

Original Research Article

Preliminary Investigation of Beagle Dog as Substitute for Humans in Bioequivalence Studies

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Abstract

Purpose: To assess the suitability of beagle dog as an animal model for the evaluation of formulations in bioavailability and bioequivalence studies.

Methods: A generic cetirizine 10 mg tablet formulation was compared with another reference formulation using beagle dog as animal model. A crossover oral comparative bioavailability study was conducted on cetirizine tablet 10 mg in healthy, male dogs under fasting conditions. The formulations were administered orally with the aid of water. Serial blood samples were collected from pre-dose to 48.0 h post-dose and plasma concentrations of cetirizine were determined using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical method. Pharmacokinetic parameters were calculated using non-compartmental analysis while bioavailability was assessed using an analysis of variance (ANOVA) model for humans and dogs.

Results: Cetirizine plasma concentrations in dog were comparatively higher, in relation to human plasma concentrations, due to the smaller blood volume in former. There was a delay in time to reach maximum plasma concentration (T_{max}) in dog. Cetirizine formulations were found to be bioequivalent in either of the species (dog and human). The ratio (test/reference) of least-squares mean for area under plasma concentration curve from time zero to last detectable concentration (AUC_{0-t}), area under plasma concentration curve extrapolated to infinity ($AUC_{0-\infty}$) and maximum plasma concentration (C_{max}), calculated for the dogs were comparable to those for humans. AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} ratios ranged within 92.81 - 106.80 % for dogs and 95.43 - 104.84 % for humans

Conclusion: The results suggest that beagle dogs can be used in place of humans in bioequivalence tests on generic products of cetirizine.

Keywords: Cetirizine, Beagle dog, Bioavailability, Bioequivalence, Pharmacokinetics, Non-compartmental.

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INTRODUCTION

Cetirizine is a selective H₁ receptor inverse agonist used in the treatment of allergies, hay fever, angioedema, and urticaria. Generic drug companies have played a significant role in the developing world by supplying cost-effective treatment regimens [1]. To make cost effective

formulation, initial development of formulations can be feasible using animal model. This can serve as guideline for planning clinical bioequivalence studies. The dog model is used frequently in formulation development and has shown utility for prediction of human bioavailability [2, 3]. During initial phase of formulation development, multiple formulations

would require to be screened out to match with reference formulation in terms of rate and extent of absorption. The current study was planned to evaluate suitability of using beagle dog as an animal model to screen out the prototype under development closer to reference product in terms of bioavailability. This provides an additional advantage in terms of cost benefit, faster execution of study and it would also result in faster formulation development process. Kevin et al performed bioavailability studies for ritonavir formulations in which 12 out of 16 experimental ritonavir formulations in dog mirrored those obtained in human (both bioequivalent and non-bioequivalent results) [4].

Cetirizine is well-tolerated in dogs [5]. In this study, we assessed and compared the relative bioavailability of generic cetirizine 10 mg tablet manufactured by Ranbaxy Laboratories Limited with that of innovator product Zyrtec 10 mg tablet (containing cetirizine) manufactured by UCB Farchim SA, Switzerland.

EXPERIMENTAL

Materials

The test formulation was generic cetirizine 10 mg tablet (batch no.1962123) manufactured by Ranbaxy Laboratories Limited, India, and reference formulation was innovator product Zyrtec 10 mg tablet (containing cetirizine 10 mg, batch no.08C26C), manufactured by UCB Farchim SA, Switzerland. These were used for bioequivalence study in beagle dogs and humans.

Subjects and criteria

A total of 12 adult male healthy dogs aged 37 - 54 months with body weight ranging from 10 - 16 kg (Source: Animal Breeding and Housing Facility, Ranbaxy Research Laboratories, Gurgaon, India) were received for study. Each animal was housed in individual pen. Temperature and relative humidity were maintained at 18 – 29 °C and 30 – 70 %, respectively. Illumination was controlled to give approximately a sequence of 14 h light and 10 h dark. Air changes of Kennel Facility were maintained at or above 12 air changes/hour. The tablets were administered orally. Approximately 10 ml of water was administered after dosing. Animals were divided into two groups (Group I and Group II), comprising of six animals each. Group I received cetirizine tablet 10 mg, a test formulation (T) and Group II received zyrtec 10 mg tablet, a reference formulation (R). After six days washout period, Group I administered orally

with Zyrtec 10 mg tablet and Group II with cetirizine tablet 10 mg.

Twenty four healthy, adult, male, Indian Asian human subjects aged 19-35 year were used. Body weight was ranging from 48-84 kg. The subjects were admitted 12 h before dose administration in each period. The tablets were administered orally and the subjects were randomized to receive a single oral dose of test formulation or reference formulation in each period.

Study design

This trial was a single-center, two-way crossover study designed to assess the bioavailability of cetirizine 10 mg tablet and Zyrtec 10 mg tablet under fasting conditions in beagle dogs. Treatments were administered with approximately 10 mL of water and separated by adequate washout period (6 days). The dogs were fed 200 - 250 g/day of standard pelleted meal (Pedigree adult dog feed, Mars Pvt Ltd, India). Food was withdrawn at least 10 h prior to commencement of dosing and up to approximately 3 h post dose. Water was restricted to 1 h pre-dose to 2 h post-dose.

The study was performed as per in-house standard operating procedures based upon the guidelines [6,7], recommendations of FELASA [8], the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for Laboratory Animal Facility [9], Guide for the Care and Use of Laboratory Animals [10]. Ethical approval was obtained from Institutional Animal Ethics Committee (IAEC protocol no 21/2010), which is monitored by CPCSEA under Ministry of Environment and Forest, Government of India.

Healthy, adult, male, human subjects aged 19-35 year were used. A bioavailability study in twenty four Asians was conducted as per the basic principles defined in US 21 CFR part 320. Ethical approval was obtained for protocol no. BE-212-CETI-2008 from Sentinel Independent Ethics Committee, India. Informed consent was obtained from subjects. This study was performed as per the principles enunciated in the Declaration of Helsinki. This study was conducted by Ranbaxy Laboratories Limited, India and the data were used for comparison with dog study data. An open label, balanced, randomized, two-treatment, two-sequence, two-period, single-dose, cross-over design was used. A single oral dose of test formulation or reference formulation of cetirizine was administered with 240 mL of drinking water at ambient temperature

after an overnight fast of at least 10 h. Each treatment was separated by a washout period of eleven days.

Bioavailability study in animals

Twelve dogs were taken for the study and all of them completed the study. Formulations were administered through mouth (oral route). Approximately 10 ml of water was administered after dosing. Animals were divided into two groups (Group I and Group II), comprising of six animals each. Group I received cetirizine tablet 10 mg, a test formulation (T) and Group II received zyrtec 10 mg tablet, a reference formulation (R). After six days wash out period, Group I administered with zyrtec 10 mg tablet and Group II with cetirizine tablet 10 mg. Blood samples approximately 0.8 ml were collected from cephalic / saphenous vein in labeled tubes containing anticoagulant 1.8 mg/ml of EDTA (K3), predose, and at 0.167, 0.500, 0.833, 1.000, 1.333, 1.667, 2.000, 2.500, 3.000, 4.000, 6.000, 8.000, 12.000, 24.000, 36.000 and 48.000 h post dose. Plasma (approximately 300 μ l) was separated by centrifugation at 3500 rpm for 10 minutes at 4°C (+ 2°C) and stored in a freezer (below -20° C).

The plasma samples were analyzed by a validated method based on the Food and Drug Administration validation guidelines [11]. The plasma concentrations of cetirizine were determined using high-performance liquid chromatography fitted with mass spectrometry (LC-MS/MS) detection. Cetirizine and the internal standard (fexofenadine) were extracted from plasma using solid-phase extraction. Internal standard working solution (50 μ L) was added to 100 μ L of each plasma sample and vortexed. The samples were transferred to pre-conditioned Oasis HLB (30 mg/cc) extraction cartridges. Analyte was eluted from cartridge with 1 mL methanol twice. Extract was evaporated and reconstituted with 200 μ L mobile phase {acetonitrile : 10 mM ammonium formate buffer pH 3.5 (90:10 V/V)}. The extracts were injected into the LC-MS/MS system, and positive ions were monitored in multiple reaction monitoring (MRM) mode. The ion transitions (m/z) 389.4/201.3 and 502.5/466.5 were monitored for cetirizine and internal standard, respectively. Instrument setup consisted of an autosampler, an API 3000 detector and a data-processing system (Analyst 1.4.1). Linearity of cetirizine was assessed by plotting area ratios versus standard concentrations and using a linear regression weighted 1/cetirizine concentration. Validated analytical range for cetirizine in plasma was 4.94 - 811.99 ng/mL. Inter batch precision (CV %) was

\leq 9.9% and accuracy (% theoretical) ranged between 95.8 and 106.2 % at low, medium and high quality control levels.

Bioavailability study in human

Twenty-two human volunteers completed the study. Two human subjects were withdrawn from study. A single oral dose of test formulation (Cetirizine Hydrochloride 10 mg tablets) or reference formulation (Zyrtec® 10 mg tablets) was administered with 240 mL of drinking water after an overnight fast of at least 10 hrs. Subjects received alternate treatment in the subsequent period in such a way that each subject received both the treatments at the end of the study.

Blood samples were collected at pre-dose and at 0.167, 0.333, 0.500, 0.667, 0.833, 1.000, 1.250, 1.500, 1.750, 2.000, 2.500, 3.000, 4.000, 5.000, 6.000, 8.000, 10.000, 12.000, 16.000, 20.000, 24.000, 35.000 and 47.000 h post-dose in each period. High performance liquid chromatography using mass spectrometric detection method was used to determine cetirizine content in human plasma.

Human plasma samples were analyzed by a validated method based on the Food and Drug Administration validation guideline [11]. The plasma concentrations of cetirizine were determined using high-performance liquid chromatography fitted with mass spectrometry (LC-MS/MS) detection. Cetirizine and the internal standard (diclofenac) were extracted from plasma using solid-phase extraction. Internal standard working solution (50 μ L) was added to 200 μ L of each plasma sample and vortexed. Samples were transferred to pre-conditioned HLB (30 mg/cc) extraction cartridges. The analyte was eluted from the cartridge with 1 mL methanol followed by 1 mL of water twice. The extract was evaporated and reconstituted with 500 μ L mobile phase {methanol : 2 mM ammonium acetate solution : acetic acid (80:20:0.02 V/V/V)}. The extracts were injected into the LC-MS/MS system, and positive ions were monitored in multiple reaction monitoring (MRM) mode. The ion transitions (m/z) 389.1/201.0 and 296.0/250.0 were monitored for cetirizine and internal standard, respectively. Instrument setup consisted of an autosampler, an API 3000 detector and a data-processing system (Analyst 1.4.1). The linearity of cetirizine was assessed by plotting area ratios versus standard concentrations and using a linear regression weighted 1/ cetirizine² concentration. Validated analytical range for cetirizine in plasma was 5.00-697.21 ng/mL. Inter batch precision

(CV %) was ≤ 9.0 % and accuracy (% theoretical) ranged between 94.4 and 98.8 % at low, medium and high quality control levels.

Pharmacokinetics analysis

Non-compartmental analysis for deriving pharmacokinetic parameters was performed with WinNonlin version 5.0.1 (PharSight Corporation, Mountain View, California) on both the species concentration data. Actual time of sample collection was used for pharmacokinetic analysis of cetirizine. AUC from time 0 to the time for the last measurable concentration (AUC_{0-t}) was calculated by linear trapezoidal method. AUC from time 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_{0-t} and ratio of last measurable plasma concentration to elimination rate constant. C_{max} was calculated as the maximum measured plasma concentration over the time span specified. T_{max} was calculated as time of maximum measured plasma concentration.

Statistical analysis

Statistical analysis was performed using Statistical Analysis System software (SAS) version 9.1. Analysis of variance (ANOVA) was performed on the log natural (ln)-transformed pharmacokinetic parameters C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ using Type III sum of squares, with the main effects of formulation, period, sequence and animal /subject nested within sequence. Sequence effect, if any, was tested at the 10 % level of significance using the animal / subject nested within sequence mean square as error term. Formulation and period effects were tested at 5% level of significance against the residual error (mean square error) from the ANOVA model as error term. Each ANOVA included calculation of least-squares means (LSMs), the difference between the adjusted formulation means and the standard error associated with the difference.

Ratio of test (T) and reference (R) formulation least square means were calculated for log-transformed pharmacokinetic parameters (AUC_{0-t} , $AUC_{0-\infty}$ and C_{max}). It was expressed as a LSM percentage of the reference formulation. 90% confidence intervals for the ratio of LSMs were also calculated for cetirizine using two one sided hypothesis at 5 % level of significance.

Test and reference formulation ratio for C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ for both species were compared statistically using the parametric t-test at 5 % level of significance. Formulations were considered bioequivalent if 90 % confidence intervals (CIs) and ratios were within the

regulatory acceptance range of 80.00 to 125.00 %.

RESULTS

Pharmacokinetics and statistics

For the beagle dogs, the arithmetic mean \pm (SD) values for T_{max} , C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ for test (T) and reference (R) formulations, respectively, were as follows: 1.8 (0.67) h and 1.9 (0.79) h; 4037.68 (527.94) and 4069.04 (621.43) ng/mL; 54532.64 (15110.48) and 55492.52 (15497.44) ng.h/mL; 61831.85 (18569.05) and 62473.60 (19254.01) ng.h/mL in dog study. The AUC extrapolated observed was < 20 % in all cases expect one, which implies that the sampling scheme was adequate for characterization of pharmacokinetic profile.

Point values of test/reference mean ratio for C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were 99.56, 98.20 and 99.17 % in the dog study. The 90 % CIs of ratios were within the acceptance limits of 80-125% for cetirizine dog study. Least square mean values, ratios and 90% CIs for pharmacokinetic variables obtained from this study are summarized in Table 1.

Table 1: Least square mean values, ratios and 90 % CIs for pharmacokinetic variables of cetirizine formulations in dogs

Parameter	Treatment		Ratio of LSM T/R (90% CIs), %CV
	Cetirizine tablet (T)	Zyrtec Tablet®	
$AUC_{0-\infty}$ (ng.h/mL)	58945.92	59436.38	99.17 (94.43-104.15), 5.8
AUC_{0-t} (ng.h/mL)	52710.10	53674.13	98.20 (93.93-102.66), 6.7
C_{max} (ng/mL)	4006.62	4024.28	99.56 (92.81-106.80), 9.1

Mean dog plasma cetirizine concentrations are represented in Figure 1. This showed that both formulations exhibit identical pharmacokinetic profile and it indicates that both formulations are similar in terms of rate and extent of absorption.

Pharmacokinetic and statistical analyses were performed on data from 22 human subjects for cetirizine. Pharmacokinetic parameters were calculated and thereafter these were subjected to statistical analysis. The arithmetic mean (SD) values for T_{max} , C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ for

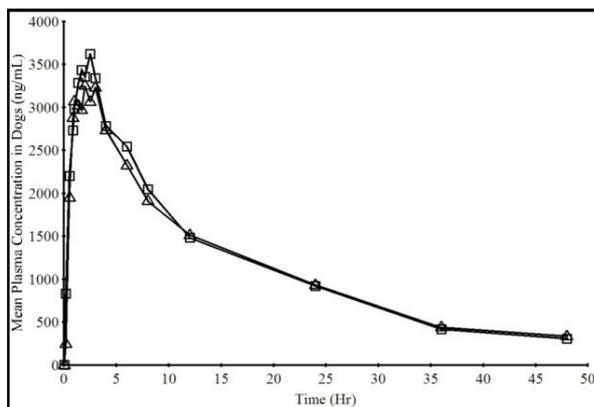


Figure 1: Linear plot of mean dog plasma cetirizine concentration (ng/mL) versus time (h) of test (□) and reference (Δ) formulations

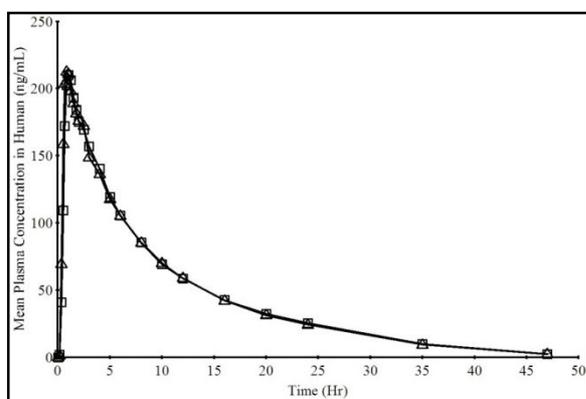


Figure 2: Linear plot of mean human plasma cetirizine concentration (ng/mL) versus time (h) of test (□) and reference (Δ) formulations

cetirizine and zyrtec formulations, respectively, were as follows: 1.1 (0.41) and 0.9 (0.43) h, 230.9 (52.53) and 228.71 (42.17) ng/mL, 2016.29 (515.37) and 2018.09 (522.42) ng.h/mL; 2140.26 (538.20) and 2143.68 (537.93) ng.h/mL in human study. AUC% extrap observed was < 20 % in all cases for cetirizine, which implied that sampling scheme, was adequate for characterization of pharmacokinetic profile. The ratios of test (T) and reference (R) formulations LSMs for C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were 100.02, 99.99 and 99.82 % in human study. The 90 % CIs for ratios were within the acceptance limits of 80 – 125 % for cetirizine

human study. Least square mean values, ratios and 90 % CIs for pharmacokinetic variables obtained from human study are summarized in Table 2.

Mean human plasma cetirizine concentrations are represented in Figure 2. This showed that both formulations exhibit identical pharmacokinetic profile and it indicates that both formulations are similar in terms of rate and extent of absorption.

A parametric t-test was performed using SAS software at 5 % level of significance (With PROC T-TEST) on T/R ratio of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of both the species (dog and human) and no statistically significant difference was observed. Table 3 represents the *p*-value observed for pharmacokinetic parameters with equal and unequal variance assumption. A *p* value > 0.05 indicates that no significant difference between the species.

DISCUSSION

The higher C_{max} in dogs compared to humans is more likely due to smaller blood volume in dogs. Certain other factors might be accounted like high destructive mechanical forces in dogs [12], different enzyme content of gut. Further, cetirizine is a BCS class I molecule with high solubility and high permeability. T_{max} was 1.8 (0.67) and 1.9 (0.79) h for test and reference formulations in dog. T_{max} was 1.1 (0.41) and 0.9 (0.43) h for test and reference formulations in human, respectively. It shows delayed T_{max} in dog. This shows that cetirizine was absorbed with a T_{max} around 2 h in dog as compared to human which was around 1 h.

Based on the ANOVA results from dog, no significant formulation, period and sequence effect was observed for log transformed PK parameters C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ at 5 % level of significance. No statistically significant difference was observed in Test/Reference ratio of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of both species at 5 % level of significance.

Table 2: Least square mean values, ratios and 90 % CIs for pharmacokinetic variables of Cetirizine formulations in humans

Parameter	Treatment		Ratio of LSM T/R (90% CIs) %CV
	Cetirizine Tablet (T)	Zyrtec Tablet (R)	
$AUC_{0-\infty}$ (ng.h/mL)	2077.13	2080.79	99.82 (96.84–102.90) 6.3
AUC_{0-t} (ng.h/mL)	1954.39	1954.63	99.99 (96.57–103.53) 6.0
C_{max} (ng/mL)	225.30	225.26	100.02 (95.43–104.84) 9.5

Table 3: Results of t-test for pharmacokinetic parameters

Variable	Method	Variance	t -value	P-value* > t
Ratio_ C _{max}	Pooled	Equal	-0.12	0.9088
Ratio_ C _{max}	Satterthwaite	Unequal	-0.12	0.9069
Ratio_ AUC _{0-t}	Pooled	Equal	-0.59	0.5606
Ratio_ AUC _{0-t}	Satterthwaite	Unequal	-0.62	0.5429
Ratio_ AUC _{0-∞}	Pooled	Equal	-0.25	0.8063
Ratio_ AUC _{0-∞}	Satterthwaite	Unequal	-0.25	0.8088

* Significantly different (if $p < 0.05$)

Comparison of least square means ratios across beagle dog and human bioavailability study had showed < 2 % variation in least square means ratios (T/R) of C_{max} and AUC in dog and human data. This indicates that least square means ratios (test/reference) dog and human are comparable to each other. Similar intra-subject variability observed for primary pharmacokinetic parameters in dog and human bioavailability studies. The intra-subject variability (%CV) of C_{max} and AUC are approximately 9% and 6%, respectively. This exhibits that formulation variability, i.e., intra-subject variability will remain the same whether bioavailability study conducted on dog or human.

Outcome of bioequivalence study is depended upon ratio and 90 % CI for primary pharmacokinetic parameter such as C_{max} and AUC. Even though differences were observed in pharmacokinetic values as discussed above but there is no significant differences were observed on absolute T/R ratio and intra-subject variability. The 90 % CI for ratios were within the acceptance limits of 80 - 125 % for cetirizine dog study as well as human study. The products were bioequivalent in both beagle dogs and humans.

CONCLUSION

The results of the preliminary investigation suggest that beagle dogs may be suitably substituted for human subjects in the bioequivalence studies of generic products with the result that study costs would be considerably lower

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DECLARATION OF INTEREST

Authors report no conflicts of interest. Authors alone are responsible for the content and writing of the paper.

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