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Pharmacokinetic Changes of Acebutolol after Orall Administration in Rabbits with Diabetes Mellitus Induced by Alloxan

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Because physiological changes that potentially alter pharmacokinetics occurs in diabetes mellitus patients, pharamacokinetics of drugs used in the treatment of hypertension was studied using acebutolol as a model anti-hypertensive drug. Thus, the pharmacokinetics of acebutolol was investigated after oral administration of acebutolol (15 mg/kg) to control rabbits and rabbits with acute or chronic diabetes mellitus induced by alloxan. Kidney and liver functions were documented for acute and chronic diabetes mellitus groups based on plasma chemistry data. After oral administration of acebutolol to acute and chronic groups, the plasma concentrations appeared higher; As a result, area under the plasma concentration-time curve from time zero to time infinity10575 and 8668 μg·h/mL for acute and chronic group, respectively. In comparison, the area was apparently smaller in the control group (i.e., 7132 μg·h/mL). The half-life in acute groups was significantly prolonged 8.45 h compared with the half-life in the control group (i.e., 6.30 h). Alteration in acebutolol pharmacokinetics was more pronounced in the acute group as evidenced by the significantly higher values the area under the plasma concentration time curve, absorption rate constant and maximum plasma concentration compared with chronic or control group. Therefore, these observations indicate that acebutolol pharmacokinetics may be affected in patients with diabetes mellitus, especially in the early stage of the disease.

Key words: Acebutolol, Pharmacokinetics, Oral administration, Diabetes mellitus

INTRODUCTION

Many diabetic patients develop complications during the course of the disease, including hypertension, cardiovascular disorders, nephropathy, neuropathy, and retinopathy (Gwilt, Nahhas *et al.*, 1991). Some physiological disorders such as gastroparesis, decreased plasma albumin level, elevated plasma free fatty acid level, glycosylation of plasma proteins, and changes in the cytochrome P-450 contents were reported to occur in diabetes mellitus patients (O'Connor and Feely, 1987; Gwilt, Nahhas *et al.*, 1991). Such physiological changes could potentially alter the pharmacokinetics and hence the pharmacodynamics of drugs in such patients. Animal models of insulin-dependent diabetes mellitus, induced by administration of several chemicals, principally alloxan, streptozotocin, and zinc chelators, have been reported (Pickup and Williams, 1991). Impacts of diabetes mellitus on the pharmacokinetics and/or pharmacodynamics of some drugs in the patients or rats with diabetes mellitus induced by alloxan (DMIA) have been reviewed (OíConnor and Feely 1987; Gwilt, Nahhas *et al.*, 1991). Changes in the pharmacokinetics and/or pharmacodynamics of furosemide (Park *et al.*, 1998), azosemide (Park *et al.*, 1996), DA-1131, a new carbapenem (Kim *et al.*, 1998), DA-125, a new anthracycline (Choi *et al.*, 1996), adriamycin (Lee *et al.*, 1995), sulfamethoxazole (Choi and Choi, 2000) and diltiazem (Choi and Kim, 2002) in rats and rabbits with DMIA have recently been reported.

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 a drug that is well absorbed from the gut following oral administration. It undergoes significant hepatic first-pass metabolism being converted first to the primary amine (Roux *et al.*, 1980, Mclean *et al.*, 1978), then acetylated to diacetolol. Acebutolol's main metabolite, diacetolol, has pharmacologic

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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.*, 1979, Gulaid *et al.*, 1981, Gabriel *et al.*, 1981). Althoug pharmacokinetic and/or pharmacodynamic changes of drugs in patients with diabetes mellitus and rats with DMIA were studied in the literature, pharmacokinetic changes of acebutolol in rabbits with DMIA seemed not to be published. Therefore, the purpose of this study was to investigate the pharmacokinetics of acebutolol and the main metabolite, diacetolol, after oral administration of acebutolol in control rabbits and acute and chronic alloxan-induced diabetes mellitus rabbits.

MATERIALS AND METHODS

Materials

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

Induction of diabetes mellitus in rabbits by alloxan

Alloxan dissolved in normal saline injectable solution (40 mg/kg) was administered intravenously via the ear vein (total injection volume was approximately 6-7 mL) for two consecutive days to the overnight-fasted rabbits (weighing 2.0-2.2 kg, obtained from Ilchull Scientific Company, Naju, Korea). On the fifth day, the blood glucose levels were measured and rabbits with blood glucose levels of greater than 230 mg/dL were chosen as the acute group. For the induction of chronic diabetes mellitus group, alloxan was further administered on sixth and tenth days. On the thirteenth day, the blood glucose levels were measured and rabbits with glucose levels of greater than 300 mg/dL were chosen as the chronic group. The same volume of normal saline injectable solution was administrated to the control group.

Pharmacokinetics of acebutolol after oral administration

On the fifth (for the control and acute groups) or thirteenth (for the chronic group) day after starting the injection of alloxan or normal saline (for the control group, just after measuring the blood glucose levels), each rabbit was anesthetized with a subcutaneous injection of 25% urethane, 4 mL/kg. The polyethylene tubing (Clay Adams, Parsippany, NJ, USA) was cannulated into each of the right femoral artery. After the catheterization, the animals were fasted overnight with a free access to water for the use in the pharmacokinetic study.

Acebutolol, 15 mg/kg, was administered orally (total oral volume was 10 mL) to the control group (n = 6), the acute (n = 6) or chronic (n = 6) group with a feed-tube. Blood samples (approximately 1.5 mL) were collected via the right femoral artery at 0 (to serve as a control), 0.25, 0.5, 1, 1.5, 2, 4, 6, 9, 12, and 24 h after oral administration of acebutolol. Heparinized normal saline injectable solution (0.25 mL; 75 units/mL) was used to flush the cannula after each blood sampling to prevent blood clotting. Collected blood samples were centrifuged immediately at 5000 rpm for 5 min and plasma samples were stored at -30°C until HPLC analysis for acebutolol.

Plasma chemistry

At the end of the experiment, plasma was stood at -30 °C for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, creatinine, and total proteins using corresponding kits and a photometer (Model 5010, Böheringer Mannheim GmbH, Mannheim, Germany).

HPLC analysis of acebutolol

Plasma concentrations of acebutolol and diacetolol were determined by a high performance liquid chromatography assay (Buskin *et al.*, 1982). 0.1 mL of triamterene (i.e., internal standard) solution (2 μg/mL) and 0.2 mL of NaOH solution (1 M) and 4 mL of ether were added to 0.5 mL of sample. The mixture was then vortex-mixed for 10 min and centrifuged at 3,000 rpm for 5 min. An aliquot (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 into 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 integrater. The detector wavelength was set at 243 nm and the separation carried out in room temperature. The stationary phase used was a Shin-Pack CLC-ODS column (4.6×250 mm, Shimadzu Co., Japan). Mobile phase consisted of acetonitrile : water : 0.1 M phosphate buffer (pH = 4) (22:68:10, v/v/v). The mobile phase was filtered with a membrane filter of the pore size of 0.45 μm. 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

Pharmacokinetic parameters were calculated assuming two compartment open model by a nonlinear least square regression analysis (MULTI, Yamaoka *et al.*, 1981). The parameter value was obtained by simplex algorithm when AIC (Akaike's information criterion) value was the lowest. The area under the plasma concentration-time curves (AUC) was calculated by trapezoidal rule and total body clearance (CLt) was calculated by CLt = 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 it was necessary to compare the concentration ratio for the parent drug to the metabolite, the following equation was used.

$$\text{Metabolite rate (\%)} = \frac{\text{plasma diacetolol}}{\text{total acebutolol (acebutolol+diacetolol)}} \times 100$$

Statistical analysis

All means were presented with their standard deviation (Mean±S.D.). Unpaired Student's *t*-test was utilized to determine a significance difference between the control and acute or chronic group. Differences were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

In rabbits with acute and chronic diabetes, we have determined whether hepatic function was altered with the induction of experimental diabetes mellitus. The activities of AST and ALT in the plasma tended to be higher in acute and chronic diabetes mellitus compared with those obtained in the control animals. However, with the limitation in the number of animals employed in this study, no statistical difference was observed (Table I). Kidney function was also studied in the disease models; the plasma concentrations of creatinine in the acute group were significantly higher ($p<0.05$) compared with the control. The creatinine concentration in the chronic was apparently comparable to the acute group, although statistical significance could not be detected. For both, acute and chronic groups urea nitrogen tended to be higher compared with the control but no significant difference was observed probably because of limited number of animals used (Table I). Despite the fact that the blood chemistry indicated apparent alteration in values without statistical significance (with an exception of plasma creatinine in the acute group), blood glucose levels was consistently elevated for both acute and chronic groups, indicating that experimental diabetes was indeed induced under the pretreatment conditions used in the study.

Mean plasma concentration-time profiles of acebutolol in rabbits exhibited a multi-exponential manner (Fig. 1). Moment and nonlinear regression analyses indicated that terminal phase slope (i.e., β), Ka, Cmax, half-life and AUC values were elevated in the acute group compared with those obtained the control group (Table II). In contrast, pharmacokinetic parameters were apparently unaffected by the induction of experimental diabetes. Since the drug was administered by the oral route, the mechanism(s) responsible for the changes in the acebutolol pharmacokinetics during the acute diabetes is difficult to identify. However, since the terminal phase slope, a parameter that is not a function of the absorption process, was elevated in the acute group, this observation suggests that acebutolol elimination from the body may be affected by the induction of acute diabetes. Additional study with intravenous administration acebutolol may be necessary to identify the underlying mechanism(s) for the changes in the pharmacokinetics.

In this study, temporal profiles of mean diacetolol concentration were studied in rabbits (Fig. 2). Again, moment and nonlinear regression analysis indicated that terminal phase slope (i.e., β), Ka, Cmax, half-life and AUC values

Table I. Mean (± standard deviation) plasma chemistry data in the control rabbits and rabbits with acute and chronic diabetes mellitus

Parameter	Control (n = 4)	Acute (n = 4)	Chronic (n = 4)
Aspartate aminotransferase (IU/L)	66 ± 21	74 ± 23	75 ± 24
Alanine aminotransferase (IU/L)	54 ± 16	66 ± 18	64 ± 20
Urea nitrogen (mg/dL)	38 ± 11	46 ± 17	48 ± 16
Creatinine (mg/dL)	0.96 ± 0.24	1.34 ± 0.28*	1.27 ± 0.31
Total protein (g/dL)	6.2 ± 0.62	5.8 ± 0.52	5.7 ± 0.61
Glucose (mg/dL)	122 ± 39	266 ± 81*	281 ± 86*

Mean±S.D. (n = 4)
*$p<0.05$ significantly different from control group.

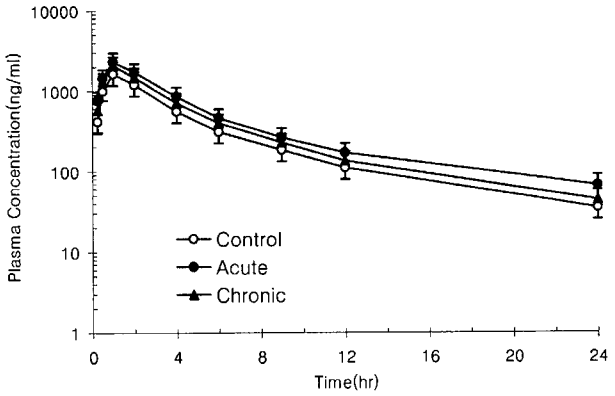

Fig. 1. Mean arterial plasma concentration-time profiles of acebutolol (ng/mL) after oral administration to control rabbits (○, n = 6) and rabbits with acute (●, n = 6) and chronic (▲, n = 6) diabetes mellitus induced by alloxan. Bars represent standard deviation.

Table II. Pharmacokinetic parameters of acebutolol after oral administration to control rabbits and rabbits with acute and chronic diabetes mellitus induced by alloxan

Parameter	Control	Acute	Chronic
β (hr^{-1})	0.11 ± 0.023	0.081 ± 0.021*	0.12 ± 0.031
Ka (hr^{-1})	1.02 ± 0.27	1.34 ± 0.322*	1.22 ± 0.362
C_{max} (ng/ml)	1611 ± 398	2127 ± 510*	1868 ± 489
T_{max} (hr)	1.31 ± 0.31	1.27 ± 0.29	1.33 ± 0.31
t(1/2) (hr)	6.30 ± 1.52	8.45 ± 2.26*	5.78 ± 1.98
AUC (ng/mL·hr)	7132 ± 2018	10575 ± 2674*	8668 ± 2112

Mean±S.D. (n = 6)
*p< 0.05 significantly different from control
Ka : absorption rate constant
β: elimination rate constant
t(1/2) : half-life
C_{max} : peak concentration
T_{max}: time to reach peak concentration
AUC_{0-24} area under the plasma concentration-time curve from 0h to 24h

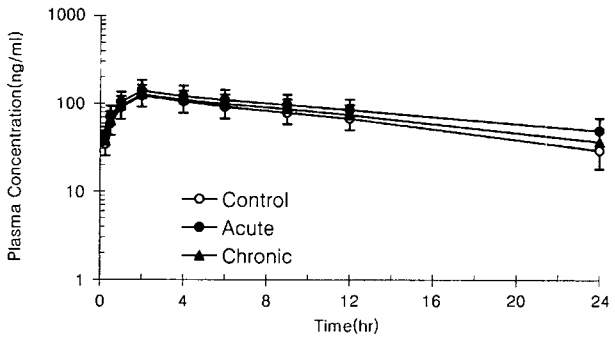

Fig. 2. Mean arterial plasma concentration-time profiles of diacetolol (ng/mL) after oral administration to control rabbits (°¤, n = 6) and rabbits with acute (°‹, n = 6) and chronic (°‚, n = 6) diabetes mellitus induced by alloxan. Bars represent standard deviation.

were elevated in the acute group compared with those obtained the control group (Table III). Similar to the pharmacokinetic study with the parent drug, the data appear to suggest that elimination of the metabolite may be affected by the induction of acute diabetes. Interestingly, however, metabolite rate (MR, Table III) was unaffected by the induction of experimental diabetes, and, thus, the induction did not affect the metabolic conversion of acebutolol to diacetolol. However, studies involving intravenous administration of acebutolol may be necessary to fully identify the pharmacokinetic alteration for the metabolite pharmacokinetics.

In this study, we have identified that the induction of acute diabetes lead to the elevation in the plasma concentration for acebutolol and the metabolite. Changes in metabolic conversion and the distribution characteristics did not appear to explain the changes in the pharmacokinetic property such as the terminal phase slope. Since

Table III. Pharmacokinetic parameters of diacetolol after oral administration of acebutolol to control rabbits and rabbits with acute and chronic diabetes mellitus induced by alloxan

Parameter	Control	Acute	Chronic
β (hr^{-1})	0.052 ± 0.007	0.043 ± 0.006*	0.049 ± 0.008
C_{max} (ng/mL)	111 ± 24.2	128 ± 31.1	119 ± 32.1
T_{max} (hr)	2.54 ± 0.48	2.57 ± 0.37	2.55 ± 0.57
t(1/2) (hr)	13.3 ± 2.14	16.1 ± 3.00*	13.8 ± 2.98
M.R. (%)	21.2 ± 5.0	19.0 ± 4.8	19.8 ± 4.5
AUC (ng/mL·hr)	2686 ± 528	2688 ± 681*	2829 ± 611

Mean±S.D. (n = 6)
*p<0.05 significantly different to control
Ka: absorption rate constant
β: elimination rate constant
t(1/2): half-life
C_{max} : peak concentration
T_{max}: time to reach peak concentration
M.R.(%): mean metabolite $rate_{0-24\ hr}$

$$= \frac{\text{diacetolol plasma concentration}}{\text{Total plasma acetutolol (acetbutolol + diacetolol)}} \times 100$$

AUC_{0-24} area under the plasma concentration-time curve from 0 h to 24 hr

blood chemistry data was clear on the elevation of creatinine in the acute diabetes group, the function of the kidney may be affected by the pre-treatment. Therefore, these observations indicated that the alteration in the kidney function is related to the changes in the excretion of the drug and the metabolite. Urinary excretion study for acebutolol and the metabolite may be necessary to confirm this hypothesis.

In summary, the induction of acute diabetes mellitus has an impact on the pharmacokinetics of acebutolol and the major metabolite, diacetolol. The pharmacokinetic alteration was apparently associated with the changes in the function of the kidney. Therefore, dose-adjustment of antihypertensive drugs such as acebutolol may be necessary for the optimal anti-hypertensive therapy in patients with early phase of diabetes.

REFERENCES

Alhenz-Gelas, F., Plouin, P. F., Ducrocq, M. B., Corvol, P. E., and Menard, J., Comparison of the antihypertensive and hormonal effects of a cardioselective beta-blocker acebutolol and diuretics in essential hypertension. *Am. J. Med.*, 64, 1005-1012 (1978).

Baker, P. G. and Goulton, J. A., Multicentre study of a once daily dosage of acebutolol in the treatment of hypertension in general practice. *J. Int. Med. Res.*, 7, 201-214 (1979).

Basil, J. and Jordan, R., Pharmacological properties of diacetolol, a major metabolite of acebutolol. *Eur. J. Pharmacol.*, 80, 47-56 (1982).

Buskin, J. N., Upton, R. A., Jones, R., and Williams, R. L., High performance liquid chromatography assay of acebutolol and

its metabolites in plasma and urine. *J. Chromatogr.*, 230, 438-442 (1982).

Choi, Y. J., Lee, H. J., Kwon, J. W., Kim, W. B., Yang, J., and Lee, M. G., Pharmacokinetic change of M1, M2, M3, and M4 after intravenous administration of a new anthracycline, DA-125, to alloxan-induced diabetes mellitus. *Res. Commun. Mol. Pathol. Pharmacol.*, 94, 181-192 (1996).

Choi, J. S. and Kim,Y.G, Pharmacokinetic changes of diltiazem and desacetyldiltiazem after oral administration of diltiazem in rabbits with diabetes mellitus induced by alloxan. *Biopharm. Drug Dispos.*, 23, 115-120 (2002)

Choi, J. S. and Choi, B. C., Pharmacokinetic changes of sulfamethoxazole in rabbits with diabetes mellitus induced by alloxan. *Res. Commun. Mol. Pathol. Pharmacol.*, 108, 332-340 (2000).

Flouvat, B., Roux, A., and Chau, N. P., Pharmacokinetics and bioavailability of diacetolol, the main matabolite of acebutolol. *Eur. J Clin. Pharmacol.*, 19, 287-292 (1981).

Gwilt, P. R., Nahhas, P. R., and Tracewell, W. G., The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clin. Pharmacokinet.*, 20, 477-490 (1991).

Gabriel, R., Kaye, C. M., and Sankey, M. G., Preliminary observations on the excretion of acebutolol and its acetyl metabolite in the urine and feces of man. *J. Pharm. Pharmacol.*, 33 386-387 (1981).

George, C. F. and Gruchy, B. S., Elimination of drugs by active intestinal transport. *J. Pharm. Pharmacol.*, 31, 643-644 (1979).

Gulaid, A. A., James, I. M., and Kaye, C. M., The Pharmacokinetics of acebutolol in man, following the oral administration of acebutolol as a single dose and during and after repeated oral dosing. *Biopharm. Drug Dispos.*, 2, 103-114 (1981).

Kim, S. H., Kim, W. B., and Lee, M. G., Pharmacokinetics of a new carbapenem, DA-1131, after intravenous administration to rats with alloxan-induced diabetes mellitus. *Biopharm. Drug Dispos.*, 19, 303-308 (1998).

Lee, H. J., Paik, W. H., and Lee, M. G., Pharmacokinetic and tissue distribution changes of adriamycin and adriamycinol after intravenous administration of adriamycin to alloxan-induced diabetes mellitus rats. *Res. Commun. Mol. Pathol. Pharmacol.*, 89, 165-178. (1995).

Martin, M. A., Phillips, C. A., and Smith, A. J., Acebutolol in hypertension a double blind trial against placebo. *Br. J. Clin. Pharmcol.*, 6, 351-356 (1978).

Mclean, A. J., Mcnamara, R. J., Souich, P. D., Gibaldi, M., and Lalka, D., Food, splanchnic blood flow and bioavailability of drugs subject to first pass metabolism. *Clin. Pharmacol. Ther.*, 24, 5-10 (1978).

O'Connor, P. and Feely, J., Clinical pharmacokinetics and endocrine disorders. Therapeutic implications. *Clin. Pharmacokinet.*, 13, 345-364 (1987).

Park, J. H., Lee, W. I., and Lee, M. G., Pharmacokinetic and pharmacodynamic changes of furosemide after intravenous and oral administration to rats with alloxan-induced diabetes mellitus. *Biopharm. Drug Dispos.*, 19, 357-364 (1998).

Park, K. J., Yoon, W. H., and Lee, M.G., Pharmacokinetics and pharmacodynamics of azosemide after intravenous and oral administration to rats with alloxan-induced diabetes mellitus. *J. Pharm. Pharmacol.*, 48, 1093-1097 (1996).

Pickup, J. C. and Williams, G., Textbook of Diabetes, Blackwell Scientific Publications, Oxford, England, 1, 151-155 (1991).

Roux, A., Flouvat, B., Chau, N. P., Letac, B. and Lucsko, B., Pharmacokinetics of acebutolol after intravenous bolus administration. *Br. J. Clin. Pharmacol.*, 9, 215-217 (1980).

Yamaoka, K., Tanigawara, Y., Nakagawa, T., and Uno, T., A pharmacokinetics analysis program for microcomputer. *J. Pharm. Dyn.*, 4, 879-883 (1971).