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Pharmacokinetic changes of oltipraz after intravenous and oral administration to rats with liver cirrhosis induced by dimethylnitrosamine

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Abstract

Pharmacokinetic changes of oltipraz were investigated after intravenous and oral administration at a dose of 30 mg/kg to control Sprague–Dawley rats and rats with liver cirrhosis induced by dimethylnitrosamine. After intravenous administration in rats with liver cirrhosis, the area under the plasma concentration–time curve from time zero to time infinity (AUC) was significantly greater (1490 μg min/ml versus 2840 μg min/ml) than that in control rats. This was due to significantly slower total body clearance (CL) (20.2 ml/(min kg) versus 10.6 ml/(min kg)) in the rats. The slower CL was due to significantly slower CL_{NR} (20.1 ml/(min kg) versus 10.5 ml/(min kg)) in rats with liver cirrhosis. The significantly slow CL_{NR} was due to slower hepatic blood flow rate and significantly slower in vitro intrinsic oltipraz disappearance clearance (CL_{int}, 77.2 ml/min per whole liver versus 11.5 ml/min per whole liver) because the free (unbound in serum proteins) fraction of oltipraz was significantly greater (15.1% versus 31.3%) in the rats. After oral administration in rats with liver cirrhosis, the AUC was also significantly greater (354 μg min/ml versus 812 μg min/ml) and this was not due to increased absorption in the rats. This also could be due to slower hepatic blood flow rate and significantly slower CL_{int} in the rats.

Keywords: Oltipraz; Pharmacokinetics; Liver cirrhosis; Dimethylnitrosamine; Rats

Abbreviations: HPLC, high-performance liquid chromatography; AUC, area under the plasma concentration–time curve from time zero to time infinity; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; MRT, mean residence time; V_{ss}, apparent volume of distribution at steady state; F, extent of absolute oral bioavailibility; $Ae_{0-24\,h}$, amount of unchanged oltipraz excreted in 24-h urine; $GI_{24\,h}$, amount of unchanged oltipraz recovered from gastrointestinal tract at 24 h; V_{max}, maximum velocity for disappearance of oltipraz; K_m, Michaelis–Menten constant for disappearance of oltipraz; CL_{int}, intrinsic oltipraz disappearance clearance

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1. 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, 1980). In contrast to decreasing glutathione on schistosomes to critical levels, oltipraz produced significant elevations in the detoxification potential of the host (Bueding et al., 1982). This finding provided the first evidence that oltipraz may be effective in its effectiveness as a cancer chemopreventive, radioprotector, and antiviral agent (Clapper, 1980). Cancer chemopreventive activities of oltipraz against aflatoxin-induced tumorigenesis in rats have been reported (Primiano et al., 1995).

High-performance liquid chromatographic (HPLC) analysis of oltipraz in biological fluids (Bae et al., 2001), stability, blood partition, and protein binding of oltipraz (Bu et al., 2001), and pharmacokinetic interaction between oltipraz and DDB in rats (Bae et al., 2003) have been reported. The following results have been reported in rats from our laboratories (our unpublished data). (1) After intravenous (at doses of 5, 10, and 20 mg/kg) and oral (at doses of 25, 50, and 100 mg/kg) administration to rats, pharmacokinetic parameters of oltipraz were dose-independent. (2) After oral administration of oltipraz at a dose of 20 mg/kg to rats, the mean extent of absolute oral bioavailability (F) was 41.2, 2.68% of oral dose was not absorbed from gastrointestinal tract, intestinal first-pass effect was approximately 32% of oral dose, and hepatic first-pass effect was approximately 25% of oral dose. (3) Rat kidney, heart, and spleen had negligible metabolic activities and liver could be the main metabolizing organ based on in vitro tissue homogenate study. (4) Mean percentage of intravenous dose (20 mg/kg) excreted in 8-h bile as unchanged oltipraz was 0.449% in five rats. (5) After consecutive 7 days oral administration of oltipraz at a dose of 50 mg/kg per day, the total area under the plasma concentration–time curve from time zero to time infinity (AUC) was significantly smaller (51.4% decrease) than that after single oral administration and this could be mainly due to enzyme induction by multiple administration of oltipraz.

Hepatic first-pass effect of oltipraz absorbed into the portal vein was approximately 40% (approximately 40% was equivalent to approximately 25% of oral dose of oltipraz at a dose of 20 mg/kg) and liver could be the main metabolizing organ for oltipraz in rats as mentioned earlier. Hence, it could be expected that the time-averaged nonrenal clearance (CL_{NR}) of oltipraz could be slower in rats with liver cirrhosis induced by dimethylnitrosamine than that in control rats. Recently, therapeutic effect of oltipraz in rats with liver cirrhosis induced by dimethylnitrosamine has been reported (Kang et al., 2002). For example, in rats with liver cirrhosis induced by dimethylnitrosamine, (1) oltipraz increased survival rates of cirrhotic rats as a result of improvement of liver function and regeneration of cirrhotic liver, (2) oltipraz reduced the intensity of liver fibrotic and cirrhotic nodules and eliminated accumulated extracellular matrix, and (3) oltipraz inactivated stellate cells in cirrhotic liver.

The purpose of this study is to report the pharmacokinetic changes of oltipraz at a dose of 30 mg/kg after intravenous and oral administration to rats with liver cirrhosis induced by dimethylnitrosamine.

2. Materials and methods

2.1. Chemicals

Oltipraz was supplied by Aventis Pharmaceutical Company (Virty-sur-Seine). Polyethylene glycol 400 (PEG 400) was purchased from Duksan Chemical Company (Seoul, South Korea). Dimethylnitrosamine, reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH, as a tetrasodium salt), ethylendiamine tetraacetatic acid (EDTA), and Tris-buffer were products from Sigma Chemical Company (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade, and therefore, were used without further purification.

2.2. Animals

Male Sprague–Dawley rats (weighing 150–180 g) were purchased from Charles River Company Korea (Biogenomics, Seoul, South Korea). All rats were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea) at a temperature between 20 and 23 °C with 12-h light and dark cy-

cles and a relative humidity of 50%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and with food (Samyang Company, Seoul, South Korea) and water ad libitum. The protocol of this study was approved by Animal Care and Use Committee of College of Pharmacy, Seoul National University.

2.3. Induction of liver cirrhosis induced by dimethylnitrosamine

Dimethylnitrosamine (dimethylnitrosamine powder was dissolved in 0.9% NaCl-injectable solution to make a 0.01 mg/ml) at a dose of 0.01 mg/kg was repeatedly injected intraperitoneally on three consecutive days a week for 4 weeks (Ohara and Kusano, 2002). For control rats, the same volume of 0.9% NaCl-injectable solution was injected. During the pretreatment, rats had free access to food and water. Seven days after the last dimethylnitrosamine injection (or 0.9% NaCl-injectable solution), the experiment was performed. Laboratory cirrhotic rats produced by dimethylnitrosamine administration simulate the clinical features of human liver cirrhosis such as mortality, hepatic parenchymal cell destruction, formation of connective tissue, and nodular regeneration (Kang et al., 2002). Liver cirrhosis in rats induced by dimethylnitrosamine was evident based on liver microscopy; there was extensive micronodular cirrhosis with regenerative hepatocellular changes and bile ductular proliferation (our unpublished data). It has been reported (Jenkins et al., 1985; Jezequel et al., 1987) that dimethylnitrosamine was used as a reproducible animal model of hepatic cirrhosis.

2.4. Preliminary study

The blood of control rats ($n = 5$) and rats with liver cirrhosis induced by dimethylnitrosamine ($n = 5$) were collected from the carotid artery for the measurement of hematocrit (Microprocessor pH/^{o}C Meter, Eutek Cybernetics, Singapore, Singapore). Serum was collected for the measurement of total proteins, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), creatinine (measured by Green Cross Reference Laboratory, Seoul, South Korea), and protein binding using an equilibrium dialysis technique (Bu et al., 2001). The whole kidney, spleen, and liver of each rat were excised, rinsed with 0.9% NaCl-injectable solution, blotted dry with tissue paper, and weighed. Small portions of each organ were fixed in 10% neutral phosphate buffered formalin and then processed for routine histological examination with hematoxylin–eosin staining.

2.5. Measurement of V_{max}, K_m, and CL_{int} for the disappearance of oltipraz in hepatic microsomes

The livers of control rats ($n = 6$) and rats with liver cirrhosis induced by dimethylnitrosamine ($n = 6$) were homogenized (Ultra-Turrax T25, Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in an ice-cold buffer of 0.154 M KCl/50 mM Tris–HCl in 1 mM EDTA, pH 7.4. The homogenate was centrifuge at 9500 rpm for 30 min and the supernatant fraction was further centrifuged at 36,000 rpm 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), a 2.5-μl aliquot of oltipraz (oltipraz powder was dissolved in dimethyl sulfoxide to have substrate concentrations of 2.5, 5, 7.5, 10, 12.5, 15, 20, and 25 μM), and 1 mM of NADPH in a final volume of 0.5 ml of 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 minute (opm). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of 1 ml of acetonitrile after 2.5-min incubation. Oltipraz was measured by the reported HPLC analysis (Bae et al., 2001). The kinetic constants (K_m and V_{max}) for the disappearance of oltipraz were calculated using the Lineweaver–Burk plot (Lineweaver and Burk, 1934) by the linear regression and the method of least squares. Intrinsic oltipraz disappearance clearance (CL_{int}) was calculated by dividing the V_{max} by the K_m.

2.6. Intravenous administration

The procedures for 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 PEG 400:distilled water = 40:60, v/v, 40% PEG) at a dose of 30 mg/kg was injected via the jugular vein over 1-min to control rats ($n = 8$) and rats with liver cirrhosis ($n = 8$). The total injection volume was approximately 0.6 ml. A 0.12-ml aliquot of blood sample 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, 90, 120, 180, 240, 360, 480, 720, and 1440 min after the beginning of the infusion. Blood samples were centrifuged immediately and a 50-μl aliquot of plasma samples was stored in a −70 °C freezer (Revco ULT 1490 D-N-S, Western Mednics, Asheville, NC) until HPLC analysis of oltipraz (Bae et al., 2001). At the end of the experiment (24 h), each 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 samples was stored in a −70 °C freezer until HPLC analysis of oltipraz (Bae et al., 2001). At the same time (24 h), the entire gastrointestinal tract 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 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 (Bae et al., 2001).

2.7. Oral administration

Oltipraz (the same suspension as used in the intravenous study) at a dose of 30 mg/kg was administered orally using a feeding tubing (total oral volume was approximately 2 ml) to control rats ($n = 8$) and rats with liver cirrhosis induced by dimethylnitrosamine ($n = 8$). Blood samples were collected at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 720, 1080, and 1440 min. Other procedures were the same as those in the intravenous study.

2.8. Tissue distribution of oltipraz after oral administration to rats

The procedures were similar to those reported previously (Yoon et al., 1998). Oltipraz (the same suspension as used in the intravenous study) at a dose of 30 mg/kg was administered orally to control rats ($n = 5$) and rats with liver cirrhosis induced by dimethylnitrosamine ($n = 5$). Total oral volume was approximately 2.0 ml. Eight hours (close to maximum plasma concentration of oltipraz) after oral administration of oltipraz, 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 was collected. Approximately 1 g of each liver, kidney, lung, spleen, heart, muscle, mesentery, large intestine, fat, and brain was excised, rinsed with 0.9% NaCl-injectable solution to minimize blood remaining in each tissue, and blotted dry with paper tissue. Each tissue was homogenized with 4-volume of 0.9% NaCl-injectable solution using a tissue homogenizer. After centrifugation, a 50-μl aliquot of the 9800 rpm supernatant was stored in a −70 °C freezer until HPLC analysis of oltipraz (Bae et al., 2001). All the procedures were conducted at 4 °C in an ice-bath.

2.9. Serum protein binding of oltipraz using an equilibrium dialysis technique

Serum protein binding of oltipraz in control rats and rats with liver cirrhosis induced by dimethylnitrosamine was measured using an equilibrium dialysis technique as reported earlier (Bu et al., 2001). The concentration of oltipraz spiked into the serum side was 5 μg/ml. The binding of oltipraz to 4% HSA was independent of oltipraz concentrations ranging from 1 to 100 μg/ml using an equilibrium dialysis technique; the mean value was 95% (Bu et al., 2001). After 24-h incubation, two 50-μl aliquots were removed from each compartment and stored in a −70 °C freezer until HPLC analysis of oltipraz (Bae et al., 2001).

2.10. HPLC analysis of oltipraz

Concentrations (or amount) of oltipraz in the above samples were analyzed by HPLC method developed from our laboratories (Bae et al., 2001). 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 13,100 rpm for 2 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 samples, and 45:55, v/v for rat urine samples), was run at a flow-rate of 1.5 ml/min and the column effluent was monitored by a UV detector set at 305 nm. Retention time of oltipraz was approximately 5.8 min in rat plasma and tissue samples and approximately 8.6 min in rat urine samples. Detection limits of oltipraz in rat plasma and urine were 20 and 50 ng/ml, respectively. The mean within-day coefficients of variation (C.V.s) 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 C.V.s of the analysis of the same samples on consecutive 3 days were 3.37% (range 1.77–4.65%) and 1.51% (range 0.389–2.90%).

Oltipraz in solution was reported to be photodegradation (Christensen and Malone, 1992), therefore, all samples in the present study were covered or wrapped with aluminum foil or kept in the dark when they are not in use.

2.11. Pharmacokinetic analysis

The AUC was calculated by the trapezoidal rule–extrapolation method; this method employed the logarithmic trapezoidal rule (Chiou, 1978) for 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 terminal rate constant. Standard methods (Gibaldi and 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), and apparent volume of distribution at steady state (V_{ss}) (Kim et al., 1993). The F was estimated by dividing the AUC after oral administration by AUC after intravenous administration. The peak plasma concentration of oltipraz (C_{max}) and the time to reach a C_{max} (T_{max}) were directly obtained from plasma concentration–time data.

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

2.12. 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 data are expressed as mean ± S.D.

3. Results

3.1. Preliminary study

Body weight, blood, serum, and urine chemistry data, protein binding, and organ weight in control rats and rats with liver cirrhosis induced by dimethylnitrosamine are listed in Table 1. In rats with liver cirrhosis, the hematocrit was significantly smaller (21.4% decrease) and the serum levels of total proteins (36.2% decrease) and albumin (37.5% decrease) were significantly lower than those in control rats. However, the levels of GOT (107% increase), GPT (220% increase), total bilirubin (538% increase), direct bilirubin (432% increase), and ALP (452% increase) were significantly higher than those in control rats. In rats with liver cirrhosis, the whole liver (63.1% decrease) and kidney (21.8% decrease) weight was significantly lighter, but the whole spleen weight was significantly heavier (68.2% increase) than those in control rats. The liver weight based on percentage of body weight was significantly lighter (46.2% decrease) in rats with liver cirrhosis. However, the kidney (19.9% increase) and spleen (150% increase) weight based on percentage of body weight was significantly heavier in rats with liver cirrhosis. The serum level of urea nitrogen and CL_{CR} were not significantly different between two groups of rats. However, serum protein binding of oltipraz in rats with liver cirrhosis was significantly smaller (19.1% decrease) than that in control rats. This could be due to significantly lower level of serum albumin and decreased affinity of oltipraz to albumin in the rats. It has been reported (Bu et al., 2001) that binding of oltipraz decreased with decreasing HSA concentrations at an oltipraz concentration of 5 μg/ml; the values were 69.9, 80.5, 88.8, 92.5, 93.9, and 94.7% for HSA concentrations of 0.5, 1, 2, 3, 5, and 6%, respectively. The data listed in Table 1 could indicate that liver function seemed to be impaired in rats with liver cirrhosis and this could be supported by liver mi-

Table 1
Body weight, blood, serum, and urine chemistry data, protein binding, and organ weight in control rats and rats with liver cirrhosis induced by dimethylnitrosamine[a]

Parameter	Control ($n = 5$)	Cirrhosis ($n = 5$)
Initial body weight (g)	160 ± 5.87	165 ± 8.10
Final body weight (g)	374 ± 8.94	248 ± 46.5[b]
Hematocrit (%)	46.7 ± 2.27	36.7 ± 6.63[b]
Serum		
Total proteins (g/dl)	6.02 ± 0.352	3.84 ± 0.844[b]
Albumin (g/dl)	3.47 ± 0.245	2.17 ± 0.490[b]
Urea nitrogen (mg/dl)	9.68 ± 2.82	14.8 ± 4.51
GOT (U/l)	98.4 ± 75.5	204 ± 65.6[c]
GPT (U/l)	43.4 ± 33.8	139 ± 54.5[c]
Total bilirubin (mg/dl)	0.160 ± 0.0548	1.02 ± 0.130[b]
Direct bilirubin (mg/dl)	0.0380 ± 0.00837	0.202 ± 0.0286[b]
ALP (U/l)	89.6 ± 15.7	495 ± 228[d]
Protein binding (%)	84.9 ± 2.65	68.7 ± 10.6[b]
CL_{CR} (ml/(min kg))	3.46 ± 0.824	4.25 ± 1.17
Liver weight (g)	12.4 ± 1.55	4.57 ± 1.93[b]
Liver weight (% of body weight)	3.33 ± 0.462	1.79 ± 0.444[b]
Kidney weight (g)	2.66 ± 0.173	2.08 ± 0.204[c]
Kidney weight (% of body weight)	0.712 ± 0.0610	0.854 ± 0.106[c]
Spleen weight (g)	0.660 ± 0.0548	1.11 ± 0.366[b]
Spleen weight (% of body weight)	0.176 ± 0.0113	0.440 ± 0.0817[b]

[a] Each value represents the mean ± S.D.
[b] Cirrhotic value was significantly different ($P < 0.001$) from control ones.
[c] Cirrhotic value was significantly different ($P < 0.05$) from control ones.
[d] Cirrhotic value was significantly different ($P < 0.01$) from control ones.

croscopy; there was extensive micronodular cirrhosis with regenerative hepatocellular changes and bile ductular proliferation. However, there were no significant findings in the liver of control rats based on liver microscopy. The data listed in Table 1 could also suggest that kidney function seemed not to be impaired considerably and this could be supported by kidney microscopy; there were no significant findings in the kidneys of both groups of rats. There were no significant findings in the spleen of both groups of rats based on spleen microscopy. Note that body weight gain decreased significantly in rats with liver cirrhosis (from 165 to 248 g) compared with that in control rats (from 160 to 374 g).

3.2. Measurement of V_{max}, K_m, and CL_{int} for the disappearance of oltipraz in hepatic microsomal fraction

The V_{max}, K_m, and CL_{int} values for the disappearance of oltipraz in hepatic microsomal fraction of both groups of rats are listed in Table 2. The V_{max} values for the disappearance of oltipraz in hepatic microsomal fraction were not significantly different between two groups of rats suggesting that the maximal ability to metabolize oltipraz in hepatic microsomal fraction was not significantly different between two groups of rats. However, K_m value for the disappearance of oltipraz in hepatic microsomal fraction in rats with liver cirrhosis was significantly higher (69.5% increase) than that in control rats suggesting that the affinity of oltipraz to the enzyme(s) decreased significantly in rats with liver cirrhosis. Therefore, CL_{int} in rats with liver cirrhosis was significantly slower (26.2 and 85.1% decrease, respectively, based on ml/min per mg protein and ml/min per total liver) than those in control rats suggesting that metabolism of oltipraz could be slower in rats with liver cirrhosis. In rats with liver cirrhosis, total liver weight (65.0% decrease) was significantly lighter and total protein in liver microsome (69.2% decrease) was significantly smaller, hence, CL_{int} based on ml/min per whole liver was sig-

Table 2
V_{max}, K_m, and CL_{int} for the disappearance of oltipraz by microsomes prepared from livers of control rats and rats with liver cirrhosis induced by dimethylnitrosamine[a]

Parameter	Control ($n = 6$)	Cirrhosis ($n = 6$)
V_{max} (nmol/min per mg protein)	78.6 ± 21.0	92.3 ± 38.5
K_m (μM)	197 ± 52	334 ± 103[b]
CL_{int} (ml/min per mg protein)	0.401 ± 0.00542	0.296 ± 0.0413[c]
Whole liver weight (g)	13.6 ± 0.990	4.76 ± 2.19[c]
Total protein in liver microsome (mg)	202 ± 35.8	62.3 ± 37.4[c]
CL_{int} (ml/min per whole liver)	77.2 ± 14.6	11.5 ± 13.9[c]

[a] Each value represents the mean ± S.D.
[b] Cirrhotic value was significantly different ($P < 0.05$) from control ones.
[c] Cirrhotic value was significantly different ($P < 0.001$) from control ones.

nificantly slower than that based on ml/min per mg protein.

3.3. Pharmacokinetics of oltipraz after intravenous administration to rats

The mean arterial plasma concentration–time curves of oltipraz after intravenous administration at a dose of 30 mg/kg to control rats and rats with liver cirrhosis are shown in Fig. 1, and some pharmacokinetic parameters are listed in Table 3. After 1-min intravenous infusion, the mean arterial plasma concentrations of oltipraz were higher in rats with liver cirrhosis. This resulted in a significantly greater AUC (90.6% increase) in rats with liver cirrhosis. In rats with liver cirrhosis, the terminal half-life (99.1% increase) and MRT (91.8% increase) were significantly longer, CL (47.5% decrease) and CL_{NR} (47.8% decrease) were significantly slower, and total amount of unchanged oltipraz excreted in 24-h urine ($Ae_{0–24\,h}$, expressed in terms of percentage of intravenous dose, 68.4% increase) were significantly greater than those in control rats. However, the CL_R and V_{ss} were not significantly different between two groups of rats.

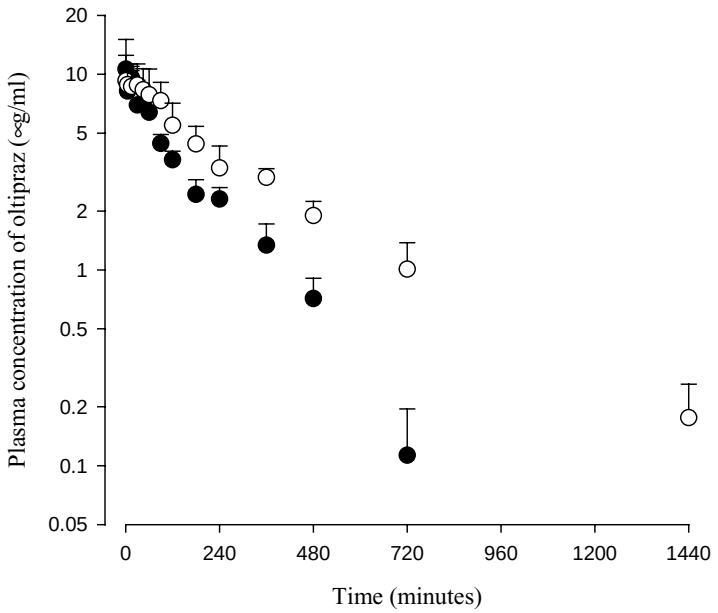

Fig. 1. Mean arterial plasma concentration–time profiles of oltipraz after 1-min intravenous infusion at a dose of 30 mg/kg to control rats (●; $n = 8$) and rats with liver cirrhosis induce by dimethylnitrosamine (○; $n = 8$). Vertical bars represent S.D.

Table 3
Pharmacokinetic parameters of oltipraz after 1-min intravenous infusion at a dose of 30 mg/kg to control rats and rats with liver cirrhosis induced by dimethylnitrosamine[a]

Parameter	Control ($n = 8$)	Cirrhosis ($n = 8$)
Initial body weight (g)[b]	164 ± 7.19	161 ± 11.4
Final body weight (g)[c]	358 ± 17.0	255 ± 50.6[d]
AUC (μg min/ml)	1490 ± 149	2840 ± 354[d]
Terminal half-life (min)	112 ± 31.5	223 ± 86.2[d]
MRT (min)	184 ± 23.8	353 ± 114[d]
CL (ml/(min kg))	20.2 ± 2.17	10.6 ± 1.26[d]
CL_R (ml/(min kg))	0.0148 ± 0.00357	0.0134 ± 0.00313
CL_{NR} (ml/(min kg))	20.1 ± 2.18	10.5 ± 1.26[d]
V_{ss} (ml/kg)	3680 ± 425	3610 ± 1110
$Ae_{0-24\,h}$ (% of dose)	0.0784 ± 0.0228	0.132 ± 0.0330[e]
$GI_{24\,h}$ (% of dose)	BD[f]	BD

[a] Each value represents the mean ± S.D.
[b] Measured just before starting the injection of dimethylnitrosamine (or 0.9% NaCl injectable solution).
[c] Measured just before starting the experiment.
[d] Cirrhotic value was significantly different ($P < 0.001$) from control ones.
[e] Cirrhotic value was significantly different ($P < 0.01$) from control ones.
[f] BD was below the detection limit.

Oltipraz was below detection limit when the entire gastrointestinal tract was collected at 24 h ($GI_{24\,h}$) for both groups of rats. Body weight gain also decreased significantly in rats with liver cirrhosis (from 161 to 255 g) compared with that in control rats (from 164 to 358 g).

3.4. Pharmacokinetics of oltipraz after oral administration to rats

The mean arterial plasma concentration–time curves of oltipraz after oral administration at a dose 30 mg/kg to both groups of rats are shown in Fig. 2, and some pharmacokinetic parameters are listed in Table 4. After oral administration to both groups of rats, oltipraz was absorbed almost completely from rat gastrointestinal tract; the percentage of oral dose of oltipraz recovered from the entire gastrointestinal tract at 24 h as unchanged oltipraz ($GI_{24\,h}$) was 2.70 and 4.83% for control rats and rats with liver cirrhosis, respectively. Oltipraz was stable for up to 48-h incubation (in a water-bath shaker kept at 37 °C and at a rate of 50 opm) in buffer solutions having pHs from 2 to 12 at an oltipraz concentration of 5 μg/ml; after 48-h incubation, 96.8, 98.2, 107, 92.6, 97.1, 99.5, 93.4, 107, 107, 96.9, and 95.9% of spiked amount of oltipraz were recovered from buffer solutions having pHs of 2–12, respectively (Bu et al., 2001). Oltipraz was also stable for up to 4-h incubation in five human gastric juices (obtained from patients before surgery at Seoul National University Hospital, Seoul, South Korea) at an oltipraz concentration of 1 μg/ml; 99.8, 100, 96.1, 93.9, and 99.8% of spiked amount of oltipraz were recovered after 4-h incubation from gastric juices having pHs of 3.7, 3.3, 6.8, 4.0, and 4.4, respectively (Bu et al., 2001). Moreover, oltipraz was stable for up to 8-h standing at room temperature in fresh rat bile juices at an oltipraz concentration of 1 μg/ml; percentage of spiked amount of oltipraz recovered after 8-h standing at room temperature in three rat bile juices was 97.1, 98.4, and 91.2% (mean value of 95.6%), respectively. It took a long time to reach peak plasma concentration of oltipraz (T_{max}); 480 and 648 min for control rats and rats with liver cirrhosis, respectively (they were significantly different). This indicated that oltipraz is continuously absorbed from rat gastrointestinal tract due to its poor water solubility. After reaching C_{max}, the mean arterial plasma concentrations of oltipraz declined in a monoexponential fashion with mean terminal half-lives of 279 and 293 min for control rats and rats with liver cirrhosis, respectively. In rats with liver cirrhosis, plasma concentrations of oltipraz were higher and C_{max} was significantly higher (81.9% increase) than those in control rats. This resulted in a significantly greater AUC (129% increase) in rats with

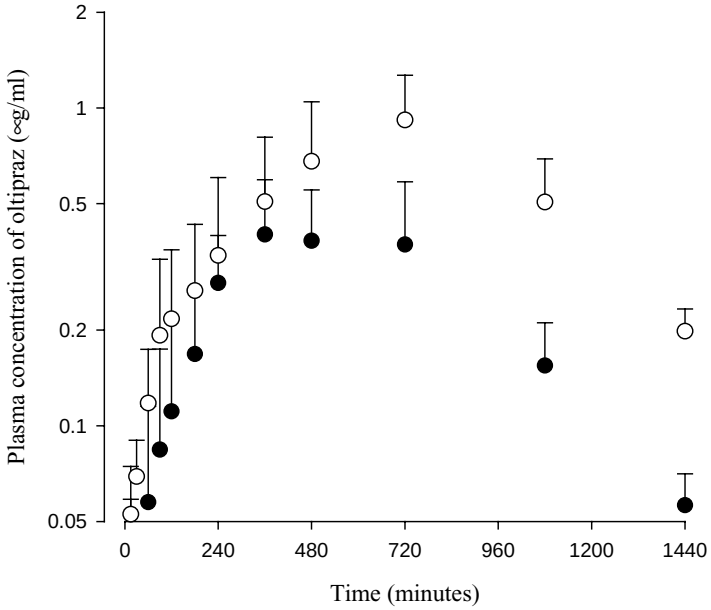

Fig. 2. Mean arterial plasma concentration–time profiles of oltipraz after oral administration at a dose of 30 mg/kg to control rats (●; $n = 8$) and rats with liver cirrhosis induce by dimethylnitrosamine (○; $n = 8$). Vertical bars represent S.D.

Table 4
Pharmacokinetic parameters of oltipraz after oral administration at a dose of 30 mg/kg to control rats and rats with liver cirrhosis induced by dimethylnitrosamine[a]

Parameter	Control ($n = 8$)	Cirrhosis ($n = 8$)
Initial body weight (g)[b]	182 ± 11.7	174 ± 9.87
Final body weight (g)[c]	381 ± 41.5	271 ± 29.6[d]
AUC (μg min/ml)	354 ± 80.5	812 ± 255[d]
Terminal half-life (min)	279 ± 68.3	293 ± 68.3
CL_R (ml/(min kg))	0.00384 ± 0.00900	0.00807 ± 0.0110
$Ae_{0-24\,h}$ (% of dose)	0.0118 ± 0.00608	0.0332 ± 0.0174[e]
$GI_{24\,h}$ (% of dose)	2.70 ± 2.51	4.83 ± 5.94
C_{max} (μg/ml)	0.536 ± 0.183	0.975 ± 0.359[f]
T_{max} (min)	480 ± 170	648 ± 116[e]
F (%)	23.7	28.6

[a] Each value represents the mean ± S.D.
[b] Measured just before starting the injection of dimethylnitrosamine (or 0.9% NaCl injectable solution).
[c] Measured just before starting the experiment.
[d] Cirrhotic value was significantly different ($P < 0.001$) from control ones.
[e] Cirrhotic value was significantly different ($P < 0.05$) from control ones.
[f] Cirrhotic value was significantly different ($P < 0.01$) from control ones.

liver cirrhosis. In rats with liver cirrhosis, $Ae_{0-24\,h}$ was significantly greater (181% increase, expressed in terms of oral dose of oltipraz) than that in control rats. The F value was 23.7 and 28.6% for control rats and rats with liver cirrhosis, respectively. The terminal half-life, CL_R, and $GI_{24\,h}$ were not significantly different between two groups of rats. Again, body weight gain decreased significantly in rats with liver cirrhosis (from 174 to 271 g) compared with that in control rats (from 182 to 381 g).

Table 5
Amount (μg/ml for plasma and μg/g for other tissues) of oltipraz recovered from tissues 8 h after oral administration at a dose of 30 mg/kg to control rats and rats with liver cirrhosis induced by dimethylnitrosamine[a]

Tissue	Control ($n = 5$)	Cirrhosis ($n = 5$)
Plasma	0.237 ± 0.109	0.529 ± 0.0491[b]
Liver	5.28 ± 1.11 (25.9 ± 13.5)	6.22 ± 1.45 (12.3 ± 3.42)
Kidney	1.97 ± 0.854 (9.43 ± 6.39)	3.06 ± 1.46 (5.96 ± 2.80)
Lung	0.621 ± 0.215 (2.82 ± 1.11)	1.58 ± 0.484 (3.17 ± 0.835)
Spleen	0.408 ± 0.151 (2.07 ± 1.44)	1.75 ± 0.608 (3.43 ± 1.09)
Heart	1.26 ± 0.178 (5.85 ± 1.58)	1.54 ± 0.811 (3.02 ± 1.74)[c]
Muscle	0.577 ± 0.144 (2.78 ± 1.75)	1.49 ± 0.329[d] (2.86 ± 0.818)
Mesentery	0.171 ± 0.0620 (0.800 ± 0.407)	3.78 ± 2.36[c] (7.22 ± 4.61)[c]
Large intestine	5.51 ± 2.94 (27.7 ± 23.2)	8.87 ± 7.96 (16.6 ± 14.9)
Fat	7.93 ± 4.86 (37.6 ± 27.3)	39.2 ± 12.5[d] (75.1 ± 25.7)
Brain	0.652 ± 0.366 (2.85 ± 1.88)	3.08 ± 0.646[d] (5.86 ± 1.45)[c]

Values in parentheses represent tissue-to-plasma (T/P) ratios.
[a] Each value represents the mean ± S.D.
[b] Cirrhotic value was significantly different ($P < 0.001$) from control ones.
[c] Cirrhotic value was significantly different ($P < 0.05$) from control ones.
[d] Cirrhotic value was significantly different ($P < 0.01$) from control ones.

3.5. Tissue distribution of oltipraz

The amount of oltipraz recovered from each gram tissue and tissue-to-plasma (T/P) ratios of oltipraz 8 h after oral administration at a dose of 30 mg/kg to control rats and rats with liver cirrhosis are listed in Table 5. Rat tissues studied had good affinity to oltipraz; the T/P ratios of oltipraz were greater-than-unity in all rat tissues studied for both groups of rats except mesentery in control rats. The results are in good agreement with high lipid solubility of oltipraz and considerably large value of V_{ss}, 3610–3680 ml/kg (Table 3). In rats with liver cirrhosis, the T/P ratio of oltipraz was significantly smaller in heart (48.4% decrease) but significantly greater in mesentery (803% increase) and brain (106% increase) than those in control rats. In rats with liver cirrhosis, the amount of oltipraz in the muscle (158% increase), mesentery (2110% increase), fat (394% increase), and brain (372% increase) was significantly greater than those in control rats. Note that the amount and T/P ratios of oltipraz in fat were the greatest for each group of rats; the amount was 7.93 and 39.2 μg/g fat for control rats and rats with liver cirrhosis, respectively, and the corresponding values for T/P ratios were 37.6 and 75.1. This suggested that fat could act as a reservoir for oltipraz in rats, especially in rats with liver cirrhosis since the amount of oltipraz in fat was significantly greater than that in control rats. Similar results could also be obtained from the muscle; although the T/P ratios in muscle were 2.78 and 2.86 for control rats and rats with liver cirrhosis, respectively, the total amount of oltipraz distributed in the entire rat muscle could be considerable considering the large contribution of entire muscle to whole body weight in rats (Davies and Morris, 1993).

4. Discussion

After intravenous administration, 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 0.0784 and 0.132% for control rats and rats with liver cirrhosis, respectively (Table 3). This indicated that the CL values of oltipraz listed in Table 3 could represent CL_{NR} values of oltipraz in rats. The contribution of gastrointestinal (including biliary) excretion of unchanged oltipraz to CL_{NR} of oltipraz was also negligible; oltipraz recovered from the entire gastrointestinal tract at 24 h as unchanged drug ($GI_{24\,h}$) was below detection limit for both groups of rats (Table 3). Moreover, after intravenous administration of oltipraz at a dose of 20 mg/kg to rats with bile duct cannulation, the biliary excretion of unchanged oltipraz were almost negligible; the percentage of intravenous dose of oltipraz excreted in 8-h bile as unchanged drug in

five rats was 0.193, 0.635, 0.576, 0.323, and 0.514% (mean value of 0.449%), respectively. Note that undetectability of oltipraz from the entire gastrointestinal tract at 24 h was not due to chemical and/or enzymatic degradation of oltipraz in the gastrointestinal tract. As mentioned earlier, oltipraz was stable in various pH solutions, rat bile juices, and human gastric juices. Hence, the CL_{NR} values of oltipraz listed in Table 3 could represent metabolic clearances of oltipraz and oltipraz was metabolized almost completely after intravenous administration in rats. It has been reported (Bieder et al., 1983) that 13 metabolites of oltipraz were obtained in rat urine.

After intravenous administration of oltipraz to rats with liver cirrhosis, significantly greater AUC was due to significantly slower CL in the rats. The slower CL could be supported by significantly longer terminal half-life and MRT (Table 3). The slower CL in rats with liver cirrhosis was due to significantly slower CL_{NR} in the rats since the CL_R was not significantly different between two groups of rats (Table 3). The slower CL_{NR} in rats with liver cirrhosis could be due to impaired hepatic function in the rats as mentioned earlier (Table 1). The slower CL_{NR} in rats with liver cirrhosis could be supported by significantly slower in vitro CL_{int} in the rats (Table 2). Hepatic first-pass effect of oltipraz absorbed into the portal vein was approximately 40% in rats. This indicates that oltipraz is an intermediate hepatic extraction ratio drug. Hence, its hepatic clearance is affected by hepatic blood flow rate, free (unbound in plasma proteins) fraction in plasma, and hepatic intrinsic clearance (Wilkinson and Shand, 1975). The significantly slower CL_{NR} of oltipraz in rats with liver cirrhosis could be due to slower hepatic blood flow rate (hepatic blood flow was slower in rats with liver cirrhosis induced by carbon tetrachloride, Goeting et al., 1986), and significantly slower CL_{int} (Table 2) since the free fraction increased significantly in the rats (Table 1).

After intravenous administration, the estimated CL_R values of oltipraz as free (unbound in plasma proteins) fraction were 0.0980 and 0.0428 ml/(min kg) for control rats and rats with liver cirrhosis, respectively, based on the CL_R values (Table 3) and protein binding values (Table 1) of oltipraz. The values (0.0428 and 0.0980 ml/(min kg)) were considerably slower than the reported glomerular filtration rate of 5.24 ml/(min kg) in rats (Davies and Morris, 1993), indicating that oltipraz is reabsorbed considerably in rat renal tubules. Considering the CL_R values of oltipraz based on plasma data (Table 3), reported kidney blood flow rate of 36.8 ml/(min kg) (Davies and Morris, 1993), and hematocrit value (Table 1), the estimated renal extraction ratios of oltipraz (CL_R of oltipraz/kidney plasma flow rate, only for urinary excretion of unchanged drug) were 0.0755 and 0.0575% for control rats and rats with liver cirrhosis, respectively. The above data indicated that oltipraz is a low renal extraction ratio drug, hence the renal extraction ratio is independent of renal blood flow. Therefore, the renal extraction ratios were comparable between both groups of rats although the renal blood flow has been reported (Atucha et al., 1993) to be decreased in rats with liver cirrhosis induced by carbon tetrachloride having ascites.

After oral administration of oltipraz to rats with liver cirrhosis, AUC was also significantly greater than that in control rats (Table 4). This could not be due to enhanced absorption of oltipraz from gastrointestinal tract in the rats. The $GI_{24\,h}$ after intravenous administration of oltipraz was below detection limit for both groups of rats (Table 3) and the values after oral administration were 2.70 and 4.83% of oral dose for control rats and rats with liver cirrhosis, respectively (Table 4). Oltipraz was stable in various pH solutions, rat bile juices, and human gastric juices as mentioned earlier. The above data indicated that more than 95% of oral dose was absorbed after oral administration for both groups of rats. As mentioned in the intravenous study, the significantly greater AUC after oral administration to rats with liver cirrhosis could also be due to slower hepatic blood flow rate (Geoting et al., 1986) and significantly slower CL_{int} (Table 2) in the rats.

In conclusion, after both intravenous and oral administration of oltipraz at a dose of 30 mg/kg to rats with liver cirrhosis, the AUC was significantly greater than that in control rats and this could be due to slower hepatic blood flow rate and significantly slower CL_{int} in the rats.

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