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Short Communication

Investigation of the pharmacokinetics of a novel sustained release metformin hydrochloride suspension with ion exchange resin as carriers in beagle dogs

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A novel metformin hydrochloride sustained-release suspension containing ion exchange resin as carriers have been reported in a previous study. In the present study, the pharmacokinetics of this sustained-release suspension in beagle dogs was investigated. A high performance liquid chromatography (HPLC) method was established to detect the amount of metformin hydrochloride *in vivo*. The pharmacokinetics parameters of the metformin hydrochloride sustained-release suspension and a reference metformin hydrochloride tablet were: $AUC_{0\sim24}$ (μg·h·ml^{-1}) 72.94 and 78.07, C_{max} (μg·ml^{-1}) 7.97 and 15.42, T_{max} (h) 4.2 and 1.83 respectively. The results indicated that the suspension was bioequivalent with the reference tablet, and had an obvious sustained-release effect.

Key words: Metformin hydrochloride, sustained-release, ion exchange resin, *in vivo*, pharmacokinetics.

INTRODUCTION

Metformin hydrochloride is an oral antihyperglycaemic agent widely used in the management of non-insulin-dependent diabetes mellitus (NIDDM or type-2 diabetes). It reduces blood glucose levels, predominantly by improving hepatic and peripheral tissue sensitivity to insulin without affecting the secretion of this hormone (Klepser and Kelly, 1997; Wadhere et al., 2010; Kay et al., 2001).

Many oral sustained-release formulations of metformin hydrochloride have been extensively investigated recently (Schwartz et al., 2006; Ouyang et al., 2005), however the simple matrix tablets which were widely used cannot meet satisfying sustained-release purpose, therefore a semi permeable membrane is often coated to improve the sustained-release profile. But if the patients break the coated tablets during taking medicine, the sustained-release effect will be jeopardized and the side effect would occur with the excess drug release. Therefore, preparation of metformin hydrochloride liquid particulate sustained release dosage form might be an alternative method which can overcome the above stated shortcoming and offer a new method for the clinical application.

In a previous study, the authors of this current study have reported the preparation of a metformin hydrochloride sustained-release suspension using ion exchange resin as carriers (Liu et al., 2006; Hu et al., 2006). The results showed that the sustained-release suspension was successfully prepared and the *in vitro* dissolution test results exhibited an obvious sustained-release characteristic. However, limited investigations have been published for sustained-release suspensions with ion exchange resin as the carrier. A simple and sensitive liquid chromatographic system may be used for the *in vivo* study for the sustained-release metformin hydrochloride suspension. The purpose of this study was to investigate the pharmacokinetics and the *in vivo* sustained-release effects of the metformin hydrochloride resinate sustained-release suspension in beagle dogs

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MATERIALS AND METHODS

Chemicals

Cation-exchange resins Amberlite® IRP69 (sodium polystyrene sulfonate) were obtained from Rohm and Haas Company (Philadelphia, USA). Metformin hydrochloride was purchased from An Kang Pharmaceutical Factory (Shanxi province, China). All reagents used were of analytical grade.

In vivo study

The *in vivo* evaluation was performed using a crossover treatment in six male beagle dogs (weighing 12 to 14 kg) with a 7 day washout period as previously reported (Sambol et al., 1996; Kumari et al., 2009). 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 formulations. The two formulations were: first, the metformin hydrochloride sustained-release suspension (test); second the conventional metformin hydrochloride tablet (reference). Both treatments contained 500 mg metformin hydrochloride. 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, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 24 h. Plasma samples were stored at 20℃ until further assay.

Assay of metformin hydrochloride in plasma samples and validation of the analysis methods

The metformin hydrochloride in the plasma was assayed with some adjustments (Liu et al., 2006; Sambol et al., 1996; Hamdan et al., 2010; Ali et al., 2007): 200 µl of plasma was sampled, then mixed with 600 µl methanol. These samples were vortexed for 5 min and centrifuged for 10 min at the speed of 10000 rpm. Thereafter, 20 µl supernatant was taken and analyzed by a high performance liquid chromatography (HPLC) equipped with a Diamonsil C_{18} column (5 µm, 200 mm; Dikma, USA). The HPLC analysis conditions were as follow: an injection loop of 20 µl, UV detector wavelength of 233 nm, column temperature of 40℃, temperature in auto sam pler 20℃ and a running time of 2 min, all reagents were of HPLC grade. The mobile phase was as follow: 500 ml of methanol and 500 of ml water containing 1.15 g ammonium dihydrogen phosphate and 0.5 g sodium dodecylsulphate. The flow rate was set at 1 ml/min.

The HPLC analysis method was validated according to established international guidelines and requirements (Validation of analytical methods: Definitions and Terminology, ICH Topic Q2A, and Validation of Analytical Procedure: Methodology, ICH Topic Q2B). No interfering peaks were detected at the retention times of metformin hydrochloride (13.4 min). A linear correlation($r > 0.999$) was obtained between the peak area and metformin hydrochloride concentration in sample between the ranges of 0.05 to 20 µg/ml. The coefficient of variation of the slope was 4.2%. The limit of quantification (10* background noise) was 0.24 ng. The precision and accuracy of the method were evaluated at concentration of 20.2 and 0.05 µg/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 percentage of these samples. The coefficient of variation of intra and inter day precision were 2.3 to 4.8% and 3.1 to 5.7% at all concentrations, respectively. The bias percentage of the intra and inter day accuracy was 1.8 to 2.7% and 1.2 to 1.5%. Moreover, no decrease in the content of quality control samples was observed in the freezer or autosampler.

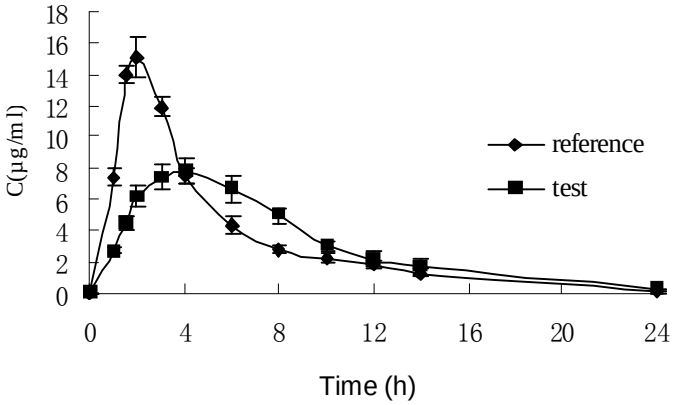

Figure 1. Plasma concentration-time curve of metformin hydrochloride following oral administration of test and reference. Each point represents the mean ± S.D. (n = 6).

Pharmacokinetics Study

Model-independent parameters, including the maximum plasma concentration (C_{max}) and time to the maximum plasma concentration (T_{max}) were observed according to values from the plasma concentration–time curve. The areas under the serum concentration-time curve (AUC_{0-24h}) were calculated by the trapezoidal method. The *in vivo* drug absorption percentages for the metformin hydrochloride, sustained-release suspension were calculated according to the method of Wagner and Nelson. The *in vivo–in vitro* correlation for the DH microspheres was obtained from the linear regression analysis between the percentage absorbed *in vivo* and the percentage released *in vitro*, at the corresponding times. Results from the two preparations were analyzed with the SPSS statistical package using an analysis of variance to assess any significant ($P < 0.05$) difference.

RESULTS AND DISCUSSION

In vivo study and pharmacokinetics study

Figure 1 shows a comparison of the plasma concentration-time profiles of metformin hydrochloride after oral administration of each formulation (containing metformin hydrochloride of 500 mg) to six beagle dogs. Table 1 summarizes the pharmacokinetic parameters obtained according to the plasma concentration-time profiles.

The comparison of the parameters between the two preparations showed no significant differences for AUC_{0-24} *and* $AUC_{0-\infty}$, but indicated a significant difference for T_{max} *and* C_{max}. The T_{max} increased from 1.83 h (reference) to 4.17 h (test), indicating a sustained-release property *in-vivo*. The C_{max} decreased from 15.42 $\mu g \cdot ml^{-1}$(reference) to 7.97 $\mu g \cdot ml^{-1}$(test), indicating a steady drug release property *in-vivo*. The test bioavailability was 94%, showed no significant differences from the reference. The two

Table 1. Pharmacokinetic parameters of metformin hydrochloride sustained-release suspension (test) and conventional tablet (reference).

Parameter	Test Mean ± SD	Reference Mean ± SD
$t_{1/2}$ (h)	4.06 ± 0.03	2.77 ± 0.21
k_e (h^{-1})	0.171 ± 0.001	0.251 ± 0.02
T_{max} (h)	4.17 ± 0.98	1.83 ± 0.26
C_{max} (μg·ml^{-1})	7.97 ± 0.26	15.42 ± 0.63
$AUC_{0\text{-}24}$ (μg·h·ml^{-1})	72.94 ± 3.54	77.52 ± 5.17
$AUC_{0\text{-}\infty}$ (μg·h·ml^{-1})	74.577 ± 3.64	77.83 ± 5.12
MRT (h)	7.73 ± 0.09	5.53 ± 0.16
Relative bioavailability (%)	94 ± 4	

Table 2. Two one-sided test and (1 - 2α) confidence interval analysis result of the main parameters between T and R (n = 6).

Parameter	t_1	t_2	90% confidence interval
ln $AUC_{0\text{-}24h}$	8.99	16.72	90.79 - 97.69%
ln C_{max}	-55.51	189.13	51.13 - 52.33%

$T_{(1\text{-}0.05)}(4)$ =2.132.

one-side test results were shown in Table 2. It was shown that the $AUC_{0\text{-}24h}$ of the sustained-release suspension located in the interval of 90.79 to 97.69% of the $AUC_{0\text{-}24h}$ of the reference table with 90% confidence level. This showed the sustained-release suspension was bioequivalent with the reference. Regarding C_{max}, it was shown that the C_{max} of the sustained-release suspension located in the interval of 51.13 to 52.33% of C_{max} of the reference table with 90% confidence level. The *in vivo–in vitro* correlation result was as follows: Fa = 1.705 F-0.32, r = 0.9852, where Fa is fraction absorbed *in vivo* and F represents fraction released *in vitro*. The results showed a good correlation between *in vivo* drug absorption and *in vitro* drug release was obtained.

Conclusion

A simple and sensitive liquid chromatographic system has been developed for the determination of metformin hydrochloride in blood samples. This method was used to investigate the pharmacokinetics of sustained-release metformin hydrochloride suspension with ion exchange resin as the carriers in beagle dogs. The results showed that compared with the commercial tablet, the test dosage forms had obvious sustained release effect, longer T_{max}, lower C_{max} and the bioavailability was 94%. The results proved that ion exchange resin may be applied as a novel carrier for the sustained release dosage forms.

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