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Enhanced Bioavailability of Ambroxol by Transdermal Administration of the EVA Matrix Containing Penetration Enhancer in Rats

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Abstract − The pharmacokinetics and bioavailability of ambroxol, an expectoration improver and mucolytic agent, were studied to determine the feasibility of enhanced transdermal delivery of ambroxol from the ethylene-vinyl acetate (EVA) matrix system containing polyoxyethylene-2-oleyl ether as an enhancer in rats. The ambroxol-010 matrix system (15 mg/kg) was applied to abdominal skin of rats. Blood samples were collected via the femoral artery for 28 hrs and the plasma concentrations of ambroxol were determined by HPLC. Pharmacokinetic parameters were calculated using Lagran method computer program. The area under the curve (AUC) was significantly higher in the enhancer group (1,678 ± 1,413.3 ng/ml·hr) than that in the control group 1,112 ± 279 ng/ml · hr), that is treated transdermally without enhancer, showing about 151% increased bioavailability ($p < 0.05$). The average C_{max} was increased in the enhancer group (86.0 ± 21.5 ng/ml) compared with the control group (59.0 ± 14.8 ng/ml). The absolute bioavailability was 13.9% in the transdermal control group, 21.1% in the transdermal enhancer group and 18.1% in the oral administration group compared with the IV group. The T_{max}, K_a, MRT and $t_{1/2}$ of ambroxol in transdermal enhancer group were increased significantly ($p < 0.01$) compared to those of oral administration. As the ambroxol-EVA matrix containing polyoxyethylene-2-oleyl ether and tributyl citrate was administered to rats via the transdermal routes, the relative bioavailability increased about 1.51-fold compared to the control group, showing a relatively constant, sustained blood concentration. The results of this study show that ambroxol-EVA matrix could be developed as a transdermal delivery system providing sustained plasma concentration.

Keywords: Bioavailability, Pharmacokinetics, EVA, Ambroxol, Enhancer, Transdermal administration

INTRODUCTION

Ambroxol, [trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexanole hydrochloride], is a metabolite of bromhexine (Ecker *et al*., 1983). It is known to have a high affinity to lung tissue. Ambroxol is used as a mucolytic drug that lowers sputum viscosity by normalizing secretion from bronchiolar glands and increases mucociliary clearance (Nagaoka and Kase, 1981; Houtmeyers *et al*., 1999), provides anti-oxidative (Nowak *et al*., 1994a, b) as well as anti-inflammatory capacity (Yang *et al*., 2002). Ambroxol, an expectoration improver and mucolytic agent, has been widely used in the treatment of acute and chronic disorders characterized by the production of excess or thick mucus (Seki *et al*., 1977; Nowak *et al*., 1994a, b).

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When administered orally, it might cause many adverse effects due to transient high blood drug concentration such as headache, drowsiness, dizziness, insomnia and languor. It might be inconvenient to administer orally due to short $t_{1/2}$. Therefore, transdermal administration with sufficient bioavailability and long $t_{1/2}$, could be more convenient compared to oral administration. Therefore, the development of transdermal drug delivery of the antihistamine without adverse effects of frequent oral administration is very important.

In my previous paper (Cho *et al.,* 2008), the ambroxol-EVA matrix system containing polyoxyethylene-2-oleyl ether as an enhancer and tributyl citrate as a plasticizer was formulated. The objective of this study was to determine the feasibility of transdermal delivery of ambroxol by studying its in vivo absorption characteristics in rats and to develop the ambroxol-EVA matrix system containing a penetration enhancer.

MATERIALS AND METHODS

Materials

Ambroxol hydrochloride and domperidone were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Ethylene vinyl acetate (EVA, 40%) of 40% VA content was purchased from Aldrich Chemical Co., Inc. (USA) and tributyl citrate was obtained from Morflex, Inc. (USA). Heparin sodium and normal saline were from Green Cross (Seoul, Korea). Acetonitrile, diethyl ether, n-butanol, n-heptane and methanol was HPLC grade and all other reagents were of analytical grade and used without further purification.

Extraction of the basic form of ambroxol

Ambroxol hydrochloride was dissolved in about 100 ml of distilled water and 100 ml of ether were added to separating funnel. Some drops of ammonia test solution was added and mechanically shaken. The ether portion was taken and dehydrated with anhydrous sodium sulfate and filtered on sintered glass before evaporation of the solvent in a rotary evaporator.

Preparation of drug-containing EVA matrix

The drug-EVA matrix containing enhancer and plasticizer were prepared using polyoxyethylene-2-oleyl ether chosen as a best effective enhancer and tributyl citrate chosen as a best effective plasticizer for EVA matrix in our previous experiments (Cho *et al.,* 2008). The EVA matrix containing ambroxol was prepared by solvent casting methods. Briefly, the weighted amount of EVA copolymer beads, plasticizer, and enhancer were dissolved in 20 ml of methylene-chloride in a beaker and the drug solution was added. This mixture was poured onto a glass plate and the solvent was allowed to evaporate off at room temperature overnight. The matrix was removed from the plate. Then, a piece of matrix was cut properly and the thickness was measured before the experiment. The drug content was calculated from the weight ratio of drug and copolymer used.

Animal experiment

Male Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research and Co. (Choongbuk, Korea), and had free access to normal standard chow diet (Jae Il Chow, Korea) and tap water. Throughout the experiments, the animals were housed, four or five per cage, in laminar flow cages maintained at $22 \pm 2^{o}C$, 50-60% relative humidity, under a 12 h light-dark cycle. The animals were kept in these facilities for at least one week before the experiment. This experiment was carried out in accordance with the “Guiding Principles in the Use of Animals in Toxicology” adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal care committee in our institution (Chosun University) approved the present study.

Rats were divided in four groups of six each: oral group (5 mg/kg of ambroxol, oral), transdermal control group (15 mg/kg of ambroxol), transdermal enhancer group (15 mg/kg of ambroxol containing enhancer) and IV group (1.25 mg/kg of ambroxol, intravenous administration). Sprague-Dawley rats were fasted for at least 24 h prior to experiments and were given water freely. Each rat was anaesthetized with ether. The right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) for blood sampling.

The transdermal dose of ambroxol-EVA matrix was chosen to keep plasma concentrations above the limit of detection. In oral group, ambroxol (5 mg/kg) was suspended in 1.5 ml of distilled water and administered orally to rat. In transdermal administration, the ambroxol-EVA matrix (15 mg/kg) was adhered on the abdominal skin, where the hair was shaved. The drug used in the IV group was prepared by adding ambroxol to injectable saline (0.5 ml/rat), and injected through the femoral vein for 1 min. To oral administration group, blood samples (0.5 ml) were withdrawn from the femoral artery at 0, 0.1, 0.25, 0.5, 1, 2, 4, 6, 8 and 12 h after the oral administration of the drug. For transdermal administration group, they were withdrawn at 0, 1, 2, 3, 4, 5, 6, 8, 12, 20 and 28 hour after administration. The blood samples were centrifuged at 5,000 rpm for 5 min to gain plasma samples (0.2 ml). The plasmas were stored at $-40^{o}C$ until the HPLC analysis.

Determination of ambroxol in rat plasma by HPLC

The plasma concentrations of ambroxol were determined by HPLC assay using a modification of the method reported Botterblom et al. (1987) Briefly, 25 μl of domperidone (2 μg/ml), as the internal standard, was added to 0.2 ml of plasma and 0.2 ml of buffer (pH 10) and 1.2 ml of diethyl ether were added. The mixture was then stirred for 2 min and centrifuged for 10 min. 1 ml of the organic layer was transferred to a clean test tube, and 0.2 ml of 0.01 N hydrochloride was added and mixed for 2 min. 50 μl of the water layer was injected into the HPLC system. The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV detector (Model SPD-10A), a system controller (Model SCL-10A), a degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The UV detector was set to 237 nm. The stationary phase was

a μ-Bondapak C_{18} column (3.9×300 mm, 10 μm, Waters Co., Ireland) and the mobile phase was methanol: acetonitrile: 0.01 M phosphate buffer (PH 7.0): tetrahydrofuran (35:35:27.5:2.5, v/v/v/v). The retention times at a flow rate of 1.5 ml/min are as follows: The internal standard at 3.7-min and ambroxol at 4.9-min (Fig. 1). The calibration curves of ambroxol were linear within the range of 10-200 ng/ml. The intra-day (n=5) and inter-day (n=5) coefficients of variation were <5% for ambroxol, and <1.5% for domperidone.

Pharmacokinetics analysis

Non-compartmental pharmacokinetic analysis was performed using the LAGRAN method computer program (Rocci and Jusko, 1983). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were obtained from the experimental data, and the half-life ($T_{1/2}$) of the drug was obtained using the formula, $0.693/K_{el}$. The absolute bioavailability (A.B.%) of ambroxol was calculated using the formula, for oral by $AUC_{oral}/AUC_{iv} \times Dose_{i.v}/Dose_{oral} \times 100$, for transdermal matrix by $AUC_{transdermal}/AUC_{iv} \times Dose_{i.v}/Dose_{transdermal} \times 100$, and the relative bioavailability (R.B.%) of ambroxol was estimated using the formula, $AUC_{transdermal\ enhancer}/AUC_{transdermal\ control} \times 100$. The mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by the area under the curve (AUC). The AUMC was determined using a plot of the plasma concentration multiplied by time (C · t) versus time and its area under the curve was calculated by the LAGRAN method.

Statistical analysis

All the means are presented with their standard deviation (Mean ± S.D.). Statistical analysis was performed using a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A p-value < 0.05 was considered statistically significant.

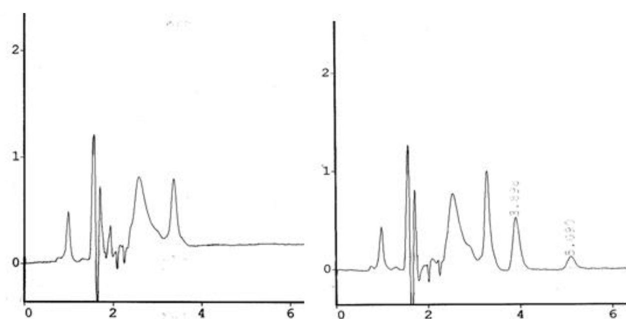

Fig. 1. Chromatograms of blank plasma and plasma spiked with domperidone (3.9 min) and ambroxol (5.1 min).

RESULTS AND DISCUSSION

Biopharmaceutical aspects of transdermal absorption of ambroxol

For the purpose of studying the biopharmaceutical aspects of transdermal absorption of ambroxol, one of the prerequisites is that the pharmacokinetic parameter after the IV administration should correlate with that after the transdermal absorption of ambroxol. The plasma-time concentration curve for ambroxol after the transdermal administration of 15 mg/kg of ambroxol comparing with oral and IV administration to rats was shown in Fig. 2.

The average areas under the serum concentration-time curves, the value of AUC was 359 ± 89.8 ng/ml · hr for oral administration, 662 ± 165.5 ng/ml · hr for intravenous administration. Following transdermal administration of a single 15 mg/kg of ambroxol to rats, the value of AUC of transdermal administration with enhancer was 1,678 ± 413.3 ng/ml·hr and that without enhancer was 1,112 ± 279 ng/ml·hr (Table I).

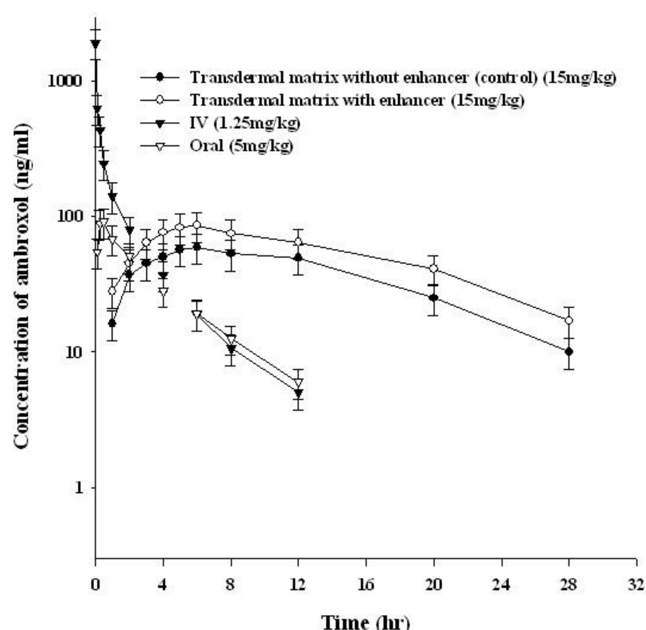

Fig. 2. Mean plasma concentration-time profile of ambroxol following the oral (5 mg/kg), the IV (1.25 mg/kg), and the transdermal administration (15 mg/kg) of the ambroxol-EVA matrix system containing an enhancer to rats (n=6). The error bar represents the standard deviation of the mean.

Table I. Pharmacokinetic parameters of ambroxol from transdermal EVA matrix system containing enhancer, oral and IV administration in rats

Parameters	Oral (5 mg/kg)	Transdermal (15 mg/kg)		IV (1.25 mg/kg)
		Control (no-enhancer)	Enhancer	
$AUC_{0-\infty}$ (ng/ml・h)	359 ± 89.8	1,112 ± 279	1,678 ± 413.3^{b}	662 ± 165.5
C_{max} (ug/ml)	91.5 ± 22.9	59.0 ± 14.8	86.0 ± 21.5^{b}	
T_{max} (h)	0.5	6.0^{a}	6.0^{a}	
K_a	5.9 ± 1.5	11 ± 2.9	18 ± 4.7^{b}	
$T_{1/2}$ (h)	3.6 ± 0.9	8.1 ± 2.0^{a}	9.0 ± 2.3^{a}	3.0 ± 0.75
MRT (h)	4.7 ± 1.2	14 ± 3.7^{a}	15 ± 3.9^{a}	2.5 ± 0.7
A.B. (%)	18.1 ± 4.7	13.9 ± 3.6	21.1 ± 5.5^{b}	
R.B. (%)		100	151	

Mean ± S.D. (n = 6), $^{a}p < 0.01$, significant difference compared to oral administration, $^{b}p < 0.05$, significant difference compared to transdermal control, $AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity, C_{max}: peak concentration, K_a: absorption rate constant, T_{max}: time to reach peak concentration, $t_{1/2}$: terminal half-life, A.B. (%): absolute bioavailability, R.B (%): AUC rate compared to AUC transdermal control, MRT: the mean residence time.

By transdermal administration of ambroxol-EVA matrix containing an enhancer, the plasma concentrations of ambroxol increased significantly ($p < 0.05$) compared to the control, the area under the plasma concentration-time curve (AUC) and the peak concentration (C_{max}) of ambroxol increased significantly ($p < 0.05$). The T_{max}, K_a, MRT and $t_{1/2}$ of ambroxol following transdermal administration of the EVA matrix increased significantly ($p < 0.01$) compared to those of oral administration.

The absolute bioavailability of AUC value of transdermal administration without an enhancer (control group) showed about 13.9%, and that of transdermal administration of ambroxol from the EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer (enhancer group) showed about 21.1%, while the oral administration group showed 18.1% compared to intravenous administration group. The transdermal administration of ambroxol from the matrix system containing polyoxyethylene-2-oleyl ether as an enhancer was higher than that from the matrix system without an enhancer. As the ambroxol-EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer was administered via the transdermal routes to rats, the AUC (%) showed about 1.51-fold compared to the control group.

When administered orally, it might bring some gastrointestinal side effects due to transient high blood drug concentration. Therefore, transdermal administration with sufficient bioavailability and long $t_{1/2}$, could be more convenient compared to oral administration. Transdermal administration of ambroxol matrix containing polyoxyethylene-2-oleyl ether to rats showed a relatively constant, sustained blood concentration and better bioavailability comparing with the control group (Fig. 1).

Peak concentration (C_{max}) and peak time (t_{max})

Statistical analysis of the C_{max} and t_{max} values observed following the transdermal administration of the ambroxol formulations shows that the enhancer group exhibited higher average C_{max} values of 86.0 ± 21.5 ng/ml than those of 59.0 ± 14.8 ng/ml which was achieved by the control group, whose differences were not significant.

The AUC (%) of the enhancer group was about 151% comparing with the control group that means the enhanced absorption ($p < 0.05$). The transdermal administration of ambroxol-EVA matrix containing enhancer showed a sustained and enhanced absorption.

CONCLUSION

When the ambroxol-EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer and tributyl citrate as a plasticizer was administered to rats via the transdermal routes, the relative AUC% increased about 1.51-fold compared to the control group, showing a relatively constant, sustained blood concentration. The results of this study show that ambroxol-EVA matrix could be developed as a transdermal delivery system providing sustained plasma concentration without causing burst absorption compared to the oral group.

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