The Investigation of the Pharmacokinetics of Pulsatile-Release Salbutamol Sulfate with pH-Sensitive Ion Exchange Resin as the Carriers in Beagle Dogs

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We previously reported the preparation of the salbutamol sulfate pulsatile-release capsules with the pH-sensitive ion exchange resin as the carriers. In the present study, we investigated the pharmacokinetics of the salbutamol sulfate pulsatile-release capsules in beagle dogs. The analysis method was established for the drugs *in vivo* by HPLC method. The pharmacokinetics parameters of pulsatile-release salbutamol sulfate and reference tablet were $AUC_{0—24}$ (ng·h/ml) 1031.8±123.1, 1112.6±118.24, C_{max} (ng/ml) 172.4±21.4, 179.3±26.1, T_{max} (h) 3.8±0.6, 1.5±0.5, T_{lag} (h) 2.7±0.5, 0.3±0.2. The results showed that the test dosage forms was bioequivalent with reference dosage form, and had an obviously pulsatile-release effect.

Key words salbutamol sulfate; pulsatile release; pH-sensitive; ion exchange resin; *in vivo*; pharmacokinetics

Salbutamol sulfate (SS) is used to treat diseases such as asthma, emphysema and bronchitis.[1] According to the human circadian rhythm,[2] asthma and related conditions often occur in the early hours of the morning and so prove very disturbing to the patient. So, if we can prepare a pulsatile-release salbutamol sulfate which can be released after a 2—3 h lag-time, this will greatly benefit the patients. But now all the SS pulsatile dosage forms are the coated tablets.[3] If the patients break the coated tablets during taking medicine, the pulsatile-release effect will disappear. So if we can prepare the particulate SS pulsatile dosage form will afford a new method for the clinical application. However, to our knowledge, few investigations have been published on the preparation of the particulate SS pulsatile dosage form and the *in vitro–in vivo* study. In our previously study, we have reported the preparation of the particulate salbutamol sulfate pulsatile-release capsules with the novel pH-sensitive ion exchange resin as the carriers.[4] The results showed that a novel pH-sensitive ion exchange resin was successfully prepared and the SS dissolution *in vitro* results exhibited an obvious pulsatile release characteristic.

The purpose of this study was to investigate the pharmacokinetics and the *in vivo* pulsatile-release effects of the particulate SS pulsatile-release capsules with the novel pH-sensitive ion exchange resin as the carriers in beagle dogs.

Experimental

Materials pH-sensitive ion exchange resin (self made). Salbutamol sulfate was purchased from Shen yang NO. 1 Pharmaceutical Factory (Shengyang, China). All reagents were of analytical grade.

***In Vivo* Study** The *in vivo* evaluation was performed by a crossover treatment in six male beagle dogs (weighing 12—14 kg) with a 7-d washout period. The beagle dogs were fasted overnight for at least 12 h, although free access to water was allowed. During the course of the experiment, water was not given until 6 h after administration of the two preparations. The two preparations were (1) the drug-loaded microspheres with SS pulsatile released *in vitro* (PM); (2) the conventional SS tablet (CT). Both treatments contained 8 mg SS. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised in 1985), and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. Blood samples were taken immediately before administering the drug and at the following times for each treatment respectively: (1) 0, 2, 3, 4, 5, 5.5, 6, 6.5, 7, 8, 9, 10, 12, 16 and 24 h; (2) 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24 h. Plasma samples were stored at −20 °C until assayed.

Assay of SS in Plasma Samples and Validation of the Analysis Methods The SS in the plasma was assayed as following[5,6]: Each plasma sample was prepared for extraction by spiking 1 ml of plasma with 15 μl of internal standard solution, atenolol in purified distilled water in concentration 100 μg/ml. First the extraction column (Bond Elute C_{18}) was activated with 1 ml methanol, followed by 1 ml of purified distilled water. The plasma sample was transferred to the top of the column and vacuum was applied. The column was washed with 1 ml of purified distilled water followed by 2 ml of 10% (v/v) of methanol in water and allowed to dry for 2 min under vacuum. Drug and internal standard were eluted from the column with 1 ml of 75% (v/v) of methanol in 0.25 mol/l ammonium acetate (Sigma Chemical Inc.) buffers. The eluent was evaporated to dryness under nitrogen at 50 °C. The residue was reconstituted with 250 μl of aqueous 10% methanol solution. Fifty microliters of this solution was injected into HPLC column (C_{18} separation column). The HPLC analysis was done as followings: a column (Lichrosorb RP-18, 5 μm, 250 mm; alltech associates, U.S.A.), an injection loop of 40 μl, UV detector wavelength of 278 nm, column temperature of 20 °C, temperature in auto sampler 10 °C, and a run time of 13 min. All reagents were of analytical grade. The mobile phase was water : acetonitril : triethylamine : phosphoric acid (92 : 8 : 0.2 : 0.12) and the flow rate was 1 ml/min.

Analysis methods were validated according to established international guidelines and requirements (Validation of analytical Methods: Definitions and Terminiology, ICH Topic Q2A, and Validation of Analytical Procedure: Methodology, ICH Topic Q2B). No interfering peaks were detected at the retention times of SS (5.7 min) and atenolol (7.3 min). A linear correlation (r>0.999) was obtained between the ratio of peak area and SS concentration between the range 5—250 ng/ml. The coefficient of variation of the shope was 6.4%. The limit of quantification (10* background noise) was 5 ng/ml. Precision and accuracy of the method were evaluated at concentration of 250, 100 and 5 ng/ml. Precision of the method was assessed on the basis of the coefficient of variation in quality control samples and accuracy was calculated as the bias % of these samples, the coefficient of variation of intra- and inter-day precision was 3.2—5.8% and 3.7—6.4% at all concentration, respectively. The bias % of the intra- and inter-day accuracy was 1.3—2.8% and 0.9—1.2%. No decrease in the content of quality control samples was observed in the freezer or autosampler.

Pharmacokinetics Study Model-independent parameters, including the maximum plasma concentration (C_{max}) and time to the maximum plasma concentration (T_{max}) were observed values from the plasma concentration–time curve. The areas under the serum concentration–time cuve ($AUC_{0—24\,h}$) were calculated by the trapezoidal method. The lag time (T_{lag}) was also observed values from the plasma concentration–time curve and calculated from the time when the drug was administered to the time when the drug was detected. The time difference between T_{max} and T_{lag} was defined as T_{psi} to compare the drug release rate of the two preparations *in vivo*. Results

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from the two preparations were analyzed with the SPSS statistical package using an analysis of variance to assess any significant ($p<0.05$) differences.

Results and Discussion

***In Vivo* Study and Pharmacokinetics Study** Figure 1 shows a comparison of the plasma concentration–time profiles of SS after oral administration of SS (8 mg) of each formulation to six beagle dogs. The CT did not exhibit any lag time before drug release. On the other hand, the PM exhibited a rapid increase in the SS plasma concentration following a lag time of about 2.7 h which approximately accords with the preparation transit time through the stomach. Table 1 summarized the pharmacokinetic parameters. The comparison of parameters with the two preparations shows no significant differences for C_{max} and $AUC_{0—24\,h}$, but shows significant difference for the T_{max} and T_{lag}. This suggests that the pulsatile release microspheres prolonged the lag time of drug release but did not lower the drug release rate.

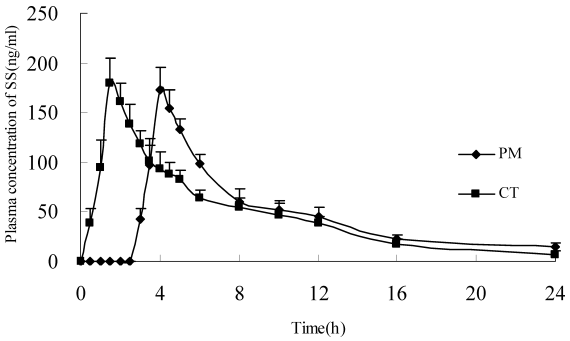

Fig. 1. Plasma Concentration–Time Curve of SS Following Oral Administration of PM and CT

Each point represents the mean±S.D. ($n=6$).

Table 1. Pharmacokinetic Parameters of SS Pulsatile Release Preparation (PM) and Conventional Tablet (CT)

	PM		CT		ANOVA
	Mean	±S.D.	Mean	±S.D.	
T_{max} (h)	3.8	0.6	1.5	0.5	$p<0.05$
T_{lag} (h)	2.7	0.5	0.3	0.2	$p<0.05$
T_{psi} (h)	1.1	0.3	1.2	0.4	$p<0.05$
C_{max} (ng/ml)	172.4	21.4	179.3	26.1	$p<0.05$
$AUC_{0—24\,h}$ (ng·h/ml)	1031.8	123.1	1112.6	118.2	$p<0.05$

Bioequivalence of the two preparations was accepted with $AUC_{0—24\,h}$ and C_{max} by the two one-side test. The relative bioavailability was 92.7% for pulsatile release microspheres to conventional tablets. This suggests that the absorption of SS was not influenced by the *in vivo* behavior of the pulsatile release preparation.

Conclusions

A simple and sensitive liquid chromatographic system had been developed for the determination of SS in blood samples. This method was used to investigate the pharmacokinetics of pulsatile-release salbutamol sulfate with pH-sensitive ion exchange resin as the carriers in beagle dogs. The results showed that the test dosage forms was bioequivalent with reference dosage form, and had an obviously pulsatile-release effect.

Acknowledgments We are grateful to Dr. David B. Jack for his valuable editing assistance.

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