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Pharmacokinetics of metoprolol enantiomers following single and multiple administration of racemate in rat

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Abstract

The chiral β-adrenergic blocking agent metoprolol (MET), which is marketed as a racemate, is a highly extracted drug with rapid absorption. The enantiomeric disposition of MET is reported following racemic administration as a single and as multiple oral dosing four times per day for four days in male Sprague–Dawley rats ($n=6$ in each group). Plasma was collected and enantiomeric concentrations of MET were determined using a stereospecific HPLC assay. The R/S ratio for AUC is not statistically different from unity either after single or after multiple administration of racemate. The oral clearance after single dose was 1.99 ± 0.87 and 2.26 ± 0.85 ml min^{-1} kg^{-1} for R- and S-MET, respectively. These values were decreased to 0.59 ± 0.21 and 0.64 ± 0.26 ml min^{-1} kg^{-1} after multiple administration of racemate. The corresponding values for the elimination half-lives were approximately 35 and 33 min after single and multiple dose administration for both enantiomers, respectively. These results may suggest a saturable first pass metabolism of MET as its enantiomers are accumulated in plasma following multiple dosing in the rat model.

Keywords: Metoprolol; Pharmacokinetics; Enantiomers; Single dose; Multiple dose

1. Introduction

Hypertension is one of the major risk factors associated with heart disease. In the therapy of hypertension different drugs with different mechanism of actions are used. β-Adrenoceptor blocking agents are now widely accepted as first line drugs in the treatment of hypertension and angina (Cruickshank et al., 1994a,b). Among them metoprolol (MET) is a cardioselective β-blocker which is marketed as a racemic mixture and administered to patients with chronic heart failure to improve cardiac function, capacity for physical exercise and lessen the symptoms of heart failure (Waagstein et al., 1998; Merit-HF Study Group, 1999). A moderate enantioselectivity of MET pharmacokinetics has been reported in humans (Lennard et al., 1983). Furthermore, the pharmacokinetic studies with metoprolol to investigate the effect of age (Quarterman et al., 1993; Vermeulen et al., 1993) and to explain the pharma-

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cokinetics and pharmacodynamics of racemic MET have been described (Benfield et al., 1986). Although the accumulation of racemic metoprolol after multiple dose administration in humans has been established (Kendall et al., 1980; Myers and Thiessen, 1980), the pharmacokinetic characteristics of MET enantiomers after multiple dosing has not been fully investigated. Consequently, they have not accounted for potential enantioselectivities in pharmacokinetic processes.

The importance of performing multiple-dose studies in assessing drugs including β-blockers has been suggested (Kendall et al., 1980). Moreover, to the best of our knowledge no studies are available dealing with the pharmacokinetics of MET enantiomers after multiple dosing in rats or in humans. We, therefore, sought to study the pharmacokinetic characteristics of MET enantiomers after single and multiple oral administration of racemate in the rat model. Because the rat is a commonly used animal to study the pharmacokinetic, pharmacology and toxicology of enantiomers, we decided to examine the feasibility of the rat as an animal model.

2. Materials and methods

2.1. Chemicals

Metoprolol tartarate was obtained from Sigma (IL, USA). The internal standard (IS), (+)-naproxen chloride, was synthesized in our laboratory. All other chemicals and reagents were HPLC or analytical grade.

2.2. Surgery and animal maintenance

Male Sprague–Dawley rats weighing between 245 and 285 g were kept on a light–dark cycle of 12 h at a room temperature of 25°C. They were fasted for about 8 h before and 2 h following drug administration for the single dose study and also before the last dose of multiple dosing, with free access to water. The animals were catheterized with silastic tubing (0.025 in i.d. × 0.037 in o.d.; Dow Corning, Midland, MI, USA) at the right jugular vein while they were under general anesthesia with pentobarbital administered via the peritoneal route. The animals were allowed to recover overnight prior to the experiments. During this time the animals were individually stored in 18″ × 95″ × 8″ in polycarbonate rodent cages.

2.3. Dosing and sample collection.

Racemic MET dissolved in normal saline was administered in doses of 20 mg kg^{-1} (1 ml kg^{-1}) either as single or as multiple doses (four times per day for 4 days) by gavage. Blood (250 μl) was collected from the jugular vein cannula just prior to and at 2, 10, 20, 30 and 45 min and 1, 1.5, 2, 2.5 and 3.5 h after drug administration. Between each blood sample collection 250 μl normal (0.9%) saline was administered via the jugular vein cannula as fluid replacement and the cannula was heparinized (10 U ml^{-1}). Blood samples were immediately centrifuged and the plasma was separated and immediately frozen at −20°C until analyzed.

2.4. Stereospecific HPLC assay of MET

Concentrations of *R*- and *S*-MET in plasma were determined utilizing a previously reported stereospecific HPLC method (Bhatti et al., 1995). Briefly, the samples were extracted with a mixture of methyl tertiary butyl ether (MTBE):isooctane (75.25, v/v), after addition of (+)-naproxen chloride as the internal standard and subsequent alkalization with 1 M sodium hydroxide. After the extract was evaporated, the resulting residues were reconstituted in the mobile phase and injected into the HPLC. The enantiomers were separated at ambient temperature on a commercially available 4.6 × 250 mm amylose carbamate-packed chiral column (Chiracel OD-H) and chromatographed via normal phase HPLC using fluorescence detection.

2.5. Pharmacokinetic data analysis

The area under the plasma concentration versus time curve (AUC) for each enantiomer was calculated by the linear trapezoidal rule. The area from the last concentration point (C_{last}) to infinity was

calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through at least three data points in the terminal elimination phase. The terminal elimination half-life ($t_{1/2}$) was calculated by $0.693/\beta$. Oral clearance ($CL_{p.o.}$) was calculated by dividing the total administered enantiomeric dose by the AUC after oral administration ($AUC_{p.o.}$). Maximum plasma concentrations (C_{max}) and the time for maximal concentrations to be reached (T_{max}) were derived from graphical analysis of plasma MET concentrations versus time. Volume of distribution (V_d/F) was calculated by dividing the corresponding $CL_{p.o.}$ by β. After multiple administration of MET the $CL_{p.o.}$ was calculated as $D/AUC_{0-\tau}$, where $AUC_{0-\tau}$ for each enantiomer was the corresponding AUC within a dosing interval at plateau. CL/F was calculated by dividing the total administered enantiomeric dose with the $AUC_{0-\tau}$ after multiple oral administration.

2.6. Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of MET enantiomers either after single or after multiple dose administration of MET were assessed utilizing a two-tailed Student's *t*-test for paired data. Comparisons of *R*- versus *R* and *S* versus *S*-MET after single and multiple dose administration were made by utilizing a two-tailed test assuming equal variance. In all tests a probability level of significance pre-set at $\alpha = 0.05$. Results are expressed as mean $\pm$ S.D.

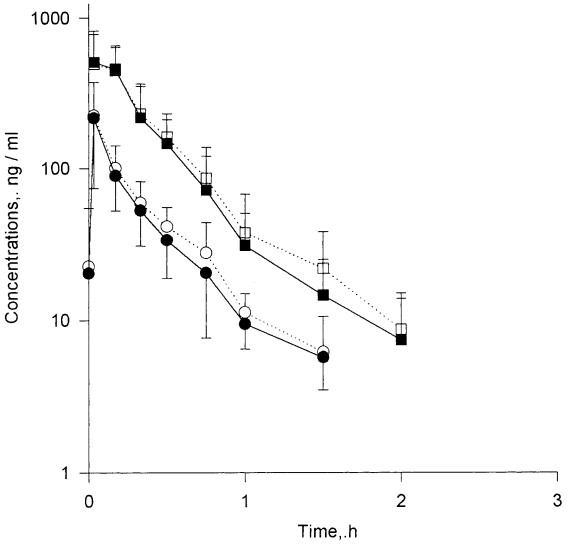

Fig. 1. Average plasma concentration vs. time profiles for *R*- and *S*-MET after administration of the racemate as a single or multiple doses. ○, *R*-MET after single dose; ●, *S*-MET after single dose; □, *R*-MET after multiple dosing; ■, *S*-MET after multiple dosing; error bars represent S.D.

3. Results

3.1. Single dose administration

Average plasma concentration of MET enantiomers after a single dose of racemate are depicted in Fig. 1. Table 1 summarises the pharmacokinetic parameters of *R*- and *S*-MET. Average maximum plasma concentrations of MET were about 235 ± 138 μg l^{-1} and 227 ± 132 μg l^{-1} for *R*- and the *S*-enantiomer, respectively. These values were attained approximately 12 min after dosing the rats and declined with a half-life of 35 min for both enantiomers. Although no significant differences were found in $AUC_{0-\infty}$ between the two enantiomers, a trend of increase in this value was observed as compared to *S*-MET (97 ± 38 μg h l^{-1}) with *R*-MET (83 ± 29 μg h l^{-1}). The mean $AUC_{0-\infty}$ value of the *R*-enantiomer was higher than that of the *S*-enantiomer (97 vs. 83 μg h l^{-1}). Consequently, the average oral clearance were greater for *S*-MET (2.66 ± 0.85) than that of *R*-MET (1.99 ± 0.87).

3.2. Multiple oral administration

The average plasma-concentration versus time curves of MET enantiomers after multiple oral administration of racemate are illustrated in Fig. 1. The average C_{max} values of the MET enantiomers reached 285 ± 142 and 289 ± 151 μg l^{-1} for *R*- and *S*-MET, respectively. The terminal half-lives did not significantly change upon repetitive dosage for both enantiomers. The mean plasma enantiomeric concentrations of MET were increased significantly than the corresponding $AUC_{0-\infty}$ after a single oral dose. Consequently, the $CL_{p.o.}$ were higher after single dose administration.

Table 1
Pharmacokinetic parameters of metoprolol enantiomers after administration of racemate to rats[a]

Pharmacokinetic parameters	Single dose		Multiple dose	
	R	*S*	*R*	*S*
$AUC_{0-\infty}$[b] (μg h l^{-1})	97*,#	83	309*	295
	38	29	95	109
$CL_{p.o.}$ (l min^{-1} kg^{-1})	1.99*,#	2.26	0.59*	0.64
	0.87	0.85	0.21	0.26
$t_{1/2}$ (min)	35	35	33	32
	11	13	9	6
V_d/F (l kg^{-1})	63	76	40	39
	19	20	25	24
Rat weight (g)	261		277	
	12		9	

[a] Data presented as mean (S.D.).
[b] $AUC_{0-\tau}$ after multiple dosing.
* Significantly different from the corresponding enantiomer, $P<0.05$.
# Significantly different from the corresponding enantiomer after multiple dose, $P<0.05$.

4. Discussion

4.1. Single dose administration

Plasma concentration-time profiles (Fig. 1) indicate that the pharmacokinetics of MET is stereoselective in rats after oral administration of racemate. Slightly but significantly greater amounts of the *R*-enantiomer of MET were seen in plasma after a 20 mg kg^{-1} p.o. dose. Consequently, the $CL_{p.o.}$ were higher for the *S*-enantiomer as compared with the *R*-MET. MET undergoes hepatic first-pass extraction and its metabolism is related to debrisoquin oxidation phenotype (Lennard et al., 1982). The higher $CL_{p.o.}$ of *S*-MET may be due to its higher intrinsic clearance, as MET is a low protein bind drug (Appelgren et al., 1974) and no differences in free fraction between the two enantiomers are expected. Our results are in agreement with the recent report on the enantiomeric disposition of MET in different age groups in the rat (Vermeulen et al,. 1993).

MET is rapidly absorbed and C_{max} was reached at 12 min post dose for both enantiomers. Further, the estimated average $t_{1/2}$ of the enantiomers were similar (35 min) for both *R*- and *S*-MET indicating that the rat is an extensive metabolizer of MET as it has been reported that the half-life of *R*-MET is longer than that of *S*-MET in poor metabolizers, whereas no differences is observed in extensive metabolizers (Lennard et al., 1986) of MET. Therefore, the rat appears to be a good animal model as the majority of Caucasians are 'extensive metabolizers' of MET.

4.2. Multiple dosing

The average plasma-concentration versus time curves of MET enantiomers after single and multiple dose administration (Fig. 1) show that the plasma concentrations at steady state were greater than that predicted by the single dose data as indicated by the comparison of the mean area under the plasma concentration–time curve for the single dose and the dosage interval areas during multiple dosing. The mean increases in this value were 62 and 74% for *R*- and *S*-MET, respectively (Table 1). The plausible explanations for this increase in the AUC during multiple dosing are: (1) a decrease in systemic clearance of MET during multiple treatment because of the potential reduction in hepatic blood flow, and/or (2) a decrease in presystemic clearance of MET upon repeated administration of the drug due to the saturation of the first-pass effect. As MET is a

low protein binding drug (10%), therefore, a change in clearance due to a change in V_d is not expected. Furthermore, the $t_{1/2}$ of MET is not changed after chronic administration as compared to the single dose treatment, therefore, a decrease in systemic clearance upon multiple dose administration of MET is unlikely.

Another explanation for the greater value for AUC during multiple dosing is the saturation of first pass-effect due to repeated administration of MET, which leads to a higher systemic availability of enantiomers. Nonetheless, other investigators have also found that the fraction of the dose available systemically after long term treatment have increased in human (Regardh et al., 1975; Jordo et al., 1980). The 26 and 10% increase in AUC after multiple dosing have been reported by these authors. Furthermore, marked effects of multiple dosing on bioavailability of metoprolol have been reported by Kendall et al. (1980) and Myers and Thiessen (1980). In these two studies the mean increase in AUC was 41 and 148% after treatment with metoprolol 100 mg twice daily for 8 days and 6–12 weeks, respectively. The pronounced increase in the latter may be explained by the small number of subjects that they included in the study. Our results, however, are more consistent with Collste et al. (1980) in which a 57% increase in the AUC during multiple dosing with MET 50 mg three times daily for at least 3 weeks has been reported. A similar increase in AUC during long term treatment for other β-blockers including acebutolol have also been reported (Mostafavi and Foster, 1998).

As many of the pharmacokinetic features of MET are comparable to those in the human, it appears that the rat is an appropriate animal model for pharmacokinetic studies of MET. Furthermore, this study illustrate the importance of considering multiple dose studies in assessing the pharmacokinetics of MET enantiomers.

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