Decreased Systemic Clearance of Diltiazem with Increased Hepatic Metabolism in Rats with Uranyl Nitrate-Induced Acute Renal Failure

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The effect of uranyl nitrate (UN)-induced acute renal failure (ARF) on the pharmacokinetics of diltiazem (DTZ) was examined in rats through *in vitro* and *in vivo* studies. *In vitro* homogenate studies demonstrated that DTZ was metabolized to deacetyl diltiazem (DAD) predominantly in the liver. Metabolism in the small intestine, kidney, or blood pool was negligible compared with that in the liver. UN-induced ARF (UN-ARF) increased the *in vitro* hepatic clearance (CL_{vit}) of DTZ 1.4-fold. *In vivo* pharmacokinetic studies following intravenous (iv) and portal venous (pv) administration revealed that UN-ARF increased the intrinsic clearance (CL_i) of DTZ from 243.0 to 414.5 ml/min/kg but decreased its total plasma clearance (CL_t) from 90.3 to 64.3 ml/min/kg. The increase in CL_i was consistent with the increase in CL_{vit} of the liver. The *in vitro* plasma free fraction of DTZ (f_p) was decreased from 0.25 to 0.14 by UN-ARF, but the *in vitro* blood/plasma partition of DTZ (R_b) remained constant at unity. From the CL_i and f_p changes, the plasma intrinsic clearance for unbound DTZ (C_{L_i}') was calculated to be increased 2.7-fold, from 1104.5 to 2960.7 ml/min/kg, by UN-ARF. The f_p decrease was also reflected in the steady-state distribution volume (Vd_{ss}) of DTZ, which was decreased significantly from 3595.5 to 2528.3 ml/kg. The absolute bioavailability of pv DTZ (F_{pv}) was decreased by UN-ARF from 37.5 to 15.5% but was still much higher than the reported oral bioavailability (6%), indicating poor absorption of DTZ from the GI tract. From the calculation based on a well-stirred pharmacokinetic model, DTZ was found to increase the hepatic blood flow (HBF) of the control rats more than twofold at doses of 3 mg/kg (iv) or 10 mg/kg (pv), possibly due to the vasodilating effect of DTZ. However, the effect of DTZ on HBF was not present in the UN-ARF rats. It is not clear at present whether this could be attributed to vasoconstricting effects of UN-ARF or blockade of the vasodilating effect of DTZ.

KEY WORDS: diltiazem; deacetyldiltiazem; uranyl nitrate acute renal failure; hepatic metabolism; tissue homogenates; total plasma clearance; intrinsic clearance; plasma protein binding; blood/plasma partition; hepatic blood flow.

INTRODUCTION

Diltiazem (DTZ) has been used as a calcium antagonist (1) in the treatment of angina pectoris, arrhythmia and hypertension (2). DTZ is metabolized to deacetyldiltiazem (DAD), which is further metabolized to various metabolites in rats, mice (3), and human subjects (4). DAD, a major metabolite of DTZ, possesses 40 to 50% of the pharmacological activity of the parent drug (5).

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DTZ is eliminated predominantly through hepatic metabolism and biliary excretion (4). Therefore, hepatic metabolism and, consequently, the total plasma clearance (CL_t) of DTZ are expected to be decreased in patients with hepatic failure. However, the effect of renal failure on hepatic drug metabolism is somewhat unpredictable, since both decreases (6–8) and increases (9–11) of hepatic clearance (CL_h) of drugs have been reported in renal failure.

The oral clearance (CL_{po}) of DTZ in the renal failure patients has previously been demonstrated to be unchanged (12). However, this does not necessarily mean that the hepatic metabolism of DTZ is unchanged. The CL_{po} is a function not only of the metabolic intrinsic clearance (CL_i), but also of the oral bioavailability (F_{po}). Moreover, F_{po} itself is sensitive to hepatic and/or gastrointestinal (GI) metabolism (first-pass effect): If renal failure alters F_{po}, CL_{po} will no longer reflect CL_i. Therefore, approaches more direct than oral administration studies are necessary to elucidate whether and how renal failure affects the hepatic metabolism of drugs.

First, we tried to elucidate in this study whether or not acute renal failure (ARF) affects the *in vitro* metabolism of DTZ in liver and other tissue homogenates. Second, we examined the effects of ARF on the plasma pharmacokinetics of DTZ and DAD after iv and pv administration of DTZ. ARF was induced by iv injection of uranyl nitrate (13) to rats.

MATERIALS AND METHODS

Chemicals

Diltiazem (DTZ) and its major metabolite, deacetyldiltiazem (DAD), were kindly provided by Han-Il Pharmaceutical Co. (Seoul, Korea). Uranyl nitrate (UN) [$UO_2(NO_3)_2$ $6H_2O$] was purchased from Sigma Co. Heparin (5000 IU/ml) was purchased from Choong-Wae Pharmaceutical Co. (Seoul, Korea). Imipramine, the internal standard, was provided by Hwan-In Pharmaceutical Co. (Seoul, Korea). Methanol (Merck), acetonitrile (Merck), and t-butyl methyl ether (Aldrich) were HPLC grade. All other reagents were analytical grade and used as purchased.

Induction of UN-ARF in Rats

Male Wistar rats weighing 200–260 g were used in all experiments. The rats were fasted for 24 hr before the experiments. ARF was induced by a single iv injection of a 0.5% (w/v) UN solution in saline (5 mg/kg) via the tail vein 5 days before the experiment (13). On the day of the experiment, serum creatinine, blood urea nitrogen (BUN), total plasma protein, hematocrit, kidney weight, sGOT, and sGPT were determined to assess the degree of ARF. Normal healthy rats served as the control group.

In Vitro Blood/Plasma Partition (R_b) of DTZ

Blood was withdrawn from each rat in the control (n = 4) and UN-ARF (n = 4) groups. A 10-μl aliquot of DTZ stock solution in saline was spiked to 500 μl of fresh blood in

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a glass test tube (Vacuject, 10 ml; Green Cross Co., Seoul, Korea) to yield 200 and 1000 ng/ml and gently mixed by hand for 30 sec. A 5-μl aliquot of heparin (150 IU/ml) was previously added to the tubes to prevent blood clotting. Then the tubes were placed in a water bath at 37°C and shaken at 75 oscillations per minute (opm). The tubes were removed at 0, 3, 6, 10, 15, 20, 30, and 60 min and centrifuged at 6000*g* for 1 min for plasma separation. DTZ in the plasma was determined by HPLC as described below. The partition ratio of DTZ between blood and plasma (R_b) was calculated from the plasma and initial blood concentrations of DTZ.

Plasma Protein Binding of DTZ

Plasma protein binding of DTZ was determined by an ultrafiltration method using a commercially available device (MPS-1, Amicon Co., USA). The membrane filter (YMT, Amicon, Co.) retains compounds with molecular weights greater than 50,000. Preliminary experiments indicated that protein leakage through the membrane and adsorption of DTZ to the device or to the membrane were negligible. An aliquot (1 ml) of fresh plasma obtained from each rat ($n = 4$ for each group) was spiked with DTZ to yield 200 and 1000 ng/ml and then poured into the upper reservoir of the filter. After gentle shaking for 3 min, the DTZ-containing plasma sample was centrifuged at room temperature for 7 min at 1000*g*. The volume of the filtrate did not exceed 20% of the initial plasma volume. The total (bound and unbound) concentration of DTZ in the spiked plasma prior to filtration and the unbound (ultrafiltrated) concentration of DTZ were measured by HPLC. Plasma samples from four rats per group were tested for their binding with DTZ.

In Vitro Metabolism of DTZ in Homogenates

Under light ether anesthesia, the carotid artery of each rat was cannulated for blood collection. After blood collection, the abdomen was opened and the portal vein was cannulated with an 18-gauge catheter placement unit. Ice-cold normal saline was perfused through the catheter at a flow rate of 40–50 ml/min. Heparin in saline (150 IU/ml) had been previously added to the tubes to prevent blood clotting. When the color of the liver became light tan, the liver, small intestine, and kidneys of the rat were excised. The feces remaining in the small intestine was removed by ice-cold saline perfusion and air injection. Each organ was cut into five or six slices and homogenized (Potter-Elvehjem) with 4 vol of ice-cold 1.15% (w/v) KCI–0.01 *M* phosphate buffer (pH 7.4) to yield 20% (w/v) homogenates. A pH of 7.4 has been reported to be the optimal pH for the activity of DTZ deacetylase (14). The homogenates were strained to remove blood vessels and connective tissue and the eluant was recovered. Each eluant was centrifuged at 100*g* for 10 min at 4°C, and the supernatants were recentrifuged at 9000*g* for 20 min at 4°C. The 9000*g* supernatant fractions were collected and stored at −40°C until use. These supernatant fractions were used within a week after collection. Protein content of the supernatants was determined by the method of Lowry *et al.* (15) using bovine serum albumin as a standard.

The *in vitro* metabolism study was conducted at 37°C in an incubation system set at 75 opm. After preincubation of the tissue homogenates in the incubator for 5 min, DTZ solution (100 μg/ml in saline) was spiked in 5 ml (=1 g wet tissue) of the homogenates to yield a final concentration of 940 ng/ml. The same study was also performed with both blood and plasma. The plasma was obtained by centrifuging the blood at 4000*g* for 10 min. Aliquots of 100 μl were sampled from the incubation medium of the liver homogenate at 0.5, 1, 2, 4, 6, 8, 10, and 15 min and from the small intestine, kidney, blood, and plasma at 10, 30, 60, 120, 180, and 240 min. All the samples were assayed for DTZ and DAD by HPLC as described below.

Administration of DTZ Through Intravenous (iv) and Portal Venous (pv) Bolus Injection

All experiments were performed on both control and UN-ARF rats. The rats were supine during the experiment. Under light ether anesthesia, the femoral artery and veins of the rats were cannulated with PE-50 polyethylene tubing. After complete recovery (1 hr) from anesthesia, a 0.3% (w/v) DTZ solution in saline was administered intravenously to the femoral vein through the catheter at a dose of 1 ml/kg (3 mg/kg). Blood samples (250 μl) were withdrawn into heparinized tubes from the femoral artery catheter at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150, and 180 min after the dose.

DTZ was also administered to another group of rats through the hepatic portal vein. Under light ether anesthesia, the abdomen was opened through a midline incision and an injection needle (25-gauge) bent at an angle of 120° and connected to PE-50 tubing was inserted into the portal vein and fixed with surgical glue (Aron Alpha, Sankyo Co., Japan). Subsequently, the femoral artery was cannulated with PE-50 for blood sampling. After closure of the incision and complete recovery from the anesthesia, a 1% (w/v) solution of DTZ in saline was administered at a dose of 1 ml/kg (10 mg/kg) through the pv catheter. Blood samples (250 μl) were withdrawn at 0, 5, 10, 20, 40, 60, 90, 120, 150, and 180 min after the dose.

Throughout the study, plasma samples were separated by centrifuging the blood samples at 6000*g* for 1 min and were stored at −20°C until HPLC assay for DTZ and DAD. DTZ was reportedly found to be stable for 3 months at −20°C (16).

HPLC Assay of DTZ and Deacetyldiltiazem (DAD)

DTZ and DAD in plasma and tissue homogenates were measured by a modified HPLC method (17). Fifty microliters of internal standard solution (2 μg/ml of imipramine in methanol) in a polypropylene tube was evaporated to dryness with a gentle stream of nitrogen. A aliquot of 100 μl of plasma sample or incubation medium was added to the residue and vortexed. The sample was then extracted with 3 ml of t-butyl methyl ether by vortexing for 5 min. After centrifugation, the tubes were placed in a dry ice bath and 2.5-ml aliquots of the unfrozen upper organic phase were transferred to other tubes. Subsequently, the organic layers were back-extracted with 100 μl of 0.01 *N* HCI by vortexing for 1 min. Aliquots of 30 μl were taken from the HCI layer and injected into HPLC system.

The HPLC system consisted of a precision isocratic

pump (Model SP 8810), a C_{18} reversed-phase column (μ-Bondapak, 10-μm silica, 300 × 3.9-mm id), and a UV absorbance detector (Model 757, Applied Biosystems). The mobile phase was a mixture of methanol, acetonitrile, 0.04 *M* ammonium bromide, and triethylamine (40:24:36:0.1 volume ratio). The pH of the mobile phase was adjusted to 6.4 using 2 *N* hydrobromide. The flow rate of the mobile phase was 1.0 ml/min and the wavelength of the detector was 237 nm. The recoveries of DTZ and DAD were more than 90% and the detection limit was 20 ng/ml.

In Vitro Pharmacokinetic Analysis

In vitro clearance (CL_{vit}) of DTZ was defined as

$$CL_{vit} = \text{Spiked Amount}/AUCH_{0-\infty} \quad (1)$$

where $AUCH_{0-\infty}$ is the area under the DTZ concentration–time curve in the homogenate from time 0 to infinity. $AUCH_{0-\infty}$ was calculated by Eq. (2).

$$AUCH_{0-\infty} = AUCH_{0-t} + CH_t/k \quad (2)$$

where $AUCH_{0-t}$ is the area under the DTZ concentration–time curve in the homogenate from time 0 to the final sampling time *t;* $AUCH_{0-t}$ was calculated by the trapezoidal method. CH_t is the DTZ concentration in the homogenate at time *t,* and *k* is the apparent degradation rate constant of DTZ in the homogenate obtained from the slope of the log-linear portion of the curve by least-squares regression analysis. The value of *t* was 15 min for the liver, 180 min for the small intestine, and 240 min for the kidney, blood, and plasma. CL_{vit} was normalized for the wet tissue weight of each organ of a 250-g rat. Normalization of CL_{vit} for a 250-g rat was performed simply by multiplying CL_{vit}/g tissue by the wet tissue weight (g) of the organ. The weights of the liver, kidney, and small intestine were experimentally determined and normalized to 250-g body weight, while the volume of the plasma was obtained from the literature (18). The volume of blood was calculated from the plasma volume and the experimentally measured hematocrit.

In Vivo Pharmacokinetic Analysis

It was assumed that the liver behaved like a well-stirred compartment and the kinetics of intrahepatic distribution and metabolic removal of DTZ were linear. Enterohepatic recycling of DTZ was not considered in this analysis. Total plasma clearance (CL_t), hepatic intrinsic clearance (CL_i), distribution volume at steady state (Vd_{ss}) for total (free plus bound form) plasma DTZ, and bioavailability of pv DTZ (F_{pv}) were calculated by Eqs. (3)–(6).

$$CL_t = D_{iv}/AUC_{iv} \quad (3)$$

$$CL_i = D_{pv}/AUC_{pv} \quad (4)$$

$$Vd_{ss} = D_{iv}\ AUMC_{iv}/AUC^2_{iv} \quad (5)$$

$$F_{pv} = AUC_{pv}D_{iv}/(AUC_{iv}\ D_{pv}) \quad (6)$$

where *D,* AUC, and AUMC, respectively, denote dose, area under the plasma DTZ concentration–time curve from time 0 to infinity, and area under the moment of the plasma DTZ concentration–time curve from time 0 to infinity. The AUC and AUMC were calculated by the trapezoidal method from 0 to 180 min and extrapolated from 180 min to infinity using the elimination rate constant (β). β is the slope of the terminal phase obtained after fitting the plasma concentration data to a conventional two-compartment model using the program MULTI (19).

Statistical Analysis

The statistical significance of the differences in the *in vitro* metabolism parameters (Table I) and *in vivo* pharmacokinetic parameters (Table II) between treatments (ie, control and UN-ARF) was determined using the one-way analysis of variance (ANOVA) for unpaired data. For both R_b and plasma protein binding studies, two-way ANOVA was used to test for effects of DTZ concentration and treatment. A *P* value of <0.05 was chosen as the level of statistical significance. All results are expressed as mean ± standard deviation.

Table I. Effect of UN-ARF on *in Vitro* Metabolism of DTZ in Various Tissue Homogenates from Rats[a]

	Liver		Small intestine		Kidney		Blood		Plasma	
Parameter	Control	ARF	Control	ARF	Control	ARF	Control	ARF	Control	ARF
Organ weight (g/250-g rat)[b]	9.40 (0.62)	9.05 (1.00)	6.26 (0.55)	6.33 (0.88)	2.07 (0.07)	2.75* (0.29)	22.46	22.46	11.25	11.25
$T_{1/2,\ deg}$ (min)	1.0 (0.3)	0.6** (0.1)	8.3 (1.5)	6.4 (1.3)	228.0 (27.3)	187.6 (37.3)	94.9 (6.5)	103.8 (21.9)	67.7 (6.2)	183.3** (76.8)
CL_{vit} (ml/min/organ)	13.90 (2.15)	18.80** (2.56)	1.14 (0.22)	1.08 (0.31)	0.01 (0.00)	0.02*** (0.00)	0.18 (0.00)	0.17 (0.03)	0.13 (0.01)	0.05**** (0.01)

[a] Data represent the mean ± SD of four experiments. DTZ was incubated with 20% (w/v) homogenates in pH 7.4 phosphate buffer or with blood or plasma at 37°C and 75 opm. CL_{vit} was calculated according to Eq. (1) and normalized to the respective organ weights of a 250-g rat.

[b] Volumes of blood and plasma were obtained from the literature (18).

* $P < 0.01$.

** $P < 0.05$.

*** $P < 0.02$.

**** $P < 0.001$.

Table II. Effect of UN-ARF on the Pharmacokinetic Parameters of DTZ in Rats[a]

Parameter	iv		pv	
	Control (n = 6)	ARF (n = 7)	Control (n = 7)	ARF (n = 12)
CL_t (ml/min/kg)	90.3 (15.0)	64.3* (15.7)	—	—
CL_i (ml/min/kg)	—	—	243.0 (91.1)	414.5** (127.0)
Vd_{ss} (ml/kg)	3595.5 (808.1)	2528.3* (490.7)		—
AUC_{DTZ} (ng min/ml)	33217.5 (5357.1)	46650.2* (490.4)	41155.7 (18183.3)	24126.8* (7256.7)
AUC_{DAD}/ AUC_{DTZ}	0.19 (0.03)	0.10** (0.06)	0.86 (0.18)	1.07 (0.33)
F_{pv} (%)	—	—	37.2	15.5

[a] Each datum was expressed as mean ± SD of the experiments. Doses of iv and pv administration were 3 and 10 mg/kg, respectively. Numbers in parentheses indicate the SD.
* $P < 0.02$.
** $P < 0.01$.

RESULTS

Induction of ARF

ARF was successfully induced by iv administration of UN (5 mg/kg) 5 days before the experiment without causing any apparent changes in physiological parameters other than serum creatinine, BUN, and kidney weight. Rats treated with UN exhibited an approximately 6-fold increase in serum creatinine (from 0.86 to 5.16 mg/100 ml), a 10-fold increase in BUN (from 22.8 to 215.6 mg/100 ml), and a 1.3-fold increase in kidney weight (from 2.07 to 2.75 g/250 g body weight), indicating severe impairment of renal function (n = 5). However, there were no changes in the serum concentrations of sGOT, sGPT, total protein, and albumin and in liver weight, indicating no significant liver damage as measured by these markers. Hematocrit values were almost identical in both groups of rats [0.49 ± 0.03 (n = 5)].

Protein contents in 9000g supernatants of liver and small intestine homogenates were 77.0 ± 10.2 and 62.5 ± 13.1 mg/g wet tissue (n = 5), respectively. They were not changed by UN-ARF, while the protein content of the kidney homogenate was decreased significantly, from 117.0 to 94.3 mg/g wet tissue (n = 5). This effect may be due to swelling of the kidney cells caused by UN-ARF (20).

Effect of UN-ARF on the Blood/Plasma Partition (R_b) and Plasma Protein Binding of DTZ

The partition of DTZ between blood and plasma (R_b) reached equilibrium within 6 min and remained constant thereafter during the 60-min experiment in rats of both groups. The steady-state values of R_b were 0.93 ± 0.08 (n = 5) and 0.97 ± 0.09 (n = 5) at DTZ concentrations of 200 and 1000 ng/ml, respectively, for the control rats. They were 0.98 ± 0.05 and 1.13 ± 0.10, respectively, for the UN-ARF rats. This result is consistent with previous results (21), where an R_b of unity for human blood was reported. Therefore, the R_b was considered unity in both groups of rats throughout the study.

Plasma protein binding of DTZ was increased by ARF: The plasma free fraction (f_p) of DTZ was 0.24 ± 0.05 (n = 4) at 200 ng/ml of DTZ and 0.26 ± 0.04 (n = 4) at 1000 ng/ml of DTZ in the control rats. It was decreased significantly by UN-ARF, to 0.12 ± 0.05 (n = 4) at 200 ng/ml DTZ and 0.16 ± 0.03 (n = 4) at 1000 ng/ml DTZ, respectively. Since binding was not affected by the concentration of DTZ, f_p was considered to be 0.25 in the control rats and to be decreased by UN-ARF to 0.14 throughout the study.

Effect of UN-ARF on *in Vitro* Metabolism of DTZ

The time courses of DTZ concentrations in various tissue homogenates, blood, and plasma are shown in Fig. 1 together with those of DAD formed from DTZ. There was an abrupt decrease in the concentrations of DTZ immediately after incubation in the liver, small intestine, and kidney homogenates but not in the blood and plasma. Thereafter the concentration of DTZ declined monoexponentially, indicating apparent first-order degradation kinetics. The initial decrease in DTZ concentration implies a possible adsorption of DTZ to a component(s) of the homogenates. Degradation was most rapid in the liver, followed by small intestine, blood, and kidney. Degradation in the kidney was much slower than that in the other organs examined.

UN-ARF accelerated the degradation of DTZ in the liver homogenate, yielding a rapid increase in DAD concentrations (Fig. 1A), but had no significant effect on the degradation in the small intestine, kidney, and blood (Figs. 1B, C, and D). In contrast, both the degradation of DTZ and the formation of DAD in the plasma were retarded by UN-ARF (Fig. 1E).

Table I summarizes the effect of UN-ARF on the *in vitro* degradation half-life ($T_{1/2,\ deg}$) of DTZ in the tissue homogenates at 37°C and their respective organ clearances (CL_{vit}) in 250-g rats. The $T_{1/2,\ deg}$ was calculated by $0.693/K_d$, where K_d is the first-order degradation rate constant of DTZ obtained by linear regression of each line in Fig. 1. The $T_{1/2,\ deg}$ of DTZ in the liver homogenate was decreased significantly by UN-ARF, while those in the small intestine, kidney, and blood were not. It is notable that UN-ARF increased the $T_{1/2,\ deg}$ in the plasma almost 2.7-fold but not in the blood. The CL_{vit} of the organs was in the rank order: liver >> small intestine >> blood > plasma >kidney after normalization to the organ weights in the control and UN-ARF rats. Table I shows significant increases in the CL_{vit} of the liver (1.4-fold) and kidney (2-fold) by UN-ARF. However, for the kidney homogenate, the significance disappeared when the clearance value was recalculated on the basis of the same kidney weight. The CL_{vit} of the plasma but not blood was decreased significantly by UN-ARF.

Effect on UN-ARF on the Pharmacokinetics of DTZ

Plasma levels of DTZ and DAD after iv administration of DTZ (3 mg/kg) to control (solid curves) and UN-ARF (dotted curves) rats were plotted as a function of time and are shown in Fig. 2. Plasma DAD had already reached its

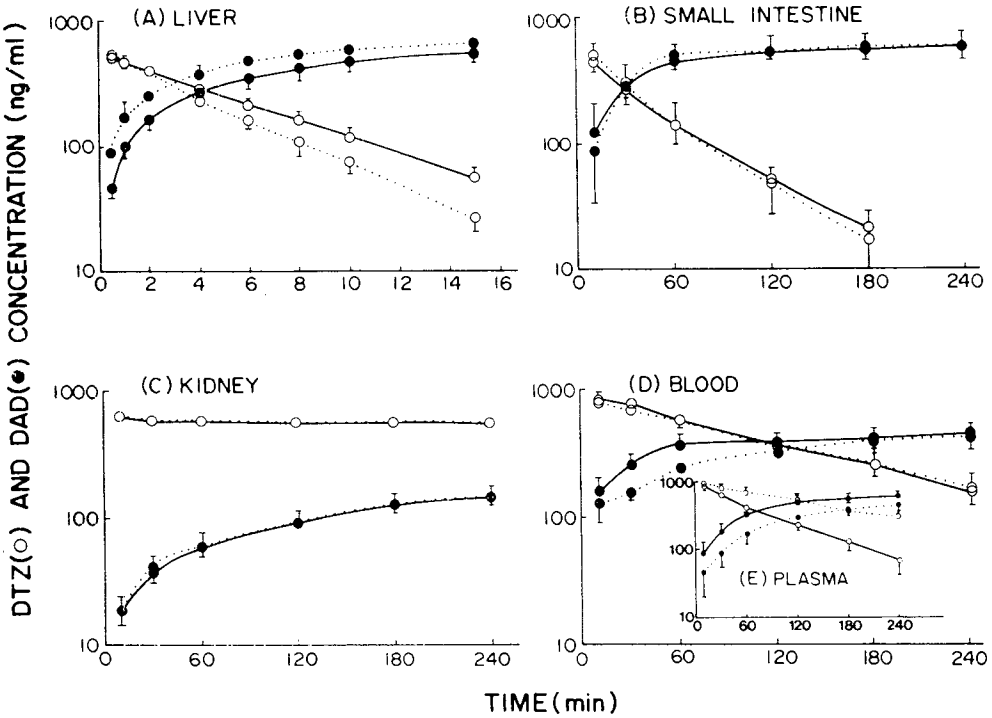

Fig. 1. *In vitro* degradation of DTZ (○) and resultant formation of DAD (●) in the 20% (w/v) homogenates of various organ tissues (A–C) and in the blood (D) and plasma (E) from control (solid lines) and UN-ARF (dotted lines) rats. DTZ was spiked into the tissue homogenates, blood, and plasma so as to yield a initial concentration of 940 ng/ml. Then the incubation was conducted at 37°C and 75 opm. Each point represents the mean ± SD of four experiments.

maximum at the first sampling time (5 min) and then decayed biexponentially in both groups. The plasma levels of DAD in both groups declined almost parallel to those of DTZ but were much lower. Figure 2 shows that UN-ARF increased plasma DTZ concentrations and decreased plasma DAD concentrations slightly. The effect of UN-ARF on the pharmacokinetic parameters of DTZ is summarized in Table II. The AUC was increased significantly, hence the CL_t was decreased by 30%. The CL_t of DTZ in control rats was much greater than the value reported for humans (21). The AUC

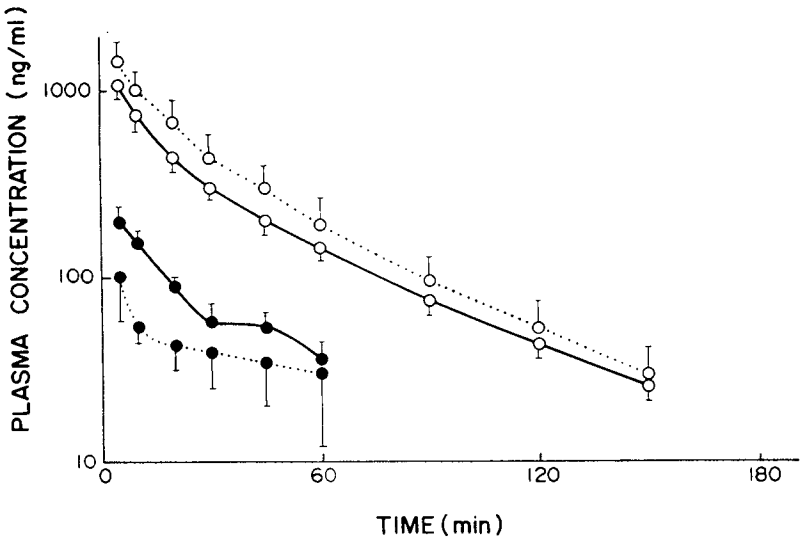

Fig. 2. Plasma concentration–time profiles of DTZ (○) and its major metabolite DAD (●) after iv bolus injection of DTZ to control (solid lines) and UN-ARF (dotted lines) rats at a dose of 3 mg/kg. Each point represents the mean ± SD of six (control) and seven (UN-ARF) experiments.

ratio between DAD and DTZ (AUC_{DAD}/AUC_{DTZ}) after iv bolus injection of DTZ decreased by one-half in UN-ARF rats. The Vd_{ss} of DTZ in control rats was consistent with results in humans (21) and was decreased by 30% in UN-ARF rats.

In the pv study (Fig. 3), the DTZ dose was increased to 10 mg/kg. The resultant plasma concentrations of DTZ were comparable to those observed for an iv dose of 3 mg/kg. As in the iv study, plasma DAD had already reached its maximum at the first sampling (5 min) and then decayed biexponentially in both groups. The plasma levels of DTZ and DAD after pv administration of DTZ were almost parallel in both groups of rats. However, the plasma concentrations of DAD were much higher than those observed after iv administration, indicating considerable first-pass metabolism of DTZ to DAD. The plasma levels of DTZ and DAD were decreased by UN-ARF. The AUC of DTZ was decreased significantly (40%) by UN-ARF, but the ratio of AUC_{DAD}/AUC_{DTZ} remained unchanged. The CL_i of DTZ from Eq. (4) was increased significantly (1.7-fold) by UN-ARF, irrespective of the decrease in CL_t (Table II). The bioavailabilities of pv DTZ (F_{pv}) were 37.2% for the control rats and 15.5% for the UN-ARF rats.

DISCUSSION

The *in vitro* degradation of DTZ occurred monoexponentially in all the homogenates examined, implying linear metabolism of DTZ at concentrations below 940 ng/ml. The organ weight-normalized CL_{vit} of the liver was much larger than that of the other organs examined in both control and ARF rats, suggesting that hepatic degradation of DTZ dominates the overall pharmacokinetics of DTZ. This is consistent with the observations of Leboeuf and Grech-Belanger (14), who reported the predominant hepatic distribution of DTZ deacetylase, an enzyme responsible for DTZ metabolism to DAD. The concentrations of DAD in all the homogenates did not decrease significantly after reaching pseudo steady state (Fig. 1), indicating negligible degradation of DAD to its secondary metabolites.

The CL_{vit} of DTZ in the liver was increased significantly (1.4-fold) by UN-ARF. This cannot be attributed to the increased content of DTZ deacetylase since the protein content in the 9000*g* supernatant of the liver homogenate remained constant in UN-ARF rats. The CL_{vit} of DTZ in the kidney homogenate was also increased significantly by UN-ARF. This could be explained solely by increased kidney weight since $T_{1/2,\,deg}$ was not changed significantly by UN-ARF. On the other hand, the CL_{vit} of DTZ in the plasma was decreased significantly by UN-ARF, and that in the whole blood remained unchanged. There is no information available at present to explain this discrepancy. Different effects of UN-ARF on the multiple metabolic pathways of DTZ in the three homogenates are one possible explanation. However, the changes in the CL_{vit} in the small intestine, kidney, and blood are not expected to influence the overall disposition of DTZ in the body significantly since CL_{vit} in each is much smaller than that in the liver.

The plasma concentrations of DAD after iv and pv administration of DTZ reached their maximum within 5 min and decayed in a profile parallel to DTZ. This parallel decay could be attributed both to the prompt metabolism of DTZ to DAD and to the similarity of the pharmacokinetics of DAD and DTZ (22). The parallel characteristics of decay were not changed by UN-ARF. The prompt metabolism of DTZ to DAD was partly supported by the low F_{pv} value of DTZ in the control rats (37.2%).

The hepatic intrinsic clearance (CL_i) of plasma DTZ was calculated to be increased 1.7-fold by UN-ARF in spite of a 30% decrease in CL_t. This means that the *in vivo* metabolism of DTZ in the liver is increased by UN-ARF, as is expected from the increased CL_{vit} of the liver in the UN-ARF rats. This is supported by the 50% decrease in the F_{pv} of DTZ in the UN-ARF rats.

The decrease in CL_t in spite of the CL_i increase in the UN-ARF rats was further analyzed according to the well-

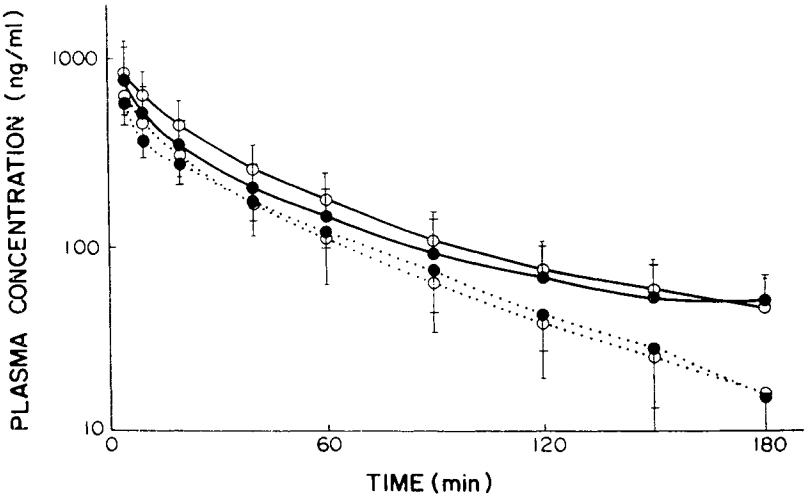

Fig. 3. Plasma concentration–time profiles of DTZ (○) and DAD (●) after pv bolus injection of DTZ to control (solid lines) and UN-ARF (dotted lines) rats at a dose of 10 mg/kg. Each point represents the mean ± SD of 7 (control) and 12 (UN-ARF) experiments.

stirred pharmacokinetic model for drugs after iv administration (23).

$$CL_h = HBF\ CL_i/(HBF + CL_i/R_b) \quad (7)$$

The blood/plasma partition (R_b) of DTZ was not affected by possible metabolites in the blood and remained constant at unity during the partition study. Thus, Eq. (7) can be simplified as

$$CL_h = HBF\ CL_i/(HBF + CL_i) \quad (8)$$

Here CL_t could approximate CL_h for DTZ (4). Using CL_t and CL_i values in Table II, HBF was calculated from Eq. (8) to be 143.7 for the control rats and 76.1 ml/min/kg for the UN-ARF rats. The HBF in the control rats was much larger than the reported value of 50–70 ml/min/kg (24). It may be somewhat overestimated since the CL_t instead of the CL_h was incorporated into the calculation. Besides, the CL_i used in this calculation might be an additional cause of overestimation since it might include the clearance by the other organs such as the small intestine. However, the HBF in the control rats in this study is certainly beyond the probable range of this overestimation. Another possible explanation is the vasodilating effect of DTZ. Indeed, Etoh *et al.* (25) reported a 50–75% increase in portal blood flow at a 313–561 ng/ml plasma DTZ level.

On the other hand, the HBF increase caused by DTZ was absent in the UN-ARF rats, the mechanism of which is unclear. A vasoconstricting effect of UN-ARF similar to that observed in glycerol-induced ARF (26,27) and blockade of the vasodilating effect of DTZ by the accumulation of unknown factors in the blood caused by UN-ARF are two possible mechanisms. Additional studies are necessary before a conclusion on the mechanism of the reduced HBF can be drawn.

The CL_i is a function of f_p and CL_i' as expressed by $f_p \times CL_i'$, where CL_i' represents the hepatic intrinsic clearance for the unbound plasma DTZ. The f_p was determined by an ultrafiltration technique. This technique requires much less time than equilibrium dialysis and, thus, is expected to suffer less interference from metabolites possibly formed during the determination. However, the f_p in the control rats was almost the same as the reported value determined by the equilibrium method at 37°C (28), suggesting that metabolites do not interfere significantly with the protein binding of the parent drug, DTZ. The f_p of DTZ was decreased significantly by UN-ARF. Uremia has been known to elevate the plasma level of α-1-acid glycoprotein (AAG) (29), which is an avid binder of many organic bases (30). UN did not interfere directly with protein binding (31). Therefore, the decrease in f_p in the UN-ARF rats might be attributed to increased binding of DTZ with AAG. The decreased f_p together with the increased CL_i (Table II) resulted in a 2.7-fold increase in the CL_i' (from 1104.5 to 2960.7 ml/min/kg) in the UN-ARF rats.

HBF and CL_i have different effects on the AUC of DTZ according to the routes of administration: HBF affects predominantly the AUC_{iv}, and CL_i affects predominantly the AUC_{pv}. This might explain the different effect of UN-ARF on the ratio of AUC_{DAD}/AUC_{DTZ} between iv and pv administrations of DTZ.

In conclusion, UN-ARF increased the *in vitro* hepatic metabolism (CL_{vit}) and *in vivo* CL_i and CL_i' of DTZ but decreased the CL_t of DTZ. The plasma protein binding of DTZ increased, but the blood/plasma partition remained unchanged (unity). From the calculation based on the well-stirred model, DTZ appeared to increase the HBF in the control rats more than two-fold at a dose of 3 mg/kg (iv) or 10 mg/kg (pv). In addition, UN-ARF reduced the HBF-increase almost to the normal range. Although extrapolation of these results to other etiologies of renal failure, human patients, other drugs, and/or enzyme systems is not guaranteed, these results may add some useful information to the body of knowledge of the effect of renal failure on HBF and the hepatic metabolism of DTZ.

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