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International Journal of Pharmaceutics 159 (1997) 105–114

international journal of pharmaceutics

Percutaneous absorption and histopathology of a poloxamer-based formulation of capsaicin analog

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Received 10 June 1997; received in revised form 18 August 1997; accepted 19 August 1997

Abstract

A new synthetic capsaicin analog (CA) modified with 4-hydroxyl and alkyl chain of capsaicin was synthesized as a potent anti-inflammatory analgesic drug and is now on clinical trial in Korea. The purpose of this study was to investigate the percutaneous absorption and histopathology of a poloxamer-based formulation of CA. A poloxamer-based gel was prepared by cold method using poloxamer 407. Vertical Franz type diffusion cells were used for skin penetration of drug against receptor phase filled with about 10 ml of 0.9% isotonic saline at 32°C. The concentration of drug was determined by the reverse phased HPLC (C18, Symmetry®) with fluorometeric detector. Total amount of CA free base permeated was higher than that of the CA salt form. Percutaneous absorption of CA was greatly enhanced in ethanol and PG than that in water, 2-hydroxypropy-β-cyclodextrin and PEG400. As ethanol concentration increased, percutaneous absorption greatly increased. The flux rate of CA increased slightly when PG was added to ethanol solution. The marked enhancing effect of the 5% fatty acid IPM in cosolvents was also noted on the percutaneous absorption of a poloxamer-based formulation of CA. Addition of 5% OA and 5% LA into the gel containing 5% IPM resulted in a slight increase in skin permeation. No significant difference in skin permeation was observed as a function of poloxamer content (20, 25 and 30%). The buffer system of 30% poloxamer-based gel slightly changed the cumulative amounts of CA penetrated for 24 h. The flux of poloxamer-based gels increased linearly as the drug concentration increased. There was a variation of percutaneous absorption of the drug, depending on the species used. The flux of a poloxamer-based formulation of CA was the highest in case of hairless mice but the lowest in hamsters. No skin erythema and histopathologic changes were observed on the dorsal site of hairless mice in six groups after a week or two months application, suggesting no skin toxicity of the poloxamer-based gel. Based on these findings, the current poloxamer-based formulation appears useful in the systemic delivery of CA as topical or transdermal patch formulations. © 1997 Elsevier Science B.V.

Keywords: Capsaicin analog; Poloxamer-based gels; Penetration enhancers; Percutaneous absorption; Histopathology

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PII S0378-5173(97)00276-7

Table 1
Formulation compositions of a poloxamer-based gel expressed as weight percentages (w/w)[a]

Codes	Poloxamer	Drug	Ethanol	PG	Fatty acid	Diluent
1	20	0.5	20		IPM (5)	water (54.25)
2	20	0.5	40		IPM (5)	water (34.25)
3	20	0.5	40		IPM (10)	water (29.25)
4	20	0.5	20	20	IPM (5)	water (34.25)
5	25	0.5	20	20	IPM (5)	water (29.25)
6	30	0.1	20	20	IPM (5)	water (24.25)
7	30	0.5	20	20	IPM (5)	water (24.65)
8	30	1.0	20	20	IPM (5)	water (23.75)
9	30	0.5	20	20	IPM (5)/LA (5)	water (19.25)
10	30	0.5	20	20	IPM (5)/OA (5)	water (19.25)
11	30	0.5	20	20	IPM (5)/OA (5)	pH 4.5 (19.25)
12	30	0.5	20	20	IPM (5)/OA (5)	pH 6.4 (19.25)
13	30	0.5	20	20	IPM (5)/OA (5)	pH 7.4 (19.25)

[a]The methyl paraben (0.15%) and propyl paraben (0.1%) were invariably added into poloxamer based gel.

1. Introduction

Capsaicin is a pungent component present in chilly peppers and related plants of the capsicum family. Capsaicin exerts its major pharmacological effects on the cardiovascular, respiratory and sensory nervous systems (Tominak and Spyker, 1987; Shim et al., 1996; Park et al., 1997). However, the therapeutic usefulness of capsaicin is restricted because of its high irritation and toxicity, including severe burning, pain and neurogenic vasodilation. Several capsaicin analogs have been synthesized to attenuate these unwanted side effects of capsaicin (Tsai et al., 1994; Fang et al., 1996a,b,c).

A new capsaicin analog (CA) modified with 4-hydroxyl and alkyl chain of capsaicin, [*N*-[3-(3,4-dimethylphenyl) propyl]-4-(2-aminoethoxy)-3-Methoxyphenyl acetamide, was synthesized as a potent nonsteroidal anti-inflammatory and analgesic drug and is now on clinical trial in Korea for its therapeutic potential in the treatment of rheumatoid arthritis, diabetic neuropathy and cancer, (Lee et al., 1994; Shim et al., 1996; Park et al., 1997).

A proper dosage form to deliver CA is of clinical value. Transdermal delivery is one of the useful routes avoiding some disadvantages of capsaicin and its analogs such as high hepatic first-pass effect and short duration (Kawada et al., 1985; Crimi et al., 1992).

Poloxamer, a nonionic polyoxyethylene-polyoxypropylene copolymer, has been widely used in pharmaceutical formulations as an emulsifying, wetting, stabilizing, solubilizing and gelling agent (Wade and Weller, 1994). One of the well-known properties of poloxamers is a sol-gel transition in the 25–35°C temperature ranges, resulting in thermogels (Saettone et al., 1988). Poloxamer has been particularly useful in topical, rectal and ocular formulations because of its low toxicity and irritation (Miller and Donovan, 1982; Miyazki et al., 1986; Gil et al., 1994).

The purpose of this study was to investigate percutaneous absorption of a poloxamer-based formulation of CA through excised hairless mouse skin as a function of varying drug form, type and concentration of penetration enhancers, poloxamer content, drug concentration and vehicle pH. The skin variations using four different animals models were also studied. The skin irritation and histopathology of the dorsal sites of hairless mouse skin were evaluated after topical application of saline, poloxamer gel only and poloxamer-based formulation of CA over a week (7 days) or 2 months (60 days).

2. Materials and methods

2.1. Materials

A new capsaicin analog (CA) was synthesized in our laboratory. Poloxamer 407 (Lutrol®F-127) was provided by the courtesy of BASF (Seoul, Korea). Oleic Acid (OA) was purchased from Showa (Tokyo, Japan). Linoleic acid (LA), propylene glycol (PG), isopropyl myristate (IPM) and propyl paraben were purchased from Sigma (St. Louis, MO). Methyl paraben and absolute ethanol were purchased from Wako (Tokyo, Japan) and Hayman (Witham, UK), respectively. Acetonitrile (HPLC grade) was purchased from EM (Gibbstown, NJ). Deionized water was used throughout the study. All other chemicals were of reagent grade and used without further purification.

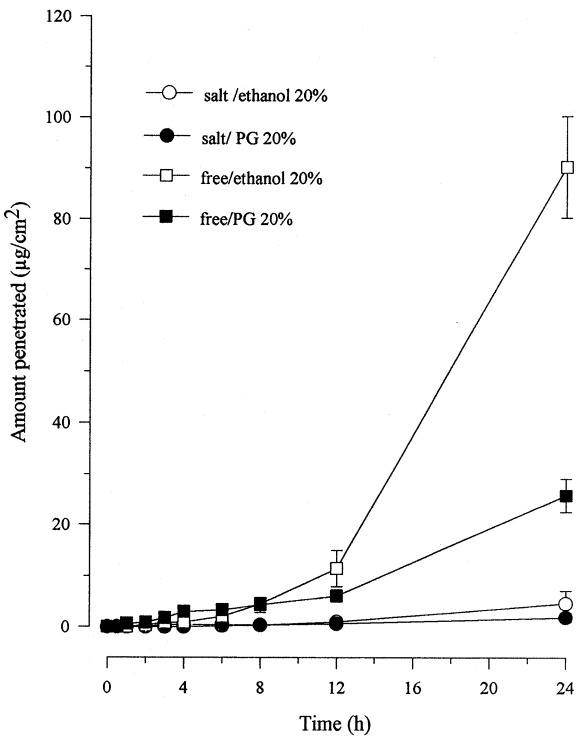

Fig. 1. Comparison of free base and salt form of drug on the percutaneous absorption through excised hairless mouse skin in a vehicle of ethanol and PG solution.

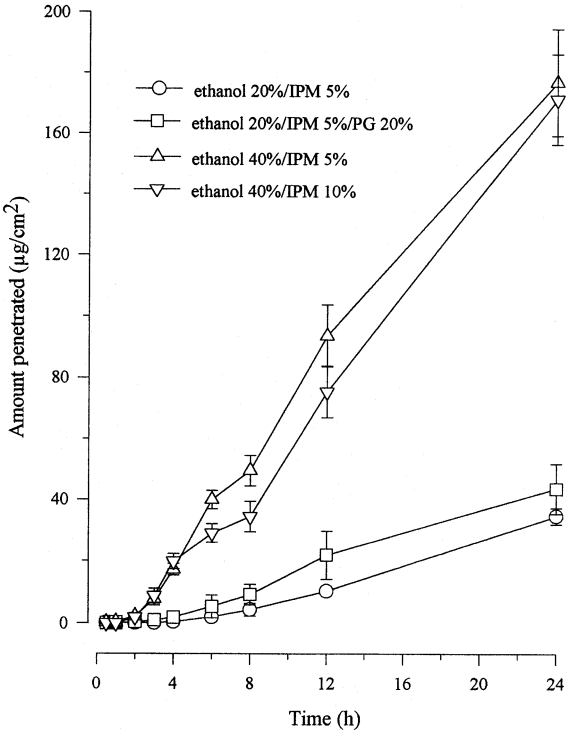

Fig. 2. Effect of various penetration enhancers on percutaneous absorption of 20% poloxamer-based formulation of capsaicin analog through excised hairless mouse skin.

2.2. Preparation of poloxamer-based gel

A poloxamer based gel was prepared according to the cold method. First, drug (0.1, 0.5 and 1.0%), methyl paraben (0.15%) and propyl paraben (0.1%) were dissolved in PG solution using a magnetic stirrer. Ethanol (20, and 40%) was added to this mixture and the final volume (20 ml) was adjusted using distilled water, acetate buffer (pH 4.5), or phosphate buffer (pH 6.4 and 7.4). Poloxamer (20, 25 and 30%, w/w) was dispersed into these mixtures and then stored in refrigerator at 4°C for 24 h. Finally, poloxamer solution was then hardened at room temperature after 5% fatty acids (OA, LA and IPM) were immediately added, resulting in transparent poloxamer gels.

Formulation parameters such as drug forms, type and concentration of penetration enhancers such as ethanol, PG and fatty acids, buffer pH

and poloxamer contents were widely varied during the preparation of poloxamer gel to investigate the percutaneous absorption of CA. The detailed formulation compositions of a poloxamer-based gel are given in Table 1. The formulation compositions are expressed as weight percentages.

2.3. Skin sources and preparation

Four different sources of skin were used in the study. Male SKH1 hairless mice (body weight 20–25 g; age, 4 weeks) were purchased from Charles River (Wilmington, MA) via Woo Jung (Seoul, Korea). Male golden Syrian hamsters (body weight 100–120 g; age, 6 weeks) and male Sprague–Dawley rats (body weight 130–150 g, 6 week old) were obtained from Dae Han (Seoul, Korea). They were housed in temperature-controlled rooms (25 ± 2°C) with a 12 h light–dark cycle (08:00–20:00 h) and allowed free access to food and tap water.

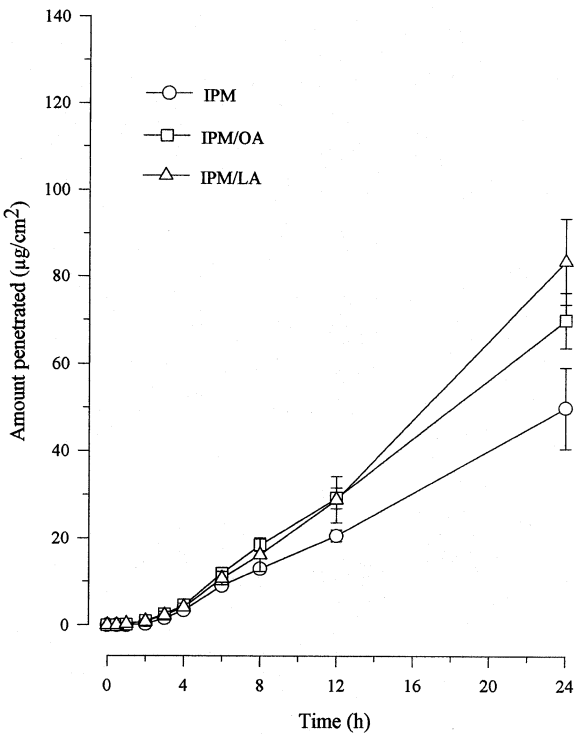

Fig. 3. The synergistic effect of fatty acids on percutaneous absorption of 30% poloxamer-based formulation of capsaicin analog through excised hairless mouse skin.

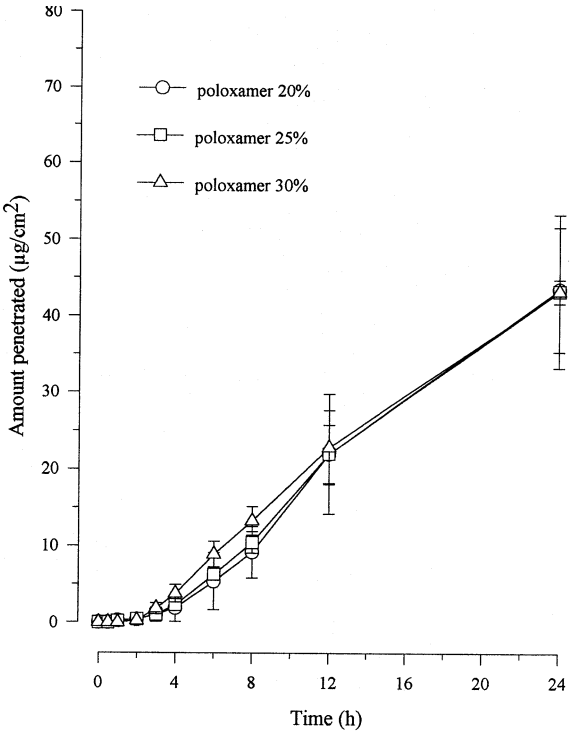

Fig. 4. The percutaneous absorption of 30% poloxamer-based formulation of capsaicin analog as a function of poloxamer content through excised hairless mouse skin.

After two weeks acclimatization period, the hairless mouse, hamster and rat were sacrificed by cervical dislocation. Hairs of the hamster and rat were removed using an electrical clipper. Full thickness abdominal skin of hamster, rat and hairless mouse was excised and the fat inside the skin was carefully removed before diffusion study. The excised human penis skin was kindly donated from Kim's Clinical Surgery (Chuncheon, Korea). The human skin was completely rinsed with 0.9% physiological saline to remove blood and other contamination before use. All skins were fresh and used immediately after pretreatment.

2.4. Diffusion studies

The excised skins were mounted on the receptor compartment with the stratum corneum side fac-

ing towards the donor compartment in Franz-type vertical diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 2.54 cm^2 and 10 ml, respectively. The temperature was maintained at 32°C (approximate skin temperature) with a circulating water bath during the entire experiment. Receptor phase filled with 0.9% physiological isotonic saline was stirred by a motor-driven magnetic bar. A portion (150 μl) of receptor solution was collected through the sampling port of the diffusion cell at a given sampling interval. The receptor phase was replenished with an equal volume of physiological saline after each sample was collected. Samples were stored in a freezer at -40°C over 24 h until HPLC analysis. The concentration of CA penetrated was determined by reverse phased HPLC (C18, symmetry®) with a fluorometeric detector at the wavelength of 270 nm excitation and 330 nm emission as reported previously (Shim et al., 1996).

2.5. Data analysis

Cumulative amounts of drug penetrated per permeation area ($\mu g/cm^2$) through skins was plotted against time and the flux ($\mu g/cm^2$ per h) and lag time (h) of CA were calculated from the slope and the intercept of this plot. All the values were expressed as mean $\pm$ standard deviation.

2.6. Skin irritation and histopathology

Thirty hairless mice were divided into six groups containing five hairless mice each. Saline solution (control), drug-free poloxamer gel and poloxamer-based formulation of CA were applied to the same dorsal site of the hairless mouse skin in six groups (five hairless mice each) for one week (7 days) or two months (60 days) for the evaluation of skin irritation and histopathology. After 1 week or 2 months application, the skin surface applied was gently washed using adsorbent cotton sucked with 0.9% physiological saline. The skin erythema of the dorsal sites of hairless mice was then visually inspected and decided by dermatologist. Thereafter, the dorsal skins applied with saline, poloxamer gel or poloxamer-based formulation of CA were excised for histopathologic evaluation. The excised skins were 10% formalin-fixed and paraffin-embedded tissues. The sections were 4 μm in thickness using microtome and then stained with Harris hematoxylin-eosin solution for the histopathologic examination with 100 times magnification.

3. Results and discussion

3.1. Effect of drug form on skin permeation

The CA has two different drug forms, free base and hydrochloride salt form. The effect of drug forms on skin permeation was compared in the presence of 20% ethanol and 20% PG solution, respectively (Fig. 1). Total amount of CA free base permeated was higher than that of the CA salt form in both 20% ethanol and 20% PG

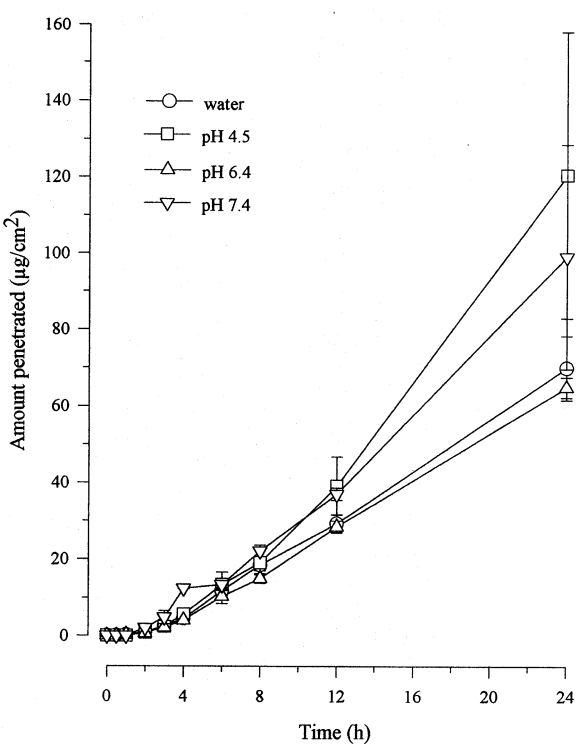

Fig. 5. Effect of pH on the percutaneous absorption of 30% poloxamer-based formulation of capsaicin analog through excised hairless mouse skin.

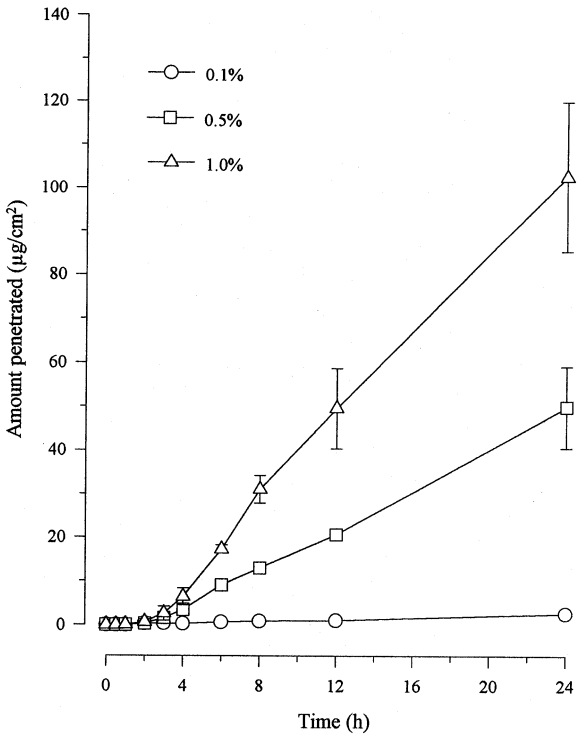

Fig. 6. Percutaneous absorption of 30% poloxamer-based formulation of capsaicin analog as a function of drug concentration through excised hairless mouse skin.

solution. Percutaneous absorption of the CA salt form was negligible when compared with the CA free base. Although the CA salt form had a higher solubility as studied previously, its skin permeation was low due to the hydrophilic property and low partitioning into the stratum corneum. Lipophilic free base form of CA was easily penetrated into the deeper layers of the skin via the lipoidal (non-polar) pathway by compensating the solubility and partitioning into the stratum corneum (Touitou and Fabin, 1988; Ohara et al., 1995). Based on these results, free base form of CA was selected for further investigation.

3.2. Effect of penetration enhancers on skin permeation

Many studies concerning penetration enhancers have been performed in order to reduce the barrier function of the skin. In our preliminary study, CA showed low solubility in distilled water (ca. 289.3 μg/ml). Therefore, other commonly used vehicles such as PG, ethanol, PEG 400 and 2-hydroxypropy-β-cyclodextrin were selected to simultaneously increase solubility and percutaneous absorption of CA. All of these vehicles increased solubility of CA. Of these, the percutaneous absorption of CA was higher in ethanol and PG than in water, 2-hydroxypropy-β-cyclodextrin and PEG 400.

The effects of penetration enhancers on percutaneous absorption of 20% poloxamer-based formulation of CA is shown in Fig. 2. PG and ethanol were effective and synergistic for skin permeation of CA. Furthermore, unsaturated fatty acid IPM resulted in a large increase in percutaneous absorption of drug. As ethanol concentration increased, percutaneous absorption increased greatly. The flux rate of CA increased slightly when PG was added to ethanol solution. PG and ethanol have been widely used as a polar

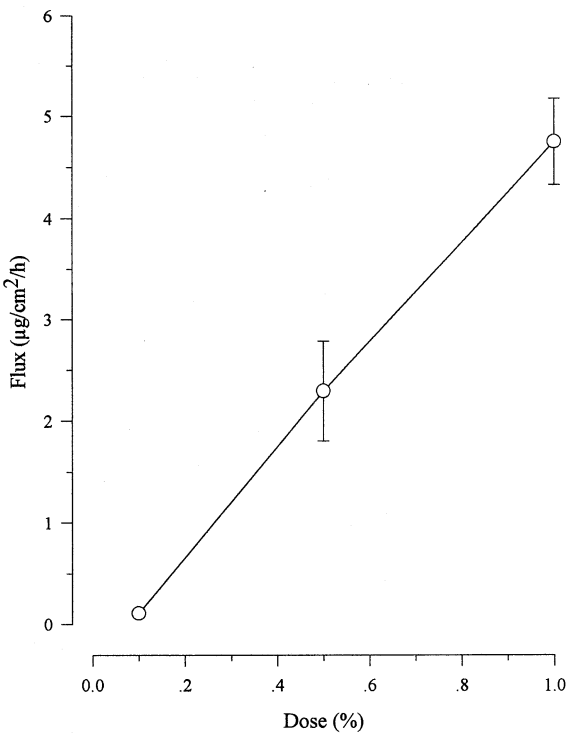

Fig. 7. The flux of 30% poloxamer-based formulation of capsaicin analog as a function of drug concentration.

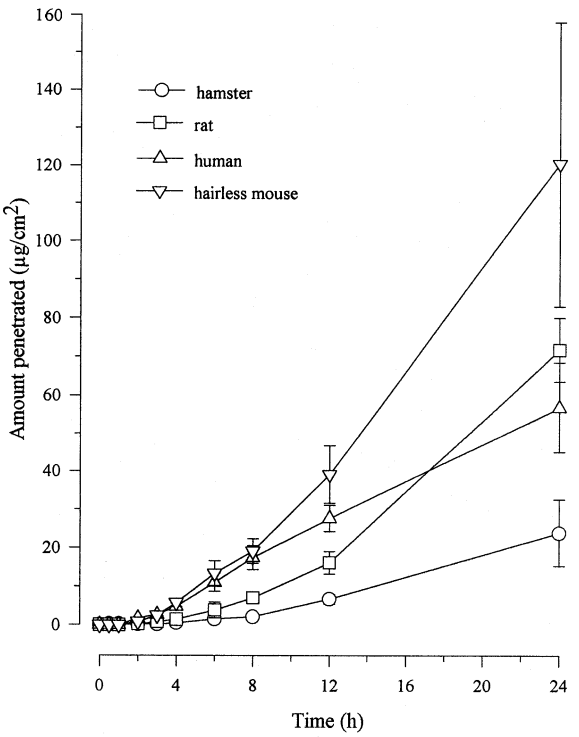

Fig. 8. The skin variations of 30% poloxamer-based formulation of capsaicin analog on the percutaneous absorption.

cosolvent in the skin delivery of various drugs. Ethanol is one of the most promising enhancer for skin permeation of lipophilic and hydrophilic compounds (Hatanaka et al., 1995). The permeation enhancing effects of these cosolvents are a result of physical perturbation of the lipoidal barrier in stratum corneum and conformational change within the keratinized protein component (Kurihara-Bergstrom et al., 1990).

The marked enhancing effect of the fatty acid IPM (5%) in cosolvents was also noted on the percutaneous absorption of a poloxamer-based gel. However, further increase in IPM content from 5 to 10% did not alter the flux rate significantly.

Other fatty acids (OA and LA) were also chosen as penetration enhancers to further increase the percutaneous absorption of CA. The synergistic effect of unsaturated fatty acids on percutaneous absorption is shown in Fig. 3. Addition of 5% OA and 5% LA into the gel containing 5% IPM resulted in a slight increase of skin permeation. No significant difference was observed between OA and LA on skin permeation of a poloxamer-based formulation of CA containing IPM. Addition of OA to PG solution is known to show increased permeation effect (Cooper, 1984). These fatty acids appear to affect the fluidity of lipid components of the stratum corneum by facilitating the permeation through lipoidal pathways (Touitou and Fabin, 1988; Ohara et al., 1995).

3.3. Effect of poloxamer contents on skin permeation

In general, the higher the topical base content, the lower the permeation rate due to high viscosity and low amounts of water. However, appropriate amounts of topical base with proper viscosity and fluidity is essential for the application onto skin surface. The percutaneous absorption of a poloxamer-based formulation of CA as a function of poloxamer content is shown in Fig. 4. No significant difference in skin permeation was observed as a function of poloxamer content (20, 25 and 30%). Type of penetration enhancers rather than the viscosity or fluidity of poloxamer gels may play a major role in permeation of CA.

3.4. Effect of buffer pH on skin permeation

Effect of pH on the percutaneous absorption of 30% poloxamer-based formulation of CA through excised hairless mouse skin is given in Fig. 5. The buffer system of 30% poloxamer-based gel slightly changed the cumulative amounts of CA at 24 h. However, no significant difference in permeation of CA was observed. Because the new CA is basic drug, it is highly ionized at acidic pH. The solubility of CA free base decreased as pH increased (i.e., 3.08 and 0.335 mg/ml at pH 1 and 8, respectively). The octanol/water partitioning ($\log P = 0.642$) was low at pH 1. As pH increased, the drug is unionized, showing higher octanol/water partitioning coefficient at pH 9 ($\log P = 2.11$) when compared with pH 1. However, the flux rate of CA appears compensated in terms of solubility and partitioning into the skin. The detailed correlation of percutaneous absorption between solubility and partitioning is under investigation.

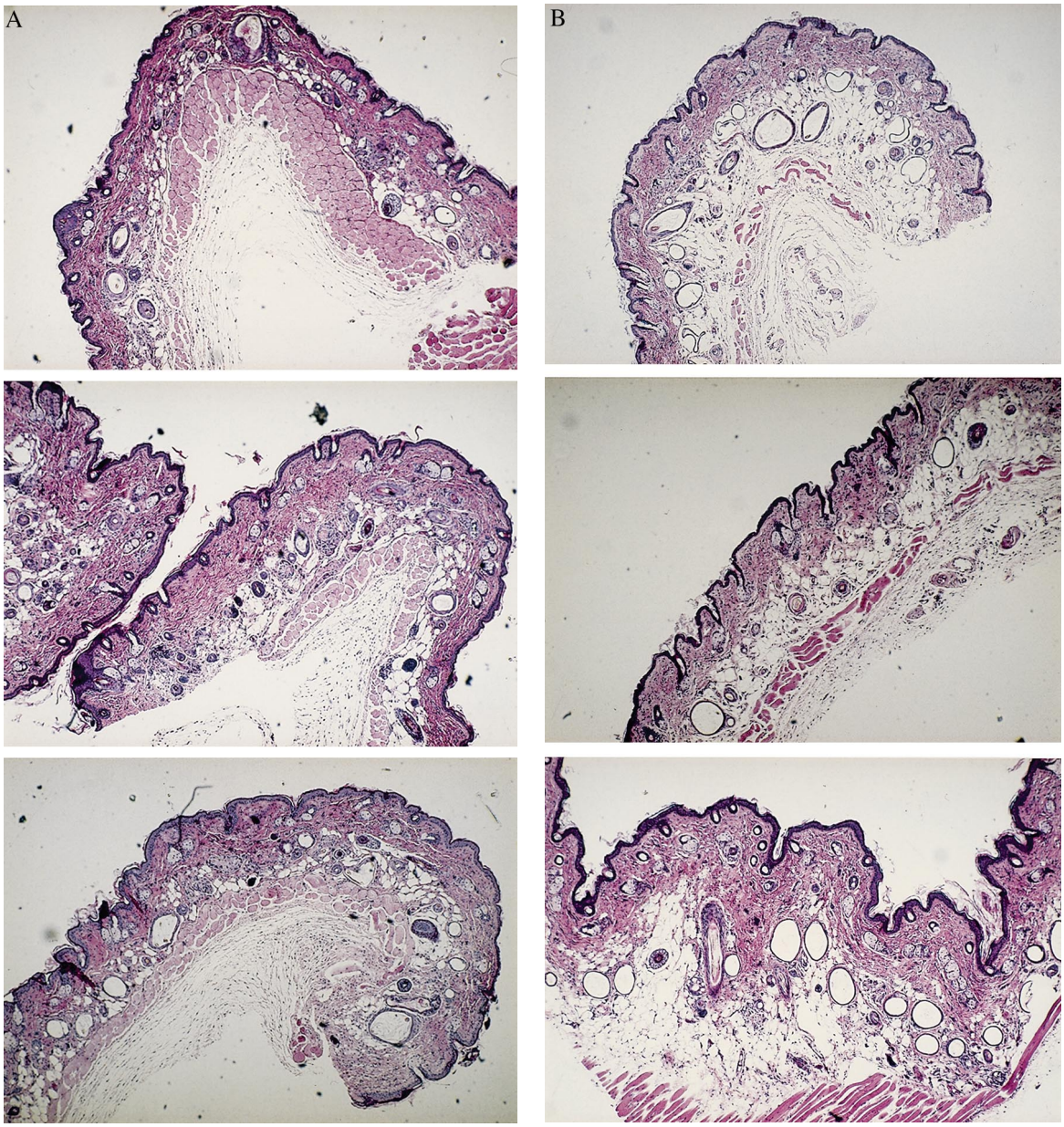

Fig. 9. Histopathologic views of one of five hairless mouse dorsal skins after topical application of saline (top), drug-free 30% poloxamer gel (middle) and 30% poloxamer-based formulation of capsaicin analog (bottom) over 1 week (A) or 2 months (B).

3.5. Effect of drug concentrations on skin permeation

The drug concentration was varied to study the dose dependency of a poloxamer-based formulation of CA on percutaneous absorption. Effect of drug concentrations on the percutaneous absorption of 30% poloxamer-based formulation of CA through excised hairless mouse skin is shown in Fig. 6. As the drug concentration (0.1, 0.5 and 1.0%) increased in poloxamer-based gels, the flux rate increased. The flux of 30% poloxamer-based

formulation of CA as a function of drug concentration is plotted in Fig. 7. The flux of poloxamer-based gels increased linearly as the drug concentration increased.

3.6. Skin variations

It has been reported that the flux rate of various drugs differs and highly depends on the type of skin models selected (Wester and Maibach, 1993). Many different animal models have been used in the diffusion studies. Although no definite correlation of skin permeation between animal models is observed, the rhesus monkey is one of good models to mimic the human skin. In this study, the four different skin models, hairless mouse, human penis, rat and hamster were used. The skin variations of 30% poloxamer-based formulation of CA on the permeation are shown in Fig. 8. There was a variation of percutaneous absorption of the drug, depending on the species used. The flux of 30% poloxamer-based formulation of CA was the highest in case of hairless mice but the lowest in hamsters. Although the hairless mouse skin showed a higher percutaneous absorption than the human penis skin (5.85 vs 3.64 μg/h per cm^2), the hairless mice is commonly used as a alternative model due to their convenience and cost. More detailed skin variations of drug needs to be further investigated.

3.7. Skin irritation and histopathology

After application of a poloxamer-based gel on the same dorsal site of hairless mouse skin in six groups (five hairless mice each) over a week (7 days) or two months (60 days), skin irritation and histopathologic changes were investigated. No noticeable skin erythema of hairless mice in six groups was visually observed, regardless of two application periods (pictures not shown). Detailed histopathologic changes of dorsal site of hairless mouse skin in six groups (five hairless mice each) were also examined using microscopy. Histopathologic views of one of five hairless mouse dorsal skins after topical application of saline, drug-free poloxamer gel and poloxamer-based formulation of CA over a week or two months are shown in Fig. 9. No histopathologic changes of the dorsal site of hairless mice in six groups were observed regardless of two application periods, suggesting no skin toxicity of a poloxamer-based gel of CA.

Based on these findings, the current poloxamer-based formulation appears useful in the systemic delivery of CA as topical or transdermal patch formulations.

Acknowledgements

This work was supported in part by the Korea Ministry of Science and Technology (HAN project). Part of this research was presented as a poster at 1996 annual meeting of the American Association of Pharmaceutical Scientists held in Seattle, WA. The authors greatly appreciate Dr. Kyung Chan Choi for his histopathologic examination.

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