

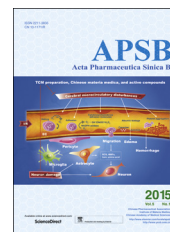
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Acta Pharmaceutica Sinica B

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ORIGINAL ARTICLE

Pharmacokinetics of propafenone hydrochloride sustained-release capsules in male beagle dogs



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Received 11 September 2014; received in revised form 11 October 2014; accepted 14 November 2014

KEY WORDS

Propafenone;
Pharmacokinetics;
Sustained-release;
Beagle dog;
Plasma

Abstract This paper describes the development and validation of a liquid chromatography–mass spectrometric assay for propafenone and its application to a pharmacokinetic study of propafenone administered as a new propafenone hydrochloride sustained-release capsule (SR-test), as an instant-release tablet (IR-reference) and as the market leader sustained-release capsule (Rythmol, SR-reference) in male beagle dogs ($n=8$). In Study A comparing SR-test with IR-reference in a crossover design T_{\max} and $t_{1/2}$ of propafenone for SR-test were significantly higher than those for IR-reference while C_{\max} and AUC were lower demonstrating the sustained release properties of the new formulation. In Study B comparing SR-test with SR-reference the observed C_{\max} and AUC of propafenone for SR-test (124.5 ± 140.0 ng/mL and 612.0 ± 699.2 ng-h/mL, respectively) were higher than for SR-reference (78.52 ± 72.92 ng/mL and 423.6 ± 431.6 ng-h/mL, respectively) although the differences were not significant. Overall, the new formulation has as good if not better sustained release characteristics to the market leader formulation.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

1. Introduction

Atrial fibrillation (AF) is a common type of arrhythmia that can cause symptoms such as palpitations, chest tightness and dizziness. It also poses a serious risk of thromboembolism which significantly increases the incidence of stroke and overall mortality. It is therefore of great importance to improve both drug and non-drug therapy of AF. Although non-drug therapy with pacemakers and defibrillators has made a significant progress, drug treatment is still the mainstay for the prevention and treatment of AF with evident advantages such as convenience, good patient compliance and reduction in the complications associated with the condition^{1,2}.

Propafenone is a potent and generally well-tolerated antiarrhythmic agent which has been shown to be effective against a variety of cardiac arrhythmias particularly AF^{3,4}. It works by slowing the influx of sodium into cardiac muscle cells with additional activity as a β -adrenergic blocker and weak calcium channel blocker. A sustained-release (SR) formulation of propafenone hydrochloride was first developed by Abbott Laboratories and approved by the FDA in September 2003. The aim of SR pharmacotherapy was to prolong the time to recurrence of symptoms in patients with episodic (most likely paroxysmal or persistent) AF who do not have structural heart disease⁵.

There are two genetically determined patterns of propafenone metabolism. In over 90% of patients, the drug is rapidly and extensively metabolized with an elimination half-life of 2–10 h by CYP2D6, CYP3A4 and CYP1A2. In less than 10% of patients, metabolism is slow with an elimination half-life of 10–32 h because of their deficiency of CYP2D6⁶. In extensive metabolizers, propafenone also undergoes saturable presystemic biotransformation such that its bioavailability is both dose and dosage form dependent^{7–10}. Propafenone exhibits a high degree of inter-subject variability in pharmacokinetic parameters following both single and multiple dose administration probably due to the wide range of CYP2D6 activity¹¹.

According to the literature, the most common adverse effects seen with propafenone are neurologic (visual blurring, paresthesias, and dizziness) and gastrointestinal (constipation and nausea)¹². As expected, these adverse effects commonly occur around the time of maximum blood concentration. An SR formulation reduces this peak concentration and thereby reduces adverse effects and the frequency of administration and increases patient compliance. The objective of this investigation was to develop an analytical method for the determination of propafenone in dog plasma and evaluate the pharmacokinetic properties of a new propafenone hydrochloride SR capsule in male beagle dogs.

2. Materials and methods

2.1. Chemicals and reagents

A propafenone hydrochloride 225 mg SR capsule (SR-test) was manufactured by Lipin Pharmaceutical Industry (Xiamen, China). Propafenone hydrochloride 50 mg immediate release tablets (IR-reference) were supplied by Xinyi Pharmaceutical Industry (Shanghai, China). The reference propafenone hydrochloride 225 mg SR capsule (SR-reference) was supplied by Abbott Laboratories (Chicago IL, USA). The propafenone hydrochloride standard (purity 99.9%) and quetiapine (purity 100.0%), for internal standard (IS) use, were purchased from the National

Institutes for Food and Drug Control (Beijing, China). HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, German). Formic acid and ammonium acetate were of analytical grade and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China).

2.2. LC-MS assay

LC-MS was performed using an Agilent Technologies Series 1100 LC/MSD SL system (Agilent Technologies, CA, USA). Isocratic chromatography was carried out on a Hedera ODS-2 column (150 mm \times 2.1 mm, 5 μ m, Jiangsu Hanbon Science & Technology Co., Ltd., China) maintained at 38 °C using a mobile phase of methanol–0.15% aqueous formic acid containing 5 mmol/L ammonium acetate (55:45, v/v) at a flow rate of 0.5 mL/min. The run time for each sample was 6 min. Detection was by selective ion monitoring (SIM) of the parent ions of propafenone at *m/z* 342.2 and IS at *m/z* 384.2.

2.3. Sample preparation

Aliquots of plasma (200 μ L) to which IS solution was added were vortex mixed for 30 s and then deproteinized by adding 1.4 mL acetonitrile. After vortexing for 3 min, samples were centrifuged at 15,600 rpm for 5 min and aliquots of supernatant (100 μ L) mixed with 100 μ L mobile phase and vortex-mixed for 5 s. The mixtures were finally transferred into autosampler vials and 5 μ L injected into the LC-MS system.

2.4. Assay validation

Assay validation was carried out according to the FDA Guidance for Validation of Bioanalytical Methods and included determination of specificity, linearity over the concentration range 1.5–1500 ng/mL, lower limit of quantitation (LLOQ), precision, accuracy, matrix effects, extraction recovery and stability. Five replicates of quality control (QC) samples at low, medium and high concentrations (4.0, 100 and 1200 ng/mL) and at the LLOQ (1.5 ng/mL) were analyzed on three consecutive days to evaluate intra- and inter-day precision and accuracy. Accuracy was determined by comparing the found concentration to its nominal concentration and expressed as a percentage; precision was expressed as relative standard deviation (RSD)^{13,14}. Recovery and matrix effects for propafenone were determined by analyzing 6 replicates of QC samples. Corresponding values for the IS at 5 ng/mL were determined in the same way.

3. Animal studies

3.1. Study design

Two crossover studies (A and B) were carried out to compare the SR-test and IR-reference formulations (Study A) and SR-test and SR-reference formulations (Study B). In Study A, 8 male beagle dogs (weight 9.9–11.2 kg) were randomly divided into two equal groups and fasted overnight for 10 h before being administered either the SR-test formulation (225 mg propafenone hydrochloride) or IR-reference formulation (200 mg propafenone hydrochloride) with 50 mL water. After a washout period of 7 days, the two groups of dogs received the other formulation. The dogs were not allowed to lie down for at least 2 h after dosing and were provided with water thereafter. Food was provided 4 h after drug

administration. Blood samples (1.5 mL) were collected from an elbow vein into heparinized tubes pre-dose and at 20, 40 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 15 and 24 h post-dose. The samples were centrifuged immediately at 4000 rpm for 10 min and plasma separated and stored at -20°C until analysis. Study B was identical to Study A except dogs received either the SR-test formulation or SR-reference formulation (both 225 mg propafenone hydrochloride).

3.2. Data analysis

Non-compartmental pharmacokinetic analysis was performed using DAS 2.0 software (DAS[®]; Professional Edition version 2.0, Drug and Statistics, Shanghai, China). The maximum plasma concentration (C_{\max}) and the time to reach C_{\max} (T_{\max}) were directly obtained from the data. AUC_{0-t} was calculated by the linear trapezoidal method and extrapolated to infinity to obtain $\text{AUC}_{0-\infty}$ using the relationship $\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t/K$, where C_t is the concentration at the last measurable time point and K is the elimination rate constant. The terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/K$. CL/F was calculated as $\text{dose}/\text{AUC}_{0-\infty}$ and V_d/F was derived from $V_d/F = \text{dose}/(K \times \text{AUC}_{0-\infty})$. The mean residence time (MRT) was estimated as $\text{MRT} = \text{AUMC}/\text{AUC}$ where AUMC is the area under the first moment curve. Differences in pharmacokinetic parameters were tested by one-way ANOVA.

4. Results and discussion

4.1. Assay validation

Precision and accuracy of the assay are shown in Table 1 and recovery and matrix effects are shown in Table 2.

The results in Table 1 demonstrate that intra- and inter-day accuracy and precision were all within acceptable limits (85%–100% for accuracy, $\pm 15\%$ for precision). The recoveries of

propafenone at three concentration levels (4, 100 and 1200 ng/mL) and of the IS at 5 ng/mL were all close to 100% (Table 2). Results of the matrix effects study for propafenone and IS reveal that there were no significant matrix effects, indicating that no co-eluting substance influenced the ionization of either the analyte or IS.

4.2. Pharmacokinetic studies

Pharmacokinetic parameters from Studies A and B are presented in Table 3 with plasma concentration–time curves of propafenone administered as SR-test and IR-reference formulations (Study A) and as SR-test and SR-reference formulations are shown in Figs. 1 and 2, respectively.

In Study A, the T_{\max} of propafenone for the IR-reference was 1.5 ± 0.7 h, indicating rapid absorption (Table 3). In comparison, the T_{\max} of analyte for the SR-test was 4.1 ± 2.6 h, representing a 2.7-fold increase. The mean $t_{1/2}$ value for SR-test was 1.77-fold greater than for IR-reference, indicating that elimination of propafenone is prolonged in the SR formulation. Consistent with these results, the C_{\max} of propafenone was considerably lower for SR-test. However, a second absorption peak of propafenone (presumably due to enterohepatic recycling) was higher for SR-test than for IR-reference. Together these results indicate that SR-test provides significantly slower release of propafenone compared with IR-reference.

Despite the evident slower release, the mean AUC_{0-24} of propafenone for SR-test was obviously lower than that for IR-reference suggesting a decrease in relative bioavailability of propafenone from SR-test of 58.2%. This is possibly due to the fact that the more gradual release of propafenone from SR-test allows time for more first pass metabolism to occur and avoids the metabolic saturation to which the first pass metabolism is susceptible^{10,15,16}.

In Study B, comparing the pharmacokinetics of propafenone after oral administration of the SR-test and SR-reference

Table 1 Precision and accuracy for the analysis of propafenone in dog plasma.

Concentration (ng/mL)		Precision (RSD%)		Accuracy (RE%)
Added	Measured	Intra-day	Inter-day	
1.533	1.582 \pm 0.079	4.4	7.5	4.0
4.088	3.952 \pm 0.196	5.2	3.5	–2.3
102.2	106.2 \pm 0.8	0.2	0.1	3.4
1226	1242 \pm 13	1.0	1.5	0.8

Data are mean \pm SD, $n=5$; RSD: relative standard deviation; RE: relative error.

Table 2 Recovery and matrix effects for the analysis of propafenone in dog plasma.

Compound	Concentration (ng/mL)	Recovery		Matrix effect	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
Propafenone	4.088	99.1	2.3	99.6	4.30
	102.2	101.3	1.0	98.2	0.70
	1226	103.0	0.8	100.6	4.10
IS	5.330	100.8	2.4	101.4	0.04

Data are mean values, $n=6$; RSD: relative standard deviation.

Table 3 Main pharmacokinetic parameters of propafenone in male beagle dogs obtained in Studies A and B.

Parameters	Study A		Study B	
	SR-test	IR-reference	SR-test	SR-reference
C_{\max} (ng/mL)	175.9 ± 112.0	886.6 ± 736.4	124.5 ± 140.0	78.52 ± 72.92
T_{\max} (h)	4.1 ± 2.6	1.5 ± 0.7	1.8 ± 1.5	3.4 ± 3.8
$t_{1/2}$ (h)	5.3 ± 4.5	3.0 ± 2.1	4.0 ± 1.9	3.1 ± 2.2
MRT (h)	6.8 ± 2.2	2.7 ± 0.9	5.9 ± 1.8	7.2 ± 3.3
CL/F (L/h)	641.6 ± 777.5	189.0 ± 153.7	846.8 ± 870.3	802.7 ± 774.3
V_d/F (L)	3227 ± 3296	738.1 ± 511.8	4300 ± 3549	9247 ± 6638
AUC ₀₋₂₄ (ng·h/mL)	753.5 ± 502.8	1804 ± 1402	612.0 ± 699.2	423.6 ± 431.6
AUC _{0-∞} (ng·h/mL)	912.7 ± 763.3	1839 ± 1436	660.4 ± 709.0	441.5 ± 437.8

Data are mean ± SD, $n=8$; RSD: relative standard deviation.

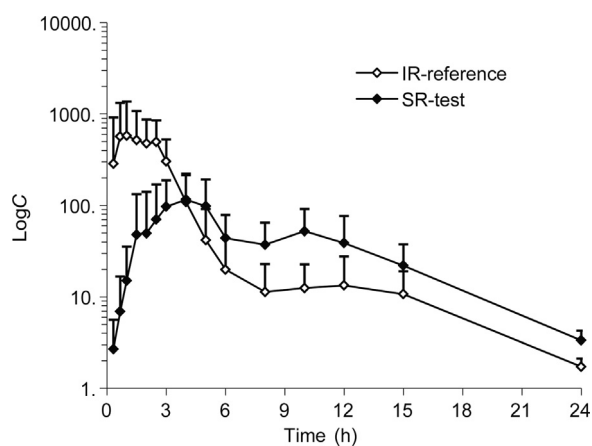


Figure 1 Plasma concentration-time curves of propafenone in a crossover study of male beagle dogs after oral administration of the SR-test and IR-reference formulations (Study A). Data are mean ± SD ($n=8$). The unit of C is ng/mL.

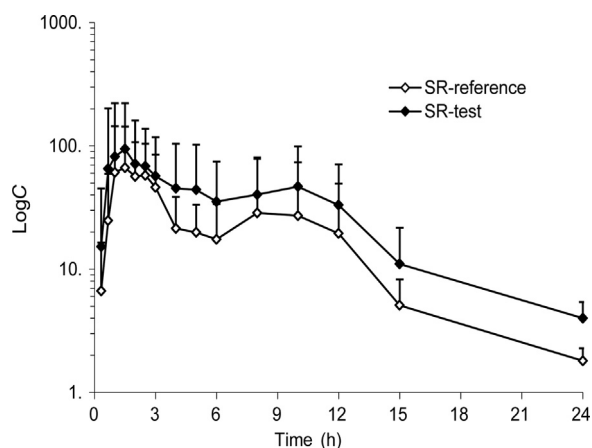


Figure 2 Plasma concentration-time curves of propafenone in a crossover study of male beagle dogs after oral administration of the SR-test and SR-reference formulations (Study B). Data are mean ± SD ($n=8$). The unit of the C is ng/mL.

formulations, the mean values of C_{\max} and AUC₀₋₂₄ for SR-test were higher than those for SR-reference but the differences were not significant. One reason for the lack of significance is because

the pharmacokinetics of propafenone in beagle dogs is nonlinear and leads to high inter-individual variation in the pharmacokinetic parameters for which the small number of dogs used in the studies is insufficient to compensate. Inter-subject variability in propafenone pharmacokinetics is known to be high in CYP2D6 extensive metabolizers suggesting the high variability seen here is due to CYP2D6 polymorphism rather than to the formulation.

The above discussion notwithstanding, the increased T_{\max} and $t_{1/2}$ of propafenone coupled with the considerably reduced C_{\max} and the maintenance of an appreciable plasma concentration demonstrate that the new SR formulation has improved sustained release characteristics. It is anticipated therefore that SR-test could offer sustained therapeutic benefits and minimize adverse effects. According to the Chinese Pharmacopoeia, a study of multiple dose administration is now needed to further develop the sustained-release formulation¹⁷.

5. Conclusions

A new propafenone hydrochloride sustained release capsule has been shown to exhibit good sustained release characteristics despite providing lower relative bioavailability than an immediate release tablet. The new formulation has also been shown to provide as good if not better sustained release characteristics than the market leader.

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