A DOUBLE-PEAK PHENOMENON IN THE PHARMACOKINETICS OF ACEBUTOLOL ENANTIOMERS AFTER ORAL ADMINISTRATION: DISCONTINUOUS ABSORPTION OF ACEBUTOLOL.

S. ABOLFAZL. MOSTAFAVI*, AND ROBERT. T. FOSTER

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2N8

ABSTRACT

Acebutolol (AC) is a chiral β-adrenergic blocking drug, which is used primarily in the treatment of hypertension. After oral administration of a racemic mixture of the drug a secondary peak is observed in plasma concentration-time profiles of both R and S-enantiomers of AC. In the present study the pharmacokinetics of AC enantiomers in rats after intraduodenal (ID) and intraileal (II) administration was investigated to inspect the generation of double peaks due to window absorption. Another possibility for the formation of double peak due to bile depletion in the gut lumen was investigated in male Sprague-Dawley rats. In a bile duct cannulated rat, the blood concentration-time profiles contained two peaks. The bioavailability after oral administration for R- and S-enantiomers of AC were 36% and 34% which increased to 47% and 46% following II administration respectively. These values increased to 69% and 68% after ID administration. While following II administration of the racemate, the double peak disappeared, it retained after ID administration . These results suggest that the double peak phenomenon observed after oral administration of AC is due to site dependent absorption of the drug.

Key Words: Acebutolol; Pharmacokinetics; Enantiomer; Site dependent; Double peaks

INTRODUCTION

Acebutolol (AC) is a cardioselective β-Adrenergic blocking agent which is used orally for the treatment of hypertension, suppression of premature ventricular contractions and other cardiac arrhythmias (1,2). It is a chiral drug and after oral administration of the racemic mixture of the drug in rats and human its concentration time profiles exhibit two maxima for both R and S- enantiomers (3-11).

Mechanisms involved for the occurrence of the double peaks in the plasma profiles of drugs include the enterohepatic recycling (12), discontinuous gastrointestinal absorption (13-15), formation of poorly absorbed micelle complex of the drug with bile salts (16,17), variation in gastric emptying time (18,19), and pH (20), and reversible metabolism (21). For AC however, it has been reported that enterohepatic recycling is not responsible for the double peaks phenomena (3) that is observed after oral administration since multiple peaks disappear after i.v. administration (22). Variable gastric emptying and gastric pH are other possibilities which might be responsible for the appearance of the double peaks in concentration-time profile of the drug. In the previous report (5), the influence of variable gastric emptying and gastric pH on double peaks formation was investigated by co-administration of cimetidine and it was suggested that these factors are not responsible for the appearance of multiple peaks.

As the double peaking phenomenon for AC was only observed after oral dosing, it is clear that the gastrointestinal tract must play an important role in generation of these phenomena. Therefore in this study the pharmacokinetics of AC enantiomers after administration of drug in different parts of the gastrointestinal tract was investigated to examine the existence of an absorption window. Furthermore, the influence of the bile depletion on the intestinal absorption of AC was investigated. The hypothesis that

Correspondance: Abolfazl. Mostafavi,Faculty of Pharmacy & Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.E-mail: mostafavi@pharm.mui.ac.ir

reversible metabolism of diacetolol (DC), a major metabolite of AC, to parent drug may be responsible for this multiple peaking was also examined.

MATERIALS AND METHODS

Chemicals

Racemic AC and the internal standard (IS), pindolol, were purchased from Sigma (Illinois, USA). The metabolite, DC (as hydrochloride salt), was obtained from Rhone-Poulenc Rorer Canada Inc. (Montreal, Que, Canada). All other chemicals and reagents were HPLC or analytical grades.

Surgery and animal maintenance

Male Sprague-Dawley rats weighing between 210-300 g were used in the study. The rats were housed under standard conditions in the animal unit where the room temperature was approximately 25 °C. Rats were deprived of food for about 8 hours prior to, and two hours following the drug administration, with free access to water. During the period that food was withheld, the rats were kept in cages with wide screen bottoms to prevent coprophagy. The day before of the experiments, animals were anesthetized by intraperitoneal injections of pentobarbital. The intrajugular, intrduodenal (ID), intraileal (II), and bile duct catheters needed in the experiments were inserted under anesthesia. Each rat had a polyethylene catheter (0.025 in. i.d. × 0.037 in. o.d.; Dow Corning, Midland, MI, USA) inserted into duodenum, ileum and bile duct. The same type of catheter was inserted into the right jugular vein for blood sampling. The catheters were passed under the skin and exteriorized at the back of the neck. In a bile duct cannulated rat the bile was collected in a vial which was installed in a pre-designed jacket on the back of the rat to prevent bile secretion into duodenum.

Drug administration

AC solution (50 mg kg^{-1}) was administered ID, or II, in two groups of rats. The same dose was administered orally to a bile duct cannulated rat by gavage. DC was administered either as a bolus injection or by the oral administration to the last group of rats by gavage to evaluate the reversible metabolism of AC. Blood samples were withdrawn from the heparinized catheter placed in the jugular vein at 0, 2, 15, 30, and 45 min,. 1, 1.5, 2, 2.5, 3.5, 5.5 and 8 h after administration of the drug to all groups.

Between each blood sample collection, 0.25 ml of normal 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 they were analyzed. Urine was collected and pooled for 24 h following drug administration. Urine samples were kept frozen at -20 °C until they were analyzed.

Measurement of AC and DC in plasma and urine

Concentrations of R- and S-AC and R- and S-DC in plasma and urine were determined by a normal phase HPLC using fluorescence detection. The analytical procedure has been described in the previous publications (23,24).

Pharmacokinetic data analysis

The area under the plasma concentration-time curve (AUC) for each individual rat was calculated by using the linear trapezoidal rule. The area to infinite time beyond the last sample was estimated by dividing the predicted plasma concentration at the last time point C_{last} to the terminal rate constant (β) which was calculated by regression through at least three data points in the terminal elimination phase. The terminal elimination half-life ($t_{1/2}$) was calculated from the formula $0.693/\beta$. Clearance (CL) was calculated as D/AUC, where D was the dose of the enantiomers that was administered and AUC was the corresponding area under the plasma concentration-time curve of enantiomers. Volume of distribution (Vd/F) was calculated by dividing corresponding CL to elimination rate constant. As the urinary excretion of AC is virtually complete within the first 24 h after single dose administration, renal clearance (CL_R) of each enantiomer was estimated by dividing the cumulative 24-hour urinary excretion of each enantiomers to the corresponding $AUC_{(0-\infty)}$ value. The bioavailability of the duodenal and ileal doses of AC enantiomers were determined from the ratio of the AUCs after the duodenal or ileal and IV doses. The AUC of the IV doses were taken from Ref. 16. The plasma AC concentration-time profiles were individually inspected to determine the maximum plasma drug concentration (C_{max}) and the time of occurrence (T_{max}) for each rat that were under study.

Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of enantiomers after administration of AC in different parts of gastrointestinal tract and oral administration of racemic mixture were determined by one-way ANOVA followed by Benferoni *post hoc test*. Assumptions of homogeneity of variance were tested by using the Levene test prior to ANOVA analysis. Comparisons between the S- and R-AC pharmacokinetic parameters within each study group were assessed by a two-tailed student's t-test for paired data. In all tests, a probability level of significance was pre-set at α=0.05. All results are presented as mean values and standard deviations unless stated otherwise.

RESULTS

Pharmacokinetic parameters of R- and S-AC after ID and II, administration as well as after oral administration of the drug in a bile duct cannulated rat are presented in Table 1. The pharmacokinetic parameters of AC after oral administration that were taken from reference 4 are also shown in this table. The individual enantiomer plasma concentration-time curves for AC enantiomers after ID administration are shown in Fig 1. The first peak appeared during the first 30 min. and the second one, was observed after 2-2.5 hr. The individual enantiomer plasma concentration-time curves for both enantiomer of AC, which were administered into the ileum, are shown in Fig 2. These profiles clearly depict that the double peaking in the plasma profiles of both enantiomers of AC disappear after II administration of the drug. By inspection of the respective $AUC_{(0-\infty)}$, it appears that the extent of AC absorption after ID administration was significantly greater ($p = 0.05$) than those after either oral or ileal administration. The absorption of AC after II administration was generally greater than that after oral administration, but the difference was not statistically significant. The CL/F values after both ID and II administration of AC as compared with oral dosing decreased but differences were significant only after duodenal administration. The mean bioavailability obtained by the AUC method increased from 36% and 34% for oral doses to 47% and 46% for ileal and 69% and 68% for duodenal dosing of the R- and S-AC respectively (Table. 1). No significant differences between these treatments were observed for CL_R or $t_{1/2}$.

A direct comparison of the T_{max} and C_{max} data after drug administration at different sites was complicated by double-peaking in the AC plasma profiles after oral and ID administrations. The T_{max} and C_{max} reported in Table 1 refer to the times and concentrations at which the peak plasma concentrations occurred irrespective of whether it was the first or second peak.

The $AUC_{(0-5.5)}$ of DC after ID administration was greater than that after either oral or ileal administration of AC. However, this difference was significant only after oral administration of both enantiomers. In all groups, the amount of R-DC recovered in urine was significantly greater than that of S-DC in urine 24 h after drug administration. The plasma concentration-time profiles of AC enantiomers after oral administration of the racemic mixture in a bile duct cannulated rat which are presented in Fig 3 clearly depict the double peaking for both R- and S- enantiomers of AC.

After either oral or iv administration of DC, no AC could be detected either in blood or in urine samples indicating no reversible metabolism for AC enantiomers.

DISCUSSION

Peritoneal surgery and cannulation of different sites of intestine are common means for determination of the absorption profile of drugs when they are administered at different sites of gastrointestinal tract. In these studies, a solution of the drug were typically injected into a particular region of the gut of the laboratory annimals through a cannula. The absorption profile of the drug was then determined by assessment of pharmacokinetic parameters of the drug. This method of evaluation of the absorption of drug has also been validated for determination of the double peak phenomena observed after oral administration of drugs (16). The reasoning for using rat in this investigation was based on reports that this animal is a good animal model for such studies (3).

Various models of discontinuous gastro-intestinal absorption have been developed to describe the multiple peaks which are observed after oral administration of drugs (13-15,25). Among these models, the existence of a non-absorbing gastro-intestinal segment between one or more absorption sites (7,9,25) is the more

Table 1. Pharmacokinetic parameters of acebutolol enantiomers after oral administration of racemate in different sites of gastrointestinal tract in rats. Data are presented as mean (SD).

Pharmacokinetic Parameters	Oral[d]		Duodenal		Ileal		Bile duct	
	R-AC	S-AC	R-AC	S-AC	R-AC	S-AC	R-AC	S-AC
AUC, μg h l^{-1}	3155[a] (1003)	3007 (895)	6113[b] (512)	6000[b] (1074)	4123 (702)	4080 (678)	4742	4550
CL, ml min^{-1} kg^{-1}	138[a] (39)	149 (42)	69[c] (6)	71[c] (14)	103 (15)	104 (15)	88	92
$t_{1/2}$, h	1.92 (0.60)	1.86 (0.60)	1.56 (0.20)	1.44 (0.05)	2.59 (0.87)	2.79 (0.91)	1.94	1.86
Cl_R, ml min^{-1} kg^{-1}	24.63 (5.56)	25.25 (6.43)	22.03 (1.1)	24.26 (1.42)	17.04 (3.31)	18.06 (3.45)	18.64	21.63
Vd / F, L kg^{-1}	22.70 (8.59)	23.61 (8.55)	9.34[b] (2.00)	8.22[b] (1.61)	23.10 (9.08)	25.18 (9.65)	14.76	14.75
$\sum X_{11}DC$	165[ab] (49)	80[b] (27)	854[ab] (269)	560[b] (219)	282[a] (84)	156 (41)	506	285
T_{max}	1.17 0.86	1.17 0.86	1.67 1.04	1.67 1.04	0.21 0.09	0.26 0.15	0.25	0.25
C_{max}	2343 2079	2270 2090	1686 206	1715 151	2220 774	2114 744	2636	2651
$Ae_{0-\infty}$,%	20.15 (10.77)	19.23 (10.60)	32.32[b] (3.31)	34.75[b] (5.07)	16.62 (2.41)	17.40 (2.62)	21.21	23.63

a. Significantly different from corresponding enantiomer, $p < 0.05$; **b.** Significantly different from corresponding enantiomer for the oral and ileal group, $p < 0.05$; **c.** Significantly different from corresponding enantiomer for the oral group $p < 0.05$

d. Adapted from reference 4.

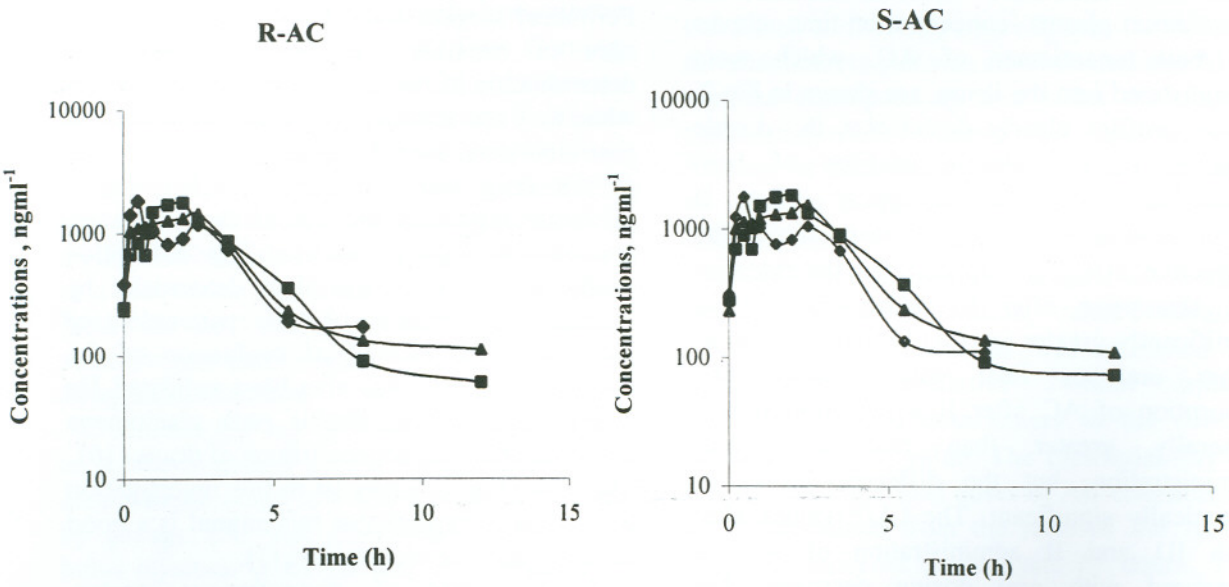

Figure 1. Individual plasma concentration of AC enantiomers following duodenal administration of a solution (50 mg kg^{-1}) to three rats.

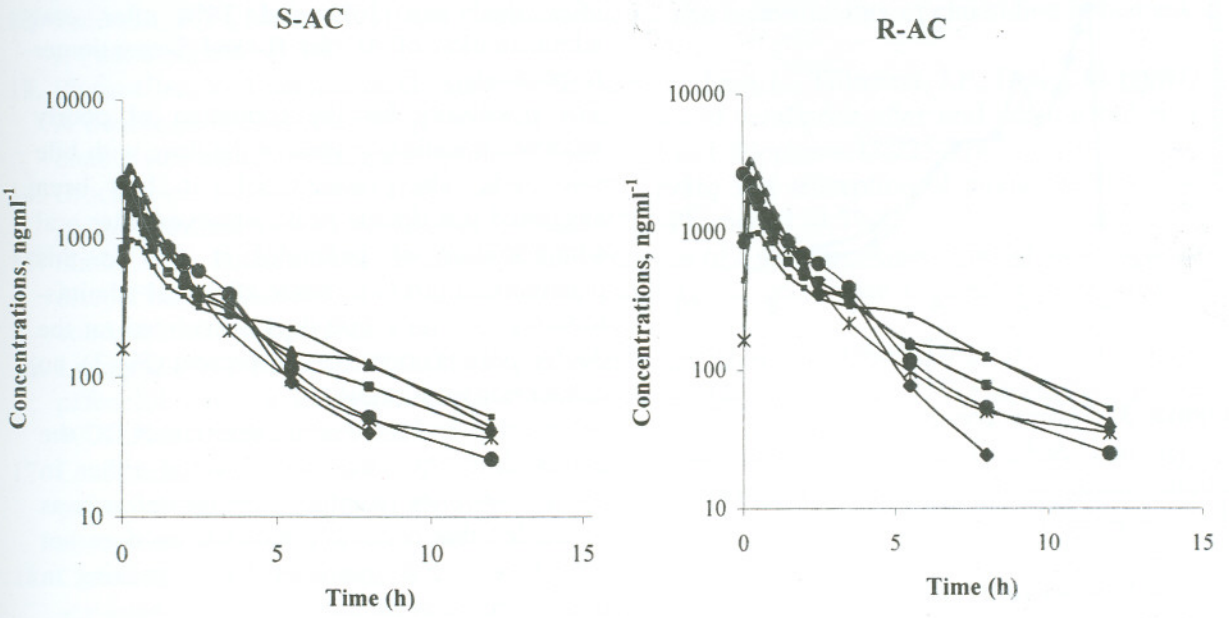

Figure 2. Individual plasma concentration of AC enantiomers following ileal administration of a solution (50 mg kg^{-1}) to six rats.

probable model, which may explain discontinuous gastrointestinal absorption. Pharmacokinetic analyses of the data demonstrate that the extent of absorption from the duodenum was significantly greater than that from ileal administration which is consistent with the absorption window theory. This theory proposes that a drug may be initially absorbed largely from a specific area within the proximal gastrointestinal tract (i.e. duodenum), resulting in the initial peak plasma concentration. A second absorption window more distal in the gastrointestinal tract (i.e., ileum or colon) may absorb some of the remaining drug and produce a smaller, second absorption peak after 2 or 3 hours.

It was found that duodenal administration of AC had no effect on double peaks in the plasma concentration time profile (Fig 1) which also confirms the previous report that gastric emptying has no effect on double peaks generation (5). Instead, the double peaks disappeared following ileal administration of AC (Fig 2) confirming the hypotheses that absorption window plays an important role in generation of the double peak phenomena in plasma profiles of AC enantiomers. Similar results have been reported for the appearance of the double peaking phenomena after oral dosing of veralipride (7).

Because of the lower $AUC_{(0-\infty)}$ after oral administration of AC as compared with ID administration, it is apparent that stomach plays a role in the absorption of AC even though it is unlikely to be due to a direct effect. Differences between the extent of absorption and the bioavailability of AC after oral administration as compared with duodenal or ileal administration may suggest that the absorption of AC in gastrointestinal tract is pH dependent. After oral administration it may be hydrolyzed in acidic pH of stomach thus, it is less available to be absorbed. Consequently, the bioavailability of both enantiomers after oral administration is lower than those after duodenal or ileal administration. The question may arise about the reasons for difference in extent of absorption and bioavailability after duodenal and ileal administration even though the pH of the ileum is more basic than duodenum. This could also be explained by the time that the drug is in contact with the absorption surface. As the time that a drug resides in absorptive surface after duodenal administration is more than that after ileal administration, the extent of absorption could be increased.

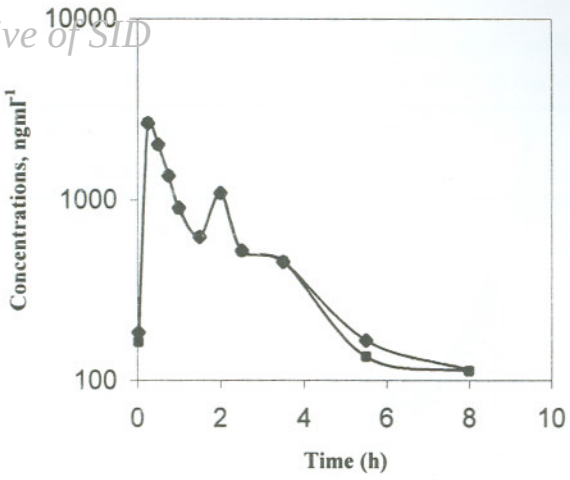

Figure 3. Plasma concentration of R- (filled circle) and S-AC (open circle) after oral administration (50 mg kg^{-1}) of racemate in a bile ducts cannulated rat.

A trend towards an increase in $AUC_{(0\text{-}5.5)}$ of DC after duodenal administration which is a reflection of increased AC availability failed to reach a significant level. Consistently, there was a trend towards an increase in the amount of AC and a significant increase in the amount of DC which were recovered in the urine. A higher recovery of AC and DC in urine after duodenal administration may explain the finding that systemic availability of duodenal administration was 69% and 68% compared to 47% and 46% after ileal and 36% and 34% after oral administration of AC for R- and S-enantiomer respectively.

The possibility for the formation of poorly absorbed micelle complex of the drug with bile salts was also investigated. It has been suggested that double peaks observed after oral administration of pafenolol is due to this phenomenon (16,17). Since after oral administration of AC to a bile duct cannulated rat the double peak phenomena was present (Fig 3), no further rats were studied.

After either oral or iv administration of DC the parent drug, AC, was not detected either in plasma or urine samples. Therefore, it was concluded that reversible metabolism does not contribute to appearance of double peaking in plasma profile of AC.

In conclusion, our study demonstrates that duodenum may be the main site for the absorption of AC after oral administration, which is probably underlying mechanism for observation of the double peak phenomena after oral administration of the drug.

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