. Each point represents the mean ± S.D. of at least three experiments.

The relationship between skin concentration and permeation rate suggests the same diffusion coefficient exists among all formulations. Fig. 7 shows lag time ($L^2/6D$) versus permeation rate. The lag times of Gel in which all IM might be solubilized was the same as that of water suspension. However, Gels which might contain some crystallized IM showed a shorter lag time than expected. This is the same phenomenon observed

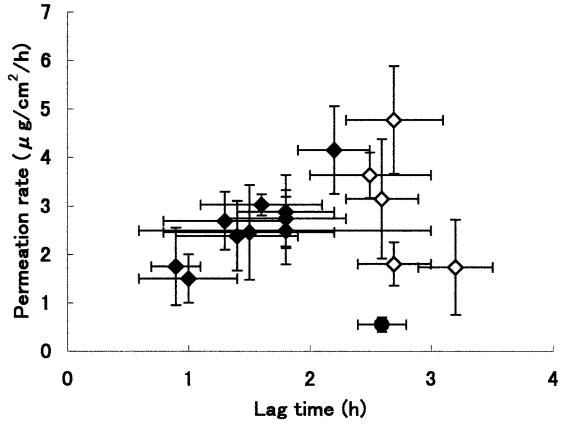

Fig. 7. Relationship between lag time and permeation rate. Gel with formulation in which all IM might be dissolved (◇), Gel with formulation in which some IM might be crystallized (◆), water suspension (●). Each point represents the mean ± S.D. of at least three experiments.

in LP suspension with PC or HPC (Fig. 1). This is due, in part, to the fact that a high concentration of IM solubilized in the vehicle was maintained in the ointment tube, whereas this concentration changed during the experiments.

4. Conclusions

The addition of PC or HPC to LP enhanced the permeation of IM. Permeation rates were proportional to skin concentration and the enhancement of permeation was dependent upon the high partition of IM from the vehicle into the skin. Skin concentration correlated with release rate, and release rate was proportional to the solubilized concentration of IM in Gel. Based on these results, we believe that phospholipids in LP improve the permeation of IM not because of the effect on skin, but due to solubilization of IM in the vehicle.

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