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Research Article

Metoprolol Exhibits Discrete Pharmacokinetics Using Various Sites of Sample Collections after Single Oral Dose Administration of Metoprolol Tartrate in SD Rats

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Abstract

IThe aim of the study was to investigate the influences of various routes of sampling on the pharmacokinetics profile metoprolol after single oral dose administration of metoprolol tartrate in rats. For this single dose parallel studies were conducted in SD rats at 5 mg/kg of metoprolol tartrate. In our investigation, significant differences were observed in the plasma PK profile of metoprolol using various routes of sample collection. The mean peak plasma metoprolol concentration obtained from jugular (C_{max}; 170.0 ng/mL) and saphenous (C_{max}; 113.2 ng/mL) routes were comparable. Similarly, retro-orbital route C_{max} (50.0 ng/mL) was comparable with C_{max} (76.2 ng/mL) of femoral route. In contrast, the C_{max} (39.3 ng/mL) obtained from tail vein sampling route was significantly different ($p \leq 0.05$) from all other sampling sites. The C_{max} obtained from tail vein sampling site was approximately 2-4 folds less compared to other routes of sampling except for retro-orbital sampling site. Corresponding differences were also observed for other PK parameters. It was concluded that the sampling sites have profound impact on the PK parameters of metoprolol after single dose oral administration in rats.

Keywords: Bioavailability; Femoral; Jugular; Metoprolol; Pharmacokinetics; Sampling site

Introduction

The variabilities in the pharmacokinetics (PK) of a drug is attributed to huge number of co-variates. Besides intersex and interspecies differences in the physiochemistry, the sampling routes have profound influences on the estimated PK parameters [1]. Most often different routes of sample collection are used during pre-clinical and clinical PK studies. Thought, there are no discrete guidelines on the selection of routes of sample collection. In order to estimate the concentration of drug at the site of action, among the body fluids blood is the sample of choice for concentration analysis. Thus, we can expect good results if the sample collection processes are standardized.

In this study, we investigated the effect of various sampling sites on the PK profile of metoprolol after single dose administration of metoprolol tartrate in rats. Five sampling routes viz. jugular vein, femoral vein, retro-orbital plexus, tail vein and saphenous vein were taken into consideration which are frequently utilized for PK studies in rodents. Subsequently, the PK parameters were calculated and compared for these routes to estimate the differences.

Material and Method

Chemicals and reagents

Acetonitrile and methanol of HPLC grade were purchased from Merck (Darmstadt, Germany). Formic acid of HPLC grade was obtained from ROE (Newark, USA). Metoprolol tartrate (purity > 98%) and internal standard (IS) Talmisartan were procured from Sigma Aldrich, USA. All other chemicals and reagents were of analytical liquid chromatographic (LC) grade. Drug free rat plasma was collected from healthy Sprague-Dawley (SD) rats obtained from *in-house* animal facility of Aragen Life Sciences Pvt. Ltd. All animal ex-

perimentation and procedures were done as per the protocols approved by Institutional Animal Ethics Committee (IAEC, Approval number: ARAGENB-IAEC-0001-01-22).

Subjects

Healthy adult SD male rats (200-250g) were acclimatized for three days prior to the study in proper ventilated polypropylene cases under controlled standard laboratory conditions of regular 12h light-dark cycle, temperature (22 ± 2°C) and relative humidity (55 ± 5%). Certified rodent diet and water was provided to *ad libitum*. Animals were kept for overnight fasting prior to studies. Animals were maintained and monitored for good health in accordance with Test Facility SOPs. Guidelines approved by the Good Laboratory Practice (GLP) were followed throughout the animal experimentation.

Study design

The studies were parallel single dose oral plasma PK, designed to estimate the influences of site of sample collection on the PK profile of metoprolol after administration of metoprolol tartrate in male SD rats. The studies were conducted in five groups of experimental animals with three animals (n = 3) in each group. Rats of all groups were dosed orally at 5 mg/kg of metoprolol tartrate. Subsequently, blood samples were withdrawn using retro-orbital plexus (group 1), jugular vein (group 2), saphenous vein (group 3), tail vein (group 4) and femoral vein (group 5) to generate PK profile of metoprolol. The sample size is based on the previous studies on the same investigational drug wherein significant inter-individual variability was not obtained.

Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 12 h post dose. The rats were surgically operated for jugular & femoral vein cannulations three days before the commencement of study and were closely observed throughout the recovery period.

Formulation

Fresh metoprolol tartrate formulations were prepared in normal saline (0.9% NaCl in water) on the day of dosing. The volume factor was 10 mL/kg at 5 mg/kg metoprolol tartrate dose. Final formulation was a clear colourless solution with final concentration of 0.5 mg/mL.

Bioanalysis

Blood samples collected from the respective experiments were immediately centrifuged to obtain plasma. The plasma samples were stored at -70^{0}C till analysis. Before analysis, sample were thawed at room temperature and 10 µL sample was aliquoted for further processing. Samples was crashed using 150 µL acetonitrile containing talmisartan (IS; 20 ng/mL) and vortexed for about 2 minutes. Resulting mixture was centrifuged at 4000 rpm for 7 minutes at 4^{0}C. From the supernatant, 100 µL volume was separated and diluted with 100 µL milli-Q water. This solution was transferred into HPLC vails and subsequently subjected to LC-MS/MS analysis. Plasma concentration was determined by using a partially validated LC-MS/MS method. Mass spectrometric detection was performed on API 6500 LC-MS/MS mass spectrometer (Applied Biosystems, Sciex, USA) with Analyst 1.7 software. Product ion transitions at m/z 268.20 to 116.20 and 515.20 to 276.20 were used for quantification of metoprolol and IS respectively. The assay was linear over the range 1.00–5000 ng/mL with LOQ 5.00 ng/mL. Coefficients of determination (r^2) were >0.990 for standard curves generated. Precision and accuracy of the method was determined by analysing QCs at 5.00, 2500 and 4200 ng/mL.

Pharmacokinetics and Statistical analysis

The primary endpoints for these studies were area under the curve (AUC), maximum plasma concentration (C_{max}), time to attain C_{max} (T_{max}), Volume of distribution (Vd), Clearance (CL), elimination half-life ($T_{1/2}$), mean residence time (MRT). The PK parameters were calculated by non-compartmental analysis using Winnonlin Phoenix (Version 8.1, Pharsight Corporation). The PK parameters were statistically compared using two-tailed *Student's t-test* for analysing variability between two groups, while *ANOVA* test was used to determine variabilities among the groups. In all the tests, a probability level of significance was kept at α = 0.05. Results were expressed as mean ± SD; n = 3).

Results

Table 1 summarizes calculated PK parameters of Metoprolol following oral dose administration of Metoprolol tartrate in male SD rats. The mean plasma concentration-time profile is shown in Figure 1.

Route of sample collection	PK Parameters						
	C_{max}(ng/mL)	$T_{1/2}$(h)	Vd_F(L/kg)	CL_F(mL/min/kg)	AUC_{0-t}(h*ng/mL)	$AUC_{0-inf.}$(h*ng/mL)	MRT_{last}(h)
Retro-orbital	50.0 ± 9.29	2.2 ± 1.23	334.2 ± 18.65	2108.4 ± 211.32	42.0 ± 11.66	43.2 ± 12.53	2.1 ± 0.43
Jugular Vein	170.0 ± 15.47	1.3 ± 0.12	65.9 ± 7.75	583.3 ± 96.68	154.0 ± 12.96	156.8 ± 47.68	1.6 ± 0.20
Saphenous Vein	113.2 ± 19.72	1.8 ± 0.15	252.4 ± 23.85	1650.7 ± 54.71	102.7 ± 21.68	105.9 ± 22.75	1.8 ± 0.11
Tail Vein	39.3 ± 7.42	1.6 ± 0.44	391.7 ± 19.86	3017.0 ± 16.96	57.0 ± 14.37	58.0 ± 6.87	2.4 ± 0.16
Femoral Vein	76.2 ± 11.48	1.9 ± 0.14	283.4 ± 9.90	1820.1 ± 45.68	61.5 ± 13.31	62.7 ± 18.38	1.7 ± 0.19

Table 1: Pharmacokinetic parameters of Metoprolol following oral dose administration of Metoprolol tartrate in SD rat (n=3; mean ± SD) at 5 mg/kg.

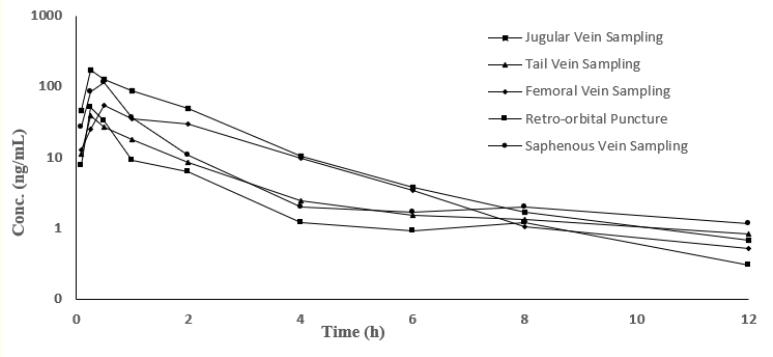

Figure 1: Concentration-Time profile of Metoprolol after oral dose administration of Metoprolol tartrate in SD rat (n = 3) at 5 mg/kg using various sites of sample collection.

Discussion

In preclinical studies, the evaluation of systemic exposure and pharmacokinetic parameters of a drug candidate are coercive measures for assessment of safety profile and associated risks of drug candidate testing in clinical settings. Most of the drugs are intended for oral dose administration because of best patient compliance. However gastrointestinal degradation, poor bioavailability, rapid first pass biotransformation etc. are the factors that limits the systemic exposure of many drug candidates [3]. It is well established that the sex and gender related characteristics, inter-individual differences in the physiochemistry etc. leads to the variations in the pharmacokinetics of a drug [4]. The differences in the estimated drug concentration between arterial and venous blood are recognized from many years [5]. The physiochemical properties of drugs, rate and extent of drug distribution also have significant influences on the drug plasma levels which ultimately affect pharmacokinetic evaluation [6]. Although, the impact of blood sampling sites on the pharmacokinetics of drugs are explored to measurable extent, but still not considered while designing the pharmacokinetic studies. Taking this into consideration, the present study was conducted to investigate the impact of various sampling routes on the pharmacokinetics of metoprolol after oral administration of metoprolol tartrate in rats.

In our investigation, significant differences were observed in the plasma PK profile of metoprolol using various routes of sample collection. The mean peak plasma metoprolol concentration obtained from jugular (C_{max}; 170.0 ng/mL) and saphenous (C_{max}; 113.2 ng/mL) routes were comparable. Similarly, retro-orbital route C_{max} (50.0 ng/mL) was comparable with C_{max} (76.2 ng/mL) of femoral route. In contrast, the C_{max} (39.3 ng/mL) obtained from tail vein sampling route was significantly different ($p \leq 0.05$) from all other sampling sites. The C_{max} obtained from tail vein sampling site was

approximately 2-4 folds less compared to other routes of sampling except for retro-orbital sampling site. Although, C_{max} (50.0 ng/mL) of retro-orbital route was around 1.3 folds higher that C_{max} (39.3 ng/mL) of tail vein sampling route (Table 1).

Significant variations were also observed in clearance (CL_F) values among various sampling site profiles. It was observed that that the retro-orbital, saphenous and femoral routes exhibited comparable CL_F values. On the other hand, CL_F estimated for tail vein sampling site was 1.5 to 2 folds higher than retro-orbital, saphenous and femoral routes. However, the CL_F (3017.0 mL/min/kg) for tail vein sampling was approximately 5-folds higher as compared to jugular vein samplings site (CL_F; 583.3 mL/min/kg). Highest systemic exposure (AUC_{0-t}; 154.0 h*ng/mL) was observed for jugular vein sampling site, followed by saphenous vein sampling (AUC_{0-t}; 102.7 h*ng/mL). For other routes of sampling, the systemic exposure was comparable with average AUC_{0-t}; 53.5 h*ng/mL. This might be possibly due to the lowest CL_F value (CL_F; 583.3 mL/min/kg) observed for jugular vein sampling site. In the similar way, the Vd (65.9 L/kg) estimated for jugular vein sampling site was approximately 5-folds lower than that observed (average Vd; 315.4 L/kg) for other sites of sample collection. The samples collected through various sampling sites represents arterial, venous, and arteriovenous blood which could be a possible reason for the observed differences in the AUC. Illum L et. Al. and Chiou WL have shown in their studies that different plasma concentrations observed between arterial and venous blood were significant to high molecule weight (>500 g/mol), high lipophilicity (Log P > 2) and high protein binding drugs (> 95%) [7]. However, in terms of metoprolol drug properties, these observations could not corroborate our observations. Metoprolol belongs to BCS class I with low molecule weight (267.36 g/mol), low lipophilicity (Log P 1.9), low plasma protein binding (~11%) [8]. Metoprolol is moderately lipid soluble acidic drug. Acidic drugs bind to albumin and are retained in the blood under physiological pH. This process facilitates fast equilibrium at steady state in plasma concentrations. In contrast, alkaline or neutral drugs bind more to tissues than plasma. The differences in the binding properties of acidic and non-acidic drugs may cause the differences in the time required to reach equilibrium between the blood and tissues. This may affect the distribution of the drug in the central and peripheral blood vessels. Consequently, the pharmacokinetic properties of the compound might be altered especially during the early stages of absorption and distribution due to differences in the sampling sites. The different metoprolol Cmax obtained from various sampling site can be explained on this basis to significant extent.

According to the literature, drugs in the arterial blood spread through the microvascular wall to the surrounding tissues of the artery for distribution and elimination. Once the drugs enter the tissues, its concentration in the arterial blood diminishes leading to lower drug concentration in the venous blood. Thus, Vd seems to be a significant factor attributing for the observed differences in the concentrations. However, Vd alone may not be the responsible factor for the observed differences in the metoprolol plasma concentrations using different sampling sites. Metoprolol undergoes a huge first pass metabolism. Approximately 50% of it is metabolized during first pass metabolism and only 3% of it is excreted through renal excretion. The possible role of first pass metabolism, differences in the body temperature and blood flow velocity at sampling sites and thereafter samples collected through various sampling sites is unknown [5,8-10].

No significant differences were observed for half-lives ($T_{1/2}$) and mean resident time (MRT) values across all the routes of sampling (Table 1). In our finding, we observed that the concentration-time curves for all the sampling sites tend to merge towards the end of plot i.e., terminal elimination phase despite of initial differences in the measured concentrations. The significant concentration differences were observed in the plasma concentrations at early time points. Post 4-6 hours of dosing, the observed plasma concentration is low and comparable for all the sampling sites (Figure 1).

Based on this study outcome, we propose that this finding could be helpful to identify relevant routes resulting into similar PK parameters. This could be an important aspect while dealing with the long-term studies on cannulated animals, where loss of patency is very common. Two or more routes of sample collections can be used in such studies without having significant difference in the actual PK parameters. We propose that meticulously identifying sampling sites during initial time points after dosing may not have significant influences on the PK parameters, while during the terminal phases of sample collection, clubbing various sampling sites does not impact PK parameters. Hence, pragmatic determination of more than one sampling sites may help to mitigate problems like loss of patency, burden on same sampling sites especially during long term studies.

Conclusion

Typically, it is assumed that the sampling sites should not influence the PK endpoints of a study. Based on our investigations, it is explicit that the sampling sites have profound impact on the PK parameters of metoprolol after single dose oral administration in rats. Statistically significant differences were observed in the estimated PK parameters for different sampling routes. Hence, careful selection of route of sample collection should be opted based on the rational of study.

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Conflicts of Interest

The authors declare that there are no competing interests to declare. The pre-print of this manuscript is available in Research Square, posted on 19th Aug, 2022 [11].

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Author's Contribution

Y.S., P.S., S.K.T. conceptualized, analysed the raw data, wrote, and reviewed the manuscript. T.T. and G.B. conducted the experiments. R.A.R. contributed to the bioanalysis of study sample. Further, the manuscript has been read and approved by all the authors and each author believes that the manuscript represents original work.

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