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Pharmacokinetic Study of Atenolol in Rabbit Blood by Capillary Electrophoresis with Laser-induced Fluorescence Detection

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A sensitive method based on the use of capillary electrophoresis with laser-induced fluorescence (CE-LIF) detection was developed and validated for determination of atenolol in plasma of rabbit. Other components including a variety of amino acids and proteins in plasma did not interfere with the determination of atenolol under the experimental condition. The assay had a wide range (1.5-500 ng/mL) of linearity and a detection limit of 0.5 ng/mL. The intra- and inter-day precisions were satisfactory with relative standard deviation (RSD) less than 10 % and accuracy within 10 %. This method was successfully applied to pharmacokinetic study of atenolol in rabbit blood.

Key Words: Atenolol, Capillary electrophoresis, Laser-induced fluorescence, Plasma, Pharmacokinetic.

INTRODUCTION

Atenolol is a representative β-blocker which has been advocated to slow heart rate and block additional effects of thyroid hormone excess on the cardiovascular system[1]. It is so sensitive that even a small oral dose of the drug gives sufficient blockade. The drug has been added to the list of forbidden drugs by the International Olympic Committee[2]. Therefore, the development of sensitive and selective analytical methods for the determination of atenolol is of great importance.

Some analytical procedures have been devised for the determination of atenolol in plasma. Several published methods were based on gas chromatography with mass spectroscopy (GC/MS)[3], high performance liquid chromatography with mass spectroscopy (HPLC/MS)[4,5], high performance liquid chromatography with variable wavelength detector[6] and liquid chromatographic method with UV detection[7]. With these methods, sensitivity levels ranging from 5 to 50 ng/mL were obtained. Capillary electrophoresis has been applied in chiral/achiral separation and determination of atenolol in some studies[8-11]. However, there was few capillary electrophoresis method exploited for the determination of atenolol in plasma[12], which is probably due to the low sensitivity of conventional detector. A method by capillary electrophoresis with diode array detection was developed for determination of atenolol in plasma and the linear range was 50-400 ng/mL[12]. Capillary electrophoresis with laser-induced fluorescence (CE-LIF) method can overcome the drawback of low sensitivity. However, there is no previous study for determination of atenolol in plasma by CE-LIF. Derivatization reaction plays an important role in CE-LIF method. Some derivative reagents have been applied in CE-LIF[13,14]. Fluorescein isothiocyanate (FITC) is an excellent derivative reagent for its derivatives have good electrophoretic property.

The present paper described a method based on the use of CE-LIF for quantification of atenolol in plasma of rabbit. The advantages of this method include high sensitivity, inexpensive chemicals used and small sample required. The detect limit was 0.5 ng/mL, which was lower than those traditional methods. In addition, the pharmacokinetics of atenolol in rabbit blood was investigated.

EXPERIMENTAL

Atenolol was obtained from the National Institute for Drugs and Bioproducts Inspection (Beijing, China). Fluorescein isothiocyanate (FITC) was purchased from Sigma (St. Louis, MO, USA). The commercial atenolol tablets were purchased from Wanma Medicine Co. Ltd. (Zhejiang, China) containing 25 mg atenolol per tablets. Acetone, hydrochloric acid, sodium hydroxide and borate were both of analytical reagent grade and purchased from Xi'an Chemical Work (Xi'an, China). The separations were made on a Beckman P/ACE 5000 system equipped with a LIF detector (Beckman Instrument, Fullerton, CA, USA). The detector was carried out with excitation at 488 nm and emission at 520 nm. All separations was carried

out with a 75 μm I.D. uncoated fused-silica capillary (length 57 cm, 50 cm to detector). The eluting peaks were processed with software, P/ACE Station Version 1.21 (Beckman Instrument, Fullerton, CA, USA). Z383 high-speed centrifuge was purchased from Hermle Labortechnik Gmbh Company. The ultrafiltration tubes were from Millipore (America). The solution of FITC was prepared by dissolving 5 mg FITC in 25 mL acetone (5.0×10^{-4} mol/L) and stored at -20 ºC in the dark. The borate solution was prepared by double-distilled water at 100 mmol/L.

Capillary electrophoresis conditions: The capillary temperature was maintained at 20 ºC. Samples were loaded by pressure injection at 3.45 kPa for 5 s. Before each electrophoretic run, the capillary was flushed (5 × 2 min) in sequence with water, 0.1 mol/L sodium hydroxide, water, 0.1 mol/L hydrochloric acid and water. Several variables were investigated, including the pH and composition of the run buffer and the applied voltage. Of these, pH and composition of run buffer were found to have the greatest effects on the separation. The best separation was obtained under the following conditions: 50 mmol buffer, pH 9.0 and 18 kV applied voltage.

Pretreatment of plasma sample: Five rabbits (2.5-3.0 kg) were used in this experiment. After oral administration of 2 mg/kg dose of atenolol to rabbits, 300 μL blood samples were drew from veins of rabbit ears twelve times in 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 10.0, 16.0, 20.0 and 24.0 h. After the blood samples were collected in polypropylene micro-centrifuge tubes and centrifuged at 900 rpm for 10 min at 5 ºC. After centrifugation, 100 μL plasmas were ultrafiltered in ultrafiltration tubes at 8000 rpm for 40 min. Then 25 μL plasma ultrafiltrate was derived with 5×10^{-4} mol/L FITC in dark at 25 ºC for 12 h.

RESULTS AND DISCUSSION

Optimization of derivatization temperature: The effect of the temperature of derivatization was studied. An increase in temperature produced an increase in rate of derivatization. However, a high temperature would result in a high volatilization of organic solvent which would lower derivatization yield. Combined rate of derivatization with reaction time, the temperature was selected at 25 ºC.

Linear range and detection limit: The method was validated in the concentration range of 1.5-500 ng/mL in blood. The corresponding regression function was as follows. Y = 0.46X + 0.11 (r = 0.9984). The detection limit of atenolol at signal-to-noise ratio of 3 was 0.5 ng/mL blood. Before oral administration of atenolol to rabbit, 3 mL blood was drawn from heart of rabbit. Then 1.5, 50 and 200 ng/mL atenolol were added to the rabbit blood, respectively. The results of recovery were shown in Table-1. It was found that atenolol could not be easily derived through the reaction when the atenolol concentration in blood was below 0.1 ng/mL. The relative standard deviation (RSD) of retention time and perk area were both within 5 %. Intra-day and inter-day variabilities were assayed on the same day and five sequential days, respectively. The intra- and inter-day precisions of the quality control samples were satisfactory with RSD less than 8.0 % (should be less than 15 % value[15]).

TABLE-1
RESULTS OF RECOVERY

Added (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (%, n = 6)
1.5	1.4	93.3	4.50
50	51.8	103.6	3.42
200	207.4	103.7	3.13

Plasma atenolol concentration: In the optimum condition, the atenolol concentrations in plasma of rabbits were measured. Representative capillary electrophoretogram was shown in Fig. 1. Fig. 1(A) was the capillary electrophoretogram of blank plasma derived with 5×10^{-4} mol/L FITC solution. In Fig. 1(A), chromatographic peak 2 represents FITC. Fig. 1(B) was the capillary electrophoretogram of FITC-atenolol derivative. Here the concentration of added atenolol was 50 ng/mL. From Fig. 1(B) we can find that the migration time of FITC-atenolol derivative and FITC were about 15 and 17 min. According to precious achievement, we knew the peaks emerged after FITC (2) were FITC-amino acids derivatives and these peaks didn't interfere with the determination of atenolol.

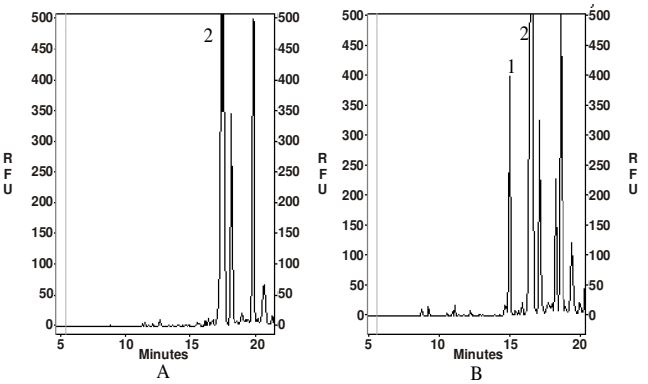

Fig. 1. Typical electropherograms of (A) blank plasma derived with 5×10^{-4} mol/L FITC solution, (B) plasma derived with 5×10^{-4} mol/L FITC solution after administration of atenolol. Peaks: (1) FITC-atenolol derivative; (2) FITC

A method based on capillary electrophoresis with laser-induced fluorescence (CE-LIF) has been developed for determination of bisoprolol in human plasma[16]. In that work, Samples in human plasma were extracted by organic solvents and then derivatized with FITC. The detection for bisoprolol was 10 ng/mL. In present study, ultrafiltration procedure was used for sample preparation. The ultrafiltration procedure offers the advantages of simplicity and high reproducibility, eliminating problems associated with the precipitation procedures[17]. Then a lower limit of detection for atenolol was obtained (0.5 ng/mL).

The plasma atenolol concentrations in rabbit plasma at different time after oral administration of suitable dose were shown in Fig. 2. The plasma atenolol concentrations were increased at first and then decreased. After 24 h, the concentrations of atenolol in rabbit plasma were inclined to zero. The individual difference of the five male rabbits could be seen clearly from Fig. 2.

Pharmacokinetic parameters: Based on the plasma atenolol concentrations in rabbit plasma, the correlative

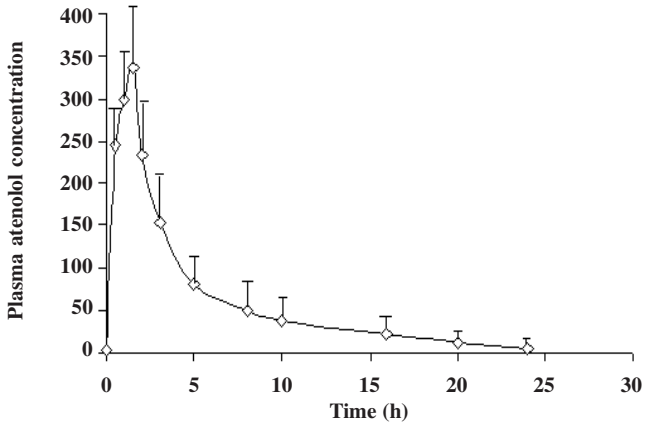

Fig. 2. Mean plasma concentration-time profile of atenolol in rabbits (n = 5) after application of atenolol tablet

pharmacokinetic data were obtained by using NDST-21 software (new drug statistical treatment) (Table-2). The areas under the plasma concentration-time curves ($AUC_{0\text{-}24\ h}$) were calculated using the trapezoid method. The $AUC_{0\text{-}\infty}$ were alculated by the trapezoidal rule and extrapolated to time infinity by the addition of $AUC_{t\text{-}\infty}$. The highest concentrations (T_{max}) were appeared during 1-2 h for the five male rabbits. The $T_{1/2}$ (h) values were from 2.5 to 3.8. The mean values of T_{max} (h) and $T_{1/2}$ (h) were 1.4 and 3.1, respectively.

TABLE-2
PHARMACOKINETIC PARAMETER OBTAINED AFTER ORAL ADMINISTRATION OF ATENOLOL

Rabbit No.	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0\text{-}24h}$ (ng h/mL)	$AUC_{0\text{-}\infty}$ (ng h/mL)
1	400.8	1.0	2.2	1552.2	1572.1
2	340.3	2.0	3.6	784.3	1809.4
3	361.4	1.5	3.8	1664.4	1697.5
4	320.8	1.0	2.5	1445.7	1511.9
5	287.7	1.5	3.5	1734.5	1792.6
Mean value	342.2	1.4	3.1	1636.2	1676.1

Conclusion

We developed a capillary electrophoresis method in combination with laser induced fluorescence detection for determination of atenolol in plasma. The plasma samples were first used ultrafiltration procedures to eliminate problems associated with the precipitation procedures, then derivatized with FITC to offer a high fluorescence intensity, finally separated by capillary electrophoresis and quantified by laser induced fluorescence. The effects of buffer concentration and the temperature of derivatization were studied. The plasma atenolol concentrations of rabbits at different time after oral administration of an appropriate dose were achieved by using this method. Then correlative pharmacokinetic data were obtained by using NDST-21 software. The method features high sensitivity and less sample consumption. Therefore the method is suitable for situations with minimal matrix volumes: *e.g.* pediatrics, patients at risk, animal-, microdialysis- and tissue-kinetic studies.

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