Effects of Solvents on Skin Permeation of Formoterol Fumarate

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**Effects of various chemicals applied as penetration enhancers on the permeation of formoterol fumarate (FF) across excised rat skin were investigated. Remarkable enhancement was noted with terpenes, fatty acid esters, and higher alcohols, whereas no significant influence was observed in the case of lower alcohols, pyrrolidones, and amines. At high concentration, a cineole/N-methyl-2-pyrrolidone (NMP) mixed solvent system slightly enhanced the skin permeation of FF compared with cineole alone, and a l-menthol/NMP mixed solvent system caused significant further increase. Maximum skin permeation of FF was seen when the ratio of l-menthol/NMP was 60/40 w/w. From the present results, l-menthol/NMP and isopropyl myristate (IPM)/NMP mixed solvent systems can be considered effective for augmenting skin permeation of FF, with potential applications in transdermal delivery of the drug.**

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Transdermal drug delivery is recognized as a desirable route for administration of systemically active drugs but the skin permeation of drugs is generally poor. Therefore, various methods for enhancing transfer have been examined using penetration enhancers, iontophoresis, and sonophoresis. In particular, many studies have been performed using terpenes, pyrrolidones, polyunsaturated fatty acids, and fatty acid esters.

Formoterol is a catecholamine analogue possessing β2-adreceptor agonist potential, with high potency and prolonged efficacy. Clinical studies have revealed that it causes bronchodilation for at least 12 h after a single oral administration. However, dosing is needed twice a day to maintain inhibition of bronchoconstriction in many asthma patients and in order to maintain effective plasma concentrations and suppress asthmatic fits, transdermal drug delivery has decided advantages.

In the present study, various chemicals were tested for their ability to enhance permeation of formoterol fumarate (FF) across excised rat skin.

**MATERIALS AND METHODS**

**Materials** FF was obtained from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals and solvents were of reagent grade quality and obtained commercially and employed without further purification.

**In Vitro Skin Permeation Study** Hair on the abdominal skin of male Wistar strain rats (Nihon SLC, body weight, 170–200 g) was shaved, and full-thickness skin was excised after 8 h, and the values were about 100-fold greater than with saline. In the case of the fatty acid esters ethyl linoleate, both cells were stirred using magnetic stirrers. At appropriate intervals, 0.5 or 0.3 ml samples were taken from the receiver solution and replaced by the same volume of fresh saline to maintain a constant volume.

Samples collected were made alkaline by the addition of 1.0 ml of 0.5 M phosphate buffer (pH 9.0) and extracted with 3.0 ml of ethyl acetate. After vigorous shaking for 10 min, the organic layer was separated by centrifugation at 3000 rpm for 10 min, and 2.5 ml of the organic layer was transferred to a centrifuge tube, and evaporated in a nitrogen stream at 40 °C. The residue was redissolved in 0.3 ml of 0.1 M of phosphate buffer (pH 3.5), and the concentration of formoterol was determined by HPLC using ECD.

**Determination of FF Concentrations** The HPLC system consisted of a Model AS-950 autosampler, a Model PU-980 pump (JASCO Co., Tokyo, Japan), and an ECD-100 glassy carbon electrochemical detector (Eicom Co., Kyoto, Japan). Detector potential was set at +650 mV versus an Ag/AgCl reference electrode. The analytical column was Nucleosil 100-5C18 (4.0×150 mm I.D.) (GL Sciences Inc., Tokyo, Japan) and the mobile phase consisted of 0.1 M phosphate buffer (pH 3.5)/acetonitrile (80:20 v/v) containing 0.5 mg of Na2EDTA, set at a flow rate of 0.6 ml/min for all separations.

**RESULTS AND DISCUSSION**

**Search for Effective Enhancers** Data for the effects of various chemicals on FF permeation across excised rat skin in horizontal diffusion cells are summarized in Table 1. The concentrations of FF and ethanol were fixed at 12.5 µg/ml and 2.5 µl/ml, respectively, in each solvent.

The skin permeation of FF was remarkably enhanced by terpenes, fatty acid esters, and higher alcohols, whereas no significant effects were observed with lower alcohols, pyrrolidones, and amines. In the case of terpenes, the greatest permeation of FF was seen with cineole, menthone, and linalyl acetate, at 34.5, 28.6, and 23.5 µg/cm², respectively, after 8 h, and the values were about 100-fold greater than with saline. In the case of the fatty acid esters ethyl linoleate,
diethyl sebacate, and isopropyl myristate (IPM), significantly higher permeations of 8.8, 8.1, and 7.8 μg/cm² were apparent after 8 h. Results with l-octanol and l-decanol were similar to those for ethyl linoleate. Although N-methyl-2-pyrrolidone (NMP) has been used as a skin permeation enhancer, the skin permeation of FF was unaffected here.

Skin permeation of lipophilic drugs such as indomethacin and ketoprofen has been reported to be remarkably enhanced by the presence of dl-limonene, and this also is the case for diclofenac sodium in the presence of l-menthol and dl-menthone. Thus, individual terpenes vary in their ability to enhance permeation of different drugs, and the activity is presumably closely related to the physicochemical properties of particular drugs. Cineole or IPM increase the fluidity of stratum corneum lipids and reduce diffusional resistance to permeation, and therefore the findings suggest that FF passes through intercellular domains.

**Synergy of Enhancers and Solvents** Skin permeation of FF was increased by using cineole and IPM, but since the solubility of FF was very low, NMP was used as a solubilizer (solubility; more than 194.6 mg/ml at 37°C). l-Menthol increases the fluidity of stratum corneum lipids and reduces diffusional resistance to permeation, as reported in cineole and IPM. The effects of l-menthol alone could not be evaluated because of its crystal state at room temperature. The effects of l-menthol in NMP solvent were therefore evaluated.

The effects of mixed solvents on the permeation of FF across the excised rat skins were then investigated with a high concentration of FF dissolved in the mixed solvents cineole/NMP, l-menthol/NMP, and IPM/NMP employing modified Frantz diffusion cells. The concentration of FF was fixed at 400 μg/ml.

Figures 1 and 2 show the cumulative amounts of FF permeating per unit area using NMP, with or without cineole or l-menthol. A significant increase was achieved by addition of cineole as compared to NMP alone, and marked elevation was noted with l-menthol in NMP. The skin permeation of FF was significantly enhanced by cineole or l-menthol alone at a high concentration. Moreover, the initial rise with cineole/NMP or l-menthol/NMP permeation tended to be earlier.
than cineole or L-menthol alone. The L-menthol/NMP mixed solvent proved significantly more effective than cineole/NMP. Skin permeation of FF was maximized when the ratio of L-menthol/NMP was 60/40 w/w.

The skin permeation of FF was only slightly enhanced by IPM or NMP alone at a high concentration (Fig. 3), but it was remarkably enhanced with the two in combination, and maximized at the ratio of 60/40 w/w of NMP with IPM.

The effect of each enhancer is greatly related to the lipophilicity of the drug. Enhancement effects of mixed solvents such as IPM/NMP and L-menthol/ethanol have already been reported.

Table 2 shows the results of pretreatment of skin with mixed solvent systems for subsequent skin permeation of FF. Pretreatment solution was applied to the skin for 2 h, followed by washing with a sufficient amount of distilled water. A remarkable increase was achieved by pretreatment with IPM as compared to saline, and similar results were obtained with IPM/NMP (70:30). Also, pretreatment with NMP resulted in significantly increased skin permeation.

The important roles of solvents such as ethanol, propylene glycol, and NMP on the skin permeation of a drug might be not only due to effects on the solubility but also of function such as a reduction of the barrier structure of skin. Pretreatment with solvents confirmed that the solvent acts directly on the stratum corneum as an enhancer, increasing the fluidity of stratum corneum lipids and reducing diffusional resistance to permeation. The results given in Table 2 clearly show the synergy of IPM and NMP.

Enhancement effects of various chemicals have been reported, but many chemicals cause skin irritation. Terpenes such as cineole and L-menthol and fatty acids, however, and their esters such as IPM are known as relatively safe compounds. Lashmar et al. reported skin irritation with NMP. blushend that cineole or L-menthol alone. The L-menthol/NMP mixed solvent proved significantly more effective than cineole/NMP. Skin permeation of FF was maximized when the ratio of L-menthol/NMP was 60/40 w/w.

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using nude mice skin based on a histopathologic study. In the present case, however, neither erythema nor edema was observed with NMP in hairless Guinea pigs. In future, the efficacy and safety of mixed solvent systems should be investigated in vivo in humans.

**Skin Permeation of Formoterol Free Base** Skin permeation of the formoterol free base was also examined in comparison with FF using \( l \)-menthol/NMP and IPM/NMP mixed solvents. Figure 4 shows data for cumulative amounts of the drug permeating per unit area, values for the formoterol free base and FF being similar. These results suggest FF in mixed solvents exists in the form of formoterol free base under the conditions used.

**CONCLUSIONS**

The skin permeation of FF is remarkably enhanced by cineole/NMP, \( l \)-menthol/NMP, and IPM/NMP mixed solvents. When the ratio of \( l \)-menthol/NMP was 60/40 w/w, maximum skin permeation of FF was obtained. The results indicate \( l \)-menthol/NMP and IPM/NMP mixed solvents are effective enhancers of FF penetration, with potential applications for transdermal delivery.

**REFERENCES**