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Enhancement of skin permeation of miconazole by phospholipid and dodecyl 2-(*N*,*N*-dimethyl amino)propionate (DDAIP)

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Abstract

Miconazole (MCZ) has very low solubility in both water and oil. Permeation rates through shed snakeskin from an aqueous suspension and a mineral oil suspension were 0.5 μg/cm^2/h and almost none, respectively. When hydrogenated phosphatidylcholine (HPC) was added to mineral oil and heated to 95 °C, the solubility of MCZ increased in proportion to the HPC concentration. DSC measurements also indicated an interaction between them. Thus, a gel formed by hydrogenated phospholipid and mineral oil, as vehicle was prepared. The solubility of MCZ in the gel was around 1% and the permeation rate was 1.3 μg/cm^2/h, which was about 2.5 times that from an aqueous suspension. As an alternative approach, a skin permeation enhancer, dodecyl 2-(*N*,*N*-dimethyl amino)propionate (DDAIP) was applied 2 h before a skin permeation study. The permeation from an aqueous suspension became 11 times that of the suspension without DDAIP pretreatment. The concentration of MCZ in the skin increased 8-fold, indicating that the enhancement effect involved high partition of MCZ into the skin. On the other hand, when a gel formulation was used, pretreatment with DDAIP was not as effective as incorporation of DDAIP in the gel formulation. Following pretreatment, permeation was only two times that of the gel without DDAIP pretreatment, and half that of the water suspension with DDAIP pretreatment. This suggested that release from the gel was the rate-limiting step with the gel formulation. When DDAIP was added to the gel, the permeation rate of MCZ was 3.3 μg/cm^2/h. It was also a release limited type permeation. The gel with DDAIP is potentially a useful formulation, because of relatively high permeation while possibly avoiding overdosing.

Keywords: Miconazole; Skin permeation; Dodecyl 2-(*N*,*N*-dimethyl amino)propionate; Phospholipid; Shed snakeskin; Enhancer

1. Introduction

Transdermal delivery is often an attractive route for local and systemic treatment. However, permeation of drugs through the skin is insufficient for many therapies, especially in cases of

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sparingly soluble drugs. Several methods for enhancing skin permeation, such as the selection of a suitable vehicle (Behl et al., 1993), co-administration of a chemical enhancer (Suhonen et al., 1999), iontophoresis (Green et al., 1993) or sonophoresis (Simonin, 1995), have been studied as approaches to obtain sufficient permeation.

Miconazole (MCZ), one of the broad spectrum antifungal agents, is a weak base that has a pK_a of 6.7, relatively high molecular weight and melting point. Thus, its solubility in water as well as in oil is extremely low. Its poor solubility reduces its efficacy for many therapeutic applications, so some attempts to improve its solubility using methods such as complexation with cyclodextrin (Pedersen et al., 1993; Tenjarla et al., 1998) have been reported.

This report describes two techniques. One is supersaturation of MCZ in a vehicle. We reported that hydrogenated soybean phospholipid (HSL) changed mineral oil into a gel (Fujii et al., 1986a). When a drug and phospholipid have some interaction, some of the drug dissolved in the gel at a high temperature and remained as a solution even when it was stored at room temperature. This technique improved the permeation of some anti-inflammatory drugs (Henmi et al., 1994) and tranilast (Hori et al., 1998). Another approach is the addition of a penetration enhancer, such as dodecyl 2-(*N*,*N*-dimethyl amino)propionate (DDAIP). It also has been reported that the biodegradable skin permeation enhancer, DDAIP, improved the permeation of several anti-inflammatory drugs, as well as clonidine, hydrocortisone, etc. (Büyüktimkin et al., 1995, 1993). This report confirms the effects of each technique, and the results of a combination of the two techniques.

2. Experimental methods

2.1. Materials

MCZ was USP grade (Spectrum Quality Products, Inc.). Hydrogenated soybean lecithin (HSL; phospholipid content was above 80%, phosphatidylcholine was about 20%) and hydrogenated phosphatidylcholine (HPC; purity was above 95%) were gifts from Nikko Chemicals (Tokyo). Mineral oil was purchased from Penreco (Drakeol 7 LT). DDAIP was received from NexMed, Inc. The other chemicals were of reagent or HPLC grade.

2.2. Thermal analysis

Thermal analysis was carried out by means of a differential scanning calorimeter (TAS-200 equipped with DSC8230, Rigaku, Tokyo). Samples were sealed in an aluminum crimp cell and heated at the rate of 10 °C/min under an atmosphere of nitrogen.

2.3. Solubility

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

An excess amount of MCZ (about 5%) was added to mineral oil with or without HPC and the mixture was heated to 95 °C, then cooled to 20 °C. The mixture became a highly viscous suspension, which was stirred to separate the mineral oil. The concentration in mineral oil was determined by HPLC and was designated as its solubility at 20 °C.

2.4. Preparation of formulations

Formulations used in this study are shown in Table 1. In the case of suspensions, MCZ at the level of 1% was added to the solvent at room temperature and kept at 37 °C overnight. The gels were prepared using the following procedure; HSL (final concentration 15%) and a fixed amount of MCZ were added to mineral oil in a flask. The mixture was heated to 95 °C with stirring until the solution became homogeneous but less than 1 h, then cooled to 20 °C. The gels obtained were then heated at 37 °C in an air incubator for 3 days, followed by storage at room temperature (Fujii et al., 1986b). For comparison,

Table 1
Formulations of MCZ used in this experiment

	Aqueous suspension	Aqueous suspension with DDAIP	Mineral oil suspension	Mineral oil suspension with DDAIP	Gel	Gel+MCZ	Gel with DDAIP
MCZ	1	1	1	1	0.2–2	1	1
HSL					15	15	15
DDAIP		5		5			5
Water	q.s.	q.s.					
Mineral oil			q.s.	q.s.	q.s.	q.s.	q.s.
	100	100	100	100	100	100	100

the gel without MCZ was prepared and MCZ was added at room temperature by mixing with a spatula (gel + MCZ).

2.5. Release study from the gel

Release from the gel was measured in a vertical-type diffusion cell apparatus. About 0.3 g of gel was spread on a membrane filter (cellulose acetate, pore size 0.45 μm), which was then mounted on the cell. Due to the low solubility of MCZ, the receptor phase was a mixture of pH 5 phosphate buffer solution (PBS) and propylene glycol (7:3). The receptor phase was maintained at 37 °C, and mixed with a magnetic stirrer. At appropriate times, 0.2 ml aliquots were withdrawn from the receptor compartment, and the same volume of fresh solution was added to the receptor compartment to keep the volume constant. Each experiment was carried out for 6 h.

2.6. Permeation study

The permeation of MCZ was measured in a similar cell apparatus as that used for the release study. Shed snakeskin was used as the model skin. The skin was soaked in saline solution for 2 h prior to the permeation study. In the case of gels, about 0.3 g of gel was spread on the skin, which was then mounted on the cell. In the case of liquid formulations, a 0.5 ml sample was added to the donor compartment after mounting the skin on the cell. At predetermined times, the receptor phase was withdrawn, and the same volume of fresh solution was added to the receptor compartment to keep the volume constant. Each experiment was carried out for 24 h. After the permeation test, the skin was washed, cut and homogenated in methanol, and then, the concentration of MCZ in the skin was determined. MCZ concentrations in the receptor phase and in the skin homogenate were determined by HPLC.

In the case of pretreatment with DDAIP, 15 μl of DDAIP was placed on the skin, spread carefully to avoiding damaging the skin, allowed to stand 2 h at room temperature, and then the skin was used for permeation studies.

2.7. Analysis of MCZ concentration

A HPLC system (Shimadzu, Kyoto) with a reverse phase column was used. A mixture of pH 3.0 PBS and acetnitrile was used as the mobile phase. MCZ was detected 225 nm.

3. Results and discussion

3.1. Interaction between MCZ and HPC

The interaction between MCZ and HPC was examined by DSC. MCZ alone showed a sharp endothermic peak at 183 °C, representing its melting and degradation point. HPC showed a broad endothermic peak from 85 to 100 °C. MCZ and HPC were mixed with a molar ratio of 1:1, heated at 100 °C for 10 min, and then cooled. When the sample was reheated, there was no peak at 183 °C and the peak of HPC appeared at a lower temperature than HPC alone (Fig. 1). This indicated that some interaction between MCZ and HPC occurred.

Table 2 shows the solubility of MCZ in mineral oil with or without HPC. MCZ scarcely dissolved in mineral oil even if it was heated at 95 °C. When HPC was added to mineral oil and the mixture was heated at 95 °C, the solubility of MCZ was increased in proportion to the HPC concentration. When the mixture and MCZ were

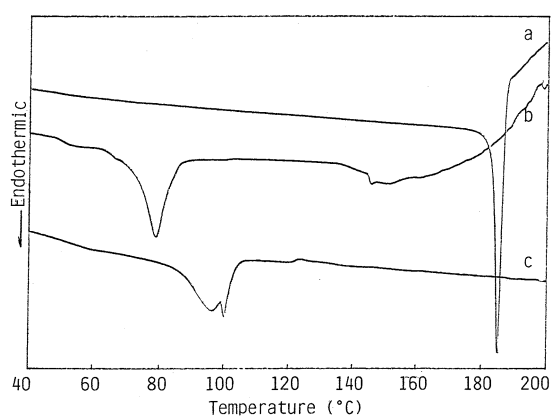

Fig. 1. The DSC curves of MCZ (a), mixture of MCZ and HPC (b) and HPC (c).

Table 2
Solubility of MCZ in mineral oil with or without HPC

Concentration of HPC (%)	Solubility (mg/ml)	
	95 °C	20 °C
0	<0.5	0.01
1	2	0.26
2	5	0.47
5	11	1.74

heated together to 95 °C, then, cooled to 20 °C, the solubility also increased in proportion to the HPC concentration. However, the reproducibility was low. Thus, the change of solubility with 2% HPC was examined for two cases: (1) excess amount of MCZ (10 mg/ml) was added so that crystals of MCZ remained even at 95 °C, and (2) MCZ was added at a concentration of 1 mg/ml so that MCZ was completely dissolved at 95 °C. In the case where an excess amount of MCZ was added, MCZ concentration at 20 °C after 1 h cooling was 1.4 mg/ml. The concentration decreased with time to 1.0 mg/ml after 1 day and 0.5 mg/ml after 8 days. In contrast, in the case where MCZ dissolved completely, MCZ concentration remained at 1.0 mg/ml up to 8 days. This suggests that the residual crystals at high concentrations of MCZ induced recrystallization. It appears that supersaturation occurred and remained relatively stable for the lower concentration (1 mg/ml) without recrystallization. However, recrystallization occurred with time for the high concentration (10 mg/ml) sample, which contained some crystals that acted as seed crystals for crystallization. Without heating, MCZ solubility showed no change with or without HPC. It was observed that HPC dissolved in a mixture of mineral oil and MCZ at 95 °C suggesting that MCZ was solubilized by HPC due to some interactions. However, at 20 °C, HPC itself scarcely dissolved in mineral oil and the solubility of MCZ did not change.

3.2. Release of MCZ from gel formulations

It was shown that MCZ has some interaction with HPC at high temperature resulting in solubilization of MCZ in mineral oil. We have reported that some drugs, which have interactions with HPC, maintain to some extent a supersaturated state in the gel formed with HSL and mineral oil (Henmi et al., 1994; Hori et al., 1998; Fujii et al., 2001). Such a gel with MCZ was prepared and the release of MCZ from the gel was determined. Plots of the square root of time versus the amount released were linear, so the release rates were calculated from the slope of these plots.

The amount of drug released at time t from solution type ointments is described as $2C(Dt/\pi)^{1/2}$, where C is the concentration in the gel, and D is the diffusion coefficient through the gel. The release rate is shown as $2C(D/\pi)^{1/2}$ (Higuchi, 1962). Thus the release rate correlates with C. If the ointment is a suspension type, the release rate is expressed as $(2CDCs)^{1/2}$, where Cs is the solubility of the drug in the ointment. Thus, the release rate correlates with $C^{1/2}$ (Higuchi, 1960). Fig. 2 shows the relationship between MCZ concentration in the gel and the release rates. The release rates were proportional to MCZ concentration at or under 1%. The release rate from the 1.5% MCZ gel was the same as that from the 1.0% MCZ gel. The release rate of MCZ from gel + MCZ was 0.5 ± 0.1 $\mu g/cm^2/h^{1/2}$. It was about 1/20 that from the gel with the same MCZ concentration. This result suggested that the dissolution rate of MCZ in the gel at 37 °C was low and the MCZ in the normal gel is probably in solution, and the solubility of MCZ in the gel was about 1%. The slope from 0 to 1% MCZ in Fig. 2

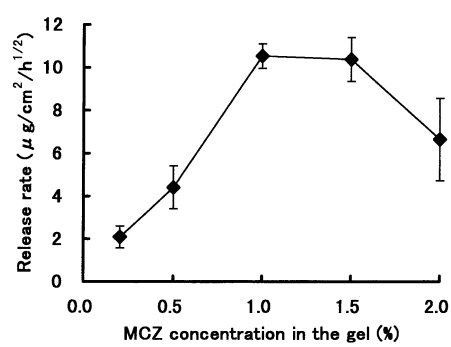

Fig. 2. The relationship between MCZ concentration in the gel and the release rate of MCZ from the gel. Each point represents the mean ± S.D. of at least three experiments.

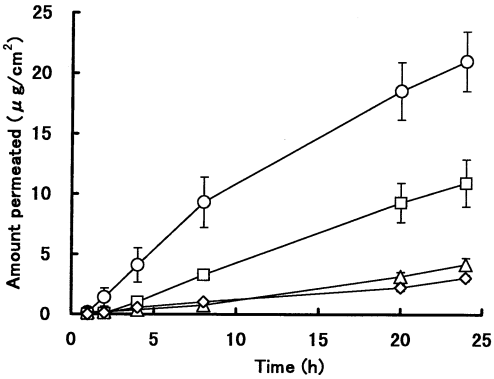

Fig. 3. Permeation profiles of MCZ from various formulations: (◇) mineral oil suspension; (△) gel + MCZ; (□) aqueous suspension; (○) gel. Each point represents the mean ± S.D. of at least three experiments.

can be expressed as $2(D/\pi)^{1/2}$ and was calculated to be 1.0×10^{-3} cm/h$^{1/2}$, so the diffusion coefficient D through the gel calculated from above equation was 8×10^{-7} cm^2/h.

3.3. Permeation of MCZ from various formulations

Permeation of MCZ from various formulations was studied and the results are shown in Fig. 3. MCZ permeated little from a mineral oil suspension. The permeation from an aqueous suspension was higher than that from the mineral oil suspension. In general, the permeation from suspensions is similar even when the type of solvent is different. It is possible that the dissolution rate of MCZ was so low in mineral oil that dissolution was the rate-limiting step of the permeation.

The permeation from gel + MCZ was almost the same as that from the mineral oil suspension. The permeation from the gel containing 1% dissolved MCZ showed about six times that from the mineral oil suspension or gel + MCZ and two times that from the aqueous suspension. This suggests that the increased permeation is not because of the effect of the vehicle on the skin, but, because of MCZ solubility in the vehicle. Higher permeation from the gel may result from MCZ interaction with HSL and its presence in a supersaturated condition.

3.4. Effect of DDAIP on the permeation of MCZ

Fig. 4 shows the permeation profiles of MCZ with or without DDAIP, a biodegradable penetration enhancer, in various formulations. When skin was pretreated with DDAIP for 2 h, the permeation from an aqueous suspension increased 11 times of that without pretreatment. It was reported (Büyüktimkin et al., 1996) that DDAIP shows high enhancement effect in the case of an acidic drug, because of interaction between DDAIP and the drug. However, relatively low enhancement effects were observed in the case of basic drugs. MCZ is also a basic drug, so the effect of DDAIP is limited. However, it appears to be adequate for topical application. The concentration of MCZ in the skin became eight times higher with pretreatment of DDAIP (31 ± 6 μg/cm^2, without DDAIP and 252 ± 62 μg/cm^2 with DDAIP). This suggests that the partitioning of MCZ to the skin was improved by DDAIP. When DDAIP (5%) was added to an aqueous suspension, the permeation was enhanced but only about half as much as pretreatment. DDAIP scarcely dissolved in water, so the transfer of DDAIP to the skin might be lower.

When MCZ was applied as a mineral oil suspension, there was no effect of pretreatment with DDAIP on the permeation profile, although the skin concentration increased 12-fold (1.4 ± 0.5 μg/cm^2, without DDAIP and 17 ± 18 μg/cm^2 with DDAIP). However, this was much less than found from aqueous suspensions suggesting that dissolution of MCZ in mineral oil is so low that the permeation is limited by dissolution even if the permeability of the skin increases.

It is possible that DDAIP increased the partitioning of MCZ to the skin resulting in increased permeation of MCZ. The enhancing effect of DDAIP for MCZ was not as high as that for indomethacin (Büyüktimkin et al., 1993), but a modest increase in skin concentration of MCZ might improve the clinical efficacy of MCZ.

3.5. Combination of the gel and DDAIP

Pretreatment with DDAIP as a skin permeation enhancer resulted in high permeation from an

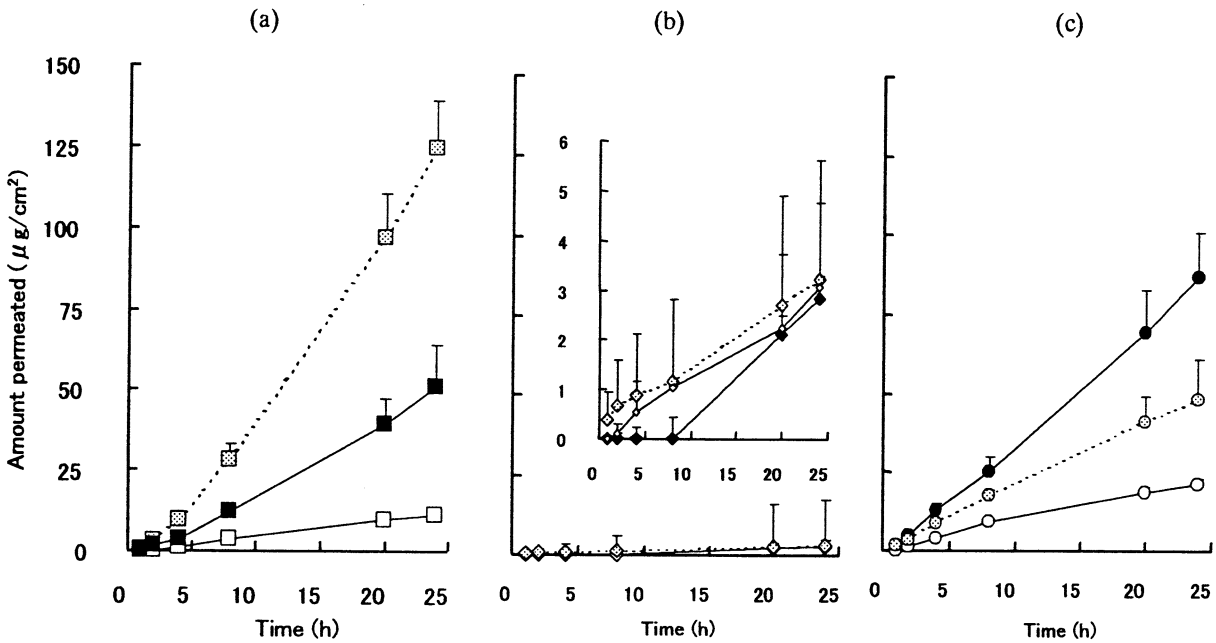

Fig. 4. Effect of DDAIP on the permeation of MCZ from an aqueous suspension (a), from mineral oil suspension (b), and from the gel (c). Open symbols, without DDAIP; Closed symbols, with DDAIP; dotted lines, pretreatment of DDAIP followed by the formulations without DDAIP. Each point represents the mean ± S.D. of at least three experiments.

aqueous suspension. Effects of DDAIP in a gel formulation were also examined. When the gel was applied after 2 h pretreatment of DDAIP, the permeation was two times higher than that without pretreatment (Fig. 4c). The permeation profile showed no linear part. Fig. 5a shows the simulated release profile and permeation profile. The release profile of MCZ from the gel and the permeation profile from the gel with pretreatment of DDAIP were similar. This suggested that DDAIP enhanced the permeation of MCZ probably the same extent as from an aqueous suspension, but that release from the gel limited the permeation.

The effect of an enhancer sometimes decreases when it is combined with a vehicle (e.g. aqueous suspension). In the case of the gel formulation, DDAIP was more effective when incorporated in the gel than when used only for pretreatment. The simulated release profile and the permeation profiles of MCZ from a gel with DDAIP are shown in Fig. 5b. The release of MCZ from the gel was also improved by DDAIP resulting in a relatively high permeation rate.

4. Conclusion

The gel formulation with mineral oil and phospholipid improved the permeation of MCZ by supersaturation of MCZ in the gel. DDAIP enhanced the permeation of MCZ about ten times.

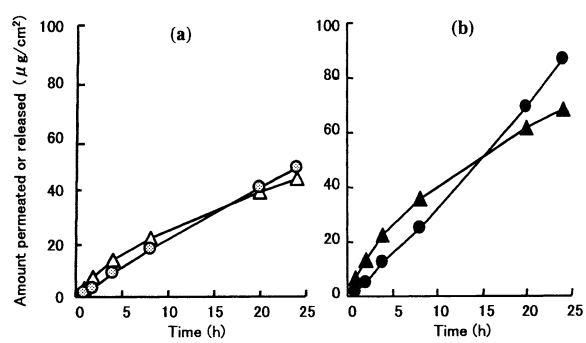

Fig. 5. The permeation profiles of MCZ and simulated release profiles of the gel and the gel with DDAIP; (△) release from the gel; (○) permeation profile from the gel with pretreatment of DDAIP; (▲) release from the gel with DDAIP; (●) permeation profile from the gel with DDAIP. Each point represents the mean of at least three experiments.

When DDAIP (5%) was included in the gel, the permeation rate was seven times that of an aqueous suspension, and permeation was limited by release from the gel. Such a formulation may offer effective topical therapy.

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