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Pharmacokinetic interaction between oltipraz and dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB) after single intravenous and oral administration to rats

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Abstract

The aim of this study was to report the pharmacokinetic interaction between oltipraz (50 mg kg^{-1}) and dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB, 10 mg kg^{-1}) after single intravenous and oral administration to rats. After intravenous administration of oltipraz plus DDB, the area under the plasma concentration–time curve from time zero to time infinity (AUC) of oltipraz was significantly greater (1440 vs 1740 μg min mL^{-1}) than that after oltipraz alone. This was not due to slower clearances of oltipraz after oltipraz plus DDB since the total body, renal and nonrenal clearances were comparable between the two groups of rats. It could be due to a decrease in tissue binding of oltipraz by DDB. The apparent volume of distribution at steady state (Vd_{ss}) of DDB was significantly smaller (7060 vs 4650 mL kg^{-1}) than after oltipraz alone. After oral administration of oltipraz plus DDB, the AUC of olitpraz was also significantly greater (479 vs 583 μg min mL^{-1}) than that after oltipraz alone. This was not due to increased absorption of oltipraz from the rat gastrointestinal tract after oltipraz plus DDB but again could be due to a decrease in Vd_{ss} of oltipraz by DDB. However, after both intravenous and oral administration, the pharmacokinetic parameters of DDB were comparable between DDB alone and DDB plus oltipraz, indicating that oltipraz did not greatly affect the pharmacokinetics of DDB in rats.

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Introduction

Oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione], a synthetic dithiolthione, has been developed by Rhône-Poulenc (Virty-sur-Seine, France) for the treatment of schistosomiasis (Clapper 1998). Thirteen metabolites of oltipraz, including its glucuronide conjugate, have been reported in rat urine (Bieder et al 1983). Effects of oltipraz on glutathione S-transferase (GST) activities (Maheo et al 1998), hepatic cytochrome P450 (CYP) 2E1, CYP1A, CYP2B, CYP2B1, CYP2B2 and CYP2B1/2 expression (Buetler et al 1995; Langouet et al 1997; Manson et al 1997; Maheo et al 1998), and benzo[a]pyrene-*trans*-7,8-dihydrodiol glucuronidating (BPD UGT) activities (Kessler & Ritter 1998) have been reported in rats. Oltipraz produced significant elevations in the detoxification potential of the host (Bueding et al 1982) and this finding provided the first evidence that oltipraz might be effective as a radioprotective, antiviral or chemopreventive agent (Clapper 1998). Recently, the therapeutic effect of oltipraz in rats with liver cirrhosis has been reported (Kang et al 2002a).

Dimethyl-4,4′-dimethoxy-5,6,5′6′-dimethylene dicarboxybiphenyl-2,2′-dicarboxylate (DDB) is a synthetic hepatoprotective agent derived from Schizandrin C, a component of *Fructus schizandrae*. It is active against toxin-induced liver injuries in animals. DDB protects the liver against CCl_4-, D-galactosamine-, thioacetamide- or prednisolone-induced injuries (Liu et al 1979, 1981, 1982; Liu & Lesca 1982; Kim et al 1995). DDB prevented aflatoxin B1-induced hepatotoxicity in rats (Liu et al 1995). DDB also improved the liver function of patients with the hepatitis B virus (Lee et al 1991). DDB was metabolized to five metabolites via CYP1A2, CYP2C9 and CYP3A4 based

on human liver microsomes (Baek et al 2001). DDB is a strong inhibitor of CYP3A4, and at higher concentrations marginally inhibited CYP1A2, CYP2C9 and CYP2D6, but had no effects on CYP2A6, CYP2C19 and CYP2E1 based on human liver microsomes (Kim et al 2001). DDB failed to alter the expression of CYP1A and CYP2C11, microsomal hydrolase and GST in rats with slight inhibition of CYP2E1 expression, but induced CYP2B1/2 (Kim et al 2000). DDB induced CYP2B1 in rat liver microsomes (Li et al 1992). DDB is currently employed as a curative agent for patients with viral hepatitis in several countries including China, Korea, Vietnam and Pakistan.

Since oltipraz has a liver antifibrotic effect in rats and DDB has a hepatoprotective effect, these two drugs could be administered together. The purpose of this study was to report the pharmacokinetic interaction between oltipraz and DDB after single intravenous and oral administration to rats. Tissue distribution of oltipraz after oral administration of oltipraz alone and oltipraz plus DDB was also reported.

Materials and Methods

Chemicals

DDB and oltipraz were supplied by the Taerim Pharmaceutical Company (Seoul, Korea) and the Aventis Pharmaceutical Company (Virty-sur-Seine, France), respectively. YH-439 ([isopropyl 2-(1,3-dithietane-2-ylidene)-2-[N-(4-methyl-2-thiazolyl)carbamoyl] acetate], an internal standard of GC/MS analysis) was donated by the Yuhan Research Center of the Yuhan Coporation (Kunpo, Korea). Polyethylene glycol 400 (PEG 400) was purchased from the Duksan Chemical Company (Seoul, Korea). Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade and therefore were used without further purification.

Animals

Male Sprague–Dawley rats (weighing 230–310 g) were purchased from Charles River Korea (Biogenomics, Seoul, Korea). Each rat was randomly divided into three groups: DDB, oltipraz and DDB plus oltipraz. Each rat was maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea) at a temperature between 20 and 23 °C with 12-h light and dark cycles and a relative humidity of 50%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) with a supply of filtered pathogen-free air and with food (Samyang Company, Seoul, Korea) and water ad libitum. The protocol of animal study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Intravenous administration

The procedures for the pretreatment of rats (including cannulation of the carotid artery and the jugular vein of each rat) were reported previously (Kim et al 1993). Oltipraz (oltipraz powder was suspended in 40% PEG 400, 50 mg kg^{-1}, n = 7), DDB (DDB powder was also suspended in 40% PEG 400, 10 mg kg^{-1}, n = 7) and oltipraz (50 mg kg^{-1}) plus DDB (10 mg kg^{-1}) (n = 9) were administered via the jugular vein over 1 min (total injection volume was approximately 0.6 mL). It has been reported from our laboratories (Kang et al 2002b) that an improved liver antifibrotic effect was observed when oltipraz (25 mg kg^{-1}) plus DDB (5 mg kg^{-1}) was administered orally to rats. Because of the assay sensitivity of DDB, the dose of DDB and oltipraz was increased twice in the present study. A 120-μL aliquot (a 220-μL aliquot for DDB plus oltipraz group) of blood was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 120, 180, 240 and 360 min. Blood samples were centrifuged immediately and a 50-μL aliquot of plasma sample (two 50-μL aliquots for the oltipraz and DDB group) was stored in a −70 °C freezer (Revco ULT 1490 D-N-S, Western Mednics, Asheville, NC) until HPLC analysis of oltipraz and GC/MS analysis of DDB. At the end of the experiment (24 h), the metabolic cage was rinsed with 10 mL of distilled water and the rinsings were combined with 24-h urine. After measuring the exact volume of the combined urine, a 50-μL aliquot of urine was stored in a −70 °C freezer until HPLC analysis of oltipraz and GC/MS analysis of DDB. At the same time (24 h), the entire gastrointestinal tract was removed, transferred into a beaker containing 100 mL of methanol (to facilitate extraction of oltipraz or DDB) and cut into small pieces using scissors. After manual shaking and stirring with a glass rod, two 50-μL aliquots of the supernatant were collected from each beaker and stored in a −70 °C freezer until HPLC analysis of oltipraz and GC/MS analysis of DDB.

Oral administration

Oltipraz (the same suspension as used in the intravenous study, 50 mg kg^{-1}, n = 8), DDB (the same suspension as used in intravenous study, 10 mg kg^{-1}, n = 7) and oltipraz (50 mg kg^{-1}) plus DDB (10 mg kg^{-1}) (n = 7) were administered orally using a feeding tube (total oral volume was approximately 2 mL). Blood samples were collected via the carotid artery at 0 (to serve as a control), 15, 30, 60, 90, 120, 240, 360, 480, 720, 1080, 1440 and 1800 min. Other procedures were similar to those in the intravenous study. Urine was collected between 0 and 30 h for oltipraz alone and oltipraz plus DDB, and between 0 and 24 h for DDB alone. The gastrointestinal tract was collected at 30 h for oltipraz alone and oltipraz plus DDB, and at 24 h for DDB alone.

Tissue distribution

The procedures were similar to those reported previously (Yoon et al 1998). At 30 min (n = 5 for each group) and 8 h (n = 5 for each group) after oral administration of oltipraz or oltipraz plus DDB (the same dose as used in the oral study), as much blood as possible was collected via the carotid artery and each rat was sacrificed by cervical dislocation. Blood samples were centrifuged immediately and

plasma samples were collected. Approximately 1 g of each liver, kidney, lung, spleen, heart, muscle, mesentery, fat, brain, and large intestine was excised, rinsed with distilled water to minimize blood remaining in the tissues, and blotted dry with paper tissue. Each tissue was homogenized with four volumes of distilled water using a tissue homogenizer (Ultra-Turrax T25, Janke and Kunkel, IKA-Labortechnik, Staufen, Germany). After centrifugation, a 50-μL aliquot of the 9000 *g* supernatant was stored in a $-70\,^{\circ}C$ freezer until HPLC analysis of oltipraz. All the procedures were conducted at $4\,^{\circ}C$ in an ice-bath.

GC/MS analysis of DDB

To determine concentrations of DDB in the above samples, a GC/MS assay procedure was developed (to be published elsewhere). A 50-μL aliquot of aqueous solution containing YH-439 (an internal standard, 1 $\mu g\,mL^{-1}$) was added to a 50-μL aliquot of plasma. A 1-mL aliquot of chloroform was added to the mixture and the resulting mixture was vortex mixed for 12 min and centrifuged. The aqueous layer was aspirated out and the remaining organic layer was collected. The organic layer was then evaporated to dryness by an evaporator (Speed Vac, Vacuum Concentration System, Hanil, Seoul, Korea). The residue was reconstituted with a 15-μL aliquot of ethylacetate and a 3-μL aliquot was injected onto a GC/MS system. The GC/MS consisted of gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA), a mass selective detector (HP5973, Hewlett Packard) and an autosampler (HP6890, Hewlett Packard). The separation of the injection mixture was carried out on a GC capillary column (Rtx-5 MS, Restek, Bellefonte, PA; 60 m, l. $\times$ 250 μm, i.d.; film thickness, 0.25 μm). The temperatures for the ion source, quadrupole, transfer line and inlet were 230, 150, 280 and $260\,^{\circ}C$, respectively. In this study, a pulsed splitless mode was used. The oven temperature was maintained at $180\,^{\circ}C$ for 1 min, then raised at a rate of $70\,^{\circ}C\,min^{-1}$ for up to $300\,^{\circ}C$. Using a selected ion monitoring mode, the detection *m/z* value was set at 418/419 for DDB and 330/332 for the internal standard. Under the assay system, DDB and the internal standard were baseline separated with endogenous peaks from the plasma. In addition, the DDB was readily quantified in the concentration ranges from 50 to 500 $ng\,mL^{-1}$ with inter- and intra-day precision of less than 8.5%. The accuracy of the assay in these ranges was less than 8.41%. For the 20 $ng\,mL^{-1}$ calibration standard sample the inter-day precision and accuracy values were 7.76% and 12.6%, respectively, while the intra-day precision and accuracy values for the sample were 17.9 and 16.5%, respectively. The limit of quantification was therefore set at 20 $ng\,mL^{-1}$ for DDB.

HPLC analysis of oltipraz

Concentrations (or amounts) of oltipraz in the above samples were analysed by the HPLC method developed by our laboratories (Bae et al 2001a). A 100-μL aliquot of acetonitrile was added to deproteinize (Chiou et al 1978) a 50-μL aliquot of biological sample. After vortex mixing and centrifugation at 14 000 rpm for 1 min, a 50-μL aliquot of the supernatant was injected directly onto the HPLC column. The mobile phase, acetonitrile:0.5 mM ammonium acetate (55:45 v/v for rat plasma and tissues, and 45:55 v/v for rat urine), was run at a flow rate of 1.5 $mL\,min^{-1}$ and the column effluent was monitored by a UV detector set at 305 nm. The retention time of oltipraz was approximately 5.8 min in rat plasma and tissues, and 8.6 min in the rat urine sample. The detection limits of oltipraz in rat plasma, urine and tissue homogenates were 20, 50 and 50 $ng\,mL^{-1}$, respectively. The mean within-day coefficients of variation (CVs) of oltipraz in rat plasma and urine were 2.29% (range 1.05–3.66%) and 1.01% (range 0.503–1.59%), respectively, and the corresponding between-day CVs of the analysis of the same samples on three consecutive days were 3.37% (range 1.77–4.65%) and 1.51% (range 0.389–2.90%).

Oltipraz in solution has been reported to photodegrade (Christensen & Malone 1992), therefore all samples were covered and wrapped with aluminum foil and kept in the dark when they were not in use.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) or up to the last measured time in plasma (8 h, AUC_{0-8h} for DDB after oral administration) was calculated by the trapezoidal rule extrapolation method, which employs the logarithmic trapezoidal rule (Chiou 1978) for the calculation of the area during the declining plasma level phase and the linear trapezoidal rule for the rising plasma level phase. The area from the last data point to time infinity (for the calculation of AUC) was estimated by dividing the last measured plasma concentration by the terminal rate constant. Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters: the time-averaged total body (CL), renal (CL_R) and nonrenal (CL_{NR}) clearances, mean residence time (MRT), first moment of AUC (AUMC) and apparent volume of distribution at steady state (Vd_{ss}) (Kim et al 1993).

The harmonic mean method was employed for the calculation of mean values of each clearance (Chiou 1980), terminal half-life, $t_{1/2}$ (Eatman et al 1977), and Vd_{ss} (Chiou 1979a).

Statistical analysis

A *P* value of less than 0.05 was considered to be statistically significant using a *t*-test between two means for unpaired data. All results are expressed as mean $\pm$ standard deviation (s.d.).

Results

Pharmacokinetics of oltipraz after intravenous administration with or without DDB

After 1 min of intravenous administration of 50 $mg\,kg^{-1}$ of oltipraz alone and 50 $mg\,kg^{-1}$ of oltipraz plus

10 mg kg^{-1} of DDB to rats, the mean arterial plasma concentrations of oltipraz were almost constant for up to 60–90 min for each group of rats (Figure 1A). This could be due to precipitation of poorly water-soluble oltipraz in blood and redissolution of oltipraz in blood (Chiou 1979b). The plasma levels of oltipraz then declined in an apparent monoexponential fashion for both groups of rats (Figure 1A), with mean terminal $t_{1/2}$ values of 168 and 105 min for oltipraz alone and oltipraz plus DDB, respectively; the half-lives were significantly different between the two groups of rats (Table 1). The mean plasma concentrations of oltipraz were higher after oltipraz plus DDB than after oltipraz alone (Figure 1A) and this resulted in a significantly greater AUC (20.8% increase) after oltipraz plus DDB (Table 1). This was not due to slower CL_R and CL_{NR} of oltipraz after oltipraz plus DDB since the CL, CL_R and CL_{NR} values of oltipraz were not significantly different between the two groups of rats (Table 1). This could be due to the significantly smaller Vd_{ss} value of oltipraz by DDB (34.1% decrease); rat tissues had greater affinity to DDB than to oltipraz (the Vd_{ss} of DDB was 5.78 times greater than that of oltipraz, Table 1). Hence, after oltipraz plus DDB, tissue distribution of oltipraz decreased. Therefore, the plasma concentrations (and the resultant AUC) of oltipraz were higher (greater) than those after oltipraz alone.

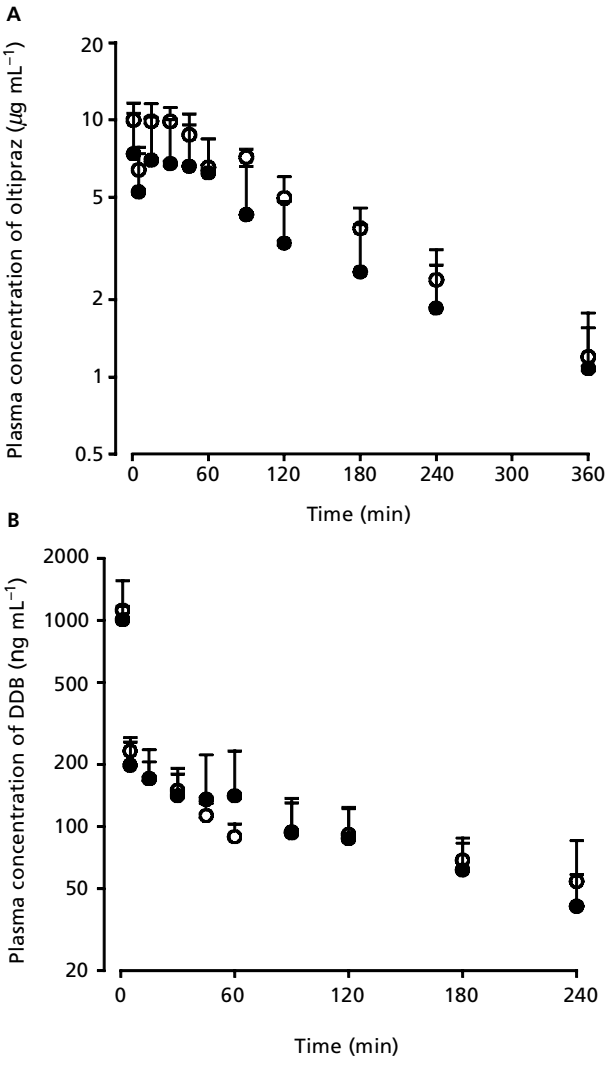

Figure 1 Mean arterial plasma concentration–time profiles of oltipraz (A) and DDB (B) after intravenous administration of 50 mg kg^{-1} of oltipraz alone or 10 mg kg^{-1} of DDB alone (closed circle) and combination of the two drugs (open circle). Bars represent s.d.

Pharmacokinetics of DDB after intravenous administration with or without oltipraz

After 1 min of intravenous administration of 10 mg kg^{-1} of DDB alone and 10 mg kg^{-1} of DDB plus 50 mg kg^{-1} of oltipraz to rats, the mean arterial plasma concentrations of DDB declined rapidly for up to 15 min and declined slowly thereafter for both groups of rats (Figure 1B), with mean terminal $t_{1/2}$ values of 92.9 and 101 min for DDB alone and DDB plus oltipraz, respectively (Table 1). Note that the pharmacokinetic parameters of DDB listed in Table 1 were not significantly different between the two groups of rats, indicating that oltipraz did not greatly affect the pharmacokinetic parameters of DDB. The Vd_{ss} values of DDB were considerable, 47 900–50 600 mL kg^{-1} (Table 1), and this supports the high lipid solubility of DDB. After intravenous administration, the contribution of CL_R to the CL of DDB was almost negligible; DDB was below detection limit in 24-h urine ($Ae_{0-24\,h}$) for both groups of rats (Table 1). This indicates that the CLs of DDB listed in Table 1 could represent the CL_{NR} of DDB in rats. The contribution of gastrointestinal (including biliary) excretion of unchanged DDB to CL_{NR} of DDB was also negligible; the amount of DDB recovered from the entire gastrointestinal tract at 24 h as unchanged drug ($GI_{24\,h}$) was also below the detection limit for both groups of rats (Table 1). DDB was stable for up to 24-h incubation in buffer solutions having pHs of 1, 2, 3, 7 and 8 and for up to 3-h incubation in four human gastric juices (pHs of 3.3, 6.8, 4.0 and 4.4). This suggests that DDB is metabolized almost completely after intravenous administration and the CLs of DDB listed in Table 1 could represent metabolic clearances of oltipraz in rats. Since the CLs of DDB were comparable between two groups of rats, the metabolism of DDB was not greatly affected by oltipraz.

Pharmacokinetics of oltipraz after oral administration with or without DDB

After oral administration of 50 mg kg^{-1} of oltipraz alone and 50 mg kg^{-1} of oltipraz plus 10 mg kg^{-1} of DDB to rats, oltipraz was absorbed slowly and almost completely from the rat gastrointestinal tract; the plasma concentration reached its peak (T_{max}) at 600 and 651 min for oltipraz alone and oltipraz plus DDB, respectively (Figure 2A and Table 2), and the percentage of oral dose of unchanged oltipraz recovered from the entire

Table 1 Pharmacokinetic parameters of oltipraz and DDB after intravenous (i.v.) administration of 50 mg kg^{-1} of oltipraz, 10 mg kg^{-1} of DDB and 50 mg kg^{-1} of oltipraz plus 10 mg kg^{-1} of DDB to rats.

Parameter	Oltipraz		DDB	
	Oltipraz alone (n = 7)	Oltipraz plus DDB (n = 9)	DDB alone (n = 7)	DDB plus oltipraz (n = 9)
Body weight (kg)	0.243 ± 0.107	0.255 ± 0.0410	0.251 ± 0.0121	0.259 ± 0.0129
AUC (μg min mL^{-1})	1440 ± 615	1740 ± 288**	33.7 ± 8.14	30.5 ± 11.4
Terminal $t_{1/2}$ (min)	168 ± 36.8	105 ± 21.9**	92.9 ± 52.5	101 ± 19.6
CL (mL min^{-1} kg^{-1})	31.1 ± 8.57	28.7 ± 4.93	297 ± 86.4	375 ± 13.6
CL_R (mL min^{-1} kg^{-1})	0.0110 ± 0.0166	0.0135 ± 0.00422		
CL_{NR} (mL min^{-1} kg^{-1})	27.0 ± 4.97	28.7 ± 4.93		
MRT (min)	194 ± 102	163 ± 26.6	161 ± 37.3	143 ± 26.5
Vd_{ss} (mL kg^{-1})	7060 ± 224	4650 ± 26.6***	47900 ± 4460	50600 ± 12800
$Ae_{0-24\,h}$ (% of i.v. dose)	0.0558 ± 0.0460	0.0500 ± 0.0143	BD	BD
$GI_{24\,h}$ (% of i.v. dose)	BD	BD	BD	BD

Values are mean ± s.d. **$P < 0.01$ and ***$P < 0.001$ vs oltipraz alone or DDB alone. BD: below detection limit.

gastrointestinal tract at 30 h (GI) was less than 2.12% for both groups of rats (hence approximately 98% of orally administered oltipraz was absorbed from the rat gastrointestinal tract) (Table 2). It took a long time to reach T_{max}, 10–11 h (Table 2), indicating that oltipraz is continuously absorbed from the rat gastrointestinal tract due to its low water-solubility. After reaching their respective peak levels, the mean arterial plasma concentrations of oltipraz declined in an apparent monoexponential fashion (Figure 2B) with mean terminal $t_{1/2}$ values of 302 and 306 min for oltipraz alone and oltipraz plus DDB, respectively; the half-lives were not significantly different between the two groups of rats (Table 2). Note that the terminal $t_{1/2}$ values after oral administration (Table 2) were considerably longer than those after intravenous administration (Table 1), and this could be due to different blood sampling time schedules. However, the plasma concentration–time profiles of oltipraz were not fitted to a flip-flop model for either group of rats.

After oral administration, plasma concentrations of oltipraz were higher for oltipraz plus DDB and this resulted in a significantly greater AUC (21.7% increase). Although the exact reason is not clear, this could not be due to an increase in the gastrointestinal absorption of oltipraz after oltipraz plus DDB: the $GI_{30\,h}$ values were comparable between the two groups of rats (Table 2). This could be due to a decrease in the volume of distribution of oltipraz, as explained in the intravenous study. Moreover, the CL_R values were not significantly different between the two groups of rats (Table 2). The F values were almost constant: 33.3 and 33.5% for oltipraz alone and oltipraz plus DDB, respectively (Table 2). The low F values suggested that the first-pass (hepatic, gastric and/or intestinal) effects of oltipraz could be considerable after oral administration, as there was approximately 98% absorption of oral dose of oltipraz. The considerable hepatic and intestinal first-pass effects of oltipraz were obtained in rats (our unpublished data).

Pharmacokinetics of DDB after oral administration with or without oltipraz

After oral administration of 10 mg kg^{-1} of DDB and 10 mg kg^{-1} of DDB plus 50 mg kg^{-1} of oltipraz to rats, DDB was absorbed rapidly and almost completely from the rat gastrointestinal tract; the drug was detected in plasma from the first blood sampling time (15 min), reached its peak (T_{max}) at 80 and 154 min for DDB alone and DDB plus oltipraz, respectively (Figure 2B), and the percentages of oral dose of DDB recovered from the entire gastrointestinal tract as an unchanged drug (GI) were 8.56 and 4.00% for DDB alone and DDB plus oltipraz, respectively (hence approximately 90% of orally administered DDB was absorbed from the rat gastrointestinal tract, Table 2). Note that the values of 8.56 and 4.00% cannot be compared directly because they were obtained at 24 and 30 h, respectively. Although the plasma levels of DDB were higher after DDB plus oltipraz (Figure 2B), the $AUC_{0-8\,h}$ of DDB was not significantly different between the two groups of rats because of considerable intersubject variations; the coefficient of variation for $AUC_{0-8\,h}$ after DDB plus oltipraz was 53.4% (Table 2).

Tissue distribution of oltipraz

Since the Vd_{ss} of oltipraz was significantly smaller after intravenous administration of oltipraz plus DDB than after oltipraz alone (Table 1), tissue distribution of oltipraz was measured for both groups of rats. The amount of oltipraz recovered from each gram of tissue and the tissue-to-plasma (T/P) ratios of oltipraz 30 min and 8 h after oral administration of 50 mg kg^{-1} of oltipraz and 50 mg kg^{-1} of oltipraz plus 10 mg kg^{-1} of DDB are listed in Table 3. The rat tissues studied had good affinity to oltipraz for both groups of rats; the T/P ratios of oltipraz were greater-than-unity in all rat tissues studied except muscle

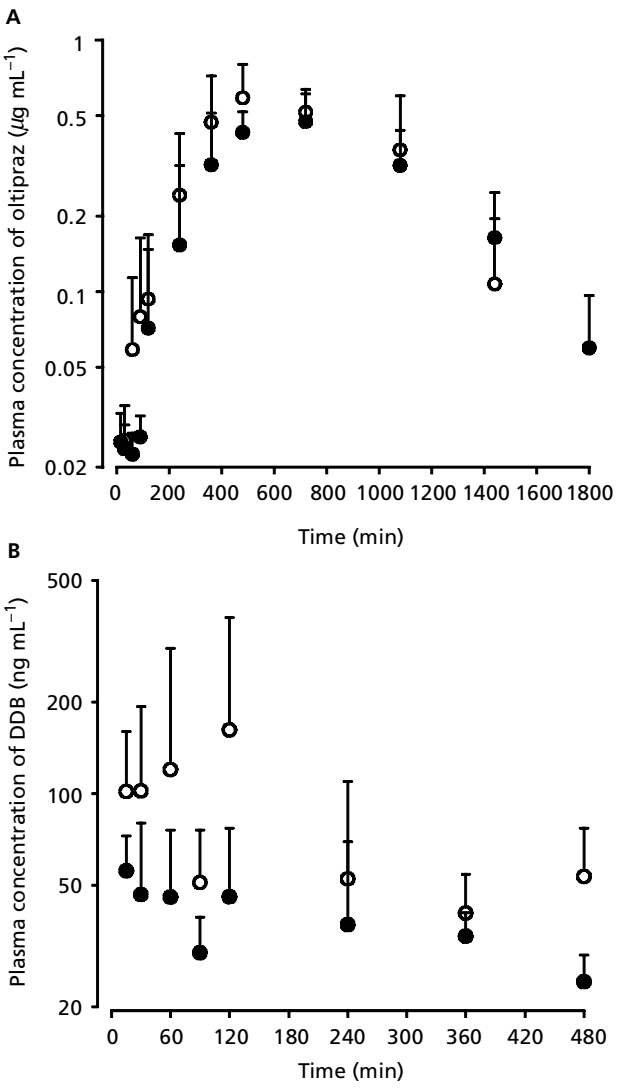

Figure 2 Mean arterial plasma concentration–time profiles of oltipraz (A) and DDB (B) after oral administration of 50 mg kg^{-1} of oltipraz alone or 10 mg kg^{-1} of DDB alone (closed circle) and combination of the two drugs (open circle). Bars represent s.d.

and fat at 30 min and spleen at 8 h (Table 3). The above results are in good agreement with the high lipid solubility of oltipraz and considerably large value of Vd_{ss}, 4650–7060 mL kg^{-1} (Table 1). In all rat tissues studied except in the muscle and fat, the T/P ratios were significantly lower at 8 h than at 30 min (Table 3), suggesting that oltipraz is rapidly distributed in rat tissues and reaches equilibrium between blood and tissues with lower T/P ratios than at 30 min.

The T/P ratios of oltipraz after oltipraz plus DDB were significantly smaller in lung and large intestine at 30 min and kidney, heart, muscle, fat, brain and large intestine at 8 h than those after oltipraz alone (Table 3). This could explain the significantly smaller Vd_{ss} of oltipraz after oltipraz plus DDB (Table 1). This could be due to a significantly smaller free (unbound in plasma proteins) fraction of oltipraz after oltipraz plus DDB [the protein binding values of oltipraz were 90.1 ± 8.16 and $94.3 \pm 3.88\%$ for oltipraz alone and for oltipraz plus DDB, respectively ($n = 9$, each) using an equilibrium dialysis technique at oltipraz and DDB concentrations of 5 and 1 μg mL^{-1}, respectively]. Note that in fat, oltipraz was below the detection limit at 30 min for both groups of rats, but at 8 h the T/P ratios were 27.2 and 16.6 for oltipraz alone and oltipraz plus DDB, respectively (Table 3). This suggests that oltipraz is slowly but markedly distributed in fat (due to high lipid solubility of oltipraz) and fat could act as a reservoir for oltipraz. Similar results were obtained from rats with liver cirrhosis (Kang et al 2002a) and from muscle; although the T/P ratios at 8 h in muscle was only 1.25 and 0.489 for oltipraz alone and oltipraz plus DDB, respectively (Table 3), the total amount of oltipraz distributed in the muscle could be considerable, in view of the contribution of muscle to whole rat body weight in rats (Davies & Morris 1993).

Discussion

After intravenous administration of oltipraz alone to control rats, the CL of oltipraz (31.1 mL min^{-1} kg^{-1} based on plasma data, Table 1) was considerably slower than the reported cardiac output of 296 mL min^{-1} kg^{-1} in rats based on blood data (Davies & Morris 1993). This suggests that the first-pass effect of oltipraz in the lung and heart could be almost negligible, if any, in rats. After intravenous administration of oltipraz alone to control rats, the contribution of CL_R to CL of oltipraz was almost negligible; the percentage of intravenous dose of oltipraz excreted in 24-h urine as unchanged drug ($Ae_{0-24\,h}$) was 0.0558% (Table 1). This indicates that the CL of 31.1 mL min^{-1} kg^{-1} (Table 1) could represent the CL_{NR} of oltipraz in rats. The contribution of gastrointestinal (including biliary) excretion of unchanged oltipraz to the CL_{NR} of oltipraz was also negligible; unchanged oltipraz recovered from the entire gastrointestinal tract at 24 h ($GI_{24\,h}$) was below detection limit (Table 1). Similar results were reported from our laboratories (our unpublished data); the $GI_{24\,h}$ was smaller than 0.0287% for the intravenous dose ranges (5–20 mg kg^{-1}) studied in rats. Moreover, the percentage of intravenous dose of oltipraz at a dose of 20 mg kg^{-1} excreted in 8 h bile as unchanged drug was less than 0.449%. Oltipraz was stable for up to 48 h incubation in buffer solutions having pHs ranging from 2 to 12 (Bae et al 2001b) and for up to 4 h incubation in five human gastric juices having pHs of 3.7, 3.3, 6.8, 4.0 and 4.4. These data suggested that oltipraz is metabolized almost completely after intravenous administration and the CL_{NR} of 27.0 mL min^{-1} kg^{-1} (Table 1) could represent metabolic clearance of oltipraz in rats. Since the CL_{NR} values of oltipraz were comparable between oltipraz alone and oltipraz plus DDB (Table 1), it could be concluded that DDB did not greatly affect the metabolism of oltipraz.

After intravenous administration of oltipraz alone to control rats, the estimated CL_R of oltipraz as free (unbound

Table 2 Pharmacokinetic parameters of oltipraz and DDB after oral administration of 50 mg kg^{-1} of oltipraz, 10 mg kg^{-1} of DDB and 50 mg kg^{-1} of oltipraz plus 10 mg kg^{-1} of DDB to rats.

Parameter	Oltipraz		DDB	
	Oltipraz alone (n = 8)	Oltipraz plus DDB (n = 7)	DDB alone (n = 7)	DDB plus oltipraz (n = 7)
Body weight (kg)	0.287 ± 0.0171	0.284 ± 0.00732	0.301 ± 0.0225	0.297 ± 0.0196
AUC[a] (μg min mL^{-1})	479 ± 103	583 ± 71.7*	17.0 ± 0.352	32.0 ± 17.1
Terminal $t_{1/2}$ (min)	302 ± 53.8	306 ± 151		
C_{max} (μg mL^{-1})	0.565 ± 0.0729	0.707 ± 0.187	0.0890 ± 0.0196	0.236 ± 0.225
T_{max} (min)	600 ± 170	651 ± 316	80.0 ± 87.8	154 ± 161
Ae[b] (% of oral dose)	0.0677 ± 0.00449	0.0273 ± 0.00161	BD	BD
CL_R (mL min^{-1} kg^{-1})	0.0497 ± 0.0682	0.0219 ± 0.00901		
GI[c] (% of oral dose)	2.12 ± 1.77	1.09 ± 0.741	8.56 ± 8.00	4.00 ± 1.51**
F (%)	33.3	33.5		

Values are mean ± s.d. [a]AUC for oltipraz and $AUC_{0-8\,h}$ for DDB; [b]0–30 h for oltipraz and 0–24 h for DDB; [c]recovered from gastrointestinal tract, 30 h for oltipraz alone and oltipraz plus DDB, and 24 h for DDB alone. *$P < 0.05$ and **$P < 0.01$ vs oltipraz alone or DDB alone. BD: below detection limit.

Table 3 Amount of oltipraz recovered from each gram of tissue (μg mL^{-1} for plasma or μg g^{-1} for other tissues) at 30 min and 8 h after oral administration of oltipraz and oltipraz plus DDB.

Tissue	30 min		8 Hours	
	Oltipraz alone (n = 5)	Oltipraz plus DDB (n = 5)	Oltipraz alone (n = 5)	Oltipraz plus DDB (n = 5)
Plasma	0.0240 ± 0.00401	0.0259 ± 0.00301	0.396 ± 0.0142	0.556 ± 0.109*
Liver	0.923 ± 0.108	0.789 ± 0.101	3.97 ± 0.894	4.79 ± 1.48
	(39.0 ± 7.43)	(31.2 ± 7.97)	(9.67 ± 2.18)†††	(8.61 ± 2.00)†††
Kidney	0.304 ± 0.131	0.275 ± 0.108	2.17 ± 0.667	1.22 ± 0.453*
	(15.3 ± 6.58)	(11.0 ± 5.69)	(5.28 ± 1.62)†	(2.42 ± 1.56)*†
Lung	0.185 ± 0.00741	0.204 ± 0.0158	0.782 ± 0.153	0.777 ± 0.173
	(9.27 ± 0.371)	(7.96 ± 0.917)*	(1.90 ± 0.372)†††	(1.43 ± 0.381)†††
Spleen	0.112 ± 0.0191	0.110 ± 0.0135	0.408 ± 0.150	0.423 ± 0.188
	(5.60 ± 0.964)	(4.21 ± 0.577)	(0.993 ± 0.365)†††	(0.839 ± 0.547)†††
Heart	0.310 ± 0.114	0.309 ± 0.0563	1.26 ± 0.178	0.824 ± 0.169**
	(15.6 ± 5.74)	(12.1 ± 2.91)	(3.08 ± 0.432)††	(1.58 ± 0.660)**†††
Muscle	BD	BD	0.512 ± 0.174	0.270 ± 0.155
			(1.25 ± 0.424)	(0.489 ± 0.301)*
Mesentery	0.652 ± 0.0475	0.751 ± 0.0916	3.09 ± 0.159	2.79 ± 0.572
	(32.8 ± 2.39)	(29.9 ± 7.63)	(7.52 ± 0.387)†††	(5.40 ± 2.35)†††
Fat	BD	BD	11.2 ± 2.14	8.80 ± 1.63
			(27.2 ± 5.52)	(16.6 ± 5.45)*
Brain	0.437 ± 0.0642	0.489 ± 0.0285	1.32 ± 0.454	1.02 ± 0.136
	(22.0 ± 3.22)	(19.1 ± 2.54)	(3.21 ± 1.10)	(1.87 ± 0.213)*†††
Large intestine	0.995 ± 0.167	0.742 ± 0.249	1.03 ± 0.269	0.742 ± 0.249
	(50.0 ± 8.41)	(30.0 ± 12.7)*	(2.52 ± 0.655)	(1.37 ± 0.482)*††

The numbers in parentheses represent tissue-to-plasma (T/P) ratios. Values are mean ± s.d. *$P < 0.05$ and **$P < 0.01$ vs oltipraz alone. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$ vs 30 min. BD: below detection limit.

in plasma proteins) drug was 0.111 mL min^{-1} kg^{-1} based on 90.1% plasma protein binding of oltipraz in fresh rat plasma, as mentioned earlier. The value, 0.111 mL min^{-1} kg^{-1}, was considerably slower than the reported glomerular filtration rate of 5.24 mL min^{-1} kg^{-1} in rats (Davies & Morris 1993), indicating that oltipraz is reabsorbed considerably in rat renal tubules. Considering the CL_R of oltipraz based on plasma data (Table 1), reported kidney blood flow rate of 36.8 mL min^{-1} kg^{-1} (Davies & Morris 1993) and hematocrit value of approximately 45% (Mitruka & Rawnsley 1982) in rats, the estimated renal extraction ratio (CL_R of oltipraz/renal plasma

flow rate, only for urinary excretion of unchanged drug) of oltipraz was 0.0543% after administration of oltipraz alone to control rats. This indicates that oltipraz is poorly excreted from rat renal tubules. The CL_{NR} of oltipraz based on plasma data, 27.0 mL min^{-1} kg^{-1} (Table 1), was close to the reported hepatic plasma flow rate of 30.4 mL min^{-1} kg^{-1} in rats [based on a hepatic blood-flow rate of 13.8 mL min^{-1} (250 g)$^{-1}$ (Davies & Morris 1993) and a hematocrit value of approximately 45% (Mitruka & Rawnsley 1982) in rats]. This suggests that rat liver could be the main metabolizing organ for oltipraz. This could be supported by in-vitro disappearance (mainly by metabolism) of oltipraz in rat tissue homogenates; liver had the greatest metabolic activities and muscle, brain and lung had some metabolic activities for oltipraz (our unpublished data). The hepatic first-pass effect of oltipraz was approximately 40% in rats based on the AUC difference between intravenous and intraportal administration (our unpublished data).

Conclusion

After both intravenous and oral administration of oltipraz plus DDB, the AUC of oltipraz was significantly greater than after oltipraz alone. This could be due to significantly smaller Vd_{ss} values of oltipraz after oltipraz plus DDB because DDB decreases tissue binding of oltipraz. After intravenous administration, the CL, CL_R and CL_{NR} of oltipraz were not significantly different between oltipraz alone and oltipraz plus DDB. After both intravenous and oral administration of DDB plus oltipraz, the pharmacokinetic parameters of DDB were comparable to those after DDB alone, indicating that oltipraz did not greatly affect the pharmacokinetics of DDB in rats.

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