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Research paper

Enhanced bioavailability of atenolol by transdermal administration of the ethylene-vinyl acetate matrix in rabbits

Sang-Chul Shin[a], Jun-Shik Choi[b,*]

[a]*College of Pharmacy, Chonnam National University, Gwangju, South Korea*
[b]*College of Pharmacy, Chosun University, Gwangju, South Korea*

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Abstract

The pharmacokinetics and bioavailability of atenolol, a antihypertensive, were studied to determine the feasibility of enhanced transdermal delivery of atenolol from the ethylene-vinyl acetate (EVA) matrix system containing polyoxyethylene-2-oleyl ether as an enhancer in rabbits. The atenolol-EVA matrix (20 mg/kg) was applied to abdominal skin of rabbits. Blood samples were collected via the femoral artery for 32 h and the plasma concentrations of atenolol were determined by high-performance liquid chromatography. Pharmacokinetic parameters was calculated using Lagran computer program. The area under the curve (AUC) was significantly higher in the enhancer group (12,402 ± 3061 ng/ml·h) than that in the control group (8507 ± 2092 ng/ml·h), showing about 46% increased bioavailability ($P < 0.05$). The average C_{max} was increased in the enhancer group (1361 ± 340 ng/ml) compared with the control group (1168 ± 293 ng/ml), but not significantly. The T_{max} was significantly decreased in the enhancer group (1.3 ± 0.36 h) compared with the control group (2.0 ± 0.51 h). The elimination time ($t_{1/2}$) and mean residence time were significantly increased in the transdermal group compared with the IV group. The absolute bioavailability was 19.7% in the control group, 28.6% in the enhancer group and 77.4% in the oral administration group compared with IV the group. As the atenolol-EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer and tributyl citrate as a plasticizer was administered to rabbits via the transdermal routes, the relative AUC% increased about 1.46-fold compared to the control group, showing a relatively constant, sustained blood concentration with minimal fluctuation. The results of this study show that atenolol-EVA matrix could be developed as a transdermal delivery system providing sustained plasma concentration.

Keywords: Bioavailability; Pharmacokinetics; Ethylene-vinyl acetate matrix; Atenolol; Enhancer; Transdermal administration

1. Introduction

Atenolol is widely used in the management of hypertension as monotherapy or in combination with other classes of antihypertensive agents. In the management of hypertension or chronic stable angina pectoris in patients with chronic obstructive pulmonary disease or type I diabetes mellitus, many clinicians prefer to use low dosages of a β_1-selective adrenergic blocking agent, rather than a non-selective agent [1–4].

The absorption of atenolol upon oral administration in humans and most laboratory animal species is rapid but incomplete [5]. Due to incomplete intestinal absorption, the systemic bioavailability is about 50–60% in the human [6,7] as well as in the rat, mouse, rabbit, and monkey [8]. Following absorption, atenolol is widely distributed to most body tissues in humans [6] and in animal species [9]. However, only a small proportion of the administered dose reaches the brain in both humans and animals [10]. Atenolol shows almost 5%-binding to plasma protein in the human [11]. In rodents, atenolol is evenly distributed between red blood cells and plasma [8]. Only about 10% of the absorbed amount is metabolized in animals and humans and the rest is excreted unchanged. The major route of excretion is through the urine in humans as well as in the animal species [5]. Since 80–90% of atenolol is excreted unchanged, there is little biotransformation.

It is reported that in case of oral administration of atenolol, it can induce side effects such as diarrhea, nausea, mesenteric arterial thrombosis, ischemic colitis and dry

* Corresponding author. College of Pharmacy, Chosun University, 375 Donggu, Gwangju 501-757, South Korea. Tel.: +82-62-230-6365; fax: +82-62-232-6414.
E-mail address: isachoi@chosun.ac.kr (J.-S. Choi).

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mouth [12]. Therefore, the development of transdermal drug delivery systems for the antihypertensives maintaining appropriate blood levels for a prolonged time without adverse effects associated with frequent oral administration is very important.

Our previous paper [13] described the formulation of an atenolol-EVA matrix system containing polyoxyethylene-2-oleyl ether as an enhancer and tributyl citrate as a plasticizer. The objective of this study was to determine the feasibility of transdermal delivery of atenolol by studying its in vivo absorption characteristics in rabbits and to develop the atenolol-EVA matrix system containing a penetration enhancer.

2. Materials and methods

2.1. Materials

Atenolol, metoprolol (internal standard), sodium dodecyl sulfate and polyoxyethylene-2-oleyl ether were purchased from Sigma Chemical Co. (ST. Louis, MO, USA), ethylene-vinyl acetate copolymer 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, South Korea). Acetonitrile, n-butanol, n-heptane and methanol was high-performance liquid chromatography (HPLC) grade and all other reagents were of analytical grade and used without further purification.

2.2. Drug-containing EVA matrix preparation

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 [13]. EVA matrix containing atenolol was prepared by a solvent casting process. Briefly, 2.0 g of EVA polymer beads and 30 mg of atenolol were dissolved in methylene chloride and anhydrous ethanol in a beaker with vigorous stirring. One hundred mg of polyoxyethylene-2-oleyl ether and 100 mg tributyl citrate were dissolved in this polymer solution. This above combined drug solution 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 and dried for 2 days at room temperature. Then, a piece of matrix was cut from the membrane and weighed accurately. The drug content was calculated from the weight ratio of drug and copolymer used.

2.3. Animal treatment

The New Zealand white, male rabbits weighing 1.8–2.0 kg were housed individually over 2 weeks in a temperature-controlled environment (20–25°C). The relative humidity varied between 50 and 60%. They had free access to a diet and water 1 week before experiments unless otherwise noted. They were fasted 24 h before experiments.

2.4. HPLC conditions

Plasma concentrations of atenolol was determined by modified HPLC methods of Winkler et al. [14]. The HPLC system consisted of a solvent delivery pump (LC-10AD, Shimadzu, Japan), a fluorescence detector (RF-10A, Shimadzu, Japan), and computing integrator. The column was u-Bondapak C_{18} column (10 μm, 3.9 × 300 mm, Waters). The mobile phases was a combination of acetonitrile: methanol: 0.02 M sodium biphosphate (pH 3.5) (35:10:55) including 0.1% sodium dodecyl sulfate and used after degasing. The fluorence detector was set at the wavelength of the Em 280 nm and Ex 300 nm, and the column temperature was maintained at ambient, and flow rate of 1.5 ml/min. Under these conditions, atenolol peak appeared at the retention time of 3.5 min at room temperature (Fig. 1).

2.5. Route of administration and withdrawal of blood samples

The New Zealand white rabbits were fixed on a plate and anesthetized by subcutaneous injection of 25% urethane-physiological saline (4 ml/kg) and the abdominal skin of rabbits was shaved 1 h prior to application. The teeth of the rabbits were fixed on the plate, while the tongue was kept tightly on the lower teeth. An infusion set equipped with a 22-gauge (0.8 mm) hypodermic needle

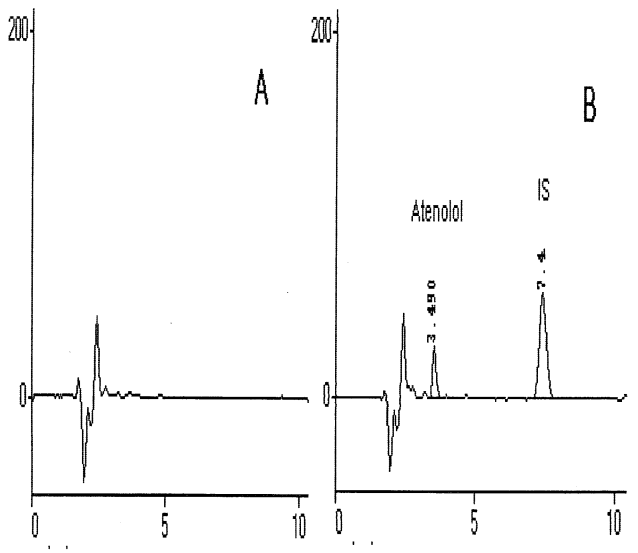

Fig. 1. Chromatograms of blank plasma (A); and plasma spiked with internal standard (7.4 min) and atenolol (3.5 min) (B).

and winged adapter was inserted into a right femoral artery to facilitate sampling of blood for drug analysis. Blood samples were collected via femoral artery for 32 h and the plasma concentrations of atenolol were determined by HPLC.

The atenolol-EVA matrix (20 mg/kg of atenolol) was applied to abdominal skin of rabbits and 1.0 ml of blood specimens was taken at specific time intervals from the cannulae inserted into the femoral artery in a haparinized-glass tubes and centrifuged at 5000 rpm for 5 min to obtain 0.5 ml of the plasma and frozen until analyzed. A single dose of 2.5 mg/kg of atenolol was administered by a rapid injection via the ear vein for the intravenous administration. Blood samples were taken before and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 32 h after transdermal administration and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after oral administration, 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after intravenous administration. After taking the blood specimen, heparinized physiological saline (75 IU/ml) was inserted into the set to prevent blood coagulation. The homeostasis of the rabbits was maintained by injection of same volume of physiological saline via the ear vein.

2.6. Determination of atenolol in rabbit plasma

The determination of atenolol in the plasma was carried out by the Winkler method [14]. A 0.5 ml aliquot of plasma was pipetted into a 15 ml centrifuge tube, 0.1 ml of 0.5N NaOH solution and 5 ml of n-butanol and n-heptan (1:1) were added and shaken for 3 min by mechanical shaking. After centrifugation for 5 min at 3000 rpm, added 0.3 ml of 0.1N HCl and vortexed for 1 min, then 50 μl of water layer was injected into the HPLC.

2.7. Pharmacokinetic data analysis

The non-compartmental pharmacokinetic analysis was performed with the Lagran computer program [15] which employs the Lagran method to calculate the area under the curve (AUC) of plasma concentration (C_p) as a function of time (t). Area under the curves were computed by Lagran method to reduce the errors by the trapezoidal rule. Mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by AUC. AUMC was determined using a plot of plasma concentration multiplied by time (C·t) versus time and calculation of its area under the curve by the Lagran. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were determined by visual inspection of the experimental data. The elimination rate constant ($_{Kel}$) was calculated by the regression analysis from the slope of the line and the half-life ($t_{1/2}$) of drug was obtained by $0.693/K_{el}$. The absolute bioavailability of atenolol after transdermal administration per the I.V. administration was calculated as following:

$$\text{Absolute } \textit{bioavailability } (A.B.) = \frac{\text{Sample AUC}}{\text{IV } \textit{AUC}} \times \frac{\text{IV dose}}{\text{Sample } \textit{dose}} \times 100$$

The statistical significance of the differences between formulations was tested by the Student's paired *t*-test. It was defined to be statistically significant when $P < 0.05$. All values were reported as mean ± standard deviation (SD).

3. Results and discussion

3.1. Area under the concentration-time curve

For the purpose of studying the biopharmaceutical aspects of transdermal absorption of atenolol, one of the prerequisites is that the pharmacokinetic parameter after the i.v. administration should correlate with that after the transdermal absorption of atenolol. The plasma-time concentration curve for atenolol after the transdermal administration of 20 mg/kg of atenolol is shown in Fig. 1 with oral and i.v. administration to rabbits.

The average areas under the serum concentration-time curves, the value of AUC was 16,760 ± 4190 ng/ml·h for oral administration, 5412 ± 1353 ng/ml·h for intravenous administration. Following transdermal administration of a single 20 mg/kg of atenolol to rabbits, the value of AUC of transdermal administration with enhancer was 12,402 ± 3061 ng/ml·h and that without enhancer was 8507 ± 2092 ng/ml (Table 1). Within each study, significant differences were observed among the formulations.

The absolute bioavailability of AUC value of transdermal administration without an enhancer showed about 19.7% compared to intravenous administration. However, the absolute bioavailability of AUC value of transdermal administration of atenolol from the EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer showed about 28.6% compared to intravenous administration. The transdermal administration of atenolol 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 atenolol-EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer was administered via the transdermal routes to rabbits, the AUC (%) showed about 1.46-fold compared to the control group ($P < 0.05$).

Transdermal administration of atenolol matrix containing polyoxyethylene-2-oleyl ether to rabbits showed a relatively constant, sustained blood concentration with minimal fluctuation without showing 'flip-flop' effect and better bioavailability comparing with control group (Fig. 2).

Table 1
Pharmacokinetics of atenolol from transdermal EVA matrix system containing enhancer, oral and IV administration in rabbits

Groups parameters	Control	Enhancer	Oral	IV
AUC (ng/ml·h)	8507 ± 2092	12,402 ± 3061*	16,760 ± 4190	5412 ± 1353
C_{max} (ng/ml)	1168 ± 293	1361 ± 340	1821 ± 455	–
T_{max} (h)	2.0 ± 0.51	1.3 ± 0.36*	1.6 ± 0.41	–
K_{el} (h^{-1})	0.67 ± 0.19*	0.69 ± 0.20*	0.91 ± 0.22	0.94 ± 0.27
$T_{1/2}$ (h)	10.35 ± 2.38*	10.05 ± 2.34*	7.61 ± 2.02	7.36 ± 1.85
MRT (h)	11.0*	12.0*	10.0	7.4
A.B. (%)	19.7	28.6*	77.4	100
AUC (%)	100	146*		–

Each value represents the mean ± SD ($n = 8$). *$P < 0.05$ compared to control or IV. AUC, area under the plasma concentration-time curve from time zero to time infinity; C_{max}, maximum plasma concentration; T_{max}, time of C_{max}; K_{el}, elimination rate constant; $T_{1/2}$, terminal half-life; MRT, mean residence time; A.B., absolute bioavailability to IV group; and AUC (%), relative percent of AUC to control group. Transdermal dose of control and enhancer group = 20 mg/kg; oral dose = 10 mg/kg; and IV dose = 2.5 mg/kg.

3.2. 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 atenolol formulations shows that the enhancer group exhibited higher average C_{max} values of 1361 ± 340 ng/ml than those of 1168 ± 293 ng/ml which was achieved by the control group, whose differences were not significant. The t_{max} of the enhancer group (1.3 ± 0.36 h) was significantly increased compared with the control group (2.0 ± 0.51 h) ($P < 0.05$) (Table 1). The AUC (%) of the enhancer group was about 146% comparing with the control group that means the enhanced absorption ($P < 0.05$). The transdermal administration of atenolol-EVA matrix containing enhancer showed a sustained and enhanced absorption.

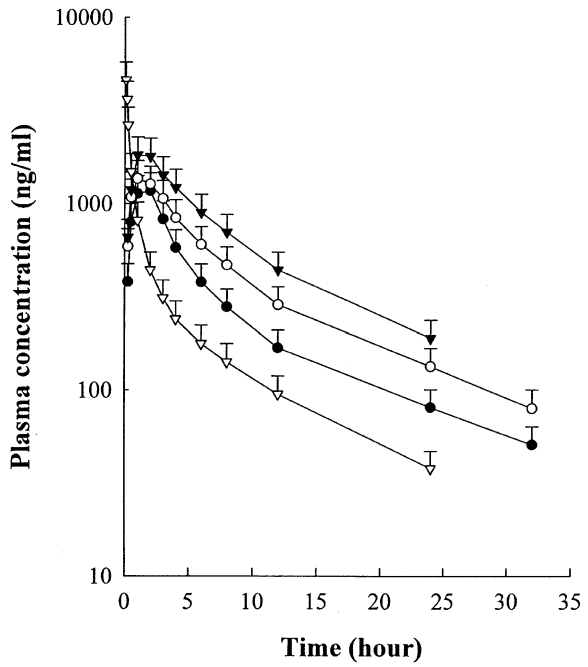

Fig. 2. Plasma concentration-time profile of atenolol following oral (10 mg/kg), IV administration (2.5 mg/kg), and transdermal administration (20 mg/kg) of the atenolol-EVA matrix system containing enhancer, to rabbits ($n = 6$). The error bar represents the standard deviation of the mean. ●, IV administration; □, oral administration; ○, transdermal administration of the matrix without enhancer(control); and ■, transdermal administration of the matrix with enhancer.

3.3. Mean residence time (MRT), K_{el} and $T_{1/2}$

The average MRT after transdermal administration was 11 h in control group and 12 h in the enhancer group. The average K_{el} of the enhancer group (0.69 ± 0.20 h^{-1}) and the control group (0.67 ± 0.19 h^{-1}) was decreased compared with the intravenous administration group (0.92 ± 0.26 h^{-1}), statistically significant. The average $T_{1/2}$ was 10.05 ± 2.34 h in the enhancer group and 10.35 ± 2.28 h in the control group and a little sustained from the transdermal administration, while 7.36 ± 2.02 h in the intravenous administration group, statistically significant.

4. Conclusions

As the atenolol-EVA matrix containing polyoxyethylene-2-oleyl ether as an enhancer and tributyl citrate as a plasticizer was administered to rabbits via the transdermal routes, the relative AUC% increased about 1.46-fold compared to the control group, showing a relatively constant, sustained blood concentration with minimal fluctuation. The results of this study show that atenolol-EVA matrix could be developed as a transdermal delivery system providing sustained plasma concentration.

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