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Research Article

Comparative bioavailability study with two amiodarone tablet formulations in healthy subjects

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ABSTRACT

The aim was to assess the comparative bioavailability of two formulations (200 mg tablet) of amiodarone in healthy volunteers. METHODS: This Open label, Randomized, One period, Two Treatment, One Sequence, Parallel Design study was conducted in 36 healthy Indian adult volunteers. Subjects received amiodarone 2 x 200 mg of either test or reference formulation. After study drug administration, serial blood samples were collected over a period of 96 hours. The samples were analyzed for amiodarone by a pre-validated HPLC method. Pharmacokinetic (PK) parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, and k_{el}, were determined for the 2 amiodarone formulations. C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were used to test for bioequivalence after log-transformation of plasma data. The formulations were to be considered bioequivalent if the log-transformed ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125%. A total of 36 healthy subjects were enrolled. No significant differences were found based on analysis of variance, with mean values and 90% confidence intervals of test/reference ratios for these parameters as follows: C_{max}, 303.68 versus 289.43 ng/mL (87.46 - 122.18); AUC_{0-t}, 3811.26 versus 3806.08 ng.hr/mL (82.87 - 118.79); and $AUC_{0-\infty}$, 4787.95 versus 4800.96 ng.hr/mL (82.79 - 117.82). In these healthy Indian volunteers, results from the PK analysis suggested that the test and reference formulations of amiodarone 200 mg tablets were bioequivalent. Both the formulations were well tolerated.

Keywords: Amiodarone; bioavailability; pharmacokinetics.

1. INTRODUCTION

Amiodarone is an effective drug in the treatment of various supraventricular and ventricular arrhythmias (Staubli, 1983; Heger, 1984; Zhang, 1996). Amiodarone is generally considered a class III antiarrhythmic drug, but it possesses electrophysiologic characteristics of all four Vaughan Williams classes. Like Class I drugs, amiodarone blocks sodium channels at rapid pacing frequencies, and like Class II drugs, it exerts antisympathetic activity. One of its main effects, with prolonged administration, is to lengthen the cardiac action potential, a Class III effect. The negative chronotropic effect of amiodarone in nodal tissues is similar to the effect of Class IV drugs.

The absorption of oral amiodarone is slow and variable, with peak serum amiodarone concentrations being attained at 3 to 12 hours after administration. Systemic bioavailability is extremely variable and ranges from 20 to 89% (Shukla, 1994; Rotmensch & Belhassen, 1988; Holt, 1983) primarily due to poor absorption and possibly high first pass metabolism. The plasma half-life of amiodarone after single-dose administration has been reported to be in the range of 3.2 to 79.7 hours. However, with prolonged use, amiodarone half-life ranges between 50 to 100 days (Zhang, 1996; Latini, 1984). Since it is a highly lipophilic drug, amiodarone is extensively distributed into tissues (Zhang, 1996; Latini, 1984). Adipose tissue and skeletal muscle accumulate large amounts of the drug during long term treatment. The prolongation in half life after chronic use may be due to accumulation of amiodarone in adipose tissue and possibly other organs (Riva, 1982).

Amiodarone is eliminated primarily by hepatic metabolism and biliary excretion. Desethyl-amiodarone is the only metabolite positively identified in the plasma of patients receiving treatment with amiodarone. Amiodarone disposition kinetics in patients with cardiac arrhythmias is not different from those in healthy volunteers.

Bioequivalence of different preparations containing the same active ingredient has gained considerable importance over the last few years because of increasing generic substitution (Hasan, 2007). It has been suggested that when a less-expensive generic equivalent becomes available, generic substitution should be con-

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sidered to achieve economic benefits (Van Wijk, 2006). However, one should expect the same quality and almost identical with original brand, because the development of generic drugs is based on pharmacological properties of the original brand (Vetchy, 2007). Reports from literature show that this may not always be so (Vetchy, 2007; Smith, 2006). The objective of this study was to compare the bioavailability of the Test Formulation of Amiodarone (Troikaa Pharmaceuticals Ltd, India) with the Innovator Product (Sanofi-Synethelabo, Guildford, Surrey).

2. SUBJECTS AND METHODS

The study was carried out at the Raptim Research Ltd, Navi Mumbai, India. All the subjects provided written informed consent to participate in the study prior to enrollment and were free to withdraw at any time during the study. The study was approved by the independent ethics committee and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

2.1. Study population and design

A total of 36 healthy Subjects were enrolled in the study with a mean age, weight and height of 25.47 years, 56.25 kg and 163.27 cm respectively. Subjects were deemed healthy on the basis of their medical history, physical examination and pathological investigation results including hematology & biochemical tests, serology, routine urine testing, urine drug screen and ECG before they were enrolled in the study. All participants provided written informed consent before inclusion in the study. Study was initiated only after approval from Ethics Committee.

Open label, Randomized, One period, Two Treatment, One Sequence, Parallel Design study was conducted in 36 healthy Indian adult volunteers under fasting conditions. The dose administration was performed as per the Randomization generated at Raptim Research Ltd, Navi Mumbai. Subjects received a single oral dose of test Formulation of Amiodarone 2 X 200 mg (Troikaa Pharmaceuticals Ltd, India) or reference Formulation (Sanofi-Synethelabo, Guildford, Surrey) with 240 mL of water after an overnight fast.

2.2. Blood sampling

Following administration of the Test/ Reference products, a total of 17 blood samples of 7 ml each were collected at 0:00hrs (pre dose), 1:00, 2:00, 3:00, 4:00, 5:00, 6:00, 7:00, 8:00, 10:00, 12:00, 18:00, 24:00, 36:00, 48:00, 72:00 and 96:00 hrs following drug administration. Prior to dosing, on the scheduled day of the study, the IV cannula was inserted in the forearm vein of the subject.

The blood samples were collected in pre-labeled centrifuge tubes containing EDTA as an anticoagulant. The plasma from blood sample was separated by centrifugation at 2,500 to 3,000 rpm for 5 minutes. The plasma from each centrifuge tube was transferred to pre labeled screw cap vials, in replicates (one set was used for analysis and the other set was kept as replicate samples, to be used for repeat analysis if required). Each vial contained approximately 1 ml plasma. Both the sets were stored at -20^{o}C ± 5 ^{o}C.

2.3. Method of analysis

Amiodarone was quantified by HPLC (Agilent 1100 series) using UV Detector. Linearity range used was 50.0 ng/mL to 750.0 ng/mL.

2.3.1. Sample Extraction

0.5 mL of blank Plasma was taken in pre-labeled vials, predetermined volume of Spiking solutions of Calibration Curve and Quality Control Samples was spiked in blank plasma. 100µL of 10ppm internal standard (Simvastatin) was added in all vials. These vials were vortexed to mix. 0.1 mL of 0.1N Hydrochloric Acid was added in all vials and vortexed for mixing. 3.0 mL of Ethyl Acetate was added in all vials and vortexed for 3 minutes. All the vials were centrifuged for 5 minutes at 2000 rpm. Organic layer was separated in another prelabeled vial and evaporated to dryness under nitrogen stream at 50˚C. Dried residue was reconstituted in 0.1mL of mobile phase (10 mM KH_2PO_4 : MeOH (20:80) v/ v and 0.05% TEA, pH of mobile phase was adjusted to 4.5 by using Ortho – phosphoric acid). 50 µL of reconstituted sample was injected in HPLC using analytical column (Merck, Purosphere 150 mm x 4.5 mm i.d. 5µm) at a flow rate of 1.5 mL/min. Wavelength of UV detector was set to 244nm. Temperature of analytical column was 30˚C.

2.3.2. Quantitation of Analyte

Calibration Curve of analyte was plotted at concentrations of 50.0 ng/mL, 100.0 ng/mL, 200.0 ng/mL, 300.0 ng/mL, 400.0 ng/mL, 500.0 ng/mL, 750.0 ng/mL while Quality control points were chosen at 150.0 ng/mL, 350.0 ng/mL and 600.0 ng/mL for Low, Middle and High Quality Control samples respectively.

2.3.3. Accuracy and Precision

Intraday Accuracy of analyte at LQC, MQC and HQC was found to be 102.78%, 103.90%, 102.33% while Intraday precision was 9.17%, 5.80%, 7.31% respectively.

Inter-day Accuracy of analyte at LQC, MQC and HQC was found to be 100.74%, 105.08%, 105.20% while Inter-day precision was 9.69%, 7.87%, 6.84%.

2.3.4. Stability of Analyte

Stock solution of analyte was found stable for 14 days at 4˚C. Samples of Amiodarone were stable for 8 hours at bench at ambient temperature while processed samples of Amiodarone were stable for 24 hours in Autosampler at 8˚C. Three freeze thaw cycles were having no impact on quantitation of Amiodarone. Samples of Amiodarone were stable for 14 days under

freezing condition at -20˚C. Method for quantitation of Amiodarone was selective as no interference from the blank plasma was observed at the retention time of the analyte (Amiodarone hydrochloride) and the Internal Standard (Simvastatin).

2.4. Pharmacokinetic analysis

The plasma pharmacokinetic parameters estimated include the observed maximum plasma concentration C_{max}, the time to reach C_{max}, (T_{max}) and the area under the plasma concentration-time curve from 0 hour to last measurable concentration (AUC_{0-t}) and 0 hour to infinity ($AUC_{0-\infty}$). The maximum plasma concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were directly determined from the plasma concentration versus time curves. The Area under the curve from 0 hour to t (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve from 0 hour to infinity ($AUC_{0-\infty}$) was estimated by summing the area from AUC_{0-t} and $AUC_{0-\infty}$, where $AUC_{0-\infty} = AUC_{0-t} + C_t / k_{el}$, with '$C_t$' defined as the last measured plasma concentration at time t, and 'k_{el}' the slope of the terminal portion of the plasma concentration versus time curve, obtained by linear regression. Logarithmic transformation was done before data analysis for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Analysis of variance (ANOVA) was used to assess effects. Intra-subject variability in terms of the overall percentage coefficient of variation (%CV), were evaluated from the ANOVA results for log transformed data. For the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ 90% confidence intervals for the ratios of Test and Reference product averages were calculated using the ANOVA of the ln-transformed data. The product was tested for bioequivalence using ratios of the Log transformed pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and its 90% confidence interval. The formulations were to be considered bioequivalent if the log transformed ratios (test/ reference) of Cmax, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125%. Pharmacokinetic output from statistical software WinNonlin - Professional version 5.0.1 was used for analysis.

2.5. Safety and tolerability

General clinical safety was assessed via physical examinations and vital signs conducted at screening and at the end of the study. Clinical laboratory tests and ECGs were also conducted at screening, before dosing within each treatment period, and at the end of the study. Adverse events were assessed for severity and relationship to treatment throughout the study.

3. RESULT

3.1 Pharmacokinetics

The amiodarone plasma concentration-time profiles of the test and reference formulations were comparable. The mean serum concentration–time curves of 2 formulations of amiodarone products each administered as a single 2 X 200 mg oral dose to healthy Indian male volunteers are shown in the figure 1. The primary PK parameters for both drugs are listed in Table 1. The mean C_{max} values of the test and reference formulations were 303.68 and 289.43 ng/mL, respectively. The mean T_{max} values of the test and reference formulations were 5.94 and 6.00 hours, respectively. Results for the extent of absorption, as determined from mean AUC_{0-t} and $AUC_{0-\infty}$ values, were 3811.26 and 4787.95 ng.hr/mL respectively after administration of the test formulation and 3806.08 and 4800.96 ng.hr/mL respectively after administration of the reference formulation. The mean $t_{1/2}$ was 15.16 hours for the test formulation and 16.34 hours for the reference formulation. ANOVA analysis for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, showed statistically non-significant difference for the treatment effect between the Test and the Reference formulation. The 90% confidence intervals of the ratios (test vs reference) for the natural log (ln)-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ are shown in Table 2. The 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 87.46 to 122.18, 82.87 to 118.79 and 82.79 to 117.82 respectively, meeting the predetermined criteria for bioequivalence.

3.2. Safety and tolerability

All 36 subjects completed the study and there were no premature withdrawals, replacements or death during the study. None of the subjects experienced or reported any adverse event, during the entire course of the study. No clinically significant abnormalities were reported in the physical examination, vital signs, ECGs and post -laboratory results. Post study physical examinations, vital signs, ECGs, and laboratory results were found to be within the normal range and not indicative of any clinical abnormality.

4. DISCUSSION

This study examined the PK properties and bioequivalence of 2 formulations of amiodarone 200 mg tablet in healthy Indian adult male volunteers. The most important objective of bioequivalence testing is to assure the safety and efficacy of generic formulations. When two formulations of the same drug are equivalent in the rate and extent to which the active drug ingredient is absorbed, and becomes equally available at the site of drug action, they are bioequivalent and thus are assumed to be therapeutically equivalent (Hassan, 2007). To demonstrate bioequivalence, certain limits should be set, depending on the nature of the drug, patient population and clinical end-points (Hassan, 2007; Abdallah, 2002). It is generally accepted that the 90% confidence interval for the ratio of averages of logarithmically transformed AUC and Cmax should lie within the range of 80 to 125 % (Hassan, 2007; Westlake, 1972).

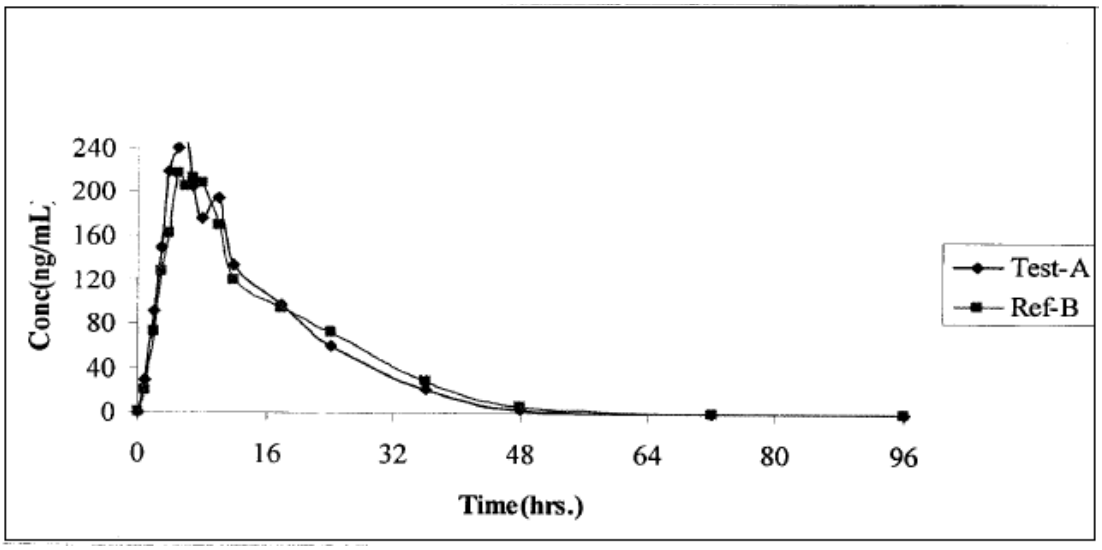

Figure 1: The mean plasma concentration time – profile for Amiodarone Test and Reference product

Table 1: Summary of pharmacokinetic parameters of amiodarone plasma, following administration of the reference and test formulations

Product	Test					
Parameter	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	$T_{1/2}$ (h)	K_{el} (h^{-1})
Mean	303.68	5.94	3811.26	4787.95	15.16	0.06
SD	96.82	1.70	1335.05	1641.63	9.73	0.03
Product	**Reference**					
Parameter	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	$T_{1/2}$ (h)	K_{el} (h^{-1})
Mean	289.43	6.00	3806.08	4800.96	16.34	0.05
SD	83.44	1.57	1260.67	1528.54	5.28	0.02

Table 2: 90 % Confidence Interval for the ratio of log - transformed data comparing Test product and Reference product

Parameter	Lower Confidence Limit	Upper Confidence Limit
C_{max}	87.46	122.18
AUC_{0-t}	82.87	118.79
$AUC_{0-\infty}$	82.79	117.82

Our study data show that both amiodarone formulations are bioequivalent for the rate and extent of absorption. The 90% confidence intervals were completely contained within the predefined bioequivalence criteria of 80% to 125% for the primary end point of C_{max} and AUC. The study results revealed that the 2 formulations of amiodarone were similar in PK characteristics among these healthy Indian male volunteers. The 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 87.46 to 122.18, 82.87 to 118.79 and 82.79 to 117.82 respectively, meeting the predetermined criteria for bioequivalence. The mean $t_{1/2}$ obtained in this study was 15.16 hours for the test formulation, which was comparable to that of the reference formulation at 16.34 hours. The mean C_{max} of the test was 303.68 ng/mL, which was comparable to that of the reference formulation 289.43 ng/mL.

Amiodarone has been associated with multiple systemic adverse effects, including bradycardia, hypothyroidism or hyperthyroidism, pulmonary toxicity, ocular deposits, and liver function derangements. Various studies showed that the incidence of adverse effects from long-term amiodarone treatment is dose dependent (Doyle & Kwok, 2009; Hu 2006; Jong, 2006). Amiodarone with dose less than 300 mg/d appears to be equally effective but with a much lower incidence of adverse effects (Triola & Kowey, 2006). In the present study both formulations were well tolerated and no adverse events were reported during the study.

5. CONCLUSIONS

In these healthy Indian volunteers, results from the pharmacokinetic analysis suggested that the test and reference formulations of amiodarone 200 mg tablets were bioequivalent. Both formulations were well tolerated.

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