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Pharmacokinetics of Tolbutamide After Oral Administration to Rabbits with Folate-Induced Renal Failure

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The pharmacokinetic of tolbutamide was studied after the oral administration to normal rabbits or rabbits with mild to medium folate-induced renal failure. The plasma concentrations of tolbutamide were significantly elevated ($p<0.05$) during 9 to 24 h in rabbits with mild or medium folate-induced renal failure. Consequently, the area under the plasma concentration-time curves (AUC) was significantly higher in mild ($p<0.05$) and medium ($p<0.01$) folate-induced renal failure rabbits (i.e., 2906 μg/mL·h for mild renal failure and 4074 μg/mL·h for moderate renal failure) than that in normal rabbits (i.e., 2295 μg/mL·h). The cumulative urinary excretion of tolbutamide was significantly depressed ($p<0.05$) in medium folate-induced renal failure rabbits (i.e., 3.3 mg) compared with that in normal rabbits (i.e., 5.9 mg). The elimination rate constant (Kel) of tolbutamide was significantly decreased in medium renal failure rabbits (i.e., 0.027 h^{-1}) than that in normal rabbits (i.e., 0.044 h^{-1}); As a result, the terminal half-life of tolbutamide in medium folate-induced renal failure rabbits (i.e., 25.5 h) was significantly longer ($p<0.01$) than that in normal rabbits (i.e., 15.7 h). The change in pharmacokinetic parameters is consistent with the hypothesis that the alteration is mediated by the depressed metabolic elimination of the drug by the induction of renal failure. Therefore, these observations indicated that the dosage adjustment may be necessary for tolbutamide in patients with renal insufficiency.

Key words: Tolbutamide, Pharmacokinetics, Oral admimstration, Renal failure

INSTRODUCTION

Sulfonylureas, such as tolbutamide, are clinically used for the management of hyperglycemia in type II diabetes. The hypoglycemic effect of sulfonylurea is thought be mediated by stimulating the β-cell of the pancreas to secret insulin in patients with non-insulin-dependent diabetes mellitus (NIDDM) (Ward *et al.*, 1981; Hosker *et al.*, 1985; Beck-nielsen *et al.*, 1984; Melander, 1987). In addition, sulfonylureas bind to surface receptors on the β-cell membrane to inhibit the ATP-sensitive potassium channel, preventing the egress of potassium and thereby depolarizing the cell membrane (Sturgess *et al.*, 1985; Bailey *et al.*, 1982).

Pharmacokinetics of tolbutamide has been well documented in the literature. For example, the drug is known to binds highly (ranging 80-99%) to serum protein (Ayanoglu, 1986; Adir, 1982). Despite the fact that the drug is significantly bound to the protein, the volume of distribution is small (*viz,* approximately 0.1 L/kg), indicating that the distribution is apparently limited to systemic circulation. When administered orally, tolbutamide is readily absorbed from the gastrointestinal track as evidenced by the fact that oral availability is over 90% (Melander *et al.*, 1978; Ferner *et al.*, 1987).

Tolbutamide is oxidized to form hydroxylated tolbutamide by liver cytochrome P-450 enzyme systems (Relling *et al.*, 1990; Veronese *et al.*, 1990). In addition to the major metabolite, tolbutamide may be metabolized to carboxyltolbutamide, a minor metabolite of the drug. The primary route of elimination for tolbutamide is *via* the hepatic metabolism as evidenced by the fact that over 75% of the administered dose may be metabolized and recovered to urine within 24 h (Knodell *et al.*, 1987; Miner *et al.*, 1988; Yamao *et al.*, 1994; Page *et al.*, 1991; Schary *et al.*, 1983; Belanger, 1991; Srivastava, 1991; Dogterom, 1993). The recovery of unchanged tolbutamide to urine is very small, indicating that the urinary elimination is minor

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pathway for tolbutamide. Based on these literature observations, it is generally considered that hepatic failure, but not the renal failure, is likely to have a clinical significance. Interestingly, however, renal insufficiency has been associated with the reduction of drug metabolism (Balant *et al.*, 1983) as evidenced by the fact the presystemic clearance of propranolol is depressed in animals with renal failure (Terao and Shen, 1983). Therefore, possibility that pharmacokinetics of tolbutamide changes during renal failure still exists. However, this aspect of tolbutamide pharmacokinetics has not been studied. The purpose of this study was to characterize and compare the pharmacokinetics of tolbutamide in normal rabbits and rabbits with renal failure.

MATERIALS AND METHODS

Materials

Tolbutamide, chlorpropamide (the internal standard in HPLC assay) and dimethylformamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol were purchased from Merck Co. (Darmstat, Germany). The other chemicals were reagent grade or better, and were used without further purification. HPLC (Model CBM-10A, Shimadzu Co., Japan), syringe pump (Model 341B, Sage Co., Japan), vortex mixer (Scientific Industries, Korea) and high speed centrifuge (Abbot Co., USA) were also used in this study.

Animals and induction of renal failure with folate

White male New Zealand rabbits, weighing 2.0-2.5 kg in body weight, were fasted at least 24 h prior to the experiment; water was given freely to animals. When it was necessary, mild or medium renal failure rabbits were induced by the intravenous administration of folate (75 mg/kg for mile renal failure; 150 mg/kg for moderated renal failure; vehicle 0.3 M $NaHCO_3$ aqueous solution) via ear vein at least 24 h before the commencement of experiment. Under 25% urethane (4 mL/kg) anesthesia, the right femoral artery and ureters were cannulated with polyethylene tubing (Clay Adams, NJ, USA) for blood sampling at room temperature. Blood chemistry data such as urea nitrogen, creatinine, aspartate aminotransferase and alanine aminotransferase were measured by photometer 5010 (Mannheim, Germany).

Oral administration of tolbutamide

Tolbutamide solution (dissolved in 5 mL of distilled water) was administered orally (50 mg/kg) to rabbits using a feeding tube. Blood samples (1.5 mL) were withdrawn from the femoral artery at 0, 0.25, 0.5, 1, 2, 3, 6, 9, 12 and 24 h after the tolbutamide administration. Plasma samples were obtained by centrifuging at 6,000 rpm for 10 min. The separated 0.5 mL plasma were stored at -30°C until HPLC analysis. Urine samples were collected between 0-2, 2-4, 4-6, 6-12 and 12-24 h after administration of the drug. After measuring the volume of urine, an aliquot of urine samples was stored at -30°C until the HPLC analysis. Physiological saline solution was infused to the animal at the rate of 1.5 mL/h via the ear vein using an infusion pump (Model 341A, Sage instrumenta, Cambridge, MA, USA) to replace the loss of blood volume using an infusion pump (Model 341A, Sage instrumenta, Cambridge, MA, USA). Each rabbit was kept in supine position during the experimental period.

HPLC analysis of tolbutamide in plasma

Plasma concentrations of tolbutamide were determined by a high performance liquid chromatography assay (Yamao, Nakagami *et al.*, 1994). Briefly, a 0.2 mL aliquot of 0.25% chlorpropamide (i.e., the internal standard), a 2 mL aliquot of 2M hydrochloric acid solution and 7 mL aliquot of ethyl acetate were added to a 0.5 mL of plasma or 0.1 mL urine samples; The mixture was vortex-mixed for 15 min. After centrifugation at 5000 rpm for 10 min, a 6 mL aliquot of organic phase was collected and transferred to a fresh tube. The organic phase was evaporated to dryness under N_2 gas at 40°C. The residue was dissolved with 0.3 mL of 10% dimethylformamide and mixed for 2 min. After centrifugation at 5,000 rpm for 5 min, a 50 μL aliquot of the aqueous layer was injected into HPLC.

The HPLC system consisted of a solvent delivery pump (Model CBM-10A, Shimadzu Co., Japan), a variable UV absorbance detector and computing intergrater. The detector wavelength was set at 254 nm and the column was used at room temperature. The column was used a μ-bondapack C_{18} column (4.6×250 mm, Shimadzu Co., Japan). Mixtures of acetonitrile : phosphate buffer (24 : 76 v/v, pH) were used as the mobile phases at a flow rate of 1.0 mL/min. The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter.

Pharmacokinetic analysis

Pharmacokinetic analysis was carried out assuming one compartment open model. Nonlinear least square regression was performed with the MULTI program (Yamaoka *et al.*, 1981) and the parameter values, i.e., the intercepts and the slopes, obtained using simplex algorithm. The area under the plasma concentration-time curves (AUC) from time zero to the last collection was calculated by trapezoidal rule and AUC from the last collection time to infinity calculated by the standard area extrapolation technique. The maximum plasma concentration (C_{max}) and time to reach the maximum plasma concentration (T_{max}) were obtained directly from plasma concentration-time curves. The terminal half-life of tolbutamide was calculated by 0.693/Kel.

Statistics

When it was necessary to compare mean values, student's *t*-test was used. In this study, $p<0.05$ was accepted as denoting statistical significance. Data are expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Clinical laboratory data

Blood chemistry data were compared for normal, mild renal failure and moderate renal failure rabbits (Table I). As expected, serum creatinine concentration (Scr) in mild and medium folate-induced renal failure rabbits increased significantly ($p<0.01$ and $p<0.05$ respectively) compared with that obtained for the normal rabbits. In addition, blood urea nitrogen (BUN) in mild and medium renal failure rabbits was elevated significantly ($p<0.01$) compared with the control value. No significant differences were ALT and AST. These observations indicated that the folate administration indeed induced renal insufficiency without hepatic damage in rabbits.

Pharmacokinetics of tolbutamide in normal or renal failure rabbits

The plasma concentration of tolbutamide after oral administered (50 mg/kg) was shown in Fig. 1. The plasma concentration of tolbutamide from 6 h to 24 h after the administration was increased ($p<0.01$), compared with that of the control, in medium renal failure rabbits; For the case of mild renal failure rabbits, the plasma concentration was similarly elevated ($p<0.05$) from 12 to 24 h after the administration. The pharmacokinetic parameters of tolbutamide in normal rabbits, mild or medium renal failure rabbits were summarized in Table II. The elimination rate constant (Kel) of tolbutamide in mild renal failure rabbit was apparently depressed without a statistical significance. However, Kel of tolbutamide in moderate renal failure was statistically decreased ($p<0.05$) for medium renal failure rabbits compared with the control. The terminal half-life of tolbutamide in mild and medium folate-induced renal failure rabbits (19.4±5.7 h, 25.5±7.4 h) was apparently longer than that in normal rabbits (15.7±4.6 h), although statistical significance was noted for the comparison between rabbits with medium renal failure and normal rabbits ($p<0.05$). The AUC of tolbutamide in mild and medium renal failure rabbits (2960±628 ng/mL·h and 4074±935 ng/mL·h) were significantly increased ($p<0.05$ for mild renal failure; $p<0.01$ for moderate renal failure) to the normal rabbits (2295±546 ng/mL·h).

Table I. Blood chemistry data in normal rabbits or rabbits with mild to moderate renal failure by folate

	Normal	Mild renal failure	Medium renal failure
Scr (mg/dL)	1.14±0.27	2.98±1.24*	4.84± 1.28**
BUN (mg/dL)	13.4 ±3.15	26.4 ±5.27**	35.6 ± 8.98**
ALT (IU/dL)	40.3 ±6.14	44.2 ±6.41	44.8 ± 8.20
AST (IU/dL)	48.9 ±8.73	51.4 ±9.12	52.6 ±11.21

Mean±S.D. (n = 6) *$p<0.05$, **$p<0.01$.
Key: Scr, serum creatinine concentration; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

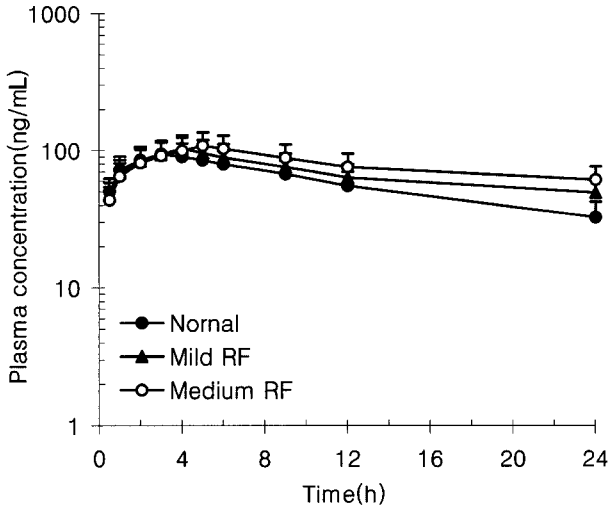

Fig. 1. Mean plasma concentration-time profiles of tolbutamide after oral administration (50 mg/kg) to normal rabbits (●) or rabbits with mild (▲) and medium (○) renal failure. Data are expressed as mean ± standard deviation of n=6 animals.

Table II. Mean (±S.D.) pharmacokinetic parameters of tolbutamide after oral administration (50 mg/kg) to normal rabbits, rabbits with mild or medium renal failure.

Rabbits Parameters	Normal (n=6)	Mild (n=6)	Medium (n=6)
Ka (h^{-1})	1.35 ± 0.42	1.14 ± 0.36	0.89 ± 0.31
Kel (h^{-1})	0.044± 0.015	0.036± 0.011	0.027± 0.008**
C_{max} (μg/mL)	94.2 ± 24.5	103.2 ± 24.5	108.4 ± 37.8
T_{max} (h)	2.62 ± 0.68	3.15 ± 0.86	4.06 ± 1.24*
$t_{1/2}$ (h)	15.7 ± 4.6	19.4 ± 5.7	25.5 ± 7.4**
AUC (μg/mL·h)	2295 ±546	2906 ±618*	4074 ±935**
AUC ratio (%)	100	126	177

Data are expressed as mean±S.D. (n=6), *$p<0.05$ and **$p<0.01$ significantly different.
Key: Ka; absorption rate constant, Kel; elimination rate constant, Cmax; maxium plasma concentration, Tmax; time of C_{max}, $t_{1/2}$; terminal half-life, AUC; area under the plasma concentration-time curve from time zero to time infinity, AUC ratio; comparative AUC in renal failure to that in normal rabbits.

Cumulative urinary excretion of tolbutamide

Temporal profile of cumulative urinary excretion of tolbutamide were shown Fig. 2. The amounts of cumulative

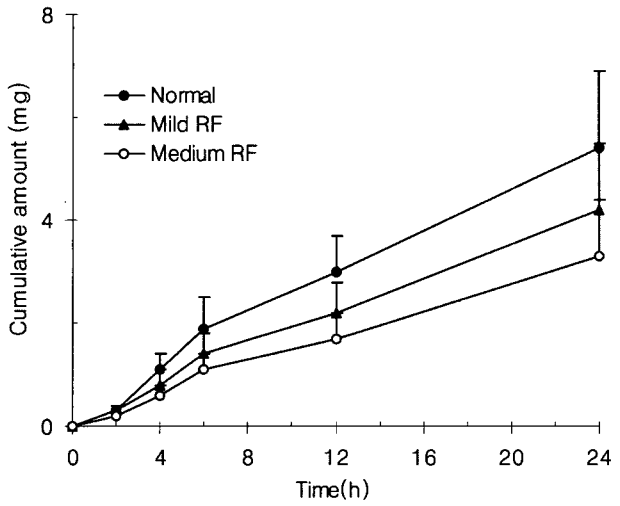

Fig. 2. Mean cumulative urinary excretion (mg) of tolbutamide after oral administration (50 mg/kg) to normal rabbits (●) or rabbits with mild (▲) and medium (○) renal failure. Data are expressed as mean ± standard deviation of n=6 animals.

urinary excretion of tolbutamide in mild and medium renal failure rabbits were 4.5±1.3 mg and 3.3±1.1 mg, respectively, indicating that the urinary recovery was probably reduced after the induction of renal failure (the urinary recovery of the drug in normal rabbits was 5.9±1.5 mg). The total amount of tolbutamide excreted in 24 hr urine as unchanged tolbutamide in medium renal failure rabbits was significantly decreased ($p<0.05$) compared with that in normal rabbits.

In this study, the plasma concentration was elevated after the induction of renal failure and the elevation was accompanied by the alteration in pharmacokinetic parameters (e.g., AUC and Kel). In general, an elevation of AUC may be manifested by increased input (i.e., elevated absorption) and/or decreased output (i.e., reduced elimination). In this study, the comparison of primary pharmacokinetic parameters (viz, the volume of distribution and the clearance) was not possible because the drug was orally given to rabbits. However, literature information clearly indicated that the absolute bioavailability for tolbutamide is almost complete and, thus, the increase in AUC is not likely to be primarily mediated by the elevated absorption. Taken together, the alteration in tolbutamide pharmacokinetics may be related to the reduced elimination after the renal failure. Consistent with this hypothesis, literature evidences have suggested that renal insufficiencies were associated with the decreased metabolism of drugs (Balant *et al.*, 1983). In addition, regardless of the source of renal insufficiency (e.g., uranyl nitrate induced renal failure or patient with renal insufficiency), hepatic metabolism was affected, indicating that folate induced renal failure, such as that used in this study, may lead to a similar reduction in the hepatic metabolism (Balant *et al.*, 1983). Secondary to the potential impact on the tolbutamide metabolism by the renal failure, the urinary excretion, the minor excretory pathway for the drug, may be decreased by the experimental disease steate. However, improvement in the experimental design (e.g., intravenous tolbutamide administrtion; sufficient urine collection time) may be necessary to confirm the involvement of reduced hepatic metabolism during mild to moderate renal insufficiency in rabbits. Therefore, these observations indicated that the dosage adjustment may be necessary for tolbutamide in patients with renal insufficiency.

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