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Penetration Enhancement of β_2-Selective Agonist, Tulobuterol, Across Hairless Mouse Skin

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ABSTRACT–The effects of various pressure sensitive adhesives (PSA) and enhancers on the percutaneous absorption of tulobuterol were investigated. The permeation rate of tulobuterol through hairless mouse skin from various adhesives was evaluated using a flow-through diffusion cell system at 37°C. The permeability of tulobuterol was variable depending on the physicochemical property of the PSA. The permeation rate of tulobuterol from polyethylene oxide grafted acrylic adhesive matrix was higher than that from other PSA matrices. The flux of tulobuterol was 4.37±0.34 μg/hr/cm^2 from polyethylene oxide grafted acrylic adhesive matrix. When the effects of various enhancers on the percutaneous absorption of tulobuterol from grafted acrylic adhesive were evaluated, Plurol oleique® showed higher flux than all other enhancers tested.

Key words–Tulobuterol, Enhancer, Pressure sensitive adhesive, Transdermal drug delivery

Tulobuterol (α-[(*tert*-butylamino)methyl] benzyl alcohol) is a novel bronchodilator; as one of the β_2-agonist agents, it has superior selective activity on the β_2- receptor[1] than other agents in this class. Oral dosage form[2] and inhaler type[3] of tulobuterol have been widely used to prevent or diminish airway obstruction of the patients. Yet, side effects such as tremor, palpitations or hypokalemia[4,5] were emerged particularly after oral administration, and these disadvantages restrict the oral use of the drug. In addition, extended duration of drug action is required to offer protection for nocturnal asthma during a whole nights sleep. Unfortunately, bronchodilating effect of β_2-agonist agents waned within 6 hr after the inhalation.[6]

The utilization of transdermal route for systemic action of drugs has brought out an important number of new clinical applications,[7] such as pain treatment, hormone therapy, smoking cessation, and etc. An application of transdermal drug delivery system (TDD) has certain benefits such as producing sustained, constant, and controlled drug plasma concentration, enhancing bioavailability and bypassing hepatic first-pass metabolism. This may be accompanied with the decrease in dose frequency required for chronic treatment and, thus, improving patient compliance. Therefore, it can be expected that an application of TDD type formulation of tulobuterol might offer several advantages over the conventional dosage forms. However, in spite of many advantages of TDD, marketed transdermal drug delivery systems are available for only a few drugs. Most of investigated drugs did not cross the skin in adequate amount to produce the therapeutic effect.[8] Thus, in an attempt to overcome the problems arising from skin impermeability and biological variability, various approaches to reduce the skin barrier resistance have been investigated. [9, 10]

In TDD applications, adhesives are used to maintain intimate contact between the patch and the skin surface. Many classes of adhesives are available that might be considered for use with TDD; particularly pressure sensitive adhesives (PSAs) are preferred.[11] Because the physicochemical properties of PSA can affect the permeation of a drug from PSA across the skin, the selection of appropriate PSA is important in designing transdermal drug delivery system.[12,13] The penetration enhancers are widely used to achieve sufficient therapeutic efficacy and account for essential components in TDD system. The permeation rate, the compatibility with incorporated components, and the skin adhesion must be considered in the selection of PSA.

In this study, we investigated the influence of the natures of pressure-sensitive adhesives and the functional groups in acrylic adhesive on the permeation rate of tulobuterol across hairless mouse skin. Moreover, we evaluated the effect of various enhancers on the penetration rate of tulobuterol from an acrylic PSA matrix.

Experimental

Materials

Tulobuterol was a gift from Jeil Pharm. Co. (Seoul, South Korea). Propylene glycol monolaurate (Lauroglycol®), Propylene glycol carprylate/caprate (Labrafac® PG), PEG-8 glyceryl caprylate/caprate (Labrasol®), PEG-8 glyceryl linoleate (Labrafil® 2609) and polyglyceryl-3 oleate (Plurol oleique® cc

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497) were purchased from Masung Co. (Seoul, South Korea). Propylene glycol dicaprylate (Miglyol® 840) was obtained from Hüls America (Somerset, NJ, USA). Cetearyl octanoate and isopropyl myristate (Crodamol® CAP), PEG-12 palm kernel glycerides (Crovol® PK40) were obtained from Croda (Parsippany, NJ, USA). Sorbitan monolaurate (Span® 20), Sorbitan monooleate (Span® 80), Oleyl alcohol and Propylene glycol were purchased from Junsei Chemical Co. Ltd (Tokyo, Japan). Acrylic, polyisobutylene and styrene-butadien-styrene block copolymer pressure-sensitive adhesive solutions in organic solvents were obtained from National Starch and Chemical Company (Bridgewater, NJ, USA). Silicone pressure sensitive adhesive was obtained from Dow Corning (Midland, MI, USA). All other chemicals were reagent grade or above and were used without further purification.

Preparation of adhesive matrices

Tulobuterol was dissolved in ethyl acetate. Then, PSA solutions were mixed well with tulobuterol solution with or without enhancers. PSA matrices were prepared by casting the above solution on a polyester release liner coated with silicone while silicone adhesive matrix was prepared on a flurocarbondiacrylate-coated release liner. They were set at room temperature for 10 min and were subsequently oven-dried at 80°C for about 20 min. The dried film was laminated onto a backing film.

***In vitro* diffusion experiment**

A flow-through diffusion cell system consisting of a multichannel peristaltic pump (205S, Watson Marlow, UK), a fraction collector (Retriever IV, ISCO Inc., NE, USA), a circulating water bath (RB-10, JeioTech, South Korea), and flow-through diffusion cells was used. The flow-through cell consisted of two side arms, which enabled conduction of receiver cell media via a peristaltic pump to a fraction collector. The temperature of receiver cell media was maintained at 37°C by circulating constant temperature water through the outer jacket of the receiver cell. The surface area of the receiver cell opening was 2 cm^2, and the cell volume was ca. 5.5 ml.

Full-thickness hairless mouse skin was excised from the fresh carcasses of animals that were humanely sacrificed with diethyl ether. Subcutaneous fat and capillary blood vessels were removed carefully with a scissors and scalpel. Preliminary experiment showed that the use of abdominal or dorsal skin had practically no influence on permeation profile of tulobuterol. Each of the flow-through diffusion cell components was connected via Teflon tubing (i.d. 0.015 inches). The receiver cell was filled with a pH 7.4 phosphate buffer solution and the media were stirred by an externally driven, Teflon-coated magnetic bar, to maintain sink condition. The hairless mouse skin was mounted on each receiver cell, and the top cell was placed onto each skin. These components were then clamped securely in place. Any air bubbles that remained in the receiver cell were removed. A disc with a surface area of 2cm^2, was cut out using a punch, and applied to the epidermal side of the skin with slight pressure before being mounted on the receiver cell. The samples were collected every 4 hr for 32 hr.

Data reduction

The following equation was used to calculate the amount of the compound permeated.[14]

$$M_n = C \times V + \frac{S_n}{2} + \sum_{i=1}^{n} S_i \quad (\text{when } n \geq 2)$$

$$M_n = C \times V + \frac{S_1}{2} \quad (\text{when } n = 1)$$

Where M_n is cumulative amount permeated; C is concentration in the receiver cell; V is volume of the receiver cell; S_n is total amount in the *n*th sample.

Assay

Tulobuterol was analyzed by an HPLC system (Shimadzu Scientific Instrument, MD, Kyoto, Japan), consisting of a detector (SPD-10A), a pump (LC-10AD), and automatic injector (SIL-10AD). The wavelength of UV detector was 210 nm and the retention time of tulobuterol was 4.3 min. A reversed-phase column (Alltima C8, Alltech Association, IL) was used. The column temperature was maintained at 30°C by a thin foil temperature controller (CH 1445, SYSTEC, MN). The flow rate was 1 ml/min. The mobile phase consisted of methanol/water/phosphoric acid (37/62.9/0.1).

Resluts and Discussion

Effect of pressure sensitive adhesive matrices

The selection of PSA is very important in the development of TDD formulation of a drug. To compare the permeation rate of tulobuterol from PSA matrices, the permeation profile of tulobuterol from polyisobutylene (PIB), styrene-butadiene-styrene (SBS), silicone, and acrylic PSA matrices were determined. Figure 1 shows the time course profiles of the cumulative amount of tulobuterol permeated across hairless mouse skin from various adhesive matrices without penetration enhancers. Among the PSA matrices tested, polyisobutylene (PIB) showed the highest degree of tulobuterol permeation. Acrylic PSA and silicone provided similar permeation rate.

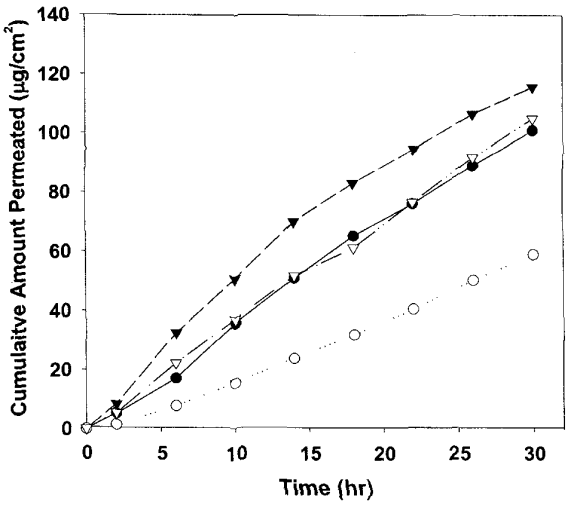

Figure 1–Effects of type of pressure sensitive adhesive on the permeation of tulobuterol across hairless mouse skin. Each point represents average of three measurements. (●) Acrylic-no functional group; (○) Styrene-butadiene-styrene; (▼) Polyisobutylene; (▽) Silicone.

Styrene-Butadiene-Styrene (SBS) adhesive matrix showed the lowest permeation rate.

It has been reported that the flux of a drug from acrylic adhesive matrix depended on the functional group of the acrylic adhesive.[13,15] The effect of chemical nature of acrylic adhesive matrix on the permeation of tulobuterol across hairless mouse skin was evaluated. Figure 2 shows the effect of functional group of acrylic adhesive matrix on the permeation of tulobuterol across hairless mouse skin. The polyethylene oxide grafted acrylic adhesive provided a higher permeation rate, followed by acrylic adhesive without a functional group. The highest permeation rate obtained from polyethylene oxide grafted acrylic adhesive may be partially due to the fact that the increased hydrophilicity due to grafted polyethylene oxide modified thermodynamic activity of tulobuterol in spite of same drug content. The highly cross-liked acrylic adhesive provided the lowest permeation rate. The cross-linking of PSA is one of several techniques used to increase the loading level of the active or excipients in the PSA matrix.[16] Though cross-linking improves cohesive strength of PSA, it could decrease the release rate of a drug from the matrix. In other words, cross-linking hinders the mobility of tulobuterol within PSA matrix, resulting in the decreased permeation rate of tulobuterol. Judging from these results, it is very important to consider the physicochemical property of the PSA and the active substance to select appropriate PSA in the development of TDD product.

Another important criterion in the selection of PSA is the adhesive force of PSA. As discussed above, although polyethylene oxide grafted acrylic adhesive showed the highest flux of tulobuterol, its relative hydrophilic nature caused peeling off from the skin when the matrix was wetted with water. Therefore, mixing with the acrylic adhesive with higher tack would be beneficial to increase adhesiveness of the matrix. Figure 3. shows the permeation of tulobuterol from the mixture of matri-

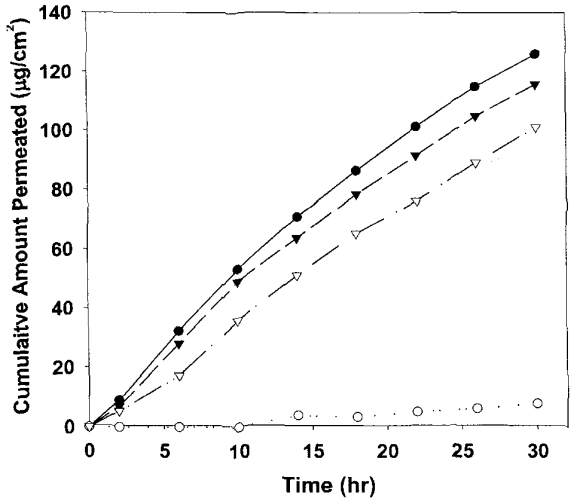

Figure 2–Effects of acrylic pressure sensitive adhesive on the permeation of tulobuterol across hairless mouse skin. Each point represents average of three measurements. (●) Acrylic-polyethylene oxide grafted; (○) Acrylic-highly cross-linked; (▼) Acrylic-no functional group; (▽) Acrylic-OH functional group.

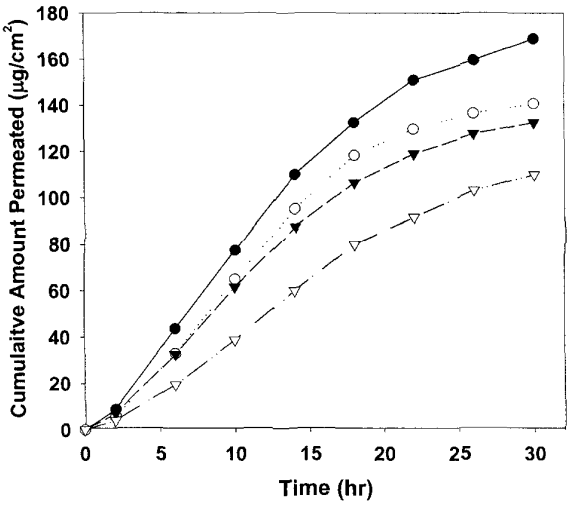

Figure 3–Effects of mixing of acrylic pressure sensitive adhesive on the permeation of tulobuterol across hairless mouse skin. Each sample contained 5% of Plurol oleique®. Each point represents average of three measurements. (●) Acrylic-polymer grafted; (○) Grafted-AA:AA-OH (7:3); (▼) Grafted-AA:AA-OH (6:4); (▽) Acrylic-OH.

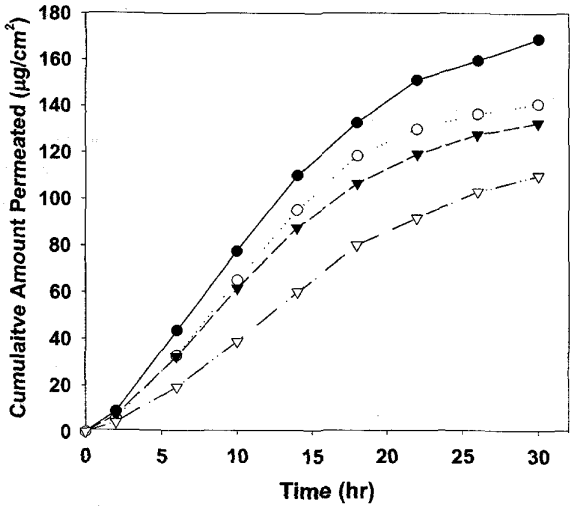

Figure 4–Effect of matrix thickness on the amount of tulobuterol permeated across hairless mouse skin. Each point represents average of three measurements. (●) 70 μm; (○) 60 μm; (▼) 50 μm; (▽) 30 μm.

ces manufactured with polyethylene oxide grafted acrylic adhesive and acrylic adhesive with hydroxyl functional group at various weight fractions. Penetration rate was gradually decreased as the amount of acrylic adhesive with hydroxyl functional group increased, while adhesive force of the matrix was improved. These results suggested that mixing various acrylic PSAs could modify the adhesive force and the permeation rate of a drug across the skin.

The effect of thickness on the amount of tulobuterol permeated across the hairless mouse skin as a function of time is shown in Figure 4. The matrix had same drug concentration (2.77% w/w); therefore, as the thickness of the adhesive matrix increased, the amount of drug within the matrix increased proportionally. Four different matrices were prepared with the thickness of 30 μm, 50 μm, 60 μm, and 70 μm, respectively. After 30 hr, the total amounts of tulobuterol permeated were 34.5±3.9 μg/cm^2, 77.1±9.3 μg/cm^2, 101.1±8.4 μg/cm^2 and 131.1±10.1 μg/cm^2 for the matrices with the thickness of 30 μm, 50 μm, 60 μm, and 70 μm, respectively. It is interesting to note that the fraction of loaded tulobuterol permeated from the adhesive matrix increased from 40% to over 60% as the thickness of the matrix increased from 30 μm to 70 μm. The tulobuterol is highly permeable compound and as the thickness of the matrix increased, it seemed that the occlusive effect of adhesive matrix increased. The occlusive effect is usually provided by backing membrane, however, as the thickness of the adhesive matrix increased, the matrix also contributes to occlusive effect to some extent. As the thickness of matrix increased, the occlusive effect of the matrix increased, resulting in the increased flux of tulobuterol.

Effect of enhancers in PSA matrix

To develop a matrix type transdermal delivery system for a drug, an appropriate enhancer is required to enhance the per-

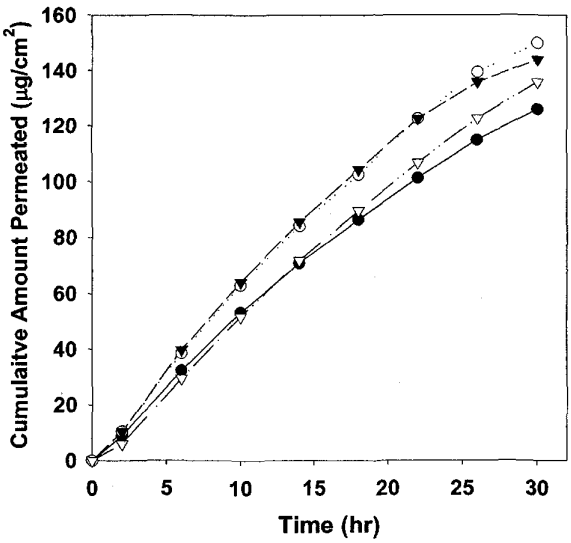

Figure 5–Effect of various vehicles on the permeation of tulobuterol across hairless mouse skin from polyethylene oxide grafted acrylic adhesive. The amount of each vehicle used was 5% of the weight of acrylic adhesive polymer. Each point represents average of three measurements. (●) Control; (○) PG; (▼) IPM; (▽) OA.

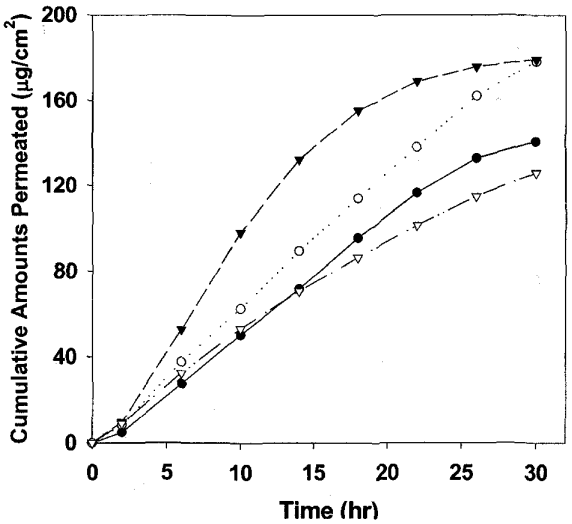

Figure 6–Effect of various vehicles on the permeation of tulobuterol across hairless mouse skin from polyethylene oxide grafted acrylic adhesive. The amount of each vehicle used was 5% of the weight of acrylic adhesive polymer. Each point represents average of three measurements. (●) Span 20; (○) Span 80; (▼) Plurol oleique; (▽) Control.

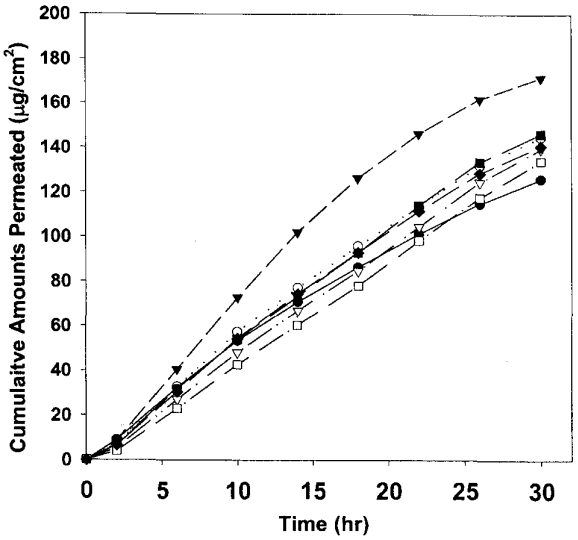

Figure 7–Effect of various vehicles on the permeation of tulobuterol across hairless mouse skin from polyethylene oxide grafted acrylic adhesive. The amount of each vehicle used was 5% of the weight of acrylic adhesive polymer. Each point represents average of three measurements. (●) Control; (○) Labrafac PG; (▼) Labrafil 2609; (▽) GTC; (■) Lauroglycol; (□) Miglyol840; (◆) Crodamol.

Table I–*Physicochemical Properties of Enhancers Used in this Study*

Enhancer	HLB	Hydrophobic portion
Crodamol CAP		C18/C16,C14
Crodamol GTC	1	C8/C10
Isopropyl Myristate		C14
Labrafac PG	2	C8/10
Labrafil 2609	8	C18:2
Lauroglycol	4	C12
Miglyol 840		C8/10
Oleyl alchol		C18
Plurol oleique cc 497	6	C18:1
Span 20	8.6	C11
Span 80	4.3	C18

meation rate and/or to solubilize the drug. The effect of some enhancers on the permeation of tulobuterol from polyethylene oxide grafted acrylic adhesive matrix was investigated to identify the optimum permeation enhancer. The effects of various enhancers on the amount of tulobuterol permeated across hairless mouse skin from grafted acrylic adhesive matrix are shown in Figures 5, 6, and 7. The physicochemical properties of the enhancers used are shown in Table I. Each tested enhancer was added to acrylic adhesive at 5%. Although tulobuterol itself is highly permeable compound, the permeation rate of tulobuterol from acrylic adhesive matrices increased by 1.06–1.42 fold higher depending on the enhancers used. Plurol oleique® showed the most potent enhancing effect followed by Span® 80 and Labrafil® 2609. The other enhancers did not provide significant enhancement effects. The flux of the tulobuterol from polyethylene oxide grafted acrylic adhesive matrix containing Plurol oleique® was fairly high (12.5±2.78 μg/hr/cm^2) initially, but it was gradually decreased (2±0.04 μg/hr/cm^2) with time. Almost 80% of tulobuterol in PSA matrix containing Plurol oleique® was penetrated in less than 20 hr, which resulted in the rapid reduction of thermodynamic activity of tulobuterol in the PSA matrix. In the other hand, the flux of polyethylene oxide grafted acrylic matrix containing span® 80 maintained pseudo steady state throughout permeation study.

It has been reported that the efficacy of enhancers depends on alkyl chain length, HLB value, and ethylene oxide chain length of surfactant. The nonionic surfactant with medium HLB, and an alkyl chain length of C18 and EO chain showed better ability to promote the penetration of piroxicam.[17] Also, Park[18] suggested that the enhancer containing EO chain length 2-5, HLB value 7-9 and an alkyl chain length C16-18 were very effective to increase the skin permeation of ibuprofen. In the present study, C18:1 (HLB 6), C18 (HLB 4.3), C18:2 (HLB 8) were more effective than C 8/10, C 8/10 (HLB 1), C8/10 (HLB 2). The enhancement of Span® 80 (C18) was higher than that of Span® 20 (C11). Though only a limited number of non-ionic surfactants were evaluated in this study for the enhancement of the permeation of tulobuterol, some surfactants having longer alkyl chain length and medium HLB enhanced the penetration of tulobuterol across hairless mouse skin better than that having shorter alkyl chain length and low HLB. These results suggested that HLB and an alkyl chain of enhancer also influenced tulobuterol absorption-enhancing ability.

In conclusion, the penetration rate of tulobuterol can be sufficiently increased by using appropriate selection of a PSA and an enhancer. The nature of PSA significantly affected the permeation rate of the drug across the skin. Thus, physicochemical properties of PSA should be considered before the selection of the matrix. HLB value and size of the alkyl chain length of surfactants were also important factors for the enhancement of skin permeation of tulobuterol. Based on the average flux of tulobuterol obtained using Plurol oleique® and the daily dose of 2 mg, the size of transdermal patch would be approximately 10 cm^2.

References

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