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Pharmacokinetics of Acebutolol and Its Main Metabolite, Diacetolol After Oral Administration of Acebutolol in Rabbits with Carbon Tetrachloride-Induced Hepatic Failure

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Pharmacokinetic characteristics of acebutolol and its main metabolite, diacetolol, following a single 10 mg/kg oral dose, were investigated in rabbits with carbon tetrachloride-induced hepatic failure. Plasma concentrations of acebutolol and diacetolol were determined by a high performance liquid chromatography assay. The area under the plasma concentration-time curves (AUC) and maximum plasma concentration (C_{max}) of acebutolol were significantly increased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. The ratio of the diacetolol to total acebutolol in plasma (i.e., metabolite percentage rate) was significantly decreased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. Volume of distribution (V_d) and total body clearance (CL_t) of acebutolol were significantly decreased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. Slope of terminal phase (β) of acebutolol was significantly decreased in hepatic failure rabbits. These findings suggest that the V_d, CL_t and β of acebutolol were significantly decreased as a result of inhibition of the hepatic metabolism in moderate to severe hepatic failure rabbits. Therefore, dose adjustment may be necessary for acebutolol in hypertensive patients with hepatic damage.

Key words: Acebutolol, Diacetolol, Pharmacokinetics, Carbon tetrachloride-induced hepatic failure

INTRODUCTION

Acebutolol is beta-adrenergic blocking agent, which is widely used in the treatment of hypertension and cardiac arrhythmias (Alhenz-Gelas *et al.*, 1978; Martin *et al.*, 1978; Baker *et al.*, 1978). Acebutolol is well absorbed from the gut following oral administration. The drug undergoes significant hepatic first-pass metabolism and is converted first to the primary amine (Roux *et al.*, 1980, Mclean *et al.*, 1978), then acetylated to diacetolol. Major metabolite of acebutolol, diacetolol, has pharmacologic properties similar to those of the parent compound (Basil *et al*, 1978, Flouvat *et al.*, 1981). Both acebutolol and diacetolol are excreted in bile and urine (George *et al.*, 1978, Gulaid *et al.*, 1981, Gabriel *et al.*, 1981).

Beta-adrenergic blocking agents are known to possess marked differences of their pharmacokinetic and pharmacodynamic properties. Particularly, differences in pharmacokinetic characteristics, such as absorption in the gastrointestinal tract, hepatic metabolism, plasma protein binding, volume of distribution and, renal and biliary elimination of the drugs play an important role. Therefore, it appears reasonable to assume that patients with altered pharmacokinetic parameters for beta-adrenergic blocking agents are likely to exhibit different pharmacodynamic properties (Borchard, 1990). Thus, liver disease may be of clinical significance since the disease may determine the choice of the appropriate drug or the dose regimen of a given drug. However, extent of pharmacokinetic change was not systematically studied for acebutolol in patients with hepatic failure.

The purpose of this study was to investigate the pharmacokinetic changes for acebutolol and the main metabolite, diacetolol, after oral administration of acebutolol in normal rabbits and rabbits with moderate to severe hepatic damage with carbon tetrachloride.

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MATERIALS AND METHODS

Materials

Acebutolol, diacetolol and an internal standard, triamterene, were purchased from Sigma Chemical Co. (St. Louis, MO). Phosphoric acid, Sodium hydroxide and KH_2PO_4 were purchased from Shinyo Pure Chemicals. Co. (Osaka, Japan). Diethyl ether and acetonitril were purchased from Merck (Darmstadt, Germany). The other chemicals were in reagent grade. HPLC (Model CBM-10A, Shimadzu Co., japan), syringe pump (Model 341B, Sage Co., Japan), vortex mixer (Scientific Industries, Korea) and centrifuger (Abbot Co., USA) were used in this study.

Animals

White male New Zealand rabbits weighing 2.0~2.5 kg were fasted at least 24hr before experiment. Free access to water was allowed. Moderate and severe hepatic failure rabbits induced with subcutaneous injection of 1.2 ml/kg and 2.0 ml/kg carbon tetrachloride (CCl_4 : olive oil=20 : 80, v/v), respectively. Under 25% urethane (4 ml/kg) anesthesia, the right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling at room temperature.

Drug administration

Acebutolol 10 mg/kg was given orally to rabbits. Blood samples (1.2 ml) were withdrawn from the femoral artery at 15, 30 min, 1, 2, 3, 4, 6, 9, 12 and 24 hr after the acebutolol administration. Plasma samples were obtained by centrifugation at 3,000 rpm for 10 min. An aliquot (0.5 ml) of plasma was stored at -70°C until analytical procedure for acebutolol. Saline was infused at the rate of 1.5 ml/hr to ear vein by syringe pump.

Assay and HPLC conditions

Plasma concentrations of acebutolol and diacetolol were determined by an HPLC assay (Buskinl *et al.*, 1982). 0.1 ml of triamterene (2 μg/ml) as an internal standard and 1 M NaOH 0.2 ml and 4 ml of ether were added to 0.5 ml of plasma sample. The mixture was then vortexed for 10 min and centrifuged at 3,000 rpm for 5 min. 3.5 ml of the organic layer was transferred to a clean test tube and evaporated to dryness under a stream of nitrogen at 40°C. The residue was then dissolved in 0.2 ml of 0.05% phosphoric acid and centrifuged at 6,000 rpm for 3 min. 50 μl of the solution was injected onto the HPLC system.

The HPLC system consisted of a solvent delivery pump (Model CBM-10A, Shimadzu Co., Japan), a variable UV absorbance detector and computing intergrater. The UV detecter wavelength was set at 243 nm and the separation was carried out at room temperature. The stationary phase was a Shin-Pack CLC-ODS column (4.6 × 250 mm, Shimadzu Co., Japan). Mixtures of acetonitrile : water : 0.1 M phosphate buffer (pH = 4) (22 : 68 : 10, v/v/v/) were used as the mobile phase. The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter. At a flow rate of 1.0 ml/min, the retention times were as follows: diacetolol, 4.2 minutes, internal standard, 7.8 minutes, and acebutolol, 10.5 minutes.

Pharmacokinetic analysis

Plasma concentration of acebutolol was fitted to two compartment open model by a nonlinear least square regression using a MULTI program (Yamaoka *et al.*, 1971). In the regression analysis study, simplex algorithm was used to obtained minimized objective function (i.e., sums of residual). The area under the plasma concentration-time curves (AUC) was calculated by trapezoidal rule and total body clearance (CL_t) was calculated by the equation CL_t=Dose/AUC. The maximum plasma concentration (C_{max}) and time to reach the maximum plasma concentration (T_{max}) were obtained directly from plasma concentration -time curves.

When necessary, ratio of diacetolol to total acebutolol in plasma (i.e., metabolite percentage rate) in rabbits was calculated as follow.

Plasma concentration of diacetolol

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When necessary, ratio of diacetolol to total acebutolol in plasma (i.e., metabolite percentage rate) in rabbits was calculated as follow.

$$\text{Metabolite percentage rate(\%)} = \frac{\text{Plasma concentration of diacetolol}}{\text{Total aceutolol(acebutolol+diacetolol)}} \times 100$$

Statistical analysis

All means were presented with the standard deviation

Table I. Laboratory data in normal rabbits and rabbits with moderate and severe carbon tetrachloride-induced hepatic failure.

Lab. data	Normal	Moderate hepatic failure	Severe hepatic failure
sGOT (unit/dl)	28 ± 3.8	168 ± 24.5**	292 ± 48.0**
sGPT (unit/dl)	22 ± 3.5	196 ± 28.5**	314 ± 52.4**
Bilirubin (mg/dl)	0.25 ± 0.043	0.34 ± 0.064*	0.46 ± 0.076**

Mean ± S.D. (n = 6) *p < 0.05, **p < 0.01

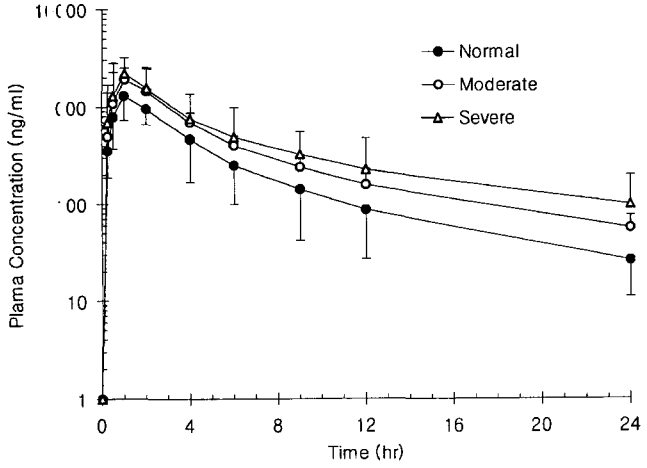

Fig. 1. Plot of plasma concentration of acebutolol in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. Mean ± S.D.(n=6)

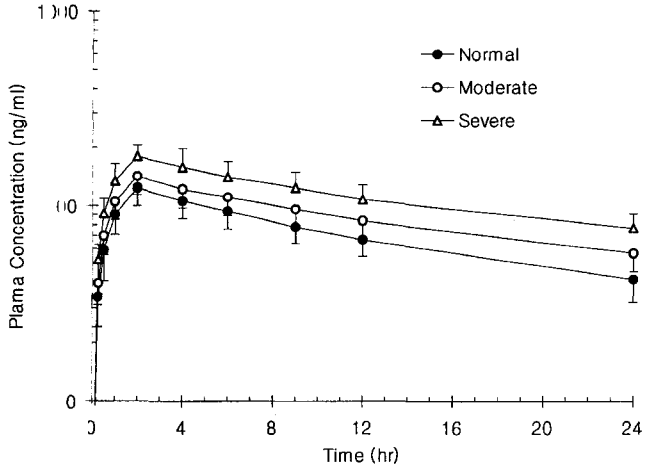

Fig. 2. Plot of plasma concentration of diacetolol in moderate and severe carbon tetrachloride-induced hepatic failure rabbits Mean ± S.D.(n=6).

(Mean ± S.D.). Unpaired student's t-test was utilized to determine difference in mean values between the normal and moderate and severe carbon tetrachloride-induced hepatic failure rabbits. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Clinical laboratory data

Clinical laboratory data in carbon tetrachloride-induced hepatic failure rabbits were shown in table I. sGOT and sGPT in moderate and severe carbon tetrachloride-induced hepatic failure rabbits increased significantly (in both cases, $p < 0.01$) from those obtained in normal rabbits. Compared with control, bilirubin was also statistically increased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits ($p < 0.05$, $p < 0.01$, respectively).

Plasma concentrations of acebutolol and diacetolol

Temporal profiles of plasma concentrations of acebutolol and diacetolol after oral administeration of acebutolol (10 mg/kg) were showed in Fig. 1 and 2, respectively. In all blood collection times, the plasma concentration of acebutolol and diacetolol in moderate and severe carbon tetrachloride-induced hepatic failure rabbits increased significantly ($p < 0.05$ and $p < 0.01$ respectively) compared with those obtained in normal rabbits.

Pharmacokinetic parameters

The pharmacokinetic parameters of acebutolol and diacetolol in normal rabbits and moderate and severe carbon tetrachloride-induced hepatic failure rabbits were shown in table I and II. The slope of the terminal phase (β) of acebutolol in moderate and severe carbon tetrachloride-induced hepatic failure rabbits (0.079 ± 0.021 hr^{-1} and 0.071 ± 0.038 hr^{-1}) decreased significantly ($p < 0.05$, respectively) from that obtained in normal rabbits (0.118 ± 0.031 hr^{-1}). Volume of distribution (V_d) and total body clearance (CL_t) of acebutolol in moderate and severe carbon tetrachloride-induced hepatic failure rabbits (2.54 ± 0.42 L/kg and 1.07 ± 0.39 L/hr/kg, 2.34 ± 0.45 L/kg and 0.85 ± 0.32 L/hr/kg) decreased significantly ($p < 0.01$) from those obtained in normal rabbits (3.78 ± 0.82 L/kg and 1.72 ± 0.28 L/hr/kg). The AUC of acebutolol in moderate and severe carbon tetrachloride-induced hepatic failure rabbits (9345 ± 1678 ng/mlhr and 11818 ± 3794 ng/mlhr) increased significantly ($p < 0.01$) from the control value (5819 ± 1148 ng/mlhr). The pharmacokinetic parameters of diacetolol were similar to those of acebutolol.

Metabolite percentage rate

The metabolite percentage rate in normal rabbits and

Table II. Pharmacokinetic parameters of acebutolol after oral administration (10 mg/kg) of the drug in normal rabbits and rabbits with moderate and severe carbon tetrachloride-induced hepatic failure.

Parameters	Normal	Moderate hepatic failure	Severe hepatic failure
α (hr^{-1})	0.716 ± 0.118	0.679 ± 0.211	0.724 ± 0.124
β (hr^{-1})	0.118 ± 0.031	0.079 ± 0.021*	0.071 ± 0.038*
K_a (hr^{-1})	0.995 ± 0.151	0.904 ± 0.262	0.975 ± 0.294
$t_{1/2\beta}$ (hr)	5.87 ± 1.12	8.77 ± 2.98*	9.72 ± 3.48*
V_d (L/kg)	3.78 ± 0.82	2.54 ± 0.42**	2.34 ± 0.45**
CL_t (L/hr/kg)	1.72 ± 0.28	1.07 ± 0.39**	0.85 ± 0.32**
AUC (ng/ml·hr)	5819 ± 1148	9345 ± 1678**	11818 ± 3794**
C_{max} (ng/ml)	1039 ± 282	1495 ± 379*	1678 ± 315*
T_{max} (hr)	1.31 ± 0.21	1.35 ± 0.31	1.21 ± 0.29

Mean ± S.D. (n=6), *p < 0.05, **p < 0.01., distribution rate constant; , slope of terminal phase; K_a, absorption rate constant; $t_{1/2}$, half life; V_d, volume of distribution at steady state; CL_t, total clearance; AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration.

Table III. Pharmacokinetic parameters of diacetolol after oral administration (10 mg/kg) of acebutolol in normal rabbits and rabbits with moderate and severe carbon tetrachloride-induced hepatic failure.

Parameters	Normal	Moderate hepatic failure	Severe hepatic failure
α (hr^{-1})	1.389 ± 0.211	1.374 ± 0.298	1.756 ± 0.328
β (hr^{-1})	0.052 ± 0.007	0.043 ± 0.006*	0.039 ± 0.005*
$t_{1/2\beta}$ (hr)	13.3 ± 2.14	16.1 ± 1.98*	17.8 ± 2.54**
V_d (L/kg)	7.08 ± 2.04	6.27 ± 2.18	4.97 ± 0.89*
CL_t (L/hr/kg)	3.72 ± 0.72	2.71 ± 0.43*	1.97 ± 0.31**
AUC (ng/mlhr)	2686 ± 528	3687 ± 611*	5078 ± 744**
C_{max} (ng/ml)	111 ± 28.2	128 ± 12.1	163 ± 32.4*
T_{max} (min)	2.54 ± 0.48	2.57 ± 0.37	2.55 ± 0.57

Mean ± S.D (n=6) *p < 0.05, **p < 0.01, distribution rate constant;, slope of terminal phase; $t_{1/2}$, half life; V_d, volume of distribution at steady state; CL_t, total clearance; AUC, area under the plasma concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration.

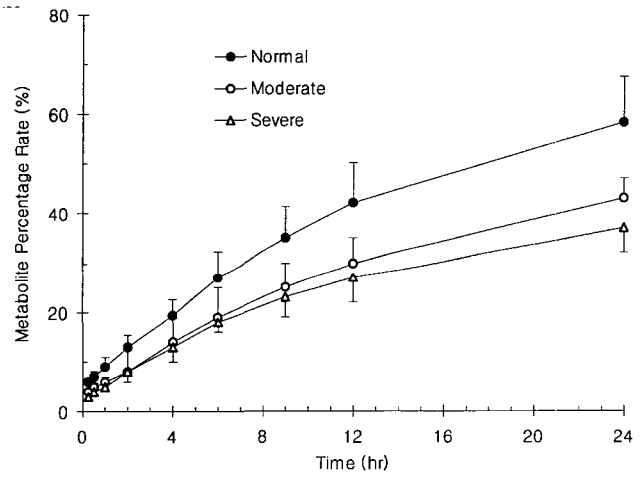

Fig. 3. Metabolite percentage rate of diacetolol to the plasma concentration of total acebutolol in moderate and severe carbon tetrachloried-induced hepatic disorder rabbits. Mean ± S.D.(n=6)
Metabolite percentage rate={diacetolol ÷ (acebutolol+diacetolol)} × 100

oderate and severe carbon tetrachloride-induced hepatic failure rabbits were shown in Fig. 3. Mean metabolite percentage rate in moderate and severe carbon tetrachloride-induced hepatic failure rabbits (17.0 ± 6.6%, 15.8 ± 5.7%) decreased significantly ($p < 0.05$ and $p < 0.01$ respectively) from that obtained in normal rabbits (24.5 ± 9.6).

A great number of beta-adrenergic blocking agents are now available for clinical use. Typically, pharmacokinetics properties of these agents are known to be diverse. In general, lipophilic beta-blockers are metabolized in the liver whereas hydrophilic agents are eliminated in the kidneys as unchanged drugs. Lejeune *et al.*(1988) reported that metabolism of acebutolol evidently slowed down in the patient with chronic liver disease. The eliminination half-lives of acebutolol and diacetolol of these patients were about twice as long as that in the control group. A decrease in hepatic function may lower the first-pass effect, a result that has been reported for other beta-blocker, propranolol, in which the plasma level in elderly patients have been reported to be fourfold higher than those in younger patients (Castleden *et al.*, 1979, Vestal *et al.*, 1979). Different conclusions were drawn from a study with acebutolol in liver disease

(Zaman *et al.*, 1985). In the previous study, no significant difference in the half-lives was found between liver patients and healthy volunteers.

In this study, the AUC of acebutolol were significantly increased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. The metabolite percentage rate were also decreased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. The terminal phase slope and total body clearance of acebutolol were significantly decreased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. These findings suggest that the hepatic metabolism of acebutolol was inhibited and, total body clearance and terminal phase slope of acebutolol were significantly decreased in moderate and severe carbon tetrachloride-induced hepatic failure rabbits. Our observations were apparently consistent with those obtained other beta-blockers that undergo significant first-pass metabolism. For propranolol, it has been shown that in liver disease the bioavaiability increase significantly due to a reduction in first-pass metabolism (Branch *et al.*, 1987). Similar pharmacokinetic charges have been noted for labetalol (Homeida *et al.*, 1988) and metoprolol (Regardh *et al.*, 1981).

In summary, our result suggests that liver disease may significantly affect the pharmacokinetics of acebutolol or its major metabolite. Therefore, dose adjustment may be necessary for acebutolol in hypertensive patients with hepatic damage.

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