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RESEARCH PAPER

Transdermal Delivery of Physostigmine: Effects of Enhancers and Pressure-Sensitive Adhesives

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ABSTRACT

The purpose of this study was to investigate the effects of various pressure-sensitive adhesives (PSA) on the percutaneous absorption of physostigmine across hairless mouse skin. In addition, the influences of various vehicles and polyvinylpyrrolidone (PVP) on the percutaneous absorption of physostigmine from PSA matrix across hairless mouse skin were evaluated using a flow-through diffusion cell system at 37°C. Physostigmine showed the highest permeability from silicone adhesive matrix, followed by polyisobutylene (PIB), styrene–isoprene–styrene (SIS), acrylic, and styrene–butadiene–styrene (SBS) matrix. Among acrylic adhesives, the permeability of physostigmine was the highest from grafted acrylic adhesive. Polyvinyl pyrrolidone inhibited the crystallization of physostigmine in the PIB adhesive matrix and enhanced the permeability of physostigmine from the PIB adhesive matrix. When esters of sorbitol and fatty acid, polyethylene glycol (PEG) alkyl esters, and caprylic/capric triglycerides were tested, the more lipophilic was a surfactant, the higher the permeation rate within the same group of surfactants. The enhancement effect of PEG derivatives was lower than that of non-PEG derivatives. Among non-linear fatty acid derivatives, linoleate derivatives showed higher permeability of physostigmine than oleate derivatives. This study showed that several non-ionic surfactants, including PEG-20 evening primrose glyceride, enhanced the permeation of physostigmine across hairless mouse skin better than oleic acid.

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INTRODUCTION

The prophylactic effect of physostigmine and pyridostigmine against organophosphate poisoning has been well established.[1–3] Physostigmine, being a tertiary amine, penetrates the blood–brain barrier and was shown to be more effective in the protection against organophosphate poisoning than the quaternary, peripherally-acting pyridostigmine.[2,4] In addition, physostigmine has been reported to improve the memory function of some patients with Alzheimer's disease.[5,6] However, physostigmine has a short plasma half-life and a narrow therapeutic window.[7,8] An intravenous infusion will be one of the most appropriate routes of administration under such conditions, but it demands the hospitalization of the patient. An alternative and promising route of administration is transdermal delivery. The delivery of a drug via the transdermal route offers the advantage of allowing prolonged and consistent blood concentration of the drug. Unlike pyridostigmine, physostigmine is an unquaternized carbamate that can cross body membranes easily, including the skin. This property makes physostigmine a good candidate for transdermal delivery.

The transdermal drug delivery system consists of several components, including active ingredient, pressure-sensitive adhesive, permeation enhancer, and backing membrane, etc. The pressure-sensitive adhesive (PSA) fulfills the adhesion-to-skin function and serves as the formulation foundation. Because the physicochemical properties of PSA can affect significantly the flux of a drug from PSA across the skin, the selection of appropriate PSA matrix is important in designing transdermal drug delivery systems.[9–11] The permeation enhancers can overcome the intrinsic resistance of the stratum corneum and increase the permeation rate of the active ingredient.[12] Althogh each individual component was described separately, a well-designed system must incorporate components that are compatible with one another. The remarkable effect of enhancers obtained from solution formulations may or may not be achievable when they are incorporated within a PSA. Also, drug, enhancer, and PSA can interact with each other in the system which may change their physicochemical properties.[11–13]

Until now, some work has been done to develop a transdermal delivery system for physostigmine. A transdermal pad was developed at the Israel Institute for Biological Research.[14–16] Also, the permeability of physostigmine and the effect of enhancers have been studied.[4,17–20] However, their studies were limited to liquid formulation and aqueous pad formulation and did not include the effect of PSA and compatibility with other components. Also, only a limited number of enhancers have been investigated.

In the present study, we investigated the effects of various PSA on the percutaneous absorption of physostigmine across hairless mouse skin. In addition, the influences of various vehicles and additives on the percutaneous absorption of physostigmine from PSA matrix across hairless mouse skin were evaluated.

MATERIALS AND METHODS

Materials

Physostigmine base was purchased from Sigma Chemical Co. (St. Louis, MO). Polyethylene glycol (PEG)-6 glyceryl menooleate (Labrafil® 1944), PEG-6 glyceryl linoleate (Labrafil® 2609), caprylic/capric triglyceride (Labrafac® CC), PG caprylate/caprate (Labrafac® PG), PEG-8 glyceryl catrylate/caprate (Labrasol®), and polyglyceryl-3 oleate (Plurol oleique® C497) were obtained from Gattepose Korea (Seoul, Korea). Propylene glycol dicaprylate/dicaprate (Miglyol® 840) was obtained from Hüls America (Edison, NJ). Polyethylene glycol-20 evening primrose glycerides (Crovol® EP40), PEG-20 almond glyceride (Crovol® A40), PEG-60 almond glyceride (Crovol® A70), corn oil, and PEG-30 castor oil (Incrocas® 30) were obtained from Croda Inc. (Parsippany, NJ). Sorbitan monolaurate (Span® 20), sorbitan monooleate (Span® 80), PEG sorbitan monolaurate (Tween® 20), PEG sorbitan monooleate (Tween® 80), oleic acid, oleyl alcohol, and propylene glycol were purchased from Junsei Chemical Co. (Tokyo, Japan). Polystyrene–polybutadiene–

polystyrene (SBS) and acrylic pressure-sensitive adhesive solutions in organic solvents were obtained from National Starch and Chemical Co. (Bridgewater, NJ). Polyisobutylene (PIB) (Vistanex LM-MH, Vistanex MML-100) were obtained from Jeil Pharm. Co. (Seoul, Korea). Polystyrene–polyisoprene–polystyrene (SIS) and silicone pressure-sensitive adhesive were obtained from Shell Chemicals (Stanlow, UK) and Dow Corning (Midland, MI), respectively. All other chemicals were reagent grade or above and used without further purification.

Preparation of Adhesive Matrices

The SBS, PIB, SIS, silicone, or acrylic adhesive solution in organic solvent mixture was mixed with physostigmine solution in ethylacetate with or without enhancer according to study protocol. A pressure-sensitive adhesive matrix was prepared by casting the above solutions in a polyester release liner using a casting knife. It was set at room temperature for 20 min and subsequently oven-dried at 80°C for about 15 min to remove the residual organic solvents. The dried film was laminated onto a backing film.

Penetration Studies

A flow-through diffusion cell system, the preparation of hairless mouse skins, the procedure of the penetration studies, and data reduction methods have been described in an earlier study.[11] The penetration samples were collected every 4 hr for 36 hr or longer.

Analytical Conditions

The high-performance liquid chromatography (HPLC) method was used to analyze physostigmine. A reverse-phase column (Alltima C8, Alltech Ass., IL) was used. The column temperature was maintained at 30°C by a thin foil temperature controller (CH1445, Systec, MN). The ultraviolet (UV) detector wavelength of was 235 nm and the mobile phase consisted of methanol/water/triethylamine/phosphoric acid (300/700/1/1). The flow rate was 1 mL/min.

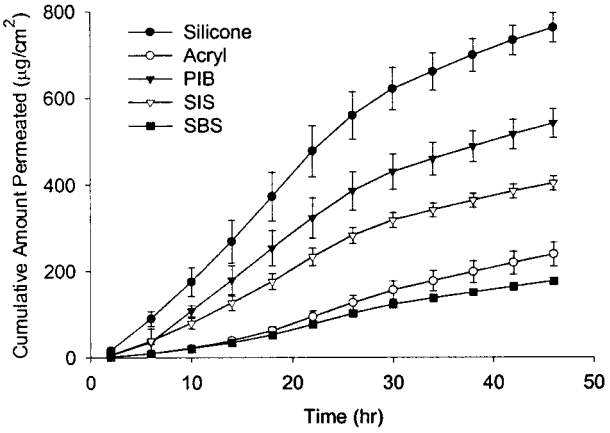

Figure 1. Effect of various adhesive matrices on the permeation of physostigmine across hairless mouse skin. The amount of physostigmine in each PSA used was 10% of the weight of the adhesive polymer. Each point represents an average of three measurements. The error bar shows standard deviation.

RESULTS AND DISCUSSION

Effect of Pressure-Sensitive Adhesive Matrix

The effect of PSA matrix on the permeation of physostigmine was investigated using SBS, PIB, SIS, silicone, and acrylic adhesive matrices. Figure 1 shows the effect of various adhesive matrices on the permeation of physostigmine across hairless mouse skin. The amount of physostigmine in each PSA tested was 10% of the weight of the adhesive polymer. The crystallization of physostigmine was observed in PIB and silicone adhesive matrices at 10% level, indicating that physostigmine is already saturated. No physostigmine crystal was found in SIS, SBS, and acrylic adhesive matrices. As shown in Fig. 1, the permeability of physostigmine was higher in silicone and PIB adhesive, saturated with physostigmine, than in SIS, acrylic and SBS adhesive. The higher thermodynamic activity of physostigmine in silicone and PIB matrices may be partially responsible for the higher flux across the skin in spite of the same drug content.[21]

To investigate the effect of the functional group of acrylic PSA on the permeation of physostigmine across hairless mouse skin, highly cross-linked enhancer-compatible acrylic adhesive (ECA), acrylic adhesives having hydroxyl group (AA-OH), without functional group (AA-none), and grafted acrylic

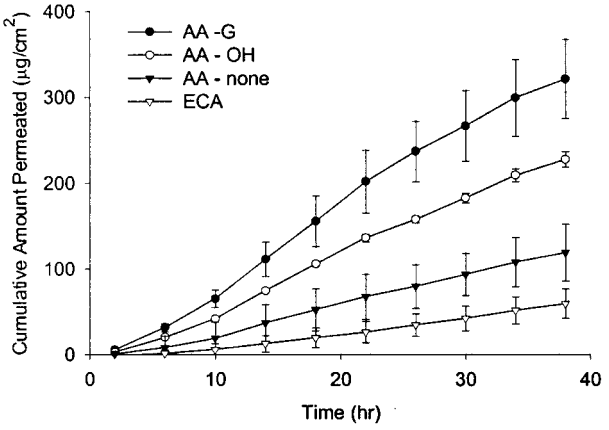

Figure 2. Effect of various acrylic adhesives on the permeation of physostigmine across hairless mouse skin. The amount of physostigmine in each PSA used was 3% of the weight of the adhesive polymer. Each point represents an average of three measurements. ECA, enhancer-compatible adhesive; AA-OH, acrylic adhesive having hydroxyl group; AA-none, acrylic adhesive without functional group; AA-G, grafted acrylic adhesive. The error bar shows standard deviation.

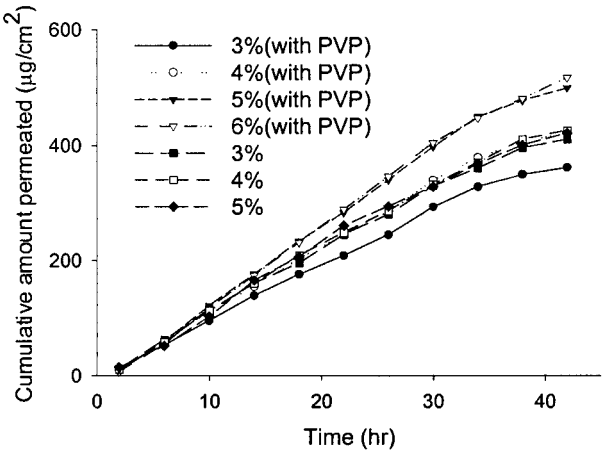

Figure 3. Effect of PVP on the permeation of physostigmine across hairless mouse skin from PIB adhesive matrix. The percentage in the key shows the amount of drug loaded in PIB adhesive polymer (% w/w). The amount of PVP loaded into the matrix was 6% of the weight of the adhesive polymer. Each point represents an average of three measurements.

adhesives (AA-G) were tested (Fig. 2). The amount of physostigmine in each PSA tested was 3% of the weight of the adhesive polymer. The highest permeability of physostigmine was obtained from AA-G, followed by AA-OH, AA-none, and ECA. Within the same type of acrylic adhesives having the same functional groups, no significant difference in the permeability of physostigmine was observed. The results clearly indicate that the functional group of acrylic adhesive significantly affects the permeability of physostigmine, and suggest that the functional group of the adhesive must be considered before the selection of the proper adhesive matrix.

Effect of Polyvinylpyrrolidone

A drug in the adhesive matrix can sometimes be crystallized during storage, and the control of drug crystallization is of particular interest for the efficiency and quality of transdermal drug delivery systems.[22] Polyvinylpyrrolidone (PVP) is commonly used to form solid dispersions, and it has been reported that it inhibits crystallization of a compound in solution.[23] It has also been shown that PVP acts as an inhibitor of drug crystallization in a transdermal matrix system.[22] The effects of PVP on the crystallization and permeation of physostigmine were investigated. The PIB patches containing 3%, 4%, or 5% of physostigmine without PVP and ones containing 3% to 6% (w/w) of physostigmine with 6% (w/w) PVP were formulated. The physostigmine crystals were observed in all of the PIB patches tested without PVP immediately after their preparation. However, in all of the PIB patches containing 6% PVP, no drug crystal was observed microscopically for over a month.

Figure 3 shows the effect of PVP on the permeation of physostigmine across hairless mouse skin from PIB adhesive matrix. Even though the drug loading increased in the PIB matrix without PVP, the flux did not change significantly. This suggests that the physostigmine was saturated in the matrix and the thermodynamic activity of the drug in the matrix became constant when its concentration was greater than 3%. On the other hand, at 6% PVP level, the permeability of physostigmine increased as the drug content increased from 3% to 5%. It seemed that the addition of PVP into the PIB matrix solubilized the physostigmine. The solubilization of the drug at the 3% level resulted in a decrease in the permeability of the drug due to the decrease in thermodynamic activity. The solubilization of the drug at the 5% level resulted in increase

in the permeability of the drug, which may be due to supersaturation of physostigmine in the PIB matrix.[22,24] At 6% PVP, no further increase in the permeability of physostigmine was observed, indicating that approximately 5% was the maximum point of supersaturation.

Effect of Enhancer

Table 1 shows the effect of various enhancers on the permeation of physostigmine across hairless mouse skin from acrylic adhesive matrix. Since steady state was not attained during the course of the diffusion study, average fluxes over 36 hr are presented. The enhancement of Crovol® EP40 from acrylic adhesive matrix was highest, followed by Incrocas® 30, Labrafil® 2609, and Span® 80. The close relationship observed between permeation enhancement and lipid bilayer fluidization in several studies suggested that lipid lamellae is the major site of the action of non-ionic surfactants.[25] The penetration of the surfactant molecule into the lipid lamellae of the stratum corneum is strongly dependent on the partitioning behavior of the surfactant. Therefore, the overall potency of a particular surfactant is likely to be a combination of the molecule's ability to penetrate into the lipid region, and to fluidize the lipid bilayers. The results of this study showed that the more lipophilic was a surfactant, the higher the permeation rate within the same group of surfactants. In the esters of sorbitol and fatty acid, the enhancement of Span® 80 (C18) was higher than that of Span® 20 (C11). Also, the enhancement of Tween® 80 (C18) was higher than that of Tween® 20 (C11). In the caprylic/capric glycerides, the enhancement of Labrafac® CC (caprylic/capric triglyceride, HLB:1) showed the highest effect, followed by Labrafac® PG (PG caprylate/caprate, HLB:2) and Labrasol® (PEG-8 glyceryl caprylate/caprate, HLB:14).

Table 1

Effect of Various Enhancers on the Permeation of Physostigmine Across Hairless Mouse Skin from Acrylic Adhesive Matrix

Enhancer	Average Flux ($\mu g/cm^2$ hr)
Control (without enhancer)	7.6 ± 1.0
Esters	
PEG alkyl esters	
Labrafil 1944	9.5 ± 2.2
Labrafil 2609	12.5 ± 0.8
Labrasol	9.4 ± 1.4
Crovol EP 40	14.2 ± 0.8
Crovol A 40	11.9 ± 0.9
Crovol A 70	9.8 ± 0.7
Incrocas 30	12.6 ± 1.9
Tween 20	9.0 ± 1.7
Tween 80	11.1 ± 1.0
Alkyl glycerides	
Labrafac CC	11.1 ± 0.8
Plurol oleique CC 497	11.5 ± 0.6
Corn oil	10.2 ± 1.5
Miglyol 840	8.2 ± 1.6
Others	
Labrafac PG	10.3 ± 1.9
Isopropyl myristate	11.5 ± 0.5
Span 20	11.3 ± 0.5
Span 80	12.2 ± 1.5
Alcohols and acid	
Propylene glycol	10.5 ± 1.9
Oleyl alcohol	11.2 ± 1.2
Oleic acid	12.0 ± 0.3

The amounts of physostigmine and enhancer in each PSA used were 3% and 5% of the weight of adhesive polymer, respectively. Each point represents an average of three measurements ± standard deviation.

Many researchers reported that the efficacy of PEG alkyl esters was structure-dependent, and the extent of penetration enhancement was dependent on both alkyl and ethylene oxide chain lengths of the surfactant.[26–28] In the present study, the effects of the ethylene oxide chain length of the surfactants were compared at a constant alkyl chain length. At 24 hr, the enhancement of Crovol® A40 (PEG-20 almond glyceride) was significantly higher than that of Crovol® A70 (PEG-60 almond glyceride). Also, the enhancement of Labrafac® CC (caprylic/capric triglyceride) and Span® series (sorbitan ester) was higher than that of Labrasol® (PEG-8 glyceryl caprylate/caprate) and Tween® series (PEG sorbitan ester), respectively. These results suggest that the proper combination of alkyl groups and the number of ethylene oxide groups could be important in determining the enhancement effect of a surfactant.

Oleic acid, linoleic acid, and linolenic acid are non-linear fatty acids, due to the presence of cis double bond(s). Due to this structural property, the insertion of oleic acid into the lipid domain of the stratum corneum is thought to open up channels for diffusion and alter the fluidity of the lipid.[4]

It is interesting to note that Crovol® EP40 (PEG-20 evening primrose glycerides) and Labrafil® 2609 (PEG-6 glyceryl linoleate), the derivatives of linoleate, showed higher enhancement in the permeability of physostigmine than Crovol® A40 (PEG-20 almond glycerides) and Labrafil® 1944 (PEG-6 glyceryl monooleat), the derivatives of oleate, respectively. It may be too early to draw a conclusion, however, a compound with two double bonds seemed to have better enhancement effect than one with one double bond.

Until now, only a limited number of vehicles excluding non-ionic surfactants have been studied for the enhancement of the permeation rate of physostigmine. The enhancement effect of oleic acid on the permeation of physostigmine has been reported in the literature.[4,16] However, this study showed that several non-ionic surfactants, including PEG-20 evening primrose glyceride, enhanced the permeation of physostigmine across hairless mouse skin better than oleic acid.

In summary, this study has shown that the proper selection of PSA and permeation enhancers is very important in the development of transdermal drug delivery systems. The functional group of PSA should also be considered, since it can significantly affect the permeation rate of a compound. The best permeation enhancer for physostigmine in acrylic adhesive matrix was found to be Crovol® EP40, and the crystallization of the drug can be prohibited by using PVP.

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