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Enhanced Skin Permeation of a New Capsaicin Derivative (DA-5018) from a Binary Vehicle System Composed of Isopropyl-myristate and Ethoxydiglycol

Bong Jin Cha[1], Eung Doo Lee[1], Won Bae Kim[1], Suk-Jae Chung[2], Min-Hwa Lee[2], and Chang-Koo Shim[2]

[1]Research Laboratories, Dong-A Pharm. Co., Ltd., Kyunggi-do 449-900, and [2]College of Pharmacy, Seoul National University, Seoul 151-742, Korea

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DA-5018, a recently synthesized capsaicin analog, appears to possess potent analgesic activity when administered topically. The objective of this study is to test the feasibility of the topical administration of this compound. Specifically, our goal was to identify vehicle system that permit a reasonable transdermal permeation of the compound in mice. Among the vehicles examined, isopropyl myristate (IPM) showed the largest in vitro permeability across the intact skin (83.6 ± 5.42 μl/cm^2/h). However, due to the limited solubility of DA-5018 in IPM (0.53 mg/ml), the maximal flux from the IPM medium remained at only 44.3 ± 2.87 μg/cm^2/hr. In order to increase the flux, addition of better solvents for DA-5018 was attempted, under the assumption that flux is the result of both solubility and permeability. Ethoxydiglycol (EG) and oleic acid (OA) were selected as examples of good solvents. The addition of EG or OA to IPM at a 1:1 volume ratio resulted in a comparable increase in the solubility of the compound (i.e., to 61.1 and 50.2 mg/ml for EG and OA, respectively). However, the addition of EG at a 1:1 volume ratio, for example, increased the flux 6.3 fold (i.e., 279 μg/cm^2/hr), while OA, at a 1:1 volume ratio, decreased the flux 5 fold (i.e., 9.26 μg/cm^2/hr). The mechanism of this discrepancy between EG and OA was investigated by measuring the permeabilty of DA-5018 across the stratum corneum-removed skin of the mouse, under the hypothesis that the viable skin layer may serve as a barrier for the permeation of lipophilic substances such as DA 5018. The permeability of DA-5018, from the medium of EG or OA, across the viable skin differed greatly for EG (0.41 μl/cm^2/hr) and OA (0.086 μl/cm^2/hr), suggesting that a higher permeability across the viable skin layer is needed for the second solvents. The maximum flux across the intact skin was achieved for DA-5018 when EG was added to IPM at a 1:1 volume ratio. Thus, the use of a binary system appears to be the best approach for realizing the transdermal delivery of DA-5018 at a reasonable rate.

Key words: DA-5018, Capsaicin, Isopropyl myristate (IPM), Ethoxydiglycol (EG), Permeability, Solubility, Flux, Binary vehicle system

INTRODUCTION

Capsaicin, a major pungent principle present in various capsicum fruits, is known to possess a potent analgesic activity, especially on the peripheral part of the sensory nervous systems. It has been demonstrated that, when applied topically, capsaicin is clinically useful in the attenuation of peripheral pains associated with diabetic neuropathy and rheumatoid arthritis (Chad *et al.*, 1991; Levy et al., 1991).

DA-5018, *N*-3-(3,4-dimethylphenyl)propyl]-4-(2-amino-ethoxy)-3-methoxy phenyl acetamide (Fig. 1), is a new capsaicin derivative that has recently been developed by the Dong-A Pharmaceutical Company. The analgesic activity of this compound has been reported to be more potent than that of capsaicin (Park *et al.*, 1991; Son *et al.*, 1997), suggesting that the compound has therapeutic potential for controlling peripheral pains. DA-5018 is presently under development for use in topical applica-

Correspondence to: Chang-Koo Shim, College of Pharmacy, Seoul National University, San 56-1, Shinlim-dong, Kwanak-gu, Seoul 151-742, Korea
E-mail: shimck@plaza.snu.ac.kr

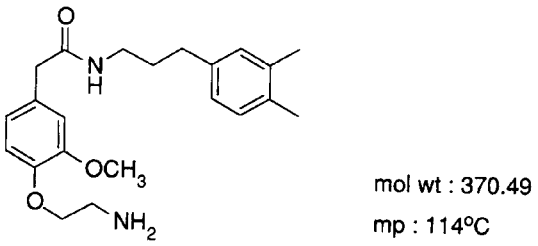

Fig. 1. The chemical structure of DA-5018, *N*-[3-(3,4-dimethylphenyl)propyl]-4-(2-aminoethoxy)-3-methoxyphenyl acetamide

tions by the Dong-A Pharmaceutical Company. In the present study, various vehicles were examined for their suitability as components of formulations that permit the substantial systemic absorption of DA-5018 after topical application.

MATERIALS AND METHODS

Materials

DA-5018 was obtained from the Dong-A Research Laboratories. Isopropyl myristate (IPM), oleyl alcohol (HD-Ocenol), oleic acid decyl ester (OADE, Cetiol V), caprylic/capric acid triglyceride (Myrithol 318) were purchased from Henkel (Dusseldorf, Germany). PEG-6 glyceryl mono oleate (Labrafil), octyldodecyl myristate, PEG-8 glyceryl caprylate/caprate (Labrasol) and ethoxydiglycol (EG, Transcutol) were supplied by Gattefosse (Saint-Priest Cedex, France). Oleic acid (OA) and glycerine were purchased from the Duksan Co. (Ansan, Korea). All other chemicals used in this study were of analytical reagent grade and were used without any further purification. Male hairless mice (aged 5-7 weeks) were purchased from Charles River Laboratories (Atsugi, Japan).

Preparation of DA-5018 suspensions for permeation studies and solubility determination

DA-5018 was added to test vehicles (see Table I, 10 ml each) in stoppered test tubes, and the tubes were agitated for 48 hr in a thermostated (32°C) water bath. The addition was continued until the amount of DA-5018 in the vehicles exceeded its solubility, in order to maintain saturated concentrations of DA-5018 in the vehicles during permeation experiments. The resultant suspensions were subjected to subsequent permeation studies. For the determination of solubility of DA-5018 in the vehicles, the suspension was filtered through a 0.45 μm pore size filter (Acrodisc LC PVDF, Gelman Science), and the concentration of DA-5018 in the filtrate was analyzed by an HPLC method (see below) after appropriate dilution with methanol.

In vitro permeation across the whole skin (intact skin) and viable skin

Hairless mice (male, 5 to 7 weeks) were sacrificed by cervical dislocation. Full-thickness skin was excised from the dorsal side of the animal and the subcutaneous fat layer was carefully removed by rubbing with a saline saturated gauze. The skin was then mounted in a modified Franz diffusion cell, within 1 h of preparation, between the donor and receptor compartments so that the epithelial side of the skin faced the donor compartment. The surface area of the cell exposed to the donor phase was 4.7 cm^2. The receptor chamber was filled with 17 ml of isotonic saline, which was stirred during the experiment by a rotating Teflon-coated magnet at 600 rpm using a synchronous motor. The temperature of the cell was maintained at 37°C using a water bath. A 2 ml aliquot of each suspension of DA-5018 was applied to the donor compartment, after which, it was immediately sealed with a paraffin film to prevent any loss of the suspension through evaporation. A 0.1 ml aliquot of the receptor phase was removed at predetermined time points for 8 h, with fresh saline replacement, and assayed for DA-5018 by the HPLC method described below. The experiment was repeated three times for each suspension (i.e., each vehicle).

For measurement of permeability across viable skin, the stratum corneum (SC) layer of the whole skin of hairless mice was removed by stripping off the SC layer 20 times with the aid of cellophane tape (Scotch, 3M). The permeability across the SC-removed skin was measured in a similar manner as described above for the whole skin.

HPLC analysis of DA-5018

The concentration of DA-5018 in each sample was assayed by HPLC (Eldex Model 9600; Can Carlos, CA, USA) by fluorescence detection (Linear Fluor LC 304; Linear, Reno NE, USA; Excitation wavelength, 270 nm; Emission wavelength, 330 nm). The HPLC system was equipped a reversed phase Symmetry C18 column (5 μm, 15 cm × 3.9 mm ID). The mobile phase consisted of 5 mM methanesulfonic acid and 10mM NaH_2PO_4 (pH 2.5, adjusted with 30% phosphoric acid): acetonitrile (70:30), and was delivered at a flow rate of 1 ml/min. The limit of quantification (LOQ) of this analytical procedure was 10 ng, as an injected amount.

Data analysis

The rate of permeation, i.e., flux, of DA-5018 across the whole skin and SC-removed skin was attained by a linear regression of the slope of the linear portion of the plot between the cumulative amount which appeared in the receptor compartment vs time. The permeability was then calculated by dividing the flux by the concentration of DA-5018 in the donor phase, which was maintained constant at the solubility of the compound in the vehicle

since the compound had been applied in the form of a suspension. The time lag for reaching steady permeation was read from the abscissa of the extrapolation of the linear portion of the cumulative amount-time curve. Student t-test was used to compare the mean values of the two groups. A value of $P<0.05$ was accepted as denoting a statistical difference.

RESULTS AND DISCUSSION

Solubility of DA-5018 in the vehicles

The solubility of DA-5018 in water at 32°C increased with decreasing pH of water, but was very low (less than 1 mg/ml for pH 1-9) at any pH (data not shown). Solubility in some of the other vehicles varied greatly depending on the vehicle types. For example, OA, EG, PEG-8 glyceryl caprylate/caprate and oleyl alcohol exhibited a relatively high solubility (i.e., > 10 mg/ml), while OADE, IPM, caprylic/capric acid triglyceride, octyldodecyl myristate and glycerine had a very low solubility (i.e., <2 mg/ml), as shown in Table I. The solubility of DA-5018 appears to be high in solvents that containing both hydrophobic (i.e., carbohydrate) and hydrophilic (i.e., either hydroxyl or carboxyl) moieties, as evidenced by the cases for oleyl alcohol and OA. This might be related with the fact that DA-5018 has both hydrophobic (i.e., a carbohydrate moiety) and hydrophilic (i.e., an amino moiety) moieties in the molecule. When the hydrophilic moiety of the solvent was blocked by esterification, the solubility decreased, as evidenced by the cases of IPM and OADE.

In vitro skin permeation of DA-5018 from vehicles

The in vitro permeation of DA-5018 across intact skin is shown in Fig. 2 for ten vehicles containing the compound in the form of a suspension. The cummulative amount of DA-5018 that permeated increased linearly as a function time, but with some time lag (2~4 min) before reaching the respective steady state permeation. This

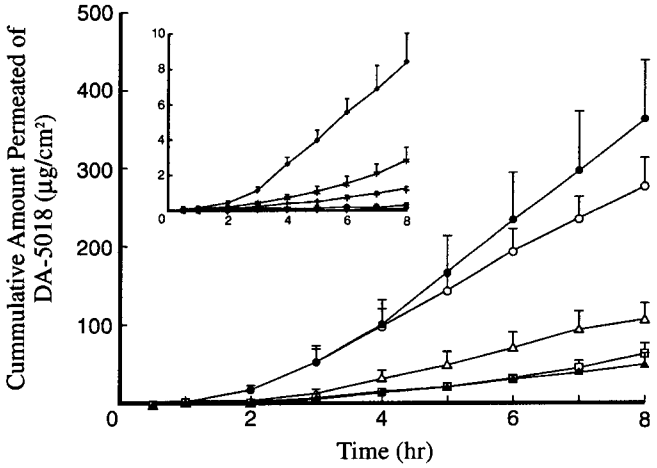

Fig. 2. Cummulative permeation (mean ± SD, n=3) of DA-5018 from various vehicles across the whole skin of hairless mice. The concentration of DA-5018 in each vehicle was maintained constant at its solubility during the experiments by suspending the compound in the tested vehicle. ● : Oleyl alcohol, ○: IPM, △: OADE, □: Labrafil, ▲: Caprylic/capric acid triglyceride, ◆ : oleic acid, ✱: octyldodecyl myristate, ✚: ethoxydiglycol, ★ : PEG-8 glyceryl caprylate/caprate, ▼ : glycerine.

Table I. Solubility, in vitro flux and permeability of DA-5018 in various vehicles

Vehicle	Solubility (A) (mg/ml)	Flux (B) (μg/cm²/h)		Permeability (B/A) (μl/cm²/h)	
		whole skin	viable skin	whole skin	viable skin
Caprylic/capric acid triglyceride	1.10	9.69 ± 2.72	nd	8.809 ± 1.081	nd
Ethoxydiglycol (EG, Transcutol)	75.9	0.23 ± 0.02	31.1 ± 1.45	0.004 ± 0.000	0.410 ± 0.019
Glycerine	1.10	ns	nd	nd	nd
Isopropyl myristate (IPM)	0.53	44.3 ± 2.87	51.8 ± 5.68	83.6 ± 5.42	97.7 ± 10.7
Octyldodecyl myristate	1.03	0.58 ± 0.16	nd	0.563 ± 0.071	nd
Oleic acid (OA)	93.2	1.47 ± 0.34	8.04 ± 0.69	0.006 ± 0.002	0.086 ± 0.007
Oleyl alcohol	10.7	66.1 ± 8.88	nd	6.178 ± 0.635	nd
Oleic acid decyl ester (OADE, Cetiol V)	0.19	19.9 ± 1.00	nd	104.7 ± 5.123	nd
PEG-6 glyceryl monooleate (Labrafil)	5.89	14.5 ± 3.42	nd	2.462 ± 0.132	nd
PEG-8 glyceryl caprylate/caprate (Labrasol)	27.9	ns	nd	nd	nd
IPM/OA (1:1)	61.1	9.26 ± 1.12	nd	5.56 ± 0.375	nd
IPM/EG (1:1)	50.2	279 ± 18.8	nd	0.152 ± 0.018	nd

Flux was determined using permeation data for each vehicle containing DA-5018 in the form of syspension. Permeability was calculated by dividing the flux by the corresponding solubility. ns: negligibly small. nd: not determined. All the data are expressed as the mean ± SD of three experiments.

time lag might be related to the adsorption and/or diffusion of the compound in the skin layer. Among the vehicles examined, IPM showed the highest flux for DA-5018 (44.3 μg/cm²/hr), followed by OADE and PEG-6 glycerylmonooleate. The permeability of DA-5018, which was calculated from dividing the flux by the solubility, was highest for the IPM suspension (i.e., 0.0836 μl/cm²/hr). Thus, IPM was selected as the first candidate, of the vehicles examined, for achieving the transdermal delivery of DA-5018. The solubility, steady state flux and the time lag to reach the steady state flux of DA-5018 are shown for each vehicle in Table I.

Effect of second solvents on the solubility and flux of DA-5018

The in vitro flux of DA-5018 across the whole skin (44.3 mg/cm²/hr) was low when the compound was applied as an IPM suspension. A larger flux would be necessary for a more efficient attenuation of pains. Generally, the flux of a substance from a vehicle is a product of solubility in the vehicle and the permeability of the substance from the vehicle. Thus, we attempted to obtain a larger flux through increasing the solubility of DA-5018 in IPM (0.53 mg/ml) by adding better solvents for DA-5018 to IPM. OA and EG were selected as the second solvents since they exhibited a higher solubility capacity, of the vehicles tested (Table I). In fact, the addition of EG or OA to IPM, at a 1:1 volume ratio, resulted in a much higher solubility of DA-5018 compared to the case of IPM only (Table I).

Transdermal delivery of a substance involves a sequence of diffusion processes, including permeation across the stratum corenum (SC) layer and then across the deep skin. For lipophilic drugs such as DA-5018, permeation across the deep skin layer (i.e., an aqueous barrier), rather than that across the SC layer, often limits overall transdermal delivery. In this case, the selection of appropriate solvents that permit a higher permeability across the viable skin would be as important as the selection of a better solvent. In order to examine the effect of the second solvent on the permeation of DA-5018 across viable skin, the permeability of the compound across SC-removed skin was compared for the best solvents, OA and EG. Despite of larger solubility capacity for EG compared to OA, a much larger permeability across the viable skin was observed for EG compared to OS (i.e., 0.410 ± 0.0191 vs 0.0863 ± 0.00740 ml/cm²/hr). On the other hand, the flux across the whole skin was larger for OA compared to EG (0.58 vs 0.28 mg/cm²/hr) (Table I).

The effect of added EG led to a maximum flux at an EG:IPM volume ratio of 1:1, indicating the presence of an optimal mixing ratio in the binary system (Fig. 3). The effect EG or OA, added at a ratio of 1:1, on the overall permeation of DA-5018 across the whole skin was then compared. Inconsistent with the solubility and permeability across the whole skin, which were larger for OA, a much larger (approximately 4 fold) flux was achieved when EG was added to IPM compared to OA (Table I). Thus, permeability across the viable skin, compared with the solubility and permeability across the whole skin, appears to be the most dominant factor for the role of the second solvent in increasing the transdermal permeability of DA-5018 from the IPM suspension.

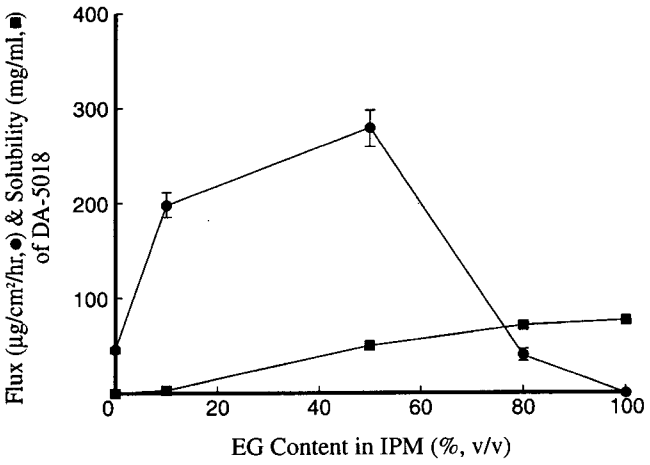

Fig. 3. Solubility and in vitro skin flux of DA-5018 in EG/IPM binary mixtures with varying EG contents in IPM. Each data point represents the mean ± SD of three runs. Key; ●, flux; ■, solubility.

For DA-5018, a lipophilic compound, permeation from the IPM vehicle across the stratum corneum and the viable layers does not appear to behave as a barrier for the overall permeation, as evidenced by the fairly large fluxes found for both layers (Table I). Rather, relatively low solubility might be a contributing factor for the low flux. Thus, the addition of good solvents to IPM appeared to be necessary requirement. Among EG and OA, EG exhibited a good permeabilty across the viable skin (31.1 μg/cm²/hr), while OA (8.04 μg/cm²/hr) did not (Table I). As a result, the EG/IPM binary mixture resulted in a 30 fold larger flux of DA-5018 compared to the OA/IPM mixture (Table I). Thus, the higher permeability of EG in the viable skin layer, as well as the good solubility, appears to contribute to the markedly increased permeation of DA-5018 across the whole skin. Considering the fact that, for permeation across the SC layer, a larger permeability was obtained from OA rather than from EG, permeation across the viable skin appears again to limit the overall rate of transdermal delivery of DA-5018 in the case of delivery from the IPM suspension.

Based on the data presented herein, a cream formulation of DA-5018 (0.3% w/w) was prepared in separate experiments (Cha, 1998), in which DA-5018 was dispersed mainly in IPM (10% w/w), EG (10% w/w) and cetostearyl alcohol (9.2% w/w). The cream exhibited an improved inhibition for croton oil-induced ear edema in

rats, and improved the analgesic effect of Freunds complete adjuvant model in rats, compared to a commercial capsaicin cream (0.075% capsaicin, Zostrix HP, GenDerm). In addition, more than 4% of the dose was recovered as intact DA-5018 from the urine in 24 hr after the topical application of the cream to rats, indicating that a substantial transdermal absorption of the compound from the formulation had occurred. All of these observation support the conclusion that the binary vehicle system of IPM and EG is a potential candidate for use in topical formulations for the transdermal delivery of DA-5018. Extrapolation of this strategy (i.e., the utilization of binary mixtures as vehicles) to other lipophilic substances is currently under examination in our laboratory.

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