ORIGINAL ARTICLE

BIOEQUIVALENCE STUDY OF PROPRANOLOL TABLETS

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The purpose of this study is to compare the bioavailability of a test tablet of propranolol (RAZA propranolol, Pharmaniaga) against an innovator product (Inderal, Astra Zeneca). The study was performed in eighteen healthy male volunteers for a single 2 x 40 mg dose of propranolol tablets. The study design used was a randomized, double blind two-period crossover design. Blood samples were collected before and within 24 hours after drug administration and plasma propranolol concentration was determined using HPLC method. Statistical analysis of the propranolol data indicated that none of the accepted parameters for drug bioavailability ($AUC^{0 \text{ to } 24hr}$, $AUC^{0 \text{ to } \infty}$, t_{max} and Cp_{max}) were significantly different between treatments for the single dose data. Parameters of $AUC^{0 \text{ to } 24hr}$ of 630.4±285.0 ng.hr/ml vs 635.6±351.0 ng.hr/ml, $AUC^{0 \text{ to } \infty}$, 670.2±299.2 ng.hr/ml vs 685.0±350.2 ng.hr/ml, t_{max} 1.3±0.5 hr vs 1.6±0.8 hr and Cp_{max} 115.3±53.4 ng/ml vs 117.5±77.3 ng/ml were obtained with test and reference formulations respectively. The 95% confidence interval of the log of ratio of $AUC^{0 \text{ to } 24hr}$, $AUC^{0 \text{ to } \infty}$ and Cp_{max} were within the range of 0.80-1.25. It can be indicated that the two tablet dosage forms (i.e. RAZA and Inderal) showed similar bioavailability and are therefore considered bioequivalent.

Keywords: propranolol, bioequivalence, bioavailability, pharmacokinetics

INTRODUCTION

The Ministry of Health is constantly emphasizing the quality, efficacy and safety of pharmaceutical products to safeguard the Malaysians public[1]. The Drug Control Authority at its 92nd meeting has decided to review the registration of generic products to include bioequivelent studies requirement for certain categories of oral immediate release products[2] Bioavailability testing of drug products in humans provides the most appropriate method available for determining bioquivalence.

Bioavailability means the rate and extent to which the active substance or therapeutic moiety is absorbed from a pharmaceutical form and becomes available at the site of action. Two medicinal products are bioequivalence if they are pharmaceutical equivalents or alternatives and if their bioavailabilities after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, are essentially the same[3,4,5].

The generic drug preparation that needs this bioequivalent study is the propranolol

tablet manufactured by RAZA Manufacturing Berhad. Propranolol (1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol) is a non-selective beta-adrenergic blocker that interacts with β_1 and β_2 receptors of the autonomic nervous system with equal affinity. It lacks intrinsic symphatomimetic activity (negative inotrophic effect) and does not block α−adrenergic receptors. Propranolol is a white solid with pK_a value of 9.4. It is not readily soluble in water except alcohol (methanol), practically insoluble in ether, benzene, and ethyl acetate[6,7].

Propranolol is a highly lipophilic substance and is almost completely absorbed following oral administration. However, most of the drug is metabolized in the liver during its first passage through the portal circulation; on average, about 25% reach the systemic circulation. The mean peak plasma concentrations of between 15 - 180 ng/ml are reached in 2-3 hours after a 80 mg dose[9]. There is great interindividual variation in the presystemic clearance of propranolol by the liver. This contributes to enormous variability in plasma concentrations (approximately 20-fold) after oral administration of the drug and in a way contributes to the wide range of doses required to produce clinical efficacy[8].

Propranolol is extensively metabolized to 4-hydroxyl-propranolol followed by conjugation with glucoronic acid[9]. The elimination half-

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life ($t_{1/2}$) of propranolol has been reported to range from between 3 hours to 6 hours or approximately 3.9 hours[3]. Propranolol has a large volume of distribution (4 L/kg) and readily enters the CNS. Approximately, 90% of the drug is bound to plasma proteins. The drug is used in the treatment of hypertension, hyperthyroidism, cirrhosis, angina pectoris, migraine and glaucoma. The adverse effects of propranolol are bronchoconstriction and disturbance in metabolism[9].

RAZA wishes to market propranolol tablets in Malaysia and overseas. It is a standard Ministry of Health, Malaysia submission requirement for them to compare the bioavailability of their generic preparation to the most commonly prescribed brand product or innovator (Inderal by Astra Zeneca). The objective of this study is to compare the bioavailability of RAZA product with Inderal according to the Ministry of Health requirement as set out in the Malaysian Guidelines for the Conduct of Bioavailability and Bioequivalence studies, 2000[2].

MATERIAL AND METHODS

This bioequivalence study was approved by the Research and Ethical Committee of the School of Medical Sciences, Universiti Sains Malaysia on 5th February 2002 (USM/PPSP®/EthicsCom./2002(74.3[6]). The study was based on a randomized, double blind, two period crossover design. Blood sampling was performed at the Clinical Trial Unit, Universiti Sains Malaysia and the analytical work was done at the Pharmacology Laboratory, The School of Medical Sciences, Universiti Sains Malaysia.

Subjects

The subjects/volunteers for bioequivalence studies were selected with the aim to minimize variability and permit the detection of differences between pharmaceutical products[2]. Eighteen (18) healthy male subjects were recruited for this study, aged between 19 and 25 years (22.8 ± 2.2 yrs), of normal weight (63.8 ± 6.8 kg), height (169.8 ± 6.2 cm) and BMI (22.0 ± 1.8) were included in the study. Each subject underwent a medical examination as well as clinical and routine laboratory evaluation tests including hematology, blood chemistry and urinalysis. One subject (AFW) smoked around five cigarettes per day.

Individuals with any significant disease history were excluded from the study. Any subjects with laboratory results more than two standard deviations from the mean value (of

each test) for laboratory performing the test (Chemical Pathology Dept., Microbiology Dept. and Hematology Dept., The School of Medical Sciences, Universiti Sains Malaysia) were excluded from the study[11]. Alcoholics, drug addicts and obese individuals were excluded from the study.

Before joining the study, all subjects were briefed on the details of the bioequivalence study, agreed and signed a consent form. All volunteers were free to leave the study at any time.

Study Design

A crossover design was used whereby equal number of subjects were taking the test preparation and reference preparation during each phase of the study. On the first dosing day each subject took on a randomized basis either two propranolol tablets (40 mg each) of Astra Zeneca (batch no. LOT OM943B, expiry date, June 2004) or two of the RAZA (batch no. 1P0378, expiry date, December 2004) propranolol tablets (40 mg each) orally with 150 ml water. After a 2 weeks wash-out period, each subject took two tablets of the other products. All subjects, physicians and drug analysts were blinded. Subjects received formulations following an overnight fast and further food or drink was withheld for at least three hours after the drug administration. After 3 hours, subjects were given a standard breakfast (of 2 slices of cheese sandwiches and a Milo drink), lunch at 6 hours (of chicken rice and orange juice) and at 10 hours post-dose, dinner (of mee soup and orange juice). Subjects were not taking any other concurrent medications[12].

Ten mililitre blood samples were collected from an indwelling venous canula or by repeated venipuncture, at predose, and at 30 minutes, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 and 24.0 hours after drug administration. Each blood sample was centrifuged immediately, plasma separated and kept frozen at -20^{0}C until analysis. Each plasma sample was analysed using high-pressure liquid chromatography (HPLC) method. The plasma concentration–time data were tabulated and the parameters derived from the profiles were analysed statistically in order that the comparative bioavailability of the two dosage forms can be determined.

Propranolol analysis and method validation

One ml of plasma and 70 μl oxprenolol, (10 μg/ml) as internal standard, was placed in a screw-capped glass tube. To each tube,

100 µl water and 50 µl 5N NaOH were added, followed by vortexing for 30 seconds. The drugs were extracted with 3.5 ml of the extraction solvent (isoamyl-alcohol [1.5 ml]: n-heptane [98.5 ml]) and shaken on a rotator mixer for twenty minutes. This was followed by centrifugation (3000 rpm) for 10 minutes. The organic phase was then transferred by aspiration to a clean glass tubes.

The extraction procedure was repeated with the remaining samples. A gentle flow of nitrogen (10 ml/min) was used to dry the organic phase. The residue was reconstituted in 70 µl of mobile phase and vortex-mixed for 20 sec. Twenty microlitres of the sample was injected directly onto Lichrosorb C18 HPLC (12.5 cm) column and detection was done by ultraviolet detector set at 254 nm[13].

The HPLC system comprised of a Gilson 307 peristaltic pump, a Gilson 115 variable wavelength UV detector, a Gilson 234 Autoinjector, and a DELL-Optiplex GX-1 computer as integrator using Unipoint software. The mobile phase consisted of a mixture of water, methanol, acetonitrile, acetic acid and triethylamine in the proportion of 160ml: 80ml: 70ml: 2.5ml: 125µl, respectively[13]. The pH was adjusted to 3.4 using 1N NaOH before the addition of triethylamine. The mobile phase was filtered off of all residues and that filtration also removed dissolved gas. The mobile phase flow rate was 0.5ml/min[14].

The main objective of method validation is to demonstrate the reliability of a particular method for the quantitative determination of an analyte concentration in a specific biological matrix[2]. This procedure took 3-6 months prior to clinical studies. The characteristic of a bioanalytical method essential to ensure the acceptability of the performance and reliability of analytical results are:
• separation and specificity
• recovery
• linearity
• accuracy and precision(interday and intraday variability must be low)
• limit of quantification (LOQ) and minimum quantified concentration (MQC) and analyte stability[10].

Under these conditions, the retention times for propranolol and oxprenolol were 9.67 and 6.86 minutes, respectively. Calibration was linear in the concentration range of 15-180 ng/ml, the regression line can be described by $y: 0.0028x - 0.006$ and the coefficient of correlation was 0.9949. The inter-day calibration curve showed consistent linearity, as seen by consistency of intercept, slope and coefficient of correlation. Intra-day precision was determined at five concentrations (15, 30, 60, 120 and 180 ng/ml) in plasma, each repeated five times (in duplicate) using the area ratio technique. Intra-day precision in this study expressed, as means of percent of coefficient of variation (CV) were 8.24%. The plot of propranolol (PRN) area ratio to concentrations (15-180 ng/ml) is linear with $R^2: 0.9948$.

Inter-day precision was determined singly at five concentrations (15-180 ng/ml) in plasma, in seven replicate runs (7 days). Inter-day precision in this study expressed, as means of percent of coefficient of variation (CV) were 9.8%. The plot of area ratio to concentrations (15-180 ng/ml) is linear with $R^2: 0.9906$. Under optimal conditions, the limit of quantification based on 3 times noise level using 1 ml plasma sample and 20 µl injection volume was 9 ng/ml.

Three concentrations of propranolol (20, 100 and 160 ng/ml) were used for stability studies showed consistent linearity (intercept, slope, and coefficient of correlation) over a six week period (CV:8.16%). Standard curves were performed daily with each volunteer's plasma samples.

Data analysis

An Excel (Microsoft) programme was used to plot the plasma concentration-time curve. Actual sampling time was used and later rounded up for easy tabulation. Pharmacokinetic parameters determined in this study were $AUC^{0 \text{ to } 24hr}$, $AUC^{0 \text{ to } \infty}$, t_{max}, Cp_{max}. AUCs are area under the plasma concentration curve from administration to 24 hours and 0 hour to infinity, respectively. The AUC reflects the total amount of active drug that reaches the systemic circulation[4]. Parameter t_{max} is the time passed since administration at which the maximum plasma concentration occurs. At t_{max}, absorption is maximum and the rate of drug absorption exactly equals the rate of drug elimination. When comparing drug products, t_{max} can be used as an approximate indication of drug absorption rate. The value for t_{max} will become smaller as the absorption rate for the drug becomes more rapid[4]. Maximal plasma concentration (Cp_{max}) provides an indication that the drug is sufficiently systemically absorbed to provide a therapeutic response. In addition, Cp_{max} provides warning of possibly toxic levels of drug.
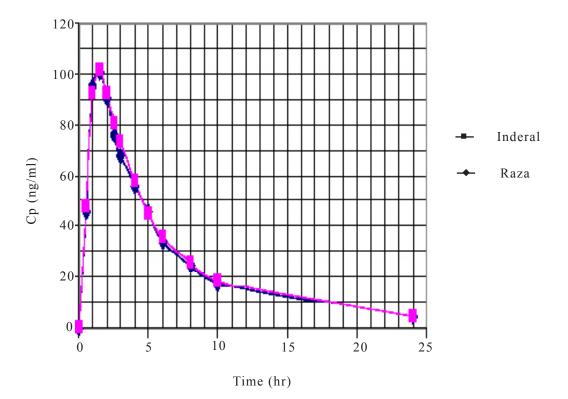
Figure 1. Mean plasma concentration of RAZA and Astra Zeneca 2 x 40 mg tablets of propranolol.

The following parameters were calculated for each subject and treatment phase: a.Maximum plasma concentration (Cp_{max}) is the observed maximum plasma concentration. b. Time of maximum plasma concentration (t_{max}) is the time of the maximum plasma concentration. c. Area under the plasma concentration-time curve (AUC) - AUC from zero time to the last data point was calculated by the trapeizoidal method (using Excel programme). It should be noted that actual sampling times rather than nominal sampling times were used in the calculation of AUC (and other relevant parameters).

Statistical method

Differences between study phases in the calculated parameters were tested for statistical significance by an analysis of variance (ANOVA for a crossover study; taking account of treatment and period effects) and confidence intervals (90%) were calculated for each of the comparisons using the following equation: A 90% confidence interval: $-t\sqrt{MS_{error}/N/2} - (X_R - X_T) \leq \ln(\mu_T/\mu_R) \leq t\sqrt{MS_{error}/N/2} - (X_R - X_T)$ where MS_{error} is mean square error from ANOVA table, X_R is mean of reference product, X_T is mean of test

product and N is the number of subjects[15].

Data for AUCs and Cp_{max} were log transformed prior to analysis. The formula was used to calculate the CV% which was then used to estimate the power of study using the tables provided by Diletti et al[15,16]. For bioequivalence, the test and reference products required that a 90% confidence interval of the ratio of means of pharmacokinetic parameters i.e. AUCs and Cp_{max} must be in the range of 0.80 – 1.25. Differences between study phases in t_{max} were tested for statistical significance by the Wilcoxon Signed Rank non-parametric analysis[17].

RESULTS

All volunteers satisfied the inclusion criteria and signed the consent forms prior to the screening tests. The study involved a 2 weeks washout period before the next crossover and each individual volunteers plasma samples (test vs reference) were assayed on the same day batch to avoid laboratory/analyst error.

No adverse effects were reported and there was a decline in blood pressure observed Drugs with significant first pass effect

Table 1. Pharmacokinetic parameters of propranolol after a single 2 x 40 mg dose of RAZA and ASTRA ZENECA (Inderal).

	RAZA 2 x 40 mg tablets			ASTRA ZENECA 2 x 40 mg tablets		
	$AUC^{0-24\ hr}$ (ng.hr/ml)	t_{max} (hr)	Cp_{max} (ng/ml)	$AUC^{0-24\ hr}$ (ng.hr/ml) (hr)	t_{max} (hr)	Cp_{max} (ng/ml)
AFA	619.91	1.6	97.01	664.55	0.98	143.92
AHH	288.22	0.97	124.86	367.65	1.97	100.70
KAJ	543.86	0.5	102.18	483.73	0.98	118.60
KNI	656.83	1.03	120.73	323.26	1.52	46.15
MIA	1061.56	0.98	173.67	693.33	1.48	115.18
MRA	648.85	0.58	134.24	487.79	1.48	108.05
RFM	854.81	1.12	156.18	741.36	1.07	132.22
AFW	485.21	2.07	81.89	530.42	4.02	48.13
MAP	297.61	2.13	42.89	239.49	1.98	38.91
MSRH	705.57	1.00	104.61	870.05	0.60	161.44
AFIM	566.16	1.03	92.18	497.93	2.03	74.84
AKAT	734.11	2.00	129.46	716.22	1.72	119.20
AFMU	583.34	0.77	136.51	1440.34	0.50	283.67
MFM	476.81	1.62	51.24	364.06	1.47	45.31
MFDB	246.57	1.50	43.31	432.83	1.63	62.12
MFZO	393.08	1.53	70.67	341.62	2.12	45.66
NFMN	1416.27	1.48	265.03	1505.53	1.13	311.20
BMS	768.27	1.55	147.94	740.04	1.20	160.31
Mean±SD	630.39 ± 284.96	1.30 ± 0.49	115.26 ± 53.41	635.57 ± 351.01	1.55 ± 0.78	117.53 ± 77.28
SEM	67.17	0.12	12.59	82.74	0.18	17
C.V.%	45.20	37.55	46.34	55.23	50.32	65.75
Range	246.57 - 1416.27	0.5 - 2.07	43.31 - 265.03	323.26 - 1505.53	0.5 - 4.02	45.31 - 311.2

like propranolol show high interindividual variation in plasma drug concentrations[8]. There was no statistically significant difference in the mean of the four parameters ($AUC^{0\ to\ 24hr}$, $AUC^{0-\infty}$, Cp_{max} and t_{max}) between the test (RAZA) and the reference product (Inderal). The level of significant (p value) obtained by ANOVA table was greater than 0.05.

DISCUSSION

In the context of bioavailability study, the "power of study" is to detect a significant of 20% difference between treatment mean, if a 20% difference or less actually existed between the two products with the specified number of subjects in the study. In the present study, the power of study for both $AUC^{0\ to\ 24}$ and Cp_{max} was 70% and that of $AUC^{0-\infty}$ was 80%.

While the 95% Confidence Interval of the ratio of $AUC^{0\ to\ 24}$, $AUC^{0-\infty}$ and Cp_{max} between RAZA and Inderal tablets were in the range of 0.80-1.25 as recommended by USP24-NF 19 guidelines for bioequivalence study[7]. Therefore, bioequivalence can be indicated between RAZA and Inderal propranolol tablets.

Statistical analysis of the propranolol data indicated that none of the accepted parameters for drug bioavailability ($AUC^{0\ to\ 24}$, $AUC^{0-\infty}$, Cp_{max} and t_{max}) were significantly different between treatments for the single dose data. Such a finding indicates that under the same experimental protocol described, the two tablet dosage forms (i.e. RAZA and Inderal, Astra Zeneca) have been shown to have similar bioavailability and are therefore considered bioequivalent with regard to extent and rate of absorption.

Table 2. Statistical analysis of pharmacokinetic parameters of propranolol (RAZA and Inderal)

	RAZA	Inderal	95% C.I	Sig (p)	NS	Power of study
AUC^{0-24hr} ng.hr/ml	630.4±285	635.6±351	0.86-1.14	0.8053	NS	70%
$AUC^{0-\infty}$ ng.hr/ml	670.2±299.2	685.0±350.2	0.87 – 1.11	0.9216	NS	80%
Cp_{max} ng/ml	115.3±53.4	117.5± 77.3	0.83 - 1.15	0.7058	NS	70%
t_{max} hr (Wilcoxon)	1.3± 0.5	1.6±0.8	-	-	NS	-

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