JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 443–452

Received September 13, 2004
Accepted December 14, 2004
DOI 10.1211/0022357055704
ISSN 0022-3573

Effect of enzyme inducers and inhibitors on the pharmacokinetics of oltipraz in rats

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Abstract

A series of in-vitro and in-vivo experiments, using various inducers and inhibitors of hepatic microsomal cytochrome P450 (CYP) isozymes, was conducted to study oltipraz pharmacokinetics in rats. In in-vivo studies, oltipraz at a dose of 10 mg kg^{-1} was administered intravenously to rats. In rats pretreated with SKF 525-A (a nonspecific CYP isozyme inhibitor in rats; n = 9), the time-averaged total body clearance (CL) of oltipraz was significantly slower (56.6% decrease) than that in untreated rats (n = 9). This indicated that oltipraz is metabolized via CYP isozymes in rats. Hence, various enzyme inducers or inhibitors were used in in-vitro and in-vivo studies in rats. In rats pretreated with 3-methylcholanthrene (n = 9 and 8 for untreated and treated groups, respectively), phenobarbital (n = 7 and 10 for untreated and treated groups, respectively) or dexamethasone (n = 7 and 12 for untreated and treated groups, respectively) (main inducers of CYP1A1/2, 2B1/2 and 3A1/2 in rats, respectively), the CL values were significantly faster (38.4, 94.4 and 33.6% increase, respectively). In rats pretreated with sulfaphenazole (n = 8 and 9 for untreated and treated groups, respectively), quinine (n = 7 and 9 for untreated and treated groups, respectively) or troleandomycin (n = 8 and 9 for untreated and treated groups, respectively) (main inhibitors of CYP2C11, 2D1 and 3A1/2 in rats, respectively), the CL values were significantly slower (31.0, 27.6 and 36.3% decrease, respectively). The in-vivo results with various enzyme inhibitors correlated well with the in-vitro intrinsic clearance for disappearance of oltipraz (CL_{int}) (n = 5, each). The above data suggested that oltipraz could be metabolized in male rats mainly via CYP1A1/2, 2B1/2, 2C11, 3A1/2 and 2D1.

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Funding: This study was supported in part by a grant 02-PJ2-PG4-PT01-0027, 2003–2004 from the Korea Ministry of Health & Welfare, Korea.

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). Oltipraz was found to significantly increase the detoxification potential of the host (Bueding et al 1982) and this finding provided the first evidence that oltipraz might be an effective radioprotective, antiviral or chemoprotective agent (Clapper 1998). The cancer chemoprotective activity of oltipraz against aflatoxin-induced tumorigenesis in rats has been reported (Primiano et al 1995). The therapeutic effect of oltipraz in rats with liver cirrhosis induced by *N*-dimethylnitrosamine has also been reported recently (Kang et al 2002). The effects of oltipraz on glutathione S-transferase (GST) activity (Maheo et al 1998), hepatic microsomal cytochrome P450 (CYP) 2E1, 1A, 2B, 2B1, 2B2 and 2B1/2 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) activity (Kessler & Ritter 1998) have also been described in rats. However, the types of CYP isozymes that are involved in the metabolism of oltipraz in man and animals are yet to be identified. Thirteen metabolites of oltipraz, including glucuronide conjugates of metabolites, were reported in rat urine (Bieder et al 1983). A phase II clinical trial of oltipraz in patients with liver fibrosis and cirrhosis induced by chronic hepatitis types B and C is being planned in Korea.

The aim of this paper was to report the types of CYP isozyme that are involved in the metabolism of oltipraz in male rats. In-vitro and in-vivo studies were performed using various CYP isozyme inducers and inhibitors in rats. The results of this study could help

explain the pharmacokinetic changes of oltipraz that have been observed in rats with acute renal failure induced by uranyl nitrate (Bae et al 2004c), protein–calorie malnutrition (Bae et al 2005a), dehydration (Bae et al 2004d), liver cirrhosis induced by dimethylnitrosamine (Bae et al 2004e) and diabetes mellitus induced by alloxan or streptozotocin (our unpublished data) where the CYP isozymes are changed (Cho et al 1999; Kim et al 2001; Moon et al 2003). Rats were used as an animal model in this study since various in-vitro and in-vivo studies can be easily performed in rats.

Materials and Methods

Chemicals

Oltipraz was donated from R & D Center of Pharmaceuticals, Institute of Science & Technology, CJ Corporation (Ichon, Korea). Furafylline (a mechanism-based inhibitor of CYP1A2 in rats (Correia 1995)), sulfaphenazole (a main inhibitor of CYP2C11 in rats (Ogiso et al 1999)), SKF 525-A (a nonspecific CYP isozyme inhibitor in rats (Correia 1995)), dexamethasone phosphate (a main inducer of CYP3A1/2 in rats (Halpert 1988)), isoniazid (a main inducer of CYP2E1 in rats (Hammond & Fry 1997)), troleandomycin (a main inhibitor of CYP3A1/2 in rats (Wrighton et al 1985)), quinine hydrochloride (a main inhibitor of CYP2D1 in rats (Tomkins et al 1997; Tyndale et al 1999)), ethylenediamine tetraacetatic acid (EDTA) and reduced β-nicotinamide adenine dinucleotide phosphate (NADPH, as a tetrasodium salt) were purchased from Sigma-Aldrich Chemical Company (St Louis, MO). 3-Methylcholanthrene (a main inducer of CYP1A1/2 in rats (Spatzenegger et al 2000)) and sodium phenobarbital (a main inducer of CYP2B1/2 in rats (Kawamura et al 1999)) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Dai Han Pharmaceutical Company (Seoul, Korea), respectively. Polyethylene glycol 400 (PEG 400) was purchased from Duksan Chemical Company (Seoul, Korea). Other chemicals were of reagent grade or high-performance liquid graphic (HPLC) grade and were used without further purification.

Animals

Male Sprague–Dawley rats, 190–300 g (Charles River Company Korea, Biogenomics, Seoul, Korea), were housed in a light-controlled room with a 12-h light–dark cycle and kept at a temperature of $22 \pm 2^{\circ}$C and a relative humidity of $55 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea). Rats were housed in metabolic cages (Tecniplast, Varese, Italy) with a supply of filtered pathogen-free air and had free access to food (Samyang Company, Seoul, Korea) and water. The protocol of this study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

In-vitro assessment of oltipraz kinetics: V_{max}, K_m, and CL_{int} values for the disappearance of oltipraz in rat hepatic microsomes with various enzyme inhibitors

The procedures for the in-vitro assessment of oltipraz kinetics were similar to those reported earlier (Bae et al 2004a). The livers of untreated rats ($n = 5$) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in an ice-cold buffer of 0.154 M KCl and 50 mM Tris-HCl in 1 mM EDTA, pH 7.4. The homogenate was centrifuge at 9000 *g* for 30 min and the supernatant fraction was further centrifuged at 100 000 *g* for 90 min. Protein content was measured using the reported method (Bradford 1976). The V_{max} (the maximum velocity) and K_m (the Michaelis–Menten constant, the concentration at which the rate is one-half of V_{max}) for the disappearance of oltipraz were determined after incubating the above microsomal fraction (equivalent to 0.5 mg protein), 5 μL of oltipraz solution (the oltipraz powder was dissolved in dimethyl sulfoxide (DMSO) to have substrate concentrations of 5, 10, 20, 50, 100 and 200 μM), with or without 5 μL of 20 μM enzyme inhibitors, such as SKF 525-A, sulfaphenazole, quinine, troleandomycin or furafylline (furafylline was dissolved in DMSO; Pan & Belpaire 1999) and 1 mM of NADPH in a final volume of 0.5 mL with 0.1 M phosphate buffer, pH 7.4, in a water-bath shaker kept at 37°C and at a rate of 50 oscillations per min. All of the above microsomal incubation conditions were linear. The dissolving media for the enzyme inhibitors were the same as those in the intravenous studies. The reaction was terminated by the addition of 1 mL of acetonitrile after 5 min incubation. The kinetic constants (K_m and V_{max}) for the disappearance of oltipraz were calculated using the Lineweaver–Burk plot (Lineweaver & Burk 1934) by the method of least squares. The intrinsic clearance for the disappearance of oltipraz (CL_{int}) was calculated by dividing the respective V_{max} by the respective K_m value.

In-vivo assessment of oltipraz pharmacokinetics: effect of enzyme inducers (3-methylcholanthrene, phenobarbital, isoniazid, dexamethasone) or enzyme inhibitors (SKF 525-A, sulfaphenazole, quinine, troleandomycin)

Rats received: a single intravenous injection of 80 mg (2 mL) kg^{-1} of sulfaphenazole (SPT group) (Arlotto et al 1987) or a single intraperitoneal injection of 50 mg (3.3 mL) kg^{-1} of SKF525-A (SKT group) (Conney 1971); 500 mg (5 mL) kg^{-1} of troleandomycin (TMT group) (Sinclair et al 2000) or 20 mg (5 mL) kg^{-1} of quinine (QNT group) (Tomkins et al 1997); three daily intraperitoneal injections of 50 mg (5 mL) kg^{-1} of dexamethasone phosphate (DXT group) (Arlotto et al 1987; Ross et al 1993) or 150 mg (3 mL) kg^{-1} of isoniazid (INT group) (Ryan et al 1985); or four daily intraperitoneal injections of 80 mg (3.3 mL) kg^{-1} of sodium phenobarbital (PBT group) (Williams et al 1979; Choi et al 1991), 20 mg (3.3 mL) kg^{-1} of 3-methylcholanthrene (MCT group) (Williams et al 1979; Choi et al 1991), isotonic saline, 5 mL kg^{-1} (untreated groups;

TMC, SKC, SPC, QNC, DXC, PBC or INC group) or corn oil, 3.3 mL kg^{-1} (untreated group; MCC group). During the pretreatment, rats had free access to food and water. Sufaphenazole was dissolved in distilled water with a minimum amount of NaOH to adjust the pH to approx. 8.0. SKF 525-A, quinine hydrochloride, dexamethasone phosphate, isoniazid and sodium phenobarbital were dissolved in isotonic saline. Troleandomycin was dissolved in isotonic saline and acidified to pH 4.0 with HCl and 3-methylcholanthrene was dissolved in corn oil.

Intravenous study

The procedures for the pretreatment of rats, including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration), were reported previously (Bae et al 2003, 2004a–e, 2005a, b). An experiment was performed just after injection of sulfaphenazole (SPC and SPT groups (Ogiso et al 1999; Bae et al 2004b)) and during the first hour for the SKT and SKC groups (Conney 1971) and QNT and QNC groups (Tomkins et at 1997), 2 h for the TMT and TMC groups (Arlotto et al 1987), 4th day for the DXT and INT groups (Ryan et al 1985; Ross et al 1993; Sinclair et al 2000) and 5th day for the PBT, MCT, DXC, PBC, INC and MCC groups (Williams et al 1979; Ryan et al 1985; Choi et al 1991; Ross et al 1993; Sinclair et al 2000) as the commencement of respective pretreatment.

Oltipraz (oltipraz powder suspended in PEG 400–distilled water, 40:60 v/v) at a dose of 10 mg kg^{-1} (Bae et al 2003, 2004a, c–e, 2005a, b) was administered intravenously over 1 min via the jugular vein in the untreated groups (QNC, DXC, INC and PBC groups, n = 7; SPC and TMC groups, n = 8; SKC and MCC groups, n = 9) and treated groups (SKT group, n = 9; SPT group, n = 9; PBT group, n = 10; TMT group, n = 8; QNT group, n = 8; DXT group, n = 12; INT group, n = 9; MCT group, n = 8). The total injection volume was approximately 0.6 mL. Blood samples (approx. 0.12 mL) were collected via the carotid artery at time 0 (to serve as a control) and at 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after the beginning of the intravenous administration. Heparinized isotonic saline (approx. 0.25 mL; 20 units mL^{-1}) was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were immediately centrifuged at 16 000 *g* for 1 min, and a 50 μL of each plasma sample was stored at −70°C in a freezer (Model DF8517; Ilshin Laboratory Company, Seoul, Korea) until HPLC analysis of oltipraz (Bae et al 2001). At the end of 24 h, the metabolic cage was rinsed with 10 mL of distilled water and the rinsed material was combined with the urine sample. After measuring the exact volume of the combined urine sample, two 50-μL volumes taken from the combined urine sample were stored at −70°C until HPLC analysis of oltipraz (Bae et al 2001). At the same time (24 h), each rat was exsanguinated and sacrificed by cervical dislocation, and the entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 100 mL of methanol (to facilitate the extraction of oltipraz) and cut into small pieces using scissors. After stirring with a glass rod, two 50 μL samples of the supernatant were collected from each beaker and stored at −70°C until HPLC analysis of oltipraz (Bae et al 2001).

In-vitro plasma protein binding of oltipraz to rat plasma

Plasma protein binding of oltipraz was measured in additional MCT, MCC, SKT and SKC groups (n = 5 per group) using an equilibrium dialysis technique (Bu et al 2001). Plasma (1 mL) was dialysed against isotonic Sørensen phosphate buffer (1 mL, pH 7.4) containing 3% dextran to minimize volume shift using a 1-mL dialysis cell (Fisher Scientific, Fair Lawn, NJ) and Spectra/Por 4 membrane (MW cutoff 12 000–14 000; Spectrum Medical Industries Inc., Los Angeles, CA). To reduce equilibrium time, oltipraz was spiked into the plasma side at an oltipraz concentration of 5 μg mL^{-1}. The spiked dialysis cell was incubated for 24 h in a water-bath shaker kept at 37°C and at a rate of 50 oscillations per min. After 24 h incubation, two 50-μL samples were removed from each compartment and stored in a −70°C freezer until HPLC analysis of oltipraz (Bae et al 2001). The binding of oltipraz to 4% human serum albumin was independent of oltipraz concentrations in the range 1–100 μg mL^{-1}; the mean value was 95% (Bu et al 2001). Hence, the concentration of oltipraz, 5 μg mL^{-1}, was arbitrarily chosen in this study.

HPLC analysis of oltipraz

The concentration of oltipraz in the above samples was analysed by an HPLC method developed in our laboratories (Bae et al 2001). Acetonitrile (100 μL) was added to deproteinize (Chiou et al 1978) the biological sample (50 μL). After vortex-mixing and centrifugation at 16 000 *g* for 1 min, 50 μL 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; 45:55 v/v for rat urine samples), 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 rat urine samples. The detection limit of oltipraz in rat plasma and urine was 20 and 50 ng mL^{-1}, respectively. The mean within-day coefficient of variation (CV) in rat plasma and urine was 2.29 and 1.01%, respectively, and the corresponding between-day CV of the analysis of the same samples on three consecutive days was 3.37 and 1.51%.

Oltipraz in solution was reported to be photodegradable (Christensen & Malone 1992), therefore all samples in this study were covered (or wrapped) with aluminium foil or kept in the dark during the experiment or when they were not in use.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule–extrapolation method; this method

utilized 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 datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the time-averaged total body clearance (CL), terminal half-life, mean residence time (MRT) and apparent volume of distribution at steady state (V_{ss}).

The mean values of CL (Chiou 1980), V_{ss} (Chiou 1979) and terminal half-life (Eatman et al 1977) were calculated by the harmonic mean method.

Statistical analysis

$P < 0.05$ was considered to be statistically significant using the unpaired *t*-test or the Duncan's multiple range test of the Statistical Package for Social Sciences (SPSS) posteriori analysis of variance among the six means for the unpaired data. All results were expressed as mean ± s.d.

Results

In-vitro assessment of oltipraz kinetics: V_{max}, K_m and CL_{int} values for the disappearance of oltipraz in hepatic microsomes with various enzyme inhibitors

The Lineweaver–Burk plots for the measurement of V_{max}, K_m and CL_{int} values for the disappearance of oltipraz in hepatic microsomes with various enzyme inhibitors are shown in Figure 1. The mean V_{max}, K_m and CL_{int} values for the disappearance of oltipraz in rat hepatic microsomes without (untreated) or treated with enzyme inhibitors (SKF 525-A, furafylline, sulfaphenazole, quinine and troleandomycin) are listed in Table 1. The V_{max} values in the presence of SKF 525-A (91.2% decrease) and quinine (82.1% decrease) were significantly slower, indicating that the maximal velocity for the disappearance (mainly due to metabolism) of oltipraz was significantly slower in the presence of SKF 525-A and quinine. The V_{max} values in the presence of furafylline, sulfaphenazole and troleandomycin were not significantly different compared with the untreated value. The K_m value in the presence of SKF 525-A (86.7% decrease) and quinine (79.9% decrease) was significantly lower, indicating that the affinity of oltipraz for the enzyme(s) increased in the presence of the SKF 525-A and quinine. However, the K_m value in the presence of furafylline, sulfaphenazole and troleandomycin was not significantly different compared with the value in untreated rats, indicating that the affinity of oltipraz to the enzyme(s) was not influenced by furafylline, sulfaphenazole or troleandomycin. The intrinsic clearance for the disappearance of oltipraz (CL_{int}) was significantly slower in the presence of SKF 525-A (33.9% decrease), furafylline (11.5% decrease) and sulfaphenazole (14.6% decrease). However, the CL_{int} in the presence of quinine (13.5% decrease; $P = 0.0575$) or troleandomycin (12.0% decrease, $P = 0.0672$) seemed to be slower than in untreated rats. The above data suggested that the metabolism of oltipraz is inhibited by the addition of various enzyme inhibitors studied.

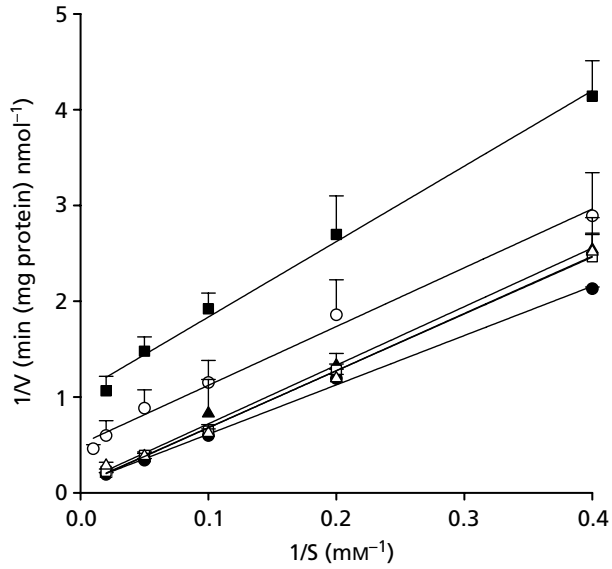

Figure 1 Lineweaver–Burk plots for the disappearance of oltipraz in male rat hepatic microsomes without (●, untreated) or with various enzyme inhibitors (○, quinine; ■, SKF 525-A; □, troleandomycin; ▲, sulfaphenazole; △, furafylline). S, concentration of oltipraz; V, velocity for the disappearance of oltipraz. Vertical bars represent s.d.

In-vivo assessment of olitpraz pharmacokinetics: effect of enzyme inducers

The mean arterial plasma concentration–time profiles of oltipraz after a 1-min intravenous administration at a dose of 10 mg kg^{-1} to male rats pretreated with 3-methylcholanthrene, phenobarbital, isoniazid and dexamethasone (MCT, PBT, INT and DXT groups), and their respective untreated rats (MCC, PBC, INC and DXC groups), are shown in Figure 2, and some relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration, the plasma concentrations of oltipraz declined in a polyexponential fashion for all groups of rats studied (Figure 2).

In rats pretreated with 3-methylcholanthrene (MCT group), the CL was significantly faster (38.4% increase), and this could be supported by a significantly shorter MRT (42.7% decrease), since V_{ss} was significantly smaller (17.3% decease) in the rats (Table 2). The smaller V_{ss} (Table 2) could be due to a significantly smaller free (unbound in plasma proteins) fraction of oltipraz in the pretreated rats; plasma protein binding was significantly greater in rats pretreated with 3-methylcholanthrene with values of 75.8 ± 8.8 and 85.80 ± 4.46% (n = 5, each) for untreated rats and pretreated rats, respectively. Note that the body weight gain was significantly smaller in rats pretreated with 3-methylcholanthrene (from 269 to 280 g) than that in untreated rats (from 264 to 293 g).

Table 1 In-vitro V_{max}, K_m and CL_{int} values for the disappearance of oltipraz after incubation of oltipraz with the hepatic microsomal fraction of male rats without (untreated) or with SKF 525-A, furafylline, sulfaphenazole, quinine or troleandomycin

Parameter	Untreated	SKF 525-A	Furafylline
V_{max} (nmol min^{-1} (mg protein)$^{-1}$)	11.00 ± 1.98	0.97 ± 0.14***	13.90 ± 5.33
K_m (μM)	57.70 ± 12.60	7.66 ± 1.55***	83.30 ± 37.00
CL_{int} (mL min^{-1} (mg protein)$^{-1}$)	0.19 ± 0.01	0.13 ± 0.02***	0.17 ± 0.01*
	Sulfaphenazole	**Quinine**	**Troleandomycin**
V_{max} (nmol min^{-1} (mg protein)$^{-1}$)	13.30 ± 9.58	1.97 ± 0.50***	14.10 ± 6.43
K_m (μM)	79.20 ± 56.30	11.60 ± 2.04***	85.50 ± 47.30
CL_{int} (mL min^{-1} (mg protein)$^{-1}$)	0.16 ± 0.02*	0.17 ± 0.02	0.17 ± 0.02

Values are mean ± s.d., n = 5. *$P < 0.05$ and ***$P < 0.001$ vs untreated group.

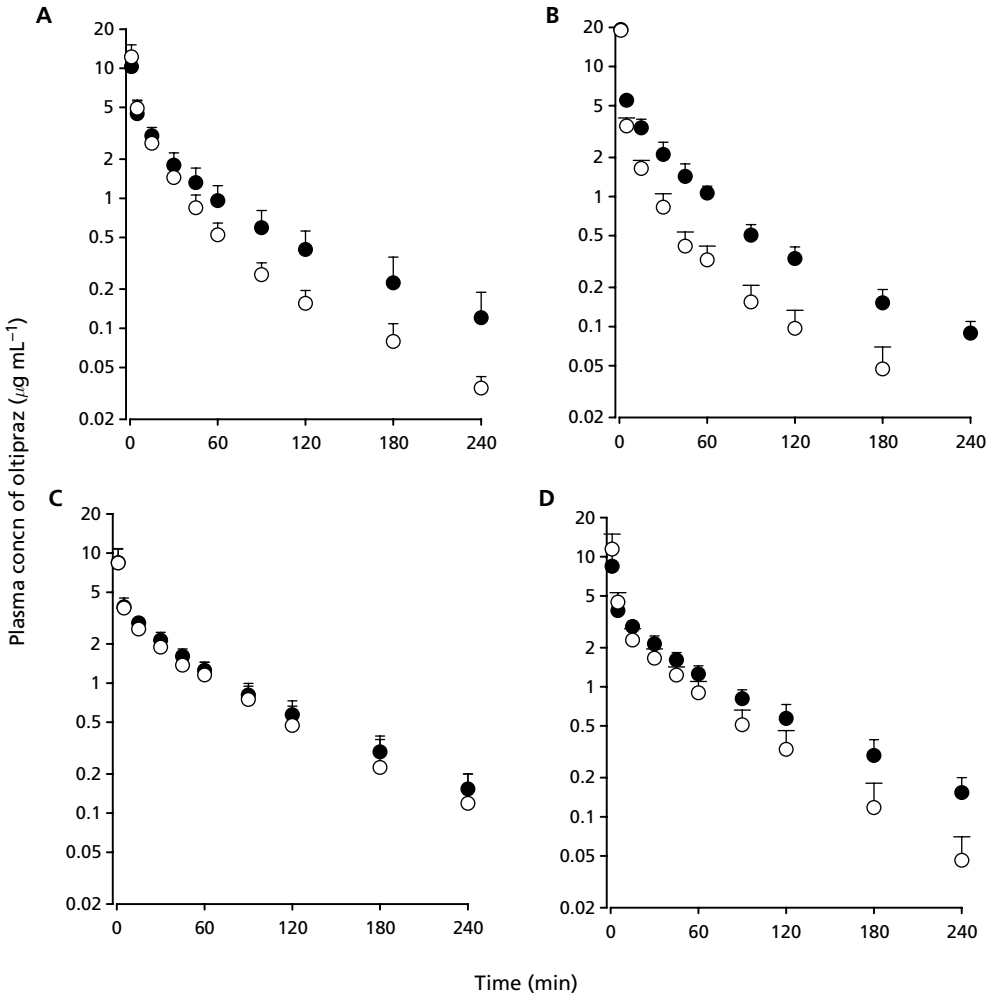

Figure 2 Mean arterial plasma concentration–time profiles of oltipraz after a 1-min intravenous administration at a dose of 10 mg kg^{-1} to male rats pretreated with enzyme inducers (open circles), 3-methaylcholanthrene (A), phenobarbital (B), isoniazid (C) and dexamethasone (D), and their respective untreated rats (closed circles). Bars represent s.d.

In rats pretreated with phenobarbital (PBT group), the CL was significantly faster (94.4% increase) than in control rats (PBC), and this could be supported by a significantly shorter MRT (40.5% decrease), since the V_{ss} values were comparable between two groups of rats (Table 2).

In rats pretreated with isoniazid (INT group), the pharmacokinetic parameters of oltipraz were not significantly

Table 2 Pharmacokinetic parameters of oltipraz after intravenous administration at a dose of 10 mg kg^{-1} to male rats pretreated with enzyme inducers, 3-methaylcholanthrene (MCT), phenobarbital (PBT), isoniazid (INT) and dexamethasone (DXT), and their respective male untreated rats

Parameter	3-Methylcholanthrene		Phenobarbital	
	Untreated (MCC; n = 9)	Treated (MCT; n = 8)	Untreated (PBC; n = 7)	Treated (PBT; n = 10)
CL (mL min^{-1}kg^{-1})	44.80 ± 12.60	62.00 ± 8.01**	39.30 ± 4.62	76.40 ± 9.37***
V_{ss} (mL kg^{-1})	2840 ± 674	2350 ± 314*	1770 ± 185	1970 ± 486
Terminal half-life (min)	65.30 ± 12.50	55.90 ± 8.06	58.20 ± 4.83	51.90 ± 7.75
MRT (min)	65.30 ± 18.00	37.40 ± 4.97***	45.40 ± 6.17	27.00 ± 6.85***
	Isoniazid		**Dexamethasone**	
	Untreated (INC; n = 7)	Treated (INT; n = 9)	Untreated (DXC; n = 7)	Treated (DXT; n = 12)
CL (mL min^{-1}kg^{-1})	39.90 ± 5.35	44.80 ± 9.73	39.90 ± 5.35	53.30 ± 7.44***
V_{ss} (mL kg^{-1})	2950 ± 295	2960 ± 401	2950 ± 295	2580 ± 454
Terminal half-life (min)	63.00 ± 8.43	57.30 ± 16.4	63.00 ± 8.43	45.80 ± 14.7*
MRT (min)	74.20 ± 8.58	67.70 ± 19.9	74.20 ± 8.58	49.20 ± 8.43***

Values are mean ± s.d. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ vs respective untreated rats.

different compared with those in untreated rats (INC) (Table 2). Note that body weight gain was significantly smaller in rats pretreated with isoniazid (from 271 to 266 g) than that in the untreated rats (from 269 to 288 g).

In rats pretreated with dexamethasone (DXT group), the CL value was significantly greater (33.6% increase), and this could be supported by a significantly shorter terminal half-life (27.3% decrease) and MRT (33.7% decrease) in the treated rats, since the V_{ss} values were not significantly different between two groups of rats (Table 2). Note that the body weight gain was significantly smaller in rats pretreated with dexamethasone (from 277 to 246 g) than that in untreated rats (from 269 to 288 g).

In-vivo assessment of oltipraz pharmacokinetics: effect of enzyme inhibitors

The mean arterial plasma concentration–time profiles of oltipraz after a 1-min intravenous administration at a dose of 10 mg kg^{-1} to male rats pretreated with SKF 525-A (SKT group), sulfaphenazole (SPT group), quinine (QNT group) and troleandomycin (TMT group), and their respective untreated control rats (SKC, SPC, QNC and TMC groups) are shown in Figure 3, and some relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration, the plasma concentrations of oltirpaz declined in a polyexponential fashion for all groups of rats (Figure 3).

In rats pretreated with SKF 525-A (SKT group), the CL was significantly slower (55.6% decrease), and this could be supported by a significantly longer terminal half-life (55.0% increase) and MRT (83.0% increase) and a significantly smaller V_{ss} (17.9% decrease) in the pretreated rats (Table 3). The smaller V_{ss} (Table 3) could be due to a significantly smaller free (unbound in plasma proteins) fraction of oltipraz; the plasma protein binding value was significantly greater in rats pretreated with SKF 525-A; the values were 75.8 ± 8.8 and 84.1 ± 1.3% (n = 5, each) for untreated rats and pretreated rats, respectively.

In rats pretreated with sulfaphenazole (SPT group), the CL value was significantly greater (31.0% decrease), and this could be supported by a significantly longer terminal half-life (74.0% increase) and MRT (45.0% increase) in the treated rats, since V_{ss} values were comparable between the untreated and pretreated groups of rats (Table 3).

In rats pretreated with quinine (QNT group), the CL value was significantly smaller (27.6% decrease), and this could be supported by a significantly longer MRT (32.6% increase) in the pretreated rats, since V_{ss} values were comparable between the untreated and pretreated groups of rats (Table 3).

In rats pretreated with troleandomycin (TMT group), the CL value was significantly smaller (36.3% decrease), and this could be supported by a significantly longer terminal half-life (79.6% increase) and MRT (34.1% increase) in the pretreated rats, since the V_{ss} values were comparable between the untreated and pretreated groups of rats (Table 3).

Discussion

After intravenous administration, the contribution of time-averaged renal clearance to the total body clearance of oltipraz was almost negligible; the percentage of intravenous dose of oltipraz excreted in 24-h urine as unchanged drug was less than 0.654% for all male rats studied. The contribution of gastrointestinal (including biliary) excretion of unchanged oltipraz to the time-averaged nonrenal clearance (CL_{NR}) of oltipraz was also negligible; oltipraz was below the detection limit in the entire gastrointestinal tract at 24 h, as unchanged drug, for all rats studied. Moreover, the percentage of the intravenous dose of oltipraz, at a dose of 20 mg kg^{-1}, excreted in 8-h

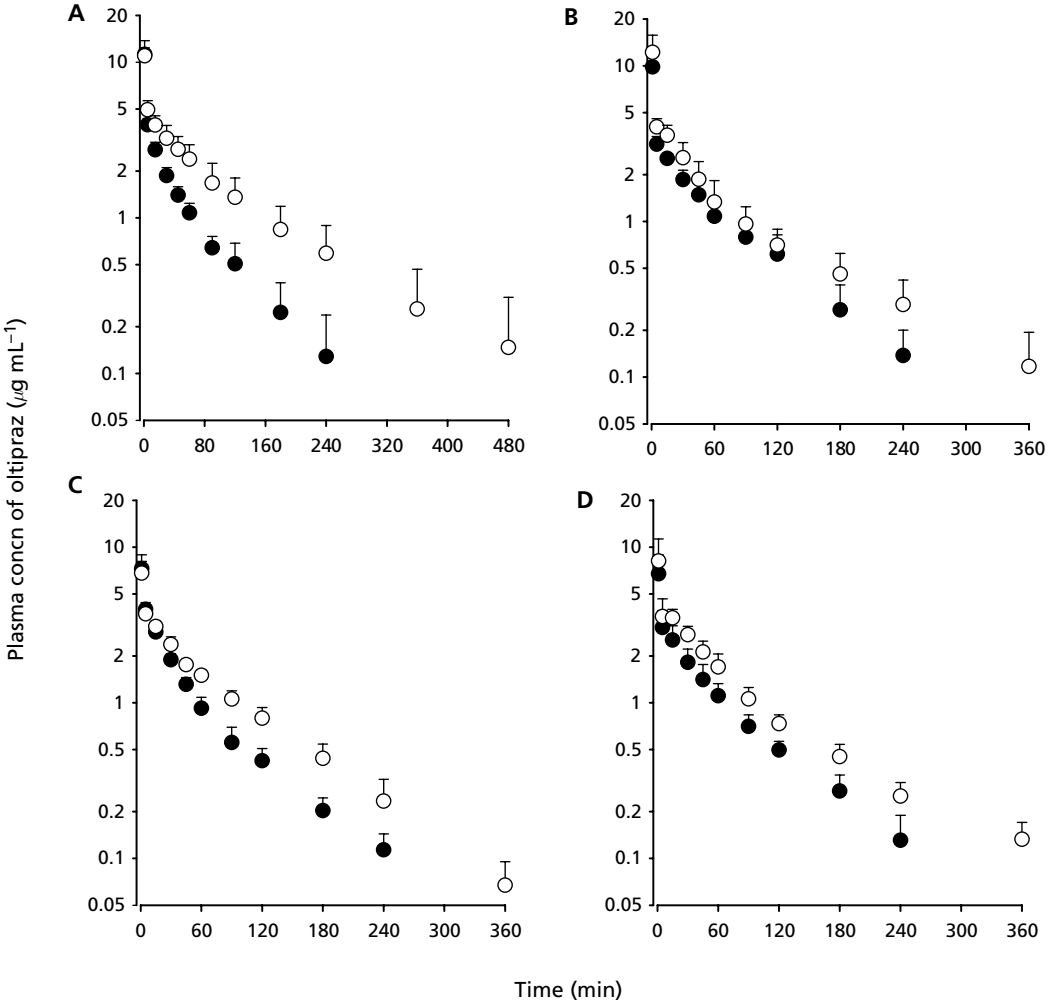

Figure 3 Mean arterial plasma concentration–time profiles of oltipraz after a 1-min intravenous administration at a dose of 10 mg kg^{-1} to male rats pretreated with enzyme inhibitors (open circles), SKF525-A (A), sulfaphenazole (B), quinine (C) and troleandomycin (D), and their respective untreated rats (closed circles). Bars represent s.d.

Table 3 Pharmacokinetic parameters of oltipraz after intravenous administration at a dose of 10 mg kg^{-1} to male rats pretreated with enzyme inhibitors, SKF525-A (SKT), sulfaphenazole (SPT), quinine (QNT) and troleandomycin (TMT), and their respective male untreated rats

Parameter	SKF 525-A		Sulfaphenazole	
	Untreated (SKC; n = 9)	Treated (SKT; n = 9)	Untreated (SPC; n = 8)	Treated (SPT; n = 9)
CL (mL min^{-1}kg^{-1})	42.80 ± 8.49	19.00 ± 5.89***	43.60 ± 6.50	30.10 ± 7.82**
V_{ss} (mL kg^{-1})	2850 ± 294	2340 ± 349**	3070 ± 448	3010 ± 659
Terminal half-life (min)	55.50 ± 21.30	86.00 ± 40.40*	47.30 ± 16.00	82.30 ± 57.20*
MRT (min)	68.30 ± 23.60	125.00 ± 4.74**	71.70 ± 13.60	104.00 ± 33.40*
	Quinine		**Troleandomycin**	
	Untreated (QNC; n = 7)	Treated (QNT; n = 8)	Untreated (TMC; n = 8)	Treated (TMT; n = 8)
CL (mL min^{-1}kg^{-1})	46.30 ± 6.13	33.50 ± 7.61***	46.30 ± 8.02	29.50 ± 4.78***
V_{ss} (mL kg^{-1})	3070 ± 438	2940 ± 409	3460 ± 884	3220 ± 580
Terminal half-life (min)	60.00 ± 12.70	65.10 ± 16.00	57.90 ± 18.20	104.00 ± 50.10*
MRT (min)	67.20 ± 9.46	89.1 ± 17.1***	76.80 ± 16.80	103.00 ± 10.50**

Values are mean ± s.d. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ vs respective untreated rats.

bile as unchanged drug was less than 0.635% in 8 rats (Bae et al 2004 g). Note that levels of oltipraz being below the detection limit in the gastrointestinal tract at 24 h was not mainly due to chemical or enzymatic degradation of oltipraz in the gastrointestinal tract. Oltipraz was stable in human gastric juices, rat bile juices and various pH solutions (Bae et al 2005b). The above data indicated that oltipraz was metabolized almost completely after intravenous administration in rats, and the CL_{NR} value of oltipraz could represent the metabolic clearance of oltipraz. Hence, the change in CL (CL_{NR}) values of oltipraz brought about by pretreatment with various enzyme inducers (Table 2) or inhibitors (Table 3) could indicate changes (slower or faster) in the metabolism of oltipraz in rats. It has been reported (Bieder et al 1983) that 13 metabolites of oltipraz are found in rat urine.

To discover whether CYP isozymes are involved in the metabolism of oltipraz in male rats, the rats were pretreated with SKF 525-A. In rats pretreated with SKF525-A (SKT group), the CL value was significantly smaller than that in untreated rats (Table 3), indicating that oltipraz is metabolized via CYP isozymes in rats. Hence, various CYP enzymes inducers or inhibitors were used for pretreatment to find out which types of CYP isozymes are involved in the metabolism of oltipraz in rats. In rats pretreated with 3-methylcholanthrene (MCT group), phenobarbital (PBT group) and dexamethasone (DXT group), the CL values were significantly greater than those in respective untreated rats (MCC, PBC and DXC groups) (Table 2). In rats pretreated with sulfaphenazole (SPT group), quinine (QNT group) and troleandomycin (TMT group), the total body clearance was significantly slower than in the respective untreated rats (SPC, QNC and TMC groups) (Table 3). The above data suggests that oltipraz is mainly metabolized via CYP1A1/2, 2B1/2, 2C11, 3A1/2 and 2D1 in male rats. Note that the various enzyme inducers and inhibitors studied acted on various CYP isozymes, hence only the main CYP isozyme is mentioned in this study and the results are confined to the main CYP isozymes. More studies are required to evaluate which other CYP isozymes are involved in the metabolism of oltipraz in rats.

The in-vitro CL_{int} values of oltipraz with various enzyme inhibitors (Table 1) correlated well with the in-vivo pharmacokinetic parameters (especially AUC and CL values) of oltipraz after pretreatment with various enzyme inhibitors (Table 3). Note that the changes in V_{max}, K_m and CL_{int} values listed in Table 1 may indicate simple changes in the relative contribution of each pathway to the overall disappearance of oltipraz, since oltipraz is metabolized by multiple CYP isozymes. Hence, the value with a specific isozyme inhibitor (Table 1) may not necessarily reflect the role of a specific isozyme.

The above results play an important role in explaining the pharmacokinetic changes seen for oltipraz in various rat disease models where the CYP isozymes are changed. For example, after intravenous administration of oltipraz at a dose of 10 mg kg^{-1} to rats with protein–calorie malnutrition (PCM), the CL_{NR} of oltipraz was significantly lower (58.0% decrease) than that in untreated rats (Bae et al 2005a), since CYP1A2, 2C11, and 3A1/2 decreased in PCM rats (Cho et al 1999). After intravenous administration of oltipraz at a dose of 30 mg kg^{-1} to rats with 72-h water deprivation (dehydration), the CL_{NR} of oltipraz was comparable with that in untreated rats (Bae et al 2004d), since CYP1A1/2, 2B1/2, 2C11 and 3A1/2 are not changed in dehydrated rats (Kim et al 2001). After intravenous administration of oltipraz at a dose of 30 mg kg^{-1} to rats with acute renal failure induced by uranyl nitrate (U-ARF), the CL_{NR} of oltipraz was significantly slower (36.2% decrease) than that in untreated rats (Bae et al 2004c), since CYP2C11 decreased in U-ARF rats (Moon et al 2003). After intravenous administration of oltipraz at a dose of 30 mg kg^{-1} to rats with liver cirrhosis induced by dimethylnitrosamine, the CL_{NR} of oltipraz was significantly slower (47.8% decrease) than that in untreated rats (Bae et al 2004e). If this rat study could be extrapolated to man, a dosage modification of oltipraz may be required in patients with various diseases who are concurrently taking drugs or foods that can induce or inhibit CYP enzymes.

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