Assessment of sex differences in Pharmacokinetics of carvedilol in human

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Abstract: Carvedilol is an anti-hypertensive agent capable of blocking both alph (α) and beta (β) receptors used to preclude cardiac arrhythmias and angina. The study was designed to evaluate the Pharmacokinetics of carvedilol in human male and female volunteers. Healthy male and female (twenty each) volunteers were finalized for the study after preliminarily clinical examination. Blood samples were collected at specific time intervals after giving an oral dose of 12.5mg carvedilol, separated the plasma and placed at -80°C until analysis. Estimation of carvedilol in human plasma was accomplished by High performance liquid chromatographic (HPLC) method using fluorescent detector. Plasma concentration-time curve was used for calculation of pharmacokinetic parameters using two-compartment open model. Mean (SD) values of AUC and C_{max} 0.076±0.021µg.h/ml and 0.024±0.005µg/mL, respectively) in male differ significantly ($P<0.05$) from the female 0.197±0.042µg.h/ml and 0.048±0.02µg/mL, respectively). Overall, bioavailability of carvedilol was somewhat higher in females than in males, but these differences could be expounded by the lower body weight of female. Conversely, no significant differences were found for t_{max}, clearance and half-life in male and female. Moreover the ethnicity had significant impact on the Pharmacokinetics of carvedilol in human.

Keywords: Carvedilol, sex, fluorescent, human, pharmacokinetics.

INTRODUCTION

Cardiovascular disease is a leading cause of death in all over the world. Carvedilol is a third generation β receptor antagonist with blocking capacity of β1, β2 and α1 receptors, widely used to treat a variety of cardiovascular ailments, including hypertension, heart failure and left ventricular dysfunction following myocardial infarction (GlaxoSmithKline, 2009). It has the ability to stabilize the membrane but lacks intrinsic sympathomimetic activity. Its antioxidant and anti-proliferative effects produce beneficial effects in treating congestive heart failure (Keating and Jarvis, 2003). It is a vasodilator with β-adrenoceptor blocking properties used in the treatment of hypertension and angina pectoris (Cubeddu *et al*., 1987; Sponer *et al*., 1987). Carvedilol is a racemic mixture, extensively metabolized in humans via both oxidative and conjugative pathways (Oldham and Clarke, 1997; Neugebauer *et al*., 1990; Neugebauer and Neubert, 1991). Several metabolites of carvedilol are pharmacologically active but 4-hydroxyphenyl carvedilol exhibits higher β-adrenoreceptor blocking potency compared to carvedilol itself (Gehr *et al*., 1999). The drug is absorbed rapidly from the gastrointestinal tract after oral administration and unchanged drug excreted (23%) in the feaces (Zhou and Wood, 1995). Carvedilol is rapidly absorbed following oral administration with peak plasma concentration occurring in 1 to 2 hour and half-life is 7-10 hours. It is highly bound to plasma protein approximately 98% and extensively metabolized in the liver (Keating and Jarvis, 2003). Due to its highly lipophilic character it is eliminated predominately by hepatic metabolism with renal excretion accounting for only 0.3% of the administered dose (Zhou and Wood, 1995).

There are many factors, which affect the Pharmacokinetics of drug such as age, gender, climate, environmental conditions and disease etc. The basic physiological difference in male and female contributes to the phenomenon that women and men frequently respond differently to cardiovascular drugs and provide basis for their difference in Pharmacokinetics and Pharmacodynamics. Difference in hormones, metabolizing enzymes, slightly slower glomerular filtration rate in females and changes in total body water can be associated with gender-specific difference in the plasma levels of cardiovascular drugs (Jochmann *et al*., 2005). Recent advance studies show that the number of specific iso-enzymes is involved in drug metabolisms, which allow for preliminary identification of enzymes systems that are affected by sex. Cytochrome P4503A4 activity is higher in women than in men whereas the activity of many other systems involved in drug metabolism may be higher in men than in women (Harris *et al*., 1995). Men have higher CYD2D6 activity than women (Jochmann *et al*., 2005). Many physiological factors also lead to gender-related Pharmacokinetic difference that may include lower body weight, organ size, high body fat (may

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increase volume of distribution of lipophilic drugs in women), low GFR and different gastric motility in women as compared to men (Schwartz, 2003).

No other study has seemed at disposition kinetic sex differences for carvedilol. Therefore it is necessary to study the disposition kinetics of carvedilol in human male and female. The intention of current study was to compare the pharmacokinetics of carvedilol in healthy male and female persons of Pakistan.

SUBJECTS AND METHODS

Blood sampling and research work was conducted in Bioequivalence Study (BeSt) Center, University of Veterinary and Animal Sciences (UVAS), Lahore. The study was accompanied in agreement with good clinical practices and approved by Ethical Committee of UVAS, Lahore. Volunteers registered for trial detailed about all traits of the trial. Persons who agreed voluntarily to participate and provide written "Informed Consent" were included in the study. Healthy male and female volunteers (twenty each) were selected after preliminary clinical examination. The Subjects were not taking any medication 10 days before reporting for experiment and were asked not to take any solid food at least 10 hours prior to the medication. They were asked to not take any fluid 1-hour before the medication and were given standard meals in equal quantities after 4 hours post-dosing and thereafter. After an overnight fast, each volunteer received one tablet of carvedilol 12.5mg orally with water, then after no drink (water) was allowed till 1 hr of drug intake. Each volunteer was given a same healthy diet after 4hr of drug administration. Body temperature, blood pressure and heartbeat were checked carefully throughout the trial.

Healthy subjects, between the age 18 and 55 years were enrolled. All subjects were in good health without significant abnormal clinical laboratory values or significant gastrointestinal, renal, hepatic, cardiovascular, hematological, or metabolic disease. Key exclusion criteria included previous history of allergy to any of the drugs under investigation, smoking and drug use or exposure to any drug within 10 days of study start. All subjects were healthy as determined by medical history, physical examination and laboratory screening by complete blood counts, serum chemistries, and urinalysis. Average demographic data of male and female volunteers are shown in table 1.

A sterile Branula (22G) was used to withdraw blood sample (5ml) vacutainers at pre-selected time intervals of 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24. Plasma was separated after centrifugation at 3000 rpm for 10 minutes and kept at -80°C till analyzed.

Chromatographic conditions

HPLC conditions were selected as used by Zargi *et al*., (2007) with little modifications. Carvedilol separation was achieved by C_{18} column manufactured by Merck (4.6 x 250mm; 5µm) using acetonitrile and potassium phosphate monobasic (0.01 M; pH 3.5) as mobile phase with the ratio of 62/38. Mobile phase was eluted in column with 1 ml/min flow rate and fixed the column temperature at 40°C. Estimation of carvedilol was monitored by fluorescent detector at 240 nm (Exi.) and 340 nm (Emm.).

Stock solution & quality control (QC) samples

Carvedilol standard was gifted by Hilton Pharmaceuticals, Pakistan. Standard stock solution of carvedilol was prepared in double distilled water and working standards were prepared in mobile phase. Calibration standards of carvedilol in plasma were prepared by spiking the working standards in plasma at 1.0, 5.0, 10, 20, 40, 80 ng/ml. QC samples were prepared at concentrations of 3.0, 15 and 60ng/ml for checking the validation parameters according to the ICH guidelines, (2005).

Carvedilol extraction

Extraction of carvedilol was achieved by taking plasma sample (500µL) in Eppendorf tubes and added acetonitrile (500µL), vortexed for 5 min followed by centrifugation at 12,000 rpm for 12 min. Supernatant was filtered by 0.22 µm filter paper using Micropore syringe filter holder and transferred into 2ml auto-sampler vial. A volume of 20µL was injected by auto-injector onto HPLC.

Process validation

Analytical method was validated by spiking the drug in plasma samples according to the ICH guideline. Selectivity and Specificity was determined using plasma by six different sources. Limit of Detection (LOD) & Quantification (LOQ) were estimated from signal-to-noise ratio with a sufficient precision and accuracy. Calibration curves were made for estimation of linearity. Recovery, precision & accuracy were estimated from low, medium and high quality control samples (n=6). Stability was checked from fresh plasma extracts (QC samples) at room temperature and stored in freezer for 3 days.

Data analysis

Pharmacokinetic parameters of carvedilol were calculated in both male and female by applying two-compartment model. The kinetic data collected from both sexes were analyzed by appropriate statistical method utilizing non-paired t-test (Steel *et al*., 1997).

RESULTS

The analytical method used was validated to assure the acceptability of the method.

Table 1: Average Demographic data of male and female volunteers

Parameters	Units	Male				Female			
		Mean	± SD	Min	Max	Mean	± SD	Min	Max
Age	Year	21.08	0.51	19.0	30.0	21.08	0.51	19.00	30.00
Weight	Kg	63.31	1.83	49.0	80.0	63.31	1.83	49.00	80.00
Height	cm	170.0	1.58	155.0	180.0	153.0	1.58	145.00	166.0
Blood Pressure	Systolic	117.5	2.24	100.0	140.0	114.0	1.82	100.00	140.0
Blood Pressure	Diastolic	73.75	1.72	60.0	90.0	79.38	1.31	70.00	90.00
Body Temperature	^{o}F	98.8	0.13	96.4	98.8	98.1	0.14	96.4	98.8

Table 2: Recovery, Precision and Accuracy of carvedilol in human plasma quality control samples (n=6)

Contents	Quality controls (ng/ml)	Recovery %	Precision RSD %	Accuracy %
Intra-day	3.0	98.81	0.68	97.08
	15	99.02	1.54	99.22
	60	99.34	0.30	99.58
Inter-day	3.0	97.98	1.11	0.68
	15	98.21	1.03	1.54
	60	99.11	1.21	0.30

Table 3: Stability (Freeze-thaw) of carvedilol in human plasma (n = 6).

Content	Added Concentration (ng/ml)	Calculated Concentration (ng/ml)	RSD %
Normal	3.0	3.1	1.95
	60	61	0.93
Refrigerated	3.0	3.0	1.85
	60	63	0.16
Freeze-thaw	3.0	3.0	0.54
	60	55	0.28

Table 4: Pharmacokinetic parameters of carvedilol in male & female healthy subjects after oral administration of 12.5 mg tablets

Parameters	Healthy male volunteers		Healthy female volunteers		P value
	Mean	± SD	Mean	± SD	
AUC (µg.h/ml)	0.076	0.021	0.197	0.042	0.03^{S}
C_{max} (µg/ml)	0.024	0.005	0.048	0.020	0.016^{S}
T_{max} (hr)	0.745	0.276	0.763	0.190	0.862^{NS}
Ka (hr^{-1})	4.248	2.312	3.591	0.157	0.346^{NS}
Vd (L)	318	102	379	286	0.646^{NS}
$t_{1/2}$ (hr)	5.205	1.824	6.933	1.438	0.699^{NS}
Clearance (l/hr)	0.140	1.320	0.063	1.561	0.06^{S}

AUC: Area Under Curve; C_{max}: Peak plasma concentration; T_{max}: Time to peak concentration; Ka: Absorption rate constant; Vd: volume of distribution; $t_{1/2}$: elimination half life

Selectivity & Specificity

The retention time of carvedilol was 7.59 and no interferences were noted around the retention time of carvedilol.

LOD & LOQ

The LOD of carvedilol was 0.5ng/ml and LOQ was 1.0 ng/ml.

Linearity

The concentration range of 1ng/ml to 80ng/ml was used for calibration curves of carvedilol. Linear relationship between peak area and drug concentration was r^2= 0.9994.

Recovery, Precision and Accuracy

The average extraction recovery of carvedilol was almost 99%. Accuracy and precision (Intra-day & inter-day) of carvedilol were also within the acceptable range as described in ICH guidelines (table 2).

Pharmacokinetics

Pharmacokinetic parameters of carvedilol in healthy male and female persons were analyzed by two compartment kinetic model, shown in table 4. The average plasma concentrations of carvedilol in both male and female subjects after oral dose of 12.5mg tablet were presented in fig. 2(a-b). Average area under curve (AUC) of carvedilol

in healthy male subjects was 0.076±0.015µg.h/ml and in female subjects was 0.197±0.042µgh/ml. The C_{max} observed in male was 0.024±0.005 µg/ml and 0.048±0.02 in female persons. Volume of distribution was 318±102 liter in male and 379±286 liter in female persons. Elimination half-life of carvedilol in male volunteer was 5.205±1.824 hour and in female was 6.733±1.438 hour. Absorption rate constant in healthy male volunteer was 4.248±2.312hr^{-1} and 3.159±0.517hr^{-1} in female. Time to peak concentration (T_{max}) was achieved in 0.735±0.252 hour in male and 0.760±0.190 hours in female subjects.

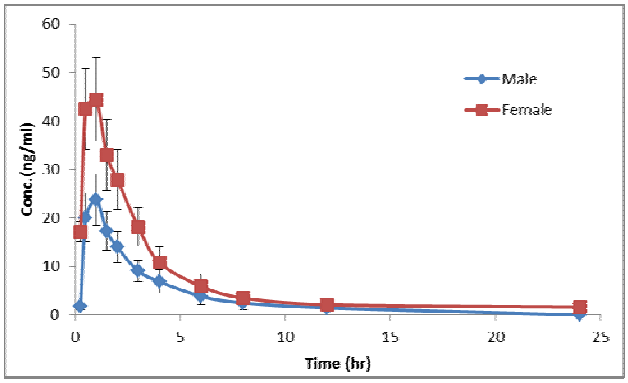

Fig. 2(a): Mean ± SD plasma concentration-time curve of carvedilol (at ordinary scale)

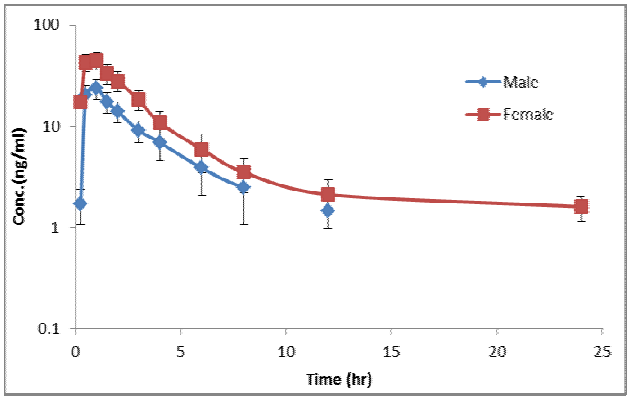

Fig. 2(b): Mean ± SD plasma concentration-time curve of carvedilol (at semi-logarithmic scale)

DISCUSSION

Gender-specific differences have been found with respect to Pharmacokinetics properties especially in case of cardio-selective drug and non-selective β-blockers. Literature showed that men have greater activity of enzyme CYP2D6 and faster clearance of β-blocker as compared to female (Jochmann *et al*., 2005). Present study was designed to study the gender difference in disposition kinetics of carvedilol in healthy male and female subjects.

AUC of carvedilol in male and female persons was different significantly might be due to gender specific effect on absorption as well as slower glomerular filtration rate in females as compared to male in which rapid elimination of carvedilol was observed, whereas result reported by Rathod *et al.* (2007) was 0.509±0.064 µg.h/ml and was not comparable to current study. Plasma concentration of carvedilol in females was significantly higher, about 50% ($P<0.05$) as compared to male volunteers in current study whereas C_{max} reported by Julie *et al.* (2000) was 80% higher in women as compared to men. This may be due to high rate of metabolism and rapid excretion of carvedilol in male as compared to females. The value of t_{max} was not different significant in both sexes, also co-relate with t_{max} (0.75±0.35 hours) reported by Rathod *et al.* (2007). Rate of absorption of carvedilol was faster in male as compared to female and half-life of carvedilol was higher in female as compared to male due to slightly slower glomerular filtration rate in female volunteers.

CONCLUSION

The current study showed that disposition kinetics of carvedilol differs significantly in both sexes. Pharmacokinetics of carvedilol in human female volunteers was slightly higher as compared to human male volunteers, indicated that the dose regimen should be define according to the gender.

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