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Purification of isopropyl fatty acids markedly changed the skin permeation of a model hydrophilic chemical

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ABSTRACT

We determined the difference in the skin-penetration enhancing effects of conventional (C-) and super-refined (SR-) isopropyl fatty acids (IPFAs) including isopropyl myristate (IPM), isopropyl palmitate (IPP), and isopropyl oleate (IPO). *In vitro* permeation experiments were performed with excised hairless rat skin using a model mal-absorbed chemical, calcein. As a result, SR-IPM pretreatment markedly increased the skin permeation of calcein from aqueous solution more than C-IPM pretreatment, in spite of a small difference in IPM purity (98.78 vs 99.96%). Similar phenomena were recorded even when using SR-IPM emulsion compared with C-IPM emulsion without pretreatment. In contrast, C-IPP pretreatment showed higher skin permeation than SR-IPP. In addition, no difference was observed in skin permeation between C-IPO and SR-IPO. Skin impedance was also determined as an index of skin barrier function. SR-IPM changed the skin barrier function more than C-IPM, which supported the penetration-enhancing order of C-IPM and SR-IPM shown above. It was unexpected to find such a big difference in the skin-penetration enhancing effect of C-IPM vs SR-IPM and C-IPP vs SR-IPP. The present results suggested that SR-IPM could be a promising ingredient in pharmaceutical and cosmetic products for topical use.

Searching for potent skin-penetration enhancers is a key issue in the development of new topical and transdermal drug delivery systems. Through many previous exploratory studies, a variety of delivery systems containing chemical enhancer(s) have been developed. However, it has become more difficult to find new potent enhancers over the past decade or two, which suggests that the present methodology and ideas are not sufficient to find good skin-penetration enhancers. Several researchers have already applied physical enhancement methods such as iontophoresis and microneedle puncture with chemical enhancers [1,2]. A combination of two or more chemical enhancers is also useful to further increase the skin permeation of drugs [3–5]. Because the purity of most enhancers in previous studies has not been 100.0%, these studies even using single enhancers may be thought of as examining the effect of a kind of enhancer mixture. We then paid attention to the purity level of the enhancers.

Isopropyl fatty acids (IPFAs) such as isopropyl myristate (IPM), isopropyl palmitate (IPP), and isopropyl oleate (IPO) are major ingredients (as skin penetration enhancers) in topical and transdermal delivery systems as well as cosmetic products. IPM is the most used among such fatty acid esters, and it was expected to especially enhance the penetration of active chemicals through the skin [4,6]. The previous experiments were done using crude IPFAs (such as IPM) as described above. Nowadays, super-refined IPFAs are available as a result of the rapid progress in purification technology for IPFAs. This technology using column chromatographic purification physically removes primary impurities to have super-refined IPFAs (SR-IPFAs). The impurities in the conventional IPFAs (C-IPFAs; such as IPM) may be related to the increased skin irritation and decreased stability as well as lowering the penetration enhancing ability of drugs through the skin.

In the present study, the effect of SR-IPFAs especially SR-IPM on the skin-penetration enhancing effect was investigated to reveal the usefulness of newly developed SR-IPFAs compared with C-IPFAs using a conventional *in vitro* skin permeation experiment and skin impedance determination. The purity of C-IPM used in this experiment was 98.78%, whereas that of SR-IPM was 99.96% (Table 2).

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1. Materials and methods

1.1. Materials

C-IPM, SR-IPM, C-IPP, SR-IPP, C-IPO, SR-IPO, and the mixture of impurities remaining in the column after the purification process were gifts from Croda Japan (Tokyo, Japan). Other reagents and solvents were of analytical grade and used without further purification. Table 1 shows the chemical structures and molecular weights (M.W.) of the chemicals analyzed. Table 2 compares impurities in the C-IPM and SR-IPM used in this experiment.

Table 2
Impurities in C-IPM and SR-IPM.

	C-IPM	SR-IPM
Benzenesulfonic acid	0	0
Dodecanoic acid	0.24	0
Tridecanoic acid	0.21	0.01
Tetradecanoic acid	0	0
Isopropyl 13-methyltetradecanoate	0	0
Butyl myristate	0.03	0
Isopropyl palmitate	0.74	0
Unidentified compounds	0	0.03
Total (impurity)	1.22%	0.04%

These values are for the C-IPM and SR-IPM used in this experiment.

1.2. Experimental animals

Male hairless rats (WBM/ILA-Ht, 7–9 weeks old, body weight: 200–250 g) were purchased from either Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experiment Animal Laboratories (Fukaya, Saitama, Japan). They were housed in temperature-controlled rooms (25 ± 2 °C) with a 12 h light-dark cycle (7:00–19:00 h). The rats were allowed free access to food (Oriental Yeast Co. Tokyo, Japan) and tap water. All breeding procedures and the experiments on animals were performed in accordance with the guideline of the Animal Experiment Committee of Josai University.

1.3. Preparation of formulations

Oil-in-water (O/W) emulsions containing calcein as a model mal-absorbed hydrophilic penetrant were prepared with 30% (v/v) C-IPFAs or SR-IPFAs and 0.1% (v/v) polysorbate (Tween) 80 as an emulsifier. The mixture of IPFA and Tween 80 was added slowly to calcein in pH 7.4 phosphate-buffered saline–EDTA solution (PBS-EDTA). The obtained solution was agitated thoroughly at 14,000 rpm for 5 min using a Polytron PT3100 (Kinematica AG, Littau-Lucerne, Switzerland) to obtain O/W emulsions.

1.4. In vitro skin permeation experiment

1.4.1. In vitro skin permeation of calcein from PBS-EDTA after pretreatment with SR-IPFA

Abdominal full-thickness skin from hairless rats was excised under anesthesia by *i.p.* injection of medetomidine (0.375 mg/kg), butorphanol (2.5 mg/kg), and midazolam (2 mg/kg), and excess subcutaneous fat and blood was carefully removed. Then, the hairless rats were sacrificed immediately by injection of pentobarbital sodium salt (40 mg/kg). The stratum corneum surface of the skin (application area; 0.95 cm^2) was pretreated with 3.0 mL of IPFAs for 1 h. Then, the pretreated skin was wiped and washed with PBS-EDTA to remove excess IPFAs from the skin surface. The skin piece was mounted in a side-by-side diffusion cell with an effective diffusion area of 0.95 cm^2 [7]. Then, 0.1 M calcein solution (3.0 mL) was placed on the stratum corneum side of the skin. The receiver solution was 3.0 mL of PBS-EDTA, which was maintained at 32 °C using a thermo-regulated water bath. A magnetic stirrer bar was added in the donor and receiver compartments, which moved at about 1200 rpm throughout the experiment. The receiver solution samples (0.5 mL) were withdrawn every 1 h, and the same volume of PBS-EDTA was added to the receiver compartment to keep the volume constant.

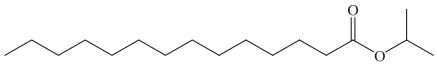
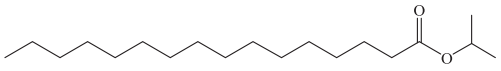
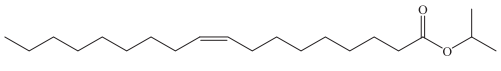
1.4.2. In vitro skin permeation experiment with calcein from O/W emulsion without pretreatment

The O/W emulsion (3.0 mL) containing calcein with C-IPFA or SR-IPFA was placed on the stratum corneum side of the skin (without pretreatment) in the side-by-side diffusion cell, as above. The other methods were the same as above.

1.4.3. Determination of calcein

Each receiver sample was examined for calcein using a fluorospectrophotometer (RF 5300PC, Shimadzu, Kyoto, Japan) at an excitation wavelength of 488 nm and fluorescent emission wavelength of 515 nm.

Table 1
Structure and molecular weight of IPFAs used in the present study.

IPFAs	Abbreviation	Molecular formula *M.W.*
Isopropyl myristate	C-IPM SR-IPM	 270.5 270.5
Isopropyl palmitate	C-IPP SR-IPP	 298.5 298.5
Isopropyl oleate	C-IPO SR-IPO	 324.5 324.5