Arch Pharm Res Vol 24, No 4, 333-337, 2001

Archives of Pharmacal Research
http://apr.psk.or.kr

Pharmacokinetics of Diltiazem and its Major Metabolite, Deacetyldiltiazem after Oral Administration of Diltiazem in Mild and Medium Folate-Induced Renal Failure Rabbits

Jun Shik Choi[1], Jin Hwan Lee[1], and Jin Pil Burm[2]

[1]College of Pharmacy, Chosun University, Kwangju 501-759, Korea and [2]Chosun Nursing College, Kwangju 501-140, Korea

(Received February 15, 2001)

The pharmacokinetic changes of diltiazem (DTZ) and its main metabolite, deacetyldiltiazem (DAD) were studied after oral administration of DTZ to normal rabbits and mild and medium folate-induced renal failure rabbits. DTZ 10 mg/kg was given to the rabbits either orally (n=6). Plasma concentrations of DTZ and DAD 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 DTZ were significantly increased in mild and medium folate-induced renal failure rabbits. The metabolite ratio of the DTZ to DAD were significantly decreased in mild and medium folate-induced renal failure rabbits. The volume of distribution (V_d) and total body clearance (CL_t) of DTZ were significantly decreased in mild and medium folate-induced renal failure rabbits. The elimination rate constant (β) of DTZ was significantly decreased in folate-induced renal failure rabbits, but that of DAD was significantly increased. These findings suggest that the hepatic metabolism of DTZ was inhibited and the V_d, CL_t and β of DTZ were significantly decreased in mild and medium folate-induced renal failure rabbits.

Key words: Diltiazem, Deacetyldiltiazem, Pharmacokinetics, Folate-induced renal failure

INTRODUCTION

Diltiazem (DTZ) inhibits voltage-dependent L-type calcium channels and leads to vascular smooth muscle relaxation and negative inotroic and chronotropic effects in the heart (Scholz, 1997). DTZ is almost completely absorbed after oral administration, but its bioavailability is reportedly low because of considerable first-pass hepatic metabolism (Bianchetti *et al.*, 1991; Eichelbaum *et al.*, 1984). The drug is able to dilate renal vasculature and can increase the glomerular filtration rate and renal sodium excretion (Epstein *et al.*, 1990; Ruilope *et al.*, 1990; Sterzel, 1987).

DTZ has a large volume of distribution because of its liphophilicity and is rapidly and extensively distributed into body tissues (Hermann *et al.*, 1983). The drug is rapidly and almost completely metabolized in the liver via deacetylation (Homsy *et al.*, 1995; Leboeuf *et al.*, 1987), N-demethylation and O-demethylation to produce a number of active and inactive metabolites via cytochrome P-450 enzyme system (Murray *et al.*, 1996; Sutton *et al.*, 1994). DTZ and its metabolites also undergo glucuronide and sulfate conjugation (Sakuma *et al.*, 1971). Urinary excretion of DTZ does not appear to play a major role in the overall elimination process. Consistent with the statement, DTZ is excreted in urine unchanged form approximately 2-4% with renal failure patients (Pozet *et al.*, 1983; Tawashi *et al.*, 1991). Based on the literature, this drug is primarily eliminated from the body via hepatic metabolism. Therefore, renal failure is not expected to affect the pharmacokinetics of DTZ to a great extent. Interestingly, however, Lee *et al.* (1992) has shown that uranyl-nitrate induced acute renal failure decreased systemic clearance of DTZ in rats. However, since uranyl-nitrate induced acute renal failure represent only one of the renal disease models, it is not clear whether the decrease is present in clinically relevant renal failure in human. Therefore, the objective of this

Correspondence to: Jin Pil Burm, Chosun Nursing College, 280 Seoseok Dong, Dong Ku, Kwangju, 501-140, Korea
E-mail: jpburm@venus.cnc.ac.kr

study was to investigate the pharmacokinetic changes of diltiazem (DTZ) and the main metabolite, deacetyldiltiazem (DAD) after oral administration of diltiazem to normal rabbits and mild and medium folate-induced renal failure rabbits (FIRRs). We were particularly interested in determining whether similar pharmacokinetic change of DTZ occurs with our model of renal disease.

MATERIALS AND METHODS

Materials

DTZ and DAD were kindly supplied Hanil Pharm. Co. (Seoul, Korea). Imipramine, tert-Butyl methyl ether, ammonium bromide and Triethylamine were purchased from Sigma Chemical Co. (St. Louis, MO). The other chemicals were reagent grade and were used without further purification. In this study, HPLC (Model CBM-10A, Shimadzu Co., japan), syringe pump (Model 341B, Sage Co., Japan), vortex mixer (Scientific Industries, Korea) and centrifuge (Abbot Co., USA) were used.

Animals

The white male New Zealand rabbits weighing 2.0~2.5kg were fasted at least 24 h before experiment with a free access to water. Renal failure was induced to rabbits by the intravenous injection of folate (75 mg/kg, mild renal failure; 150 mg/kg, moderate renal failure). 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

DTZ 10 mg/kg was given to rabbits orally. Blood samples (1.2 ml) were withdrawn from the femoral artery at 7.5, 15, 30min, 1, 2, 3, 4, 6, 9, 12 and 24 h after the administration. Plasma was obtained by a centrifugation (6,000 rpm, 10 min). An aliquot (0.5 ml) of plasma was stored at -20°C until the analysis. Saline was infused at the rate of 1.5 ml/h to ear vein by infusion pump.

Assay and HPLC conditions

Plasma concentrations of DTZ and DAD were determined by a high performance liquid chromatography assay (Goebel *et al.*, 1985) with a slight modification. Briefly, 0.2 ml of 0.25% imipramine (the internal standard) and 5 ml of tert-Butyl methyl ether were added to 0.5 ml of sample. The mixture was vortex-mixed for 5 min and centrifuged at 6,000 rpm for 5 min. The organic layer (4.5 ml) was transferred to a clean test tube containing 0.3 ml of 0.01N hydrochloric acid, vortexed and centrifuged as above. The upper layer was discarded and 50 μl of the aqueous layer 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 an integrator. The detecter wavelength was set at 254 nm and the separation carried out at room temperature. A Shin-pack CLC-ODS column (4.6 × 250 mm, Shimadzu Co., Japan) was used in this study. Mobile phase consisted of methanol, acetonitrile, 0.04 M ammonium bromide and triethylamine (24:31:45: 0.1, v/v/v/v, pH 6.43). The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter. At a flow rate of 1.5 ml/min, relevant peaks were adequately separated (i.e., retention times: DAD, 5.3 min; internal standard, 8.2 minutes; DTZ, 10.5 minutes).

Pharmacokinetic analysis

Pharmacokinetic analysis was carried out assuming two compartment open mamillary model. When necessary, a nonlinear least square analysis was carried out using a MULTI program (Yamaoka *et al.*, 1971). The parameter value was obtained by a fitting to the pharmacokinetic model using Simplex algorithm. The area under the plasma concentration-time curves (AUC) was calculated by trapezoidal rule. Cardinal pharmacokinetic parameters were calculated using moment analysis. The maximum plasma concentration (C_{max}) and time to reach the maximum plasma concentration (T_{max}) were obtained directly from plasma concentration-time curves.

Statistical analysis

Data are presented as mean ± standard deviation. Unpaired Student's t-test was utilized to determine a statistical significance between the normal and mild or medium folate-induced renal failure rabbits. A $p<0.05$ was accepted as denoting statistical significance.

RESULTS AND DISCUSSION

Clinical laboratory data

Clinical laboratory data in folate-induced renal failure rabbits were shown in Table I. Serum creatinine concentration (S_{cr}) in mild and medium folate-induced renal failure rabbits increased significantly ($p<0.01$ and $p<$

Table I. Clinical laboratory data in normal and folate-induced renal failure rabbits

Lab. data	Normal	Mild renal failure	Medium renal failure
S_{cr} (mg/dl)	1.65 ± 0.27	2.78 ± 1.24*	4.24 ± 1.28**
BUN (mg/dl)	13.4 ± 3.15	26.4 ± 5.27**	34.6 ± 8.98**
sGOT (unit/dl)	43.3 ± 6.14	45.2 ± 3.41	38.7 ± 4.20
sGOT (unit/dl)	61.9 ± 9.73	63.4 ± 5.59	62.4 ± 9.88

Mean ± S.D. (n=6) *$p<0.05$, **$p<0.01$, S_{cr}; Serum creatinine concentration, BUN; Blood urea nitrogen

0.05 respectively) compared with that in normal rabbits. Blood urea nitrogen (BUN) in mild and medium folate-induced renal failure rabbits increased significantly ($p<0.01$) to the normal rabbits. No significant differences were sGOT and sGPT. These data indicated that folate injection created a renal failure without apparent hepatic damage in rabbits used in this study.

Plasma concentrations of DTZ and DAD

The plasma concentration of DTZ and DAD after oral administered diltiazem (10 mg/kg) were showed in Fig. 1 and 2, respectively. The plasma concentration of DTZ and DAD in mild and medium folate-induced renal failure rabbits increased significantly ($p<0.05$ and $p<0.01$ respectively) to the normal rabbits.

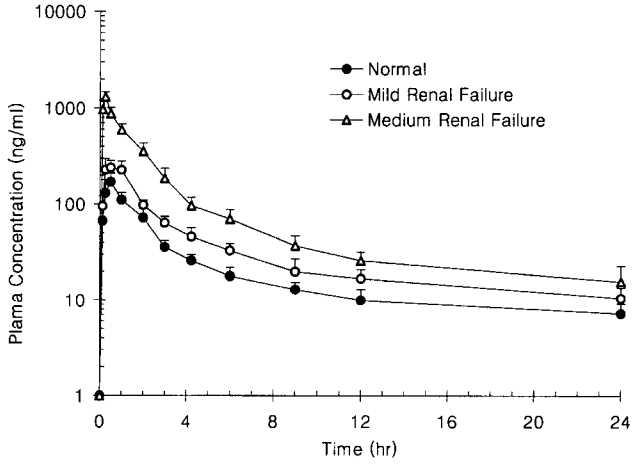

Fig. 1. Plasma concentration-time profiles of diltiazem after oral administration (10 mg/kg) in normal and folate-induced renal failue rabbits. Bars represent Mean ± S.D. (n=6)

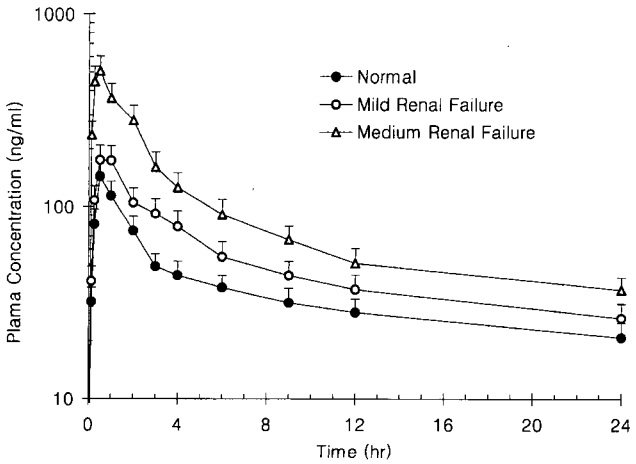

Fig. 2. Plasma concentration-time profiles of deacetyldiltiazem after oral administration of diltiazem (10 mg/kg) in normal and folate-induced renal failure rabbits. Bars represent Mean ± S.D. (n=6).

Pharmacokinetic parameters

The pharmacokinetic parameters of DTZ in normal rabbits and mild and medium folate-induced renal failure rabbits were shown in Table II. The elimination rate constant (β) of DTZ in mild and medium folate-induced renal failure rabbits (0.056 ± 0.012 h^{-1} and 0.038 ± 0.022 h^{-1}) decreased significantly ($p<0.05$ and $p<0.01$ respectively) compared with that in normal rabbits (0.071 ± 0.021 h^{-1}). The volume of distribution (V_d) and total body clearance (CL_t) of DTZ in mild and medium folate-induced renal failure rabbits (19.4 ± 2.81 L/kg and 8.20 ± 1.98 L/h/kg, 8.60 ± 3.11 L/kg and 3.98 ± 0.59 L/h/kg) decreased significantly ($p<0.01$) compared with those found in normal rabbits (42.0 ± 9.85 L/kg and 13.7 ± 3.11 L/h/kg). The AUC of DTZ in mild and medium folate-induced renal failure rabbits (1220 ± 218 ng/ml·h and 2510 ± 311 ng/ml·hr) increased significantly ($p< 0.01$) to the normal rabbits (726 ± 128 ng/ml·h). The β of DAD in mild and medium folate-induced renal failure rabbits (0.031 ± 0.006 and 0.069 ± 0.014 h^{-1}) increased significantly ($p<0.05$ and $p<0.01$ respectively) to the normal rabbits (0.017 ± 0.003 h^{-1}).

Metabolite ratio of DTZ to DAD

Metabolite ratio was obtained from the ratio of the plasma concentration of DAD and that of DTZ. The ratio for normal rabbits and mild or medium folate-induced renal failure rabbits were shown in Fig. 3. The metabolite ratio of DTZ in mild and medium folate-induced renal failure rabbits (1.45 ± 0.33, 1.10 ± 0.39) decreased significantly ($p<0.05$ and $p<0.01$ respectively) to the normal rabbits (1.73 ± 0.41).

Tawashi *et al* (1991) reported that patients with chronic

Table II. Pharmacokinetic parameters of diltiazem after oral administration of the drug in normal and folate-induced renal failure rabbits

Parameters	Normal	Mild renal failure	Medium renal failure
α (h^{-1})	0.999 ± 0.218	1.398 ± 0.311**	0.872 ± 0.124*
β (h^{-1})	0.071 ± 0.021	0.056 ± 0.012*	0.038 ± 0.022**
K_a (h^{-1})	2.71 ± 0.51	2.39 ± 0.62	3.16 ± 0.94
$t_{1/2\beta}$ (h)	9.78 ± 2.12	12.25 ± 3.98*	18.08 ± 2.48**
V_d (L/kg)	42.0 ± 9.82	19.4 ± 2.81**	8.60 ± 3.11**
CL_t (L/h/kg)	13.7 ± 3.11	8.20 ± 1.98**	3.98 ± 0.59**
AUC (ng/ml·h)	726 ± 128	1220 ± 218**	2510 ± 311**
C_{max} (ng/ml)	175 ± 28.2	323 ± 48.2**	1375 ± 282**
T_{max} (min)	52.5 ± 9.21	31.3 ± 5.11**	15.0 ± 2.41**

Mean ± S.D. (n=6), *$p<0.05$, **$p<0.01$. α, distribution rate constant; β, elimination rate constant; K_a, absorption rate constant; $t_{1/2\beta}$, 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.

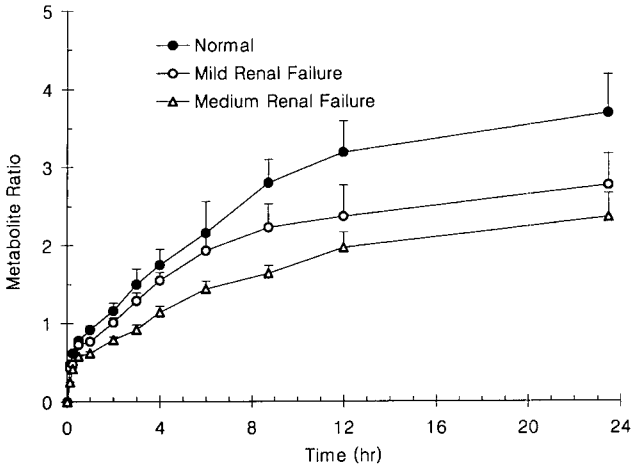

Fig. 3. Metabolite ratio of diltiazem after oral administration (10 mg/kg) in normal and folate-induced renal failure rabbits. Bars represent Mean ± S.D. (n=6), Metabolite ratio divided the plasma concentration of deacetyldiltiazem by that of diltiazem.

renal failure had lower amounts of unchanged DTZ and of its main metabolite in urine and a trend to have slightly higher values of plasma concentration. Since the terminal elimination phase was not affected by chronic renal failure, they concluded that the trend was probably the result of alterations in the volume of distribution of DTZ in these patients. In contrast, Grech-Belanger *et al.*, (1988) reported that the pharmacokinetic parameters of DTZ did not differ between healthy volunteers and patients suffering from end-stage renal disease. Pozet *et al.*, (1983) also reported that DTZ and its main metabolite, DAD had a pharmacokinetic profile similar to that in severe renal failure patients with normal renal function (peak plasma concentration, half-life and urinary excretion). DTZ is normally eliminated in the urine to a negligible extent and, thus, the small, if any, difference found in renal failure patients for the DTZ and DAD elimiantion does not appear to affect DTZ pharmacokinetics.

In summary, the AUC of DTZ were significantly increased in mild and medium folate-induced renal failure rabbits. The metabolite ratio of the DAD to DTZ were significantly decreased in mild and medium folate-induced renal failure rabbits. The elimination rate constant and total body clearance of DTZ were significantly decreased in mild and medium folate-induced renal failure rabbits. These findings suggest that the hepatic metabolism of DTZ was inhibited and total body clearance and elimination rate constant of DTZ were significantly decreased in mild and medium folate-induced renal failure rabbits. Taken together data obtained with the uranyl nitrate induced renal failure model, renal failure may generally decrease elimination of DTZ from the body. Therefore, the decrease in elimination of DTZ during renal failure may be clinically relevant.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Chosun University.

REFERENCES

Bianchetti, G., Regazzi, M., Rondanelli, R., Ascalone, V., and Morselli, P. L., Bioavailabiltity of diltiazem as a function of the administered dose. *Biopharm. Drug Dispos.*, 12, 391-401 (1991).

Eichelbaum, M. and Echizen, H. l., Clinical pharmacology of calcium antagonists. *J. Cardiovasc. Pharmacol.*, 6, 963-967 (1984).

Epstein, M. and Loutzenhiser, R. D., Effects of calcium antagonists on renal hemodynamics. *Am. J. Kidney Dis.*, 16, 10-14 (1990).

Goebel, K. J. and Kolle, E. U., High performance liquid chromatographic dertermination of diltiazem and four of its metabolites in plasma. *ibid,* 345, 355-363 (1985).

Grech-Belanger, O., Langlois, S. and LeBoeuf, E., Pharmacokinetics of diltiazem in patients undergoing continuous ambulatory peritoneal dialysis. *J. Clin. Pharmacol.*, 28, 477-480 (1988).

Hermann, P., Rodger, S. D., Remones, G., Thenot, J. P., London, D. R., and Morselli, P. L., Pharmcokinetics of diltiazem after intravenous and oral administration. *Eur. J. Clin. Pharmacol.*, 24, 349-352, (1983).

Homsy, W., Lefebvre, M., Caille, G., and du Souich P. l., Metabolism of diltiazem in hepatic and extrahepatic tissues of rabbits. *Pharm. Res.*, 12. 609-614 (1995).

Leboeuf, F. and Grech-Belanger, O. l., Deacetylation of diltiazem by rat liver. *Drug Metab. Dispos.*, 15, 122-126 (1987).

Lee, Y. -H., Lee, M. -H., and Shim, C. -K., Decreased systemic clearance of diltiazem with increased hepatic metabolism in rats with uranyl nitrate-induced acute renal failure. *Pharm. Res.*, 12, 1599-1606 (1992).

Murray, M. and Butler, A. M., Enhanced inhibition of microsomal cytochrome P-450 in rat liver during diltiazem biotransformation. *J. Pharmco.l Exp. Ther.*, 279, 1447-1452 (1996).

Pozet, N., Brazier, J. L., Aissa, A. H., Khenfer, D., Faucon, G., Apoil, E., and Traeger, J., Pharmacokinetics of diltiazem in severe renal failure. *Eur. J. Pharmacol.*, 24, 635-638 (1983).

Ruilope, L. M. and Alcazar, J. M., Renal effects of calcium entry blockers. *Cardiovasc. Drugs Ther.*, 4, 979-982 (1990).

Sakuma, M., Yoshikawa, M., and Sato, Y., The whole body autoradiographic studies on the disposition of 14C-labeled new 1,5-benzothiazepine derivative(14C-CRD -401) in mice. *Chem. Pharm. Bull.*. 19, 995-1005 (1971).

Scholz, H., Pharmacological aspects of calcium channel

blockers. *Cardiovasc. Drugs Ther.,* 10, 869-872 (1997).
Sterzel, P. B., Renal actions of calcium antagonists. *J. Cardiovasc. Pharmacol.,* 10, 17-22 (1987).
Sutton, D., Butler, A. M., Nadin, L., and Murray, M. I., Role of CYP3A4 in human hepatic diltiazem N-demethylation: inhibition of CYP3A4 activity by oxidized diltiazem metabolite. *J. Pharmacol. Exp. Ther.,* 282, 294-300 (1994).
Tawashi, M., Marc-Aurele, J., Bichet, D., Spenard, J., Lariviere, L., Plante, D., and Caille, G. E., Pharmacokinetics of intravenous diltiazem and five of its metabolites in patients with chronic renal failure and in healthy volunteers. *Biopharm. Drug Dispos.,* 12, 105-112 (1991).
Tawashi, M., Marc-Aurele, J., Bichet, D., Spenard, J., Lariviere, L., Plante, D., and Caille, G. E., Pharmacokinetics of oral diltiazem and five of its metabolites in patients with chronic renal failure. *Biopharm. Drug. Dispos.,* 12, 95-104 (1991)
Yamaoka, K., Tanigawara, Y., Nakagawa, T., and Uno, T., A pharmacokinetics analysis program for microcomputer. *J. Pharm. Dyn.,* 4, 879-883 (1971).