

The Effect of Enhancers on the Penetration of Albuterol through Hairless Mouse Skin

Han-Gon Choi, Jong-Dal Rhee, Bong-Kyu Yu, Jung-Ae Kim, Mi-Kyung Kwak, Jong-Soo Woo, Dong-Hun Oh, Myo-Jung Han, Jun-Young Choi, Mingguan Piao and Chul Soon Yong[†]

College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea

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ABSTRACT – Albuterol, a selective β_2 -adrenergic receptor stimulant, has been introduced as a potent bronchodilator for patients with bronchial asthma, chronic obstructive bronchial disease, chronic bronchitis and pulmonary emphysema. The percutaneous permeation of albuterol sulfate was investigated in hairless mouse skin in vitro with and without pretreatment with enhancers. The enhancing effects of ethanol and various penetration enhancers such as terpenes, non-ionic surfactants, pyrrolidones, and fatty acids on the permeation of albuterol sulfate were evaluated using Franz diffusion cells. Among terpenes studied, 1,8-cineole was the most effective enhancer, which increased the permeability of albuterol sulfate approximately 33-fold compared with the control without enhancer pretreatment, followed by *d*-limonene with enhancement ratio of 21.79. 2-Pyrrolidone-5-carboxylic acid increased the permeability of albuterol sulfate approximately 5.5-fold compared with the control. Other pyrrolidones tested showed only slight permeability enhancing effect with enhancement ratio less than 2.8. Nonionic surfactants showed moderate enhancing effects. Lauric acid increased the permeability of albuterol sulfate approximately 30-fold with decreasing the lag time from 2.85 to 0.64 hr. Oleic acid and linoleic acid showed enhancement ratio of 24.55 and 22.91, respectively. These findings would allow a more rational approach for designing formulations for the transdermal delivery of albuterol sulfate and similar drugs.

Key words – Albuterol, Enhancer, Transdermal drug delivery systems, Permeability

Albuterol is a relatively selective β_2 -adrenergic agonist and is currently one of the most prescribed bronchodilators in the treatment of bronchial asthma, chronic bronchitis and emphysema.¹⁾ Albuterol is effective by oral and inhalation routes. The drug undergoes extensive first-pass metabolism and has short elimination half-life, and thus requires frequent oral administrations.²⁾ The relatively short-acting injectable and aerosol dosage forms of albuterol are recommended for prompt relief of severe asthmatic attacks. Albuterol is on the market in the form of aerosols with strength of 100-200 μ g. The recommended dose in adults and children is 2-3 inhalations every 4-6 h. More frequent administration is not recommended.

For the last two decades, the skin has become an important route for drug delivery for topical, regional or systemic effects. However, skin is remarkably resistant to the permeation of most chemicals and drugs, thus causing difficulties for transdermal delivery of therapeutic agents since few drugs have the characteristics required to permeate sufficiently across the stratum corneum to reach therapeutic blood concentration. A variety of physical and chemical methods have been implemented in order to increase permeation of drugs through the skin.³⁾

These include chemical enhancers,⁴⁾ therapeutic and low frequency ultrasound,⁵⁾ iontophoresis,⁶⁾ and electroporation.⁷⁾

The chemical methods involve incorporation of specific chemicals in transdermal drug formulations to increase the penetration of the drug. The penetration enhancers facilitate the absorption of penetrant through the skin by temporarily increasing the permeability of the skin. Some of the important penetration enhancers as classified by Sinha and Kaur⁸⁾ are terpenes and terpenoids, pyrrolidinones, fatty acids and esters etc.

The utilization of penetration enhancers is a long-standing and widely employed approach to enhance the permeability of drugs in transdermal formulations.⁹⁾ To date, a variety of chemicals has been developed and evaluated as enhancers, yet their inclusion into topical or transdermal formulations have certain limitations since the underlying mechanisms of enhancing activity of these agents are not clearly elucidated.

A controlled release dosage form might have advantages over conventional oral dosage forms and inhalers because of providing prolonged therapeutic concentrations in the systemic circulation. Since asthma is a chronic disease and most of the patients suffer from nocturnal attacks,¹⁰⁾ it is necessary to develop drug delivery systems which could maintain therapeutic concentrations for long duration. Transdermal drug delivery systems offer many advantages¹¹⁾ over other classical

[†]본 논문에 관한 문의는 이 저자에게로
Tel : 053)813-0065, E-mail : csyong@yu.ac.kr

oral, injectable and inhaler systems with systemic activity. The most important advantages are enhanced bioavailability of drugs by avoiding first-pass metabolism and the controlled constant drug delivery profile. Other benefits include longer duration of therapeutic action from a single application, and the ease of use and withdrawal, in case of side effects.

Albuterol has a pK_a and log P value of 9.2 and 0.11, respectively.¹²⁾ It has been reported that albuterol could permeate through different skin models.^{13,14)} The transport may be through the porous and parallel pathway of the skin.

The purpose of this work was to investigate the effect of various enhancers on the permeability of albuterol sulfate through hairless mouse skin. Terpenes, pyrrolidinones, fatty acids and nonionic surfactants were selected as enhancer, trying to cover different groups of chemical enhancers that have proved to increase transdermal absorption.¹⁵⁻²¹⁾

These results could be utilized for future development of transdermal controlled delivery systems for albuterol sulfate.

Experimental

Materials and equipments

Albuterol sulfate ($M_w=576.7$), metaproterenol sulfate and enhancers such as caproic acid, lauric acid, myristic acid, oleic acid, linoleic acid, caproic acid, 2-pyrrolidone-5-carboxylic acid (PCA), Span 20, Span 80, Span 85, Tween 20, Tween 65, Tween 85, N-methyl-2-pyrrolidone (NMP), N-lauryl-2-pyrrolidone (NLP), and N-ethyl-2-pyrrolidone (NEP) were purchased from Sigma (St. Louis, MO, USA). Caprylic acid, capric acid, palmitic acid, stearic acid, menthol, thymol, carvone, menthone, *d*-limonene, and 1,8-cineole were obtained from Fluka (Buchs, Switzerland). Triethylamine and phosphoric acid, boric acid were obtained from Yakuri Pure Chemicals Co. Ltd. (Osaka, Japan) and acetic acid were from Junsei Chemical Co. Ltd. (Tokyo, Japan). Acetonitrile was purchased from Riedel-deHaen Co. (Seelze, Germany). Sodium hydroxide was from Kanto Chemical Co. Inc. (Tokyo, Japan). All other chemicals were commercial products of reagent grade. All the buffer solutions were prepared with double distilled water using Milli Q (Millipore Co., U.S.A). HPLC system was Hitachi Co. (Japan) and other equipments employed in this work were magnetic stirrer (Corning, USA), sonicator (Branson, USA), aspirator (Eyela, Japan), microcentrifuge (Eppendorf 5415C, Germany), shaking water bath (KMC-1205SW1, Vision Co., Korea), multi-point magnetic stirrer (Variomag, USA), pH meter (Orion Research Inc., USA), immersion circulator (Jeio Tech Co. Ltd., Korea) and Franz diffusion cells.

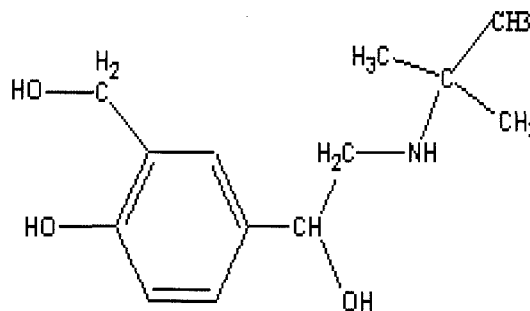


Figure 1—The structure of albuterol.

In vitro diffusion study

Male hairless mice strain SKH1, 8 weeks old, were obtained from Chungang animal laboratories, Inc. (Seoul, Korea). Animals were sacrificed by CO_2 asphyxiation and full-thickness abdominal and dorsal skin was excised. Any extraneous subcutaneous fat were removed from the dorsal surface. The skins were stored at $-80^\circ C$ until utilized. Skins were then slowly thawed, cut into small pieces. *In vitro* skin permeation studies were carried out with Franz diffusion cells. Hairless mouse skin was mounted between the donor and receptor cells with the effective diffusion area of 2.14 cm^2 . The dermal side of the skin was exposed to the receptor solution (saline buffered solution pH 7.4), which was stirred magnetically and kept at a constant temperature of $37^\circ C$. Then, skin was pretreated for 12 hr either with 1 ml of saline buffered solution pH 7.4 (buffer control), ethanol (vehicle), or a 5% (w/v) chemical enhancer solution in ethanol to determine whether ethanol or any of the compounds enhanced the percutaneous permeation of albuterol sulfate compared to the control. After equilibration with the receptor solution for at least 30 min, the donor cells, faced with the stratum corneum surface, was filled with each saturated solution of albuterol sulfate (250 mg/ml) in 3 ml of isotonic buffer solution. The receptor compartment was filled with the same isotonic buffer pH 7.4. One-ml samples from the receptor chamber were taken at appropriate time intervals for 24 hr. The sample volume taken was replaced with buffer pH 7.4. The drug contained in each sample taken has been considered in order to calculate the accumulated amount of albuterol sulfate in the receptor compartment. The donor cells were occluded with parafilm to prevent the invasion of other materials and vehicle evaporation. The samples were analyzed by HPLC.

Analytical method for albuterol sulfate

Albuterol sulfate was assayed by HPLC with slight modifications of previously published methods.²²⁾ The chromatographic system consisted of Hitachi D-7000 system manager

software (Hitachi, Ibaraki, Japan), Model 7725 injector (Rheodyne, Cotati, CA, U.S.A.) fitted with 50- μ l sample loop, Model L-7100 pump (Hitachi, Ibaraki, Japan), D-7000 interface module (Hitachi, Ibaraki, Japan) and Model L-7450 photodiode array detector (Hitachi, Ibaraki, Japan). The wavelength of the UV detector was set at 265 nm. (0.005AUFS). HPLC separation was performed on a Crestpak C18S analytical column (250 \times 4.6 mm I.D.), packed with 5 μ m diameter particles (Jasco, Tokyo, Japan). The mobile phase consisted of a mixture of 0.02 M sodium dihydrogen phosphate buffer (pH 3.0 with phosphoric acid) containing 750 μ l triethylamine/1 L buffer and acetonitrile (97 : 3 v/v). This mobile phase was filtered through a 0.45- μ m HV filter (Millipore, Bedford, MA, USA), then deaerated ultrasonically prior to use. The eluent was pumped through the column at a flow-rate of 1.5 ml/min. Injection volume was 50 μ l, and metaproterenol sulfate (1.3 mg/ml in water) was used as an internal standard. A guard column (Whatman column survival kit) containing identical packing material to that in the analytical column was used. All chromatographic operations were carried out at ambient temperature. The retention times of albuterol sulfate and internal standard under these conditions were 12.6 and 6.2 min, respectively. For the quantification of albuterol sulfate, a calibration graph was constructed in the range of 0.1-20 μ g/ml by plotting peak area ratios of albuterol sulfate to the internal standard vs albuterol sulfate concentration.

Data analysis

Blood concentration of albuterol should be maintained in the appropriate ranges to be effective after percutaneous absorption. The total mass of drug transported across the skin was determined by HPLC. Linear regression analysis of pseudo steady-state diffusion data allowed calculation of J_s , the steady-state flux (μ g \cdot cm⁻² \cdot h⁻¹).²³⁾

The flux equation gives;

$$J_s = \frac{1}{S} \left(\frac{dQ}{dt} \right)_{ss} = K_p C_d$$

where, S is cross-sectional area of the skin membrane (cm²), K_p is the apparent permeability coefficient (cm \cdot h⁻¹), and C_d is the concentration gradient. In this experiment, C_d was taken as the saturated concentration (given infinite dose and sink conditions), and dQ/dt was averaged as the total mass transport over the time course of the experiment.

The permeation lag time (T_L) which indicates the time taken by the drug to saturate the skin and to reach the receiving compartment, was calculated from the x-axis intercept values of the regression lines. Enhancement ratios (ER),²⁴⁾ expressing the

relative activity of each enhancer, were calculated from the equation below;

$$ER = (K_p \text{ with pretreatment} / K_p \text{ without pretreatment}).$$

When statistically differences were detected by means of the ANOVA test ($P < 0.05$) the permeation enhancing activities, expressed as enhancement ratio of permeability (ER), were calculated as the quotient of the permeability coefficient value obtained with the enhancer to that found with control (buffer) or vehicle (ethanol), respectively. The results are the mean and standard deviations of at least three determinations.

Results and Discussion

The effect of various enhancers on the permeability of albuterol sulfate through hairless mouse skin *in vitro* was investigated to develop the transdermal delivery system for albuterol sulfate.

Selection of the appropriate vehicles and enhancers would be the first step toward developing transdermal delivery system and solubility of a drug is one of the most important physicochemical properties in screening proper vehicles and enhancers. Final selection of the vehicle, however, should be made mainly based on the results of skin permeability study. In this regard, various classes of enhancers were evaluated to be included in the formulations of transdermal delivery system for albuterol sulfate.

Due to the continuous lipid regions in the stratum corneum, it is believed that passive transdermal diffusion occurs predominantly through the lipid phase of the skin.^{11,25)} For this reason, hydrophobic drugs generally have better transport through skin while water-soluble ionic drugs, such as albuterol sulfate, might very limited permeability.²⁶⁾ Understanding the effects of combining enhancers can also aid the development of improved multi-component transdermal formulations.²⁷⁾

Cumulative amounts of albuterol sulfate in the receptor compartment as a function of time for all the conditions assayed are plotted in Figure 2, 3, 4 and 5. As can be seen, all the conditions used in the pretreatment of skin, ethanol and all the enhancers formulated at 5% (w/v) in ethanol increase the penetration of albuterol sulfate through the hairless mouse skin.

The ethanol itself significantly increases albuterol sulfate flux with enhancement ratio of 2.7. These results confirm that ethanol would permeate through the stratum corneum²⁸⁾ and disrupt the organization of its intercellular lipids, culminating in increment of skin permeability.²⁹⁾ Although high concentrations of ethanol have been known to reduce the transdermal absorption of some penetrant because of its capability for

dehydrating the skin,³⁰⁾ the solutions of the enhancers in absolute ethanol were prepared since some of them were insoluble in water-ethanol mixture. The concentration of enhancer was fixed at 5%(w/v) with consideration of previous reports that the enhancing effect is concentration-dependent.³¹⁻³³⁾

As the concentration of enhancer goes higher, the enhancing effect does not increase in proportion to concentration. It, however, would instead reach a plateau level³⁴⁾ and even in certain cases, the enhancing activity has shown to be reduced. Furthermore, high concentrations of enhancers might cause some problems, such as skin irritation and toxicity.^{35,36)}

In recent years, terpenes have been extensively investigated as penetration enhancers with little skin irritation.³⁷⁻³⁹⁾ Carveol, menthol, thymol, carvone, menthone, 1,8-cineole and *d*-limonene were selected for our studies.

Some authors have established certain drug-specific structure-activity relationship for the terpenes.⁴⁰⁻⁴⁴⁾ Such a relationship tends to imply that one mechanism by which terpenes operate is by modifying the solvent nature of the stratum corneum, improving drug partitioning into the tissue.⁴⁵⁾ According to this hypothesis and in the case of albuterol sulfate, a hydrophilic drug, the expected enhancement activities for these compounds would be higher for hydrophilic 1,8-cineole. Table I and Figure 2 represent the effect of pretreatment with various terpenes on the permeability of albuterol sulfate through hairless mouse skin *in vitro*. Among the terpenes tested in this study, 1,8-cineole showed the most permeability enhancing effect with ER of 32.45 and about 3-fold reduction in lag time. Compared with control, carveol, menthol, thymol, carvone, and menthone enhanced the permeability of albuterol sulfate 7.15, 5.48, 3.49, 7.50 and 16.43-fold, respectively and reduced the lag time 1.63, 1.52, 1.34, 1.69 and 2.24-fold, respectively.

It was expected that 'polar' terpenes, such as 1,8-cineole

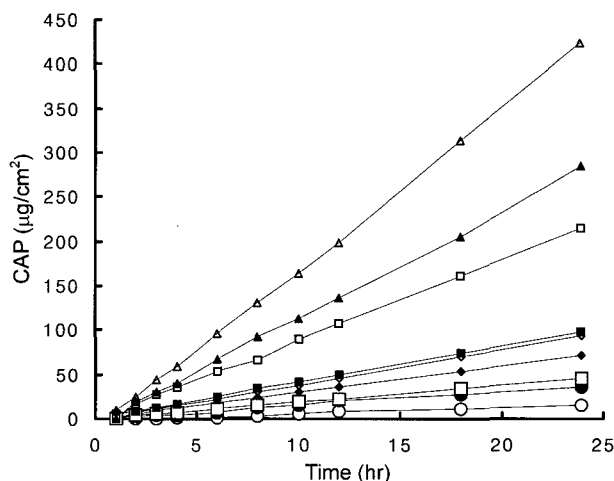


Figure 2—Permeation profiles of albuterol sulfate from isotonic buffer solution (pH 7.4) pretreated with various terpenes for 12 h. Concentration of each enhancer was 5% (w/v). Symbols represent the average of data (n=3-4). (△); cineole, (▲); *d*-limonene, (□); menthone, (■); carvone, (◇); carveol, (◆); menthol, (◻); thymol, (●); ethanol, (○); control. CAP; cumulative amount penetrated.

would provide better enhancement for albuterol sulfate than hydrocarbon terpenes, such as *d*-limonene.^{46,47)} However, our results had shown that *d*-limonene also has higher activity in comparison with 1,8-cineole (Table I). The enhancing effect of this compound is comparable to lauric, linoleic acid and oleic acid but it is better than nonionic surfactants and pyrrolidones (Table I, II, III, IV). *d*-Limonene has shown higher enhancing activity among terpenes tested. This terpene multiplies transdermal flux of albuterol sulfate by about 22 and 8 times respective to control and ethanol, respectively.

According to Barry,⁴⁸⁾ permeation enhancers would enhance the permeability by disrupting the ordered lipid structure in stratum corneum with ease of diffusion, interacting with intra-

Table I—Effect of Pretreatment with Terpenes in Ethanol on Albuterol Sulfate Skin Permeation Parameters. Concentration of Each Enhancer was 5% (w/v)

Enhancer	Lag time (hr)	J_s ($\mu\text{g}/\text{cm}^2/\text{hr}$)	$K_p \times 10^3$ (cm/hr)	ER respective to control	ER respective to vehicle
Buffer(pH 7.4), control	2.85±0.92	0.61±0.17	0.17±0.09	-	-
Absolute ethanol, vehicle control	1.93±0.75	1.47±0.21	0.43±0.13	2.71±0.32	-
Carveol	1.75±0.67	3.89±0.72	1.14±0.37	7.15±1.19	2.65±0.31
Menthol	1.88±0.72	2.98±0.45	0.87±0.23	5.48±0.95	1.98±0.27
Thymol	2.13±0.69	1.90±0.33	0.56±0.27	3.49±0.81	1.34±0.18
Carvone	1.69±0.54	4.08±0.76	1.19±0.40	7.50±0.93	2.87±0.42
Menthone	1.27±0.55	8.95±1.21	2.62±0.35	16.43±3.12	6.15±1.04
<i>d</i> -Limonene	1.34±0.73	11.86±1.85	3.47±0.54	21.79±3.47	8.23±1.79
1,8-Cineole	0.97±0.49	17.67±2.23	5.28±1.13	32.45±4.51	13.30±2.87

Values are the mean±S.D. of three to four determinations at 37°C.

cellular proteins, or increasing the partition of drug, coenhancer and cosolvent into the stratum corneum. Terpenes attributed their enhancing activity to improvement of drug diffusion inside the stratum corneum,⁴⁹⁾ possibly due to disruption of intercellular lipid barrier.⁴⁵⁾ Lipophilicity of enhancer greatly affects the diffusion of enhancer itself or other penetrants into the skin. The effect of ketone or epoxide terpenes on percutaneous absorption of 5-fluorouracil showed good correlation between ER and partition coefficient.⁴⁵⁾ Relatively lipophilic terpenes would enhance the permeability of drugs. On the contrary, terpenes with too high lipophilicity might decrease the percutaneous absorption,⁵⁰⁾ probably ascribed to thermodynamic activity of enhancer in vehicle.⁵¹⁾ In general, terpenes are too lipophilic to interact with proteins.

The prerequisite for an ideal enhancer would be without skin irritation. The ideal surfactant for enhancer should have short enhancing activity, allowing for immediate recovery of skin after removal of surfactant. However, increment of permeability would entail a variety of unwanted damages to the skin. Especially, anionic surfactants leave greater skin damages behind in comparison with nonionic surfactants. Table II lists the results of permeability study of albuterol sulfate pretreated with nonionic surfactants, and Figure 3 represents these results. As shown in Table II, Span 20 increased permeability of albuterol sulfate 3.22-fold compared with ethanol. It affects the intercellular lipids of the stratum corneum by incorporating its C12 alkyl chain between them,⁵²⁻⁵⁴⁾ leading to disruption of their packed structure and making it less rigid. Consequently, the permeability of stratum corneum and the diffusivity of the permeant would increase.⁵⁵⁻⁵⁷⁾ Nonionic surfactants tend to permeate stratum corneum swiftly without strong interactions with constituents of stratum corneum compared to ionic surfactants. Ionic surfactants, however, have lower self-perme-

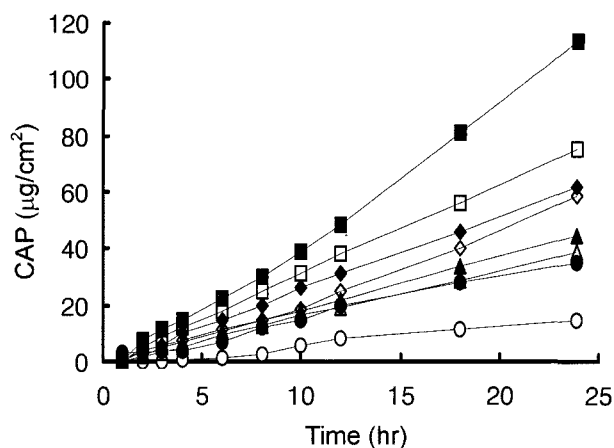


Figure 3—Permeation profiles of albuterol sulfate from isotonic buffer solution (pH 7.4) pretreated with various nonionic surfactants for 12 h. Concentration of each enhancer was 5% (w/v). Symbols represent the average of data ($n=3-4$). (■); Span 20, (□); Span 80, (◆); Tween 65, (◇); Span 85, (▲); Tween 20, (△); Tween 80, (●); ethanol, (○); control. CAP; cumulative amount penetrated.

ability and increase the percutaneous absorption through strong interactions with intracellular constituents of stratum corneum.⁵⁸⁾ Shokri *et al.*⁵⁹⁾ have reported that several surfactants could enhance the permeability of diazepam. Other surfactants used in this work only showed moderately small enhancing activity with ER of less than 5.8 compared to control (Table II). It might be necessary to carry out permeability study with more diverse surfactants to elucidate the underlying action mechanisms.

Figure 4 represents the effect of several pyrrolidones on the permeability of albuterol sulfate. Percutaneous absorption parameters obtained from these results are listed in Table III. Pyrrolidones can reside in a specific domain of stratum corneum or show different enhancing activity dependent on phys-

Table II—Effect of Pretreatment with Nonionic Surfactants in Ethanol on Albuterol Sulfate Skin Permeation Parameters. Concentration of Each Enhancer was 5% (w/v)

Enhancer	Lag time (hr)	J_s ($\mu\text{g}/\text{cm}^2/\text{hr}$)	$K_p \times 10^3$ (cm/hr)	ER respective to control	ER respective to vehicle
Buffer(pH 7.4), control	2.85±0.92	0.61±0.17	0.17±0.09	-	-
Absolute ethanol, vehicle control	1.93±0.75	1.47±0.21	0.43±0.13	2.71±0.32	-
Span 20	0.94±0.45	4.74±0.53	1.39±0.27	8.73±1.21	3.22±0.44
Span 80	1.58±0.51	3.14±0.47	0.93±0.15	5.76±0.75	2.13±0.27
Span 85	1.87±0.73	2.44±0.25	0.78±0.19	4.49±0.41	1.66±0.33
Tween 20	2.03±0.97	1.87±0.29	0.55±0.14	3.43±0.52	1.26±0.32
Tween 65	1.69±0.57	2.58±0.32	0.74±0.23	4.74±0.58	1.75±0.29
Tween 80	1.88±0.69	1.61±0.24	0.47±0.18	2.95±0.45	1.09±0.17

Values are the mean±S.D. of three to four determinations at 37°C.

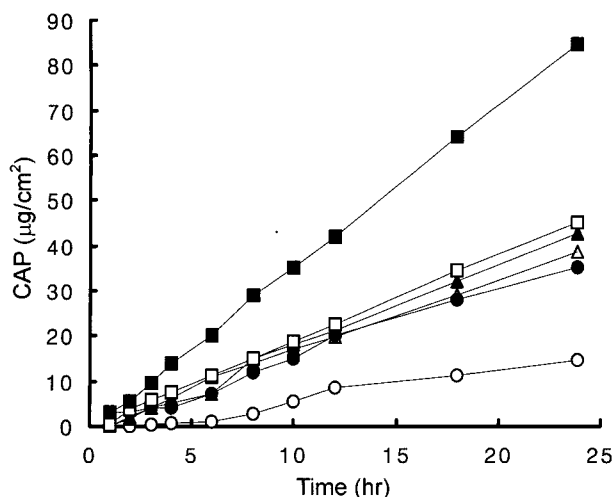


Figure 4—Permeation profiles of albuterol sulfate from isotonic buffer solution (pH 7.4) pretreated with various pyrrolidones for 12 h. Concentration of each enhancer was 5% (w/v). Symbols represent the average of data ($n=3-4$). (■); PCA, (□); NEP, (▲); NLP, (△); NMP, (●); ethanol, (○); control. CAP; cumulative amount penetrated.

icochemical properties, leading to percutaneous absorption enhancement or toxicity on skin. Among the pyrrolidones employed in this experiments, PCA showed some permeability enhancing activity with ER of 5.45 and 2.36-fold reduction in lag time. Other pyrrolidones showed ER of around 2.5. These results might implicate that pyrrolidones affect percutaneous absorption of albuterol sulfate depending on their hydrophilicities.⁶⁰

In recent years, many authors have reported the effect and mechanism of fatty acids on the percutaneous absorption enhancement.⁶¹⁻⁶³ Table IV and Figure 5 represent the effect of various fatty acids on the percutaneous absorption of albuterol sulfate. Lauric acid increased the permeability of albuterol sulfate most effectively among the fatty acids tested in our work with ER of 29.32 and 4.55-fold reduction in lag time. Oleic acid, linoleic acid and capric acid also increased the permeability of albuterol sulfate significantly compared to control with ER of 24.55, 22.91 and 14.32, respectively. Our results are consistent with the report that after investigating the effect

Table III—Effect of Pretreatment with Pyrrolidones in Ethanol on Albuterol Sulfate Skin Permeation Parameters. Concentration of Each Enhancer was 5% (w/v)

Enhancer	Lag time (hr)	J_s ($\mu\text{g}/\text{cm}^2/\text{hr}$)	$K_p \times 10^3$ (cm/hr)	ER respective to control	ER respective to vehicle
Buffer(pH 7.4), control	2.85±0.92	0.61±0.17	0.17±0.09	-	-
Absolute ethanol, vehicle control	1.93±0.75	1.47±0.21	0.43±0.13	2.71±0.32	-
N-Lauryl-2-pyrrolidone	1.59±0.48	1.78±0.33	0.52±0.20	2.83±0.41	1.04±0.24
N-Methyl-2-pyrrolidone	1.76±0.43	1.61±0.17	0.47±0.18	2.32±0.26	1.10±0.29
N-Ethyl-2-pyrrolidone	1.51±0.64	1.88±0.21	0.55±0.23	2.56±0.32	0.98±0.31
2-Pyrrolidone-5-carboxylic acid	1.21±0.54	3.52±0.42	1.03±0.31	5.45±0.27	2.45±0.37

Values are the mean±S.D. of three to four determinations at 37°C.

Table IV—Effect of Fatty Acids in Ethanol on Albuterol Sulfate Skin Permeation Parameters. Concentration of Each Enhancer was 5% (w/v)

Enhancer	Lag time (hr)	J_s ($\mu\text{g}/\text{cm}^2/\text{hr}$)	$K_p \times 10^3$ (cm/hr)	ER respective to control	ER respective to vehicle
Buffer(pH 7.4), control	2.85±0.92	0.61±0.17	0.17±0.09	-	-
Absolute ethanol, vehicle control	1.93±0.75	1.47±0.21	0.43±0.13	2.71±0.32	-
Caproic acid(6)	1.26±0.58	1.71±0.23	0.50±0.15	3.12±0.27	1.13±0.15
Caprylic acid(8)	1.05±0.47	4.14±0.46	1.21±0.19	7.56±0.65	2.77±0.37
Capric acid(10)	0.96±0.53	7.76±0.85	2.27±0.23	14.32±2.05	6.12±0.85
Lauric acid(12)	0.64±0.32	15.90±2.78	4.65±0.54	29.32±4.33	11.32±1.91
Myristic acid(14)	1.89±0.86	2.53±0.22	0.74±0.14	4.65±0.51	1.53±0.41
Palmitic acid(16)	1.93±0.94	2.84±0.32	0.83±0.12	5.20±0.47	2.03±0.39
Stearic acid(18)	1.75±0.69	3.66±0.38	1.07±0.19	6.73±0.83	2.54±0.44
Oleic acid(18,1)	0.81±0.78	13.33±2.53	3.90±0.47	24.55±3.13	10.43±1.54
Linoleic acid (18,2)	0.79±0.63	12.45±2.01	3.64±0.41	22.91±2.98	9.53±2.02

Values are the mean±S.D. of three to four determinations at 37°C.

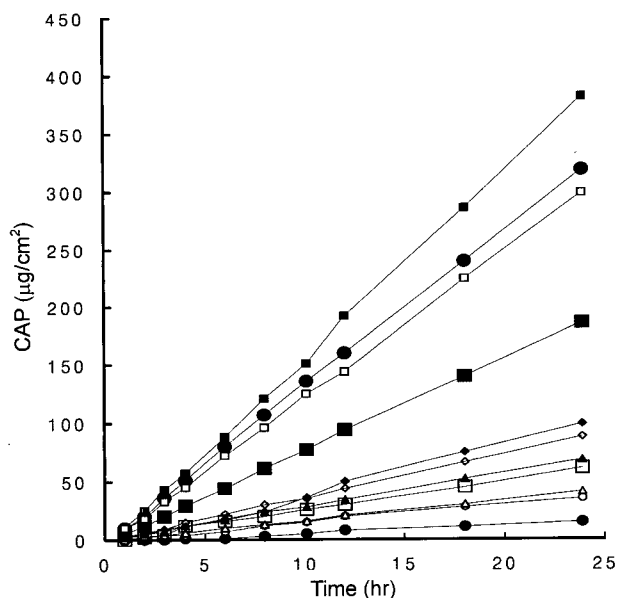


Figure 5—Permeation profiles of albuterol sulfate from isotonic buffer solution (pH 7.4) pretreated with various fatty acids for 12 h. Concentration of each enhancer was 5% (w/v). Symbols represent the average of data (n=3-4). (■); lauric acid, (●); oleic acid, (□); linoleic acid, (■); capric acid, (◆); caprylic acid, (◇); stearic acid, (▲); palmitic acid, (◻); myristic acid, (△); caproic acid, (●); ethanol, (○); control. CAP; cumulative amount penetrated.

of fatty acids on the percutaneous absorption of molsidomine in rats, lauric acid was found to have the most pronounced enhancing activity.⁶⁴ Caproic acid, caprylic acid, myristic acid, stearic acid and palmitic acid showed less significant enhancing activity than lauric acid (Table IV). Long chain fatty acids with high melting point may have relatively low solubility in ethanol, possibly leading to low permeability enhancing activity. Short chain fatty acids seem to have less effect on the structure and barrier characteristics of skin than middle chain fatty acids. Stearic acid, saturated fatty acid with 18 carbon chain, showed ER of 6.73. Oleic acid and linoleic, having the same numbers of carbon chain as stearic acid, showed greater ER than that of stearic acid, because they have double bond and efficiently disrupt the lipid layer.⁶⁵ Oleic acid significantly increases the flux of albuterol sulfate across the skin compared to the control and vehicle groups (ER of 24.55 and 10.43, respectively). It has been found to increase the transdermal absorption through a mechanism involving the stratum corneum lipid membranes. It is incorporated into intercellular lipids, resulting in disruption of molecular packaging and alteration of the hydration level, thus allowing drug penetration.⁶⁶ It has also been reported that oleic acid would exist as a separate phase within the lipid double layers at high concentrations.^{67,68}

Development of transdermal delivery systems would require most appropriate selection of drug, vehicle and percutaneous enhancer. Further experiments await investigating the mechanism of enhancing activity and finding out best combination of drug, vehicle and enhancer to formulate albuterol transdermal delivery systems.

Conclusions

Transdermal delivery of albuterol sulfate appears to be relatively a better route for patients who respond reasonably to the β -agonists.

Albuterol sulfate permeates through the skin by passive diffusion. Pretreatment of skin for 12 h with Span 20, oleic acid, linoleic acid, lauric acid, 1,8-cineole, and *d*-limonene significantly increase the flux of albuterol sulfate across the skin. However, further work with these enhancers are necessary to elucidate the permeability enhancing activity and to ensure that therapeutic blood levels by transdermal administration of albuterol sulfate can be achieved.

In the light of the results of the present work, the feasibility of developing transdermal delivery system for albuterol sulfate was confirmed.

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