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Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous ipriflavone in rats

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Abstract

In order to find out what types of the hepatic microsomal cytochrome P450 (CYP) isozymes are involved in the metabolism of ipriflavone, ipriflavone at a dose of 20 mg kg^{-1} (or 15 mg kg^{-1}) was infused in male Sprague–Dawley rats. In rats pretreated with SKF 525-A (a non-specific CYP isozyme inhibitor in rats), the total body clearance (CL) of ipriflavone was significantly slower (29.9% decrease) than that in control rats. This indicates that ipriflavone is metabolized via CYP isozymes in rats, hence various enzyme inducers and inhibitors were used in in-vitro or in-vivo studies in rats. In rats pretreated with 3-methylcholanthrene and phenobarbital (main inducers of CYP1A1/2 and 2B1/2 in rats, respectively), the CL values were significantly higher (153 and 67.2% increases, respectively). In rats pretreated with sulfaphenazole (a main inhibitor of CYP2C11 in rats), the CL was significantly slower (22.5% decrease) than that in control rats. On addition of furafylline (a main inhibitor of CYP1A2 in rats), the in-vitro intrinsic clearance for the disappearance of ipriflavone was significantly slower (50.8% decrease) than that without furafylline. However, the CL values were not significantly different in rats pretreated with orphenadrine and isoniazid (a main inducer of CYP2E1 in rats), and quinine and troleandomycin (main inhibitors of CYP2D1 and 3A23/2 in rats, respectively) compared to controls. These data suggest that ipriflavone could be metabolized mainly via CYP1A1/2, 2B1/2 and 2C11 in rats.

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Introduction

Ipriflavone (7-isopropoxy-3-phenyl-4H-1-benzopyran-4-one), a derivative of naturally occurring isoflavones, modulates mitochondrial phosphorylation with growth-promoting properties and is devoid of intrinsic estrogen activity (Brandi 1992). Ipriflavone increases the uterotrophic activities of estrogens and stimulates estrogen-induced calcitonin secretion but lacks the toxic, mutagenic or carcinogenic effects (Rohatagi & Barrett 1997). It is therefore expected to inhibit bone resorption in animal models of experimental osteoporosis and in osteoporotic patients (Kakai et al 1992). Hence, it is used orally in the treatment of osteoporosis (Reginster 1993).

Ipriflavone is extensively metabolized in rats, dogs and humans, and undergoes an extensive first-pass metabolism (Levai & Szatmari 1995). The considerable intestinal first-pass effect of ipriflavone was reported in rats (Kim & Lee 2002); the extent of absolute oral bioavailability (F) was approximately 24% and the unabsorbed fraction from the gastrointestinal tract was approximately 12% of dose. The low F was mainly due to a considerable intestinal first-pass effect in rats (Kim & Lee 2002). Seven metabolites of ipriflavone (M1–M7) were identified in animals and humans (Reginster 1993; Rohatagi et al 1997). Ipriflavone is metabolized mainly in the liver by oxidation of the isopropyl group or hydroxylation of the β-ring followed by phase II glucuronidation or sulfation. Ipriflavone is present in human plasma and urine in very small quantities and the most frequent metabolites are M1, M2 and M5, suggesting that the pharmacological action of ipriflavone is presumed to be represented by the total potencies of unchanged ipriflavone and its metabolites (Shino 1985; Rondelli et al 1991). M1 and M2 circulate in blood as conjugated forms, but M5 and ipriflavone are recovered as unconjugated forms, suggesting that M5 may greatly contribute to the action of ipriflavone (Rondelli et al 1991; Reginster 1993; Rohatagi et al 1997). Although the studies on the metabolism of ipriflavone in humans and animals were

reported as mentioned above, what types of hepatic microsomal cytochrome P450 (CYP) isozymes are involved in the metabolism of ipriflavone in humans and rats seemed not to be published.

The aim of this paper was to report the effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous ipriflavone in rats. Ipriflavone was infused over 15 min via the jugular vein of rats pretreated with enzyme inducers, such as 3-methylcholanthrene, phenobarbital, orphenadrine, isoniazid and dexamethasone (main inducers of CYP1A1/2 (Spatzenegger et al 2000) and 2B1/2 (Kawamura et al 1999), a selective inducer of 2B1/2 (Murray et al 2003), a main inducer of 2E1 (Hammond & Fry 1997) and 3A23/2 (Halpert 1988), respectively, in rats), and enzyme inhibitors, such as SKF 525-A, sulfaphenazole, troleandomycin and quinine (an inhibitor of non-specific CYP isozymes (Correia 1995), and main inhibitors of CYP 2C11 (Ogiso et al 1999), 3A23/2 (Wrighton et al 1985) and 2D1 (Tomkins et al 1997), respectively, in rats) and their respective control rats. In-vitro hepatic microsomal studies were also performed with furafylline (a mechanism-based inhibitor of CYP1A2 in rats (Correia 1995)).

Materials and Methods

Chemicals

Teobone tablets (containing 200 mg as ipriflavone) were from the Kukje Pharmaceutical Company (Seongnam, South Korea). Ipriflavone powder was supplied from the Research Laboratory of the Dong-A Pharmaceutical Company (Yongin, South Korea). Polyethylene glycol 400 (PEG 400) was purchased from the Duksan Chemical Company (Seoul, South Korea). Testosterone (an internal standard of HPLC analysis of ipriflavone), dimethylacetamide (DMA), dimethylsulfoxide (DMSO), sulfaphenazole, SKF 525-A, dexamethasone phosphate, orphenadrine citrate, isoniazid, troleandomycin, quinine hydrochloride, ethylenediamine tetraacetatic acid (EDTA), tri(hydroxymethyl)aminomethane (Tris)-buffer and a reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt) were purchased from the Sigma–Aldrich Corporation (St Louis, MO, USA). 3-Methylcholanthrene and phenobarbital sodium were products from the Wako Pure Chemical Industries (Osaka, Japan) and Dai Han Pharmaceutical Company (Seoul, South Korea), respectively. Other chemicals were of reagent grade or HPLC grade.

Measurement of V_{max}, K_m and CL_{int} for the disappearance of ipriflavone in rat hepatic microsomes with or without the presence of furafylline

The procedures were similar to those reported earlier (Bae et al 2005). The livers of control rats ($n = 4$) 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 centrifuged 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 ipriflavone were determined after incubating the above microsomal fraction (equivalent to 0.5 mg protein), a 5-μL aliquot of ipriflavone (the ipriflavone powder was dissolved in DMSO to give substrate concentrations of 1, 2, 5, 10, 50 and 100 μM), with or without the presence of 20 μM (5 μL) (Pan & Belpaire 1999) of furafylline (dissolved in DMSO), and a 10-μL (1.2 mM) aliquot 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 a rate of 50 oscillations per min (opm). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of 1 mL of acetonitrile after 15 min of incubation. The V_{max} (the maximum velocity) and K_m (Michaelis–Menten constant) for the disappearance of ipriflavone were calculated using the non-linear regression method (Duggleby 1995). The intrinsic clearance (CL_{int}) for the disappearance of ipriflavone was calculated by dividing the V_{max} by the K_m.

Pretreatment with enzyme inducers and inhibitors

Male Sprague–Dawley rats (weighing 190–300 g) purchased from the Charles River Company Korea (Orient, Seoul, South Korea) were housed in a light-controlled room (light: 0700–1900; dark: 1900–0700) and kept at a temperature of 22 ± 2°C and a relative humidity of 55 ± 5% (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under a supply of filtered pathogen-free air and with food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water ad libitum. The protocol of this study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Rats received a single intravenous injection of 80 mg (2 mL) kg^{-1} of sulfaphenazole (dissolved in distilled water with a minimum amount of NaOH to make a pH of approximately 8.0; SPT group; Arlotto et al 1987), a single intraperitoneal injection of 50 mg (3.3 mL) kg^{-1} of SKF525-A (dissolved in 0.9% NaCl-injectable solution; SKT group; Conney 1971), 500 mg (5 mL) kg^{-1} of troleandomycin (dissolved in 0.9% NaCl-injectable solution acidified to pH 4.0 with HCl; TMT group; Sinclair et al 2000) or 20 mg (5 mL) kg^{-1} of quinine hydrochloride (dissolved in 0.9% NaCl-injectable solution; QNT group; Tyndale et al 1999), three daily intraperitoneal injections of 50 mg (5 mL) kg^{-1} of dexamethasone phosphate (dissolved in 0.9% NaCl-injectable solution; DXT group; Arlotto et al 1987), 150 mg (3 mL) kg^{-1} of isoniazid (dissolved in 0.9% NaCl-injectable solution; INT group; Ryan et al 1985) or four daily intraperitoneal injections of 80 mg (3.3 mL) kg^{-1}

of phenobarbital sodium (dissolved in 0.9% NaCl-injectable solution; PBT group; Williams et al 1979; Choi et al 1991), 60 mg (5mL) kg^{-1} of orphenadrine citrate (dissolved in 0.9% NaCl-injectable solution; OPT group; Murray et al 2003), 20 mg (3.3 mL) kg^{-1} of 3-methylcholanthrene (dissolved in corn oil; MCT group; Williams et al 1979; Choi et al 1991), 5 mL kg^{-1} of 0.9% NaCl-injectable solution (TMC, SKC, SPC, QNC, DXC, PBC, OPC or INC group) or 3.3 mL kg^{-1} of corn oil (MCC group). During the pretreatment, rats had free access to food and water.

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 (Kim et al 1993). An experiment was performed just after for the SPC and SPT groups (Ogiso et al 1999; Bae et al 2004), during the first hour for the SKT and SKC groups (Arlotto et al 1987), and QNT and QNC groups (Tomkins et al 1997), 2 h after for the TMT and TMC groups (Conney 1971), on the fourth day for the OPT and OPC groups (Murray et al 2003), DXT and INT groups (Ryan et al 1985; Sinclair et al 2000), and on the fifth day for the PBT, MCT, DXC, PBC, INC and MCC groups (Williams et al 1979; Ryan et al 1985; Choi et al 1991) as the commencement of respective pretreatment.

Ipriflavone (the teobone tablets were ground into fine powder and suspended in DMA:PEG 400, 50:50 v/v) at a dose of 20 mg kg^{-1} (15 mg kg^{-1} for SKC and SKT groups) was administered intravenously (Kim et al 2002) over 15 min via the jugular vein of control groups (n = 6 for MCC group; n = 7 for OPC and SPC groups; n = 8 for SKC, QNC and TMC groups; n = 10 for PBC and INC groups; n = 13 for DXC group) and treated groups (n = 5 for INT group; n = 6 for MCT group; n = 8 for PBT, SPT, QNT and TMT groups; n = 10 for SKT group; n = 11 for OPT group; n = 18 for DXT group). The total infusion volume was approximately 0.6 mL. Blood samples (approximately 0.12 mL) were collected via the carotid artery at 0 (to serve as a control), 7.5, 15 (at the end of the infusion), 16, 20, 30, 45, 75, 105, 195, 315, 435, 555 and 735 min after infusion of ipriflavone. An approximately 0.25-mL aliquot of heparinized 0.9% NaCl-injectable solution (20 U mL^{-1}) was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were immediately centrifuged and a 50-μL aliquot of each plasma sample was stored in a −70°C freezer (Model DF8517; Ilshin Laboratory Company, Seoul, South Korea) until the HPLC analysis of ipriflavone. At the end of 24 h, the metabolic cage was rinsed with 10 mL of distilled water and the rinsed material was combined with a 24-h urine sample. After measuring the exact volume of the combined urine sample, a 50-μL aliquot of the combined urine sample was stored in a −70°C freezer until the HPLC analysis of ipriflavone. At the same time, each rat was sacrificed by cervical dislocation and then the entire gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker containing 100 mL of methanol (to facilitate the extraction of ipriflavone) and cut into small pieces using scissors. After stirring with a glass rod, a 50-μL aliquot of the supernatant was collected from each beaker and stored in a −70°C freezer until the HPLC analysis of ipriflavone.

Measurement of protein binding of ipriflavone to rat plasma

Plasma protein binding of ipriflavone to additional SPC and SPT rats (n = 5 each) was determined at an ipriflavone plasma concentration of 5 μg mL^{-1} using an equilibrium dialysis technique (Kim et al 1999). One millilitre of plasma was dialyzed against 1 mL of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% dextran, with a 1-mL dialysis cell (Fisher Scientific, Fair Lawn, NJ, USA) and Spectra/Por 4 membrane (mol. wt. cut-off of 12 000–14 000; Spectrum Medical Industries, Los Angeles, CA, USA). The spiked dialysis cell was incubated for 24 h in a water-bath shaker kept at 37°C and at a rate of 50 opm (Kim et al 1999). Protein binding of ipriflavone to 4% human serum albumin was constant, 96.6 ± 0.407%, at ipriflavone concentrations ranging from 2 to 100 μg mL^{-1} (Kim et al 1999). Hence, an ipriflavone plasma concentration of 5 μg mL^{-1} was arbitrarily chosen in the present study.

HPLC analysis of ipriflavone

Concentrations of ipriflavone in the above biological samples were analysed by the reported HPLC method (Kim et al 1997). A 100-μL aliquot of acetonitrile containing 1 μg mL^{-1} of testosterone (an internal standard) was added to deproteinize (Chiou et al 1978) a 50-μL aliquot of biological sample. After vortex-mixing and centrifugation, a 0.05-mL aliquot of supernatant was injected directly onto the reversed-phase (C_{18}) HPLC column. The mobile phase, 0.05 M acetate buffer (pH 3):acetonitrile:methanol (40:35:25 v/v/v) was run at a flow rate of 1.5 mL min^{-1}. A UV detector set at 254 nm monitored the column effluent. The retention times of testosterone and ipriflavone were approximately 6 and 12 min, respectively. The detection limit of ipriflavone in rat plasma was 20 ng mL^{-1}, and the corresponding values in rat urine and tissue homogenates were 50–100 ng mL^{-1}. The coefficients of variation of the assay (within- and between-day) were below 9.84%.

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 uses 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, total area under the first moment of the plasma concentration–time curve from time zero to time infinity

(AUMC), mean residence time (MRT) and apparent volume of distribution at steady state (V_{ss}) (Kim et al 1993).

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

A P value of less than 0.05 was considered to be statistically significant using the unpaired t-test. All results were expressed as mean ± s.d.

Results

Measurement of V_{max}, K_m and CL_{int} for the disappearance of ipriflavone in rat hepatic microsomes with or without the presence of furafylline

The mean V_{max}, K_m and CL_{int} values for the disappearance of ipriflavone in rat hepatic microsomes without (control) or with the presence of furafylline (FF), a mechanism-based inhibitor of CYP1A2 in rats (Spatzenegger et al 2000), are listed in Table 1. The V_{max} and K_m values were not significantly different with or without the presence of furafylline. This suggests that the maximum velocity for the disappearance of ipriflavone and the affinity of ipriflavone to the enzyme(s) were not affected by furafylline. However, in the presence of furafylline, the intrinsic clearance (CL_{int}) for the disappearance of ipriflavone was significantly slower (50.8% decrease) than that without furafylline, suggesting that the metabolism of ipriflavone was significantly slower with furafylline. The above data suggest that ipriflavone is metabolized via CYP1A2 in rats.

Pharmacokinetics of ipriflavone in rats after pretreatment with enzyme inducers

The mean arterial plasma concentration–time profiles of ipriflavone after 15-min intravenous infusion at a dose of 20 mg kg^{-1} in rats pretreated with 3-methylcholanthrene (MCT), phenobarbital (PBT), isoniazid (INT) and dexamethasone (DXT), and their respective control (MCC, PBC, INC and DXC) rats are shown in Figure 1, and some relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration, the plasma concentrations of ipriflavone declined in a polyexponential fashion for all groups of rats (Figure 1).

In the MCT group, the plasma concentrations of ipriflavone were lower than those in control rats (MCC) (Figure 1A). This resulted in a significantly smaller AUC of ipriflavone (59.6% decrease) than that in control rats, which could be due to significantly faster CL (153% increase) in rats with MCT (Table 2). In the PBT group, the plasma concentrations of ipriflavone were also lower than those in control rats (PBC) (Figure 1B). This resulted in a significantly smaller AUC (40.2% decrease) than that in control rats, and this could be due to significantly faster CL (67.2% increase) in rats with PBT (Table 2). However, in the OPT group the plasma concentrations and pharmacokinetic parameters of ipriflavone were comparable to their control rats (OPC) (data not shown). In the INT group, the plasma concentrations of ipriflavone shown in Figure 1C and the pharmacokinetic parameters of ipriflavone listed in Table 2 were not significantly different compared with those in control rats (INC). Note that body-weight gain decreased significantly in rats with INT (from 267 to 263 g) compared with that in control rats (from 260 to 281 g) (Table 2). In the DXT group, the plasma concentrations of ipriflavone were higher than those in control rats (DXC) (Figure 1D). This resulted in a significantly greater AUC (59.8% increase) than that in control rats, and this could be due to significantly slower CL (37.6% decrease) in rats with DXT (Table 2).

Table 1 V_{max}, K_m and CL_{int} values for the disappearance of ipriflavone after incubation of ipriflavone with hepatic microsomal fractions of rats without (control) or with furafylline (FF)

Parameter	Control	FF
V_{max} (nmol min^{-1} mg protein^{-1})	0.256 ± 0.0552	0.249 ± 0.0733
K_m (μM)	3.70 ± 2.48	6.55 ± 3.68
CL_{int} (mL min^{-1} mg protein^{-1})	0.0862 ± 0.0253	0.0424 ± 0.00880*

Values are mean ± s.d., n = 4; *P < 0.05 compared with control.

Pharmacokinetics of ipriflavone in rats after pretreatment with enzyme inhibitors

The mean arterial plasma concentration–time profiles of ipriflavone after 15-min intravenous infusion at a dose of 20 mg kg^{-1} (15 mg kg^{-1} for SKC and SKT groups) in rats pretreated with SKF 525-A (SKT), sulfaphenazole (SPT), quinine (QNT) and troleandomycin (TMT), and their respective control (SKC, SPC, QNC and TMC) rats are shown in Figure 2, and some relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration, the plasma concentrations of ipriflavone declined in a polyexponential fashion for all groups of rats (Figure 2).

In the SKT group, the plasma concentrations of ipriflavone were higher than those in control rats (SKC) (Figure 2A). This resulted in a significantly greater AUC of ipriflavone (51.6% increase) than that in control rats, which could be due to significantly slower CL (29.9% decrease) in rats with SKT (Table 3). In the SPT group, the plasma concentrations of ipriflavone were higher than those in control rats (SPC) (Figure 2B). This resulted in a significantly greater AUC (29.1% increase) than that in control rats, which could be due to significantly slower CL (22.5% decrease) in rats SPT (Table 3). In rats with SPT, the V_{ss} was significantly smaller (40.7% decrease) than that in control rats (SPC) (Table 3). Although the exact reason is not clear, this could not be due to an increase in free (unbound to plasma proteins) fractions of ipriflavone in plasma in rats with SPT. The protein binding values of ipriflavone were 98.7 ± 0.450% and 98.1 ± 0.461% for control rats (SPC) and rats with SPT, respectively; they

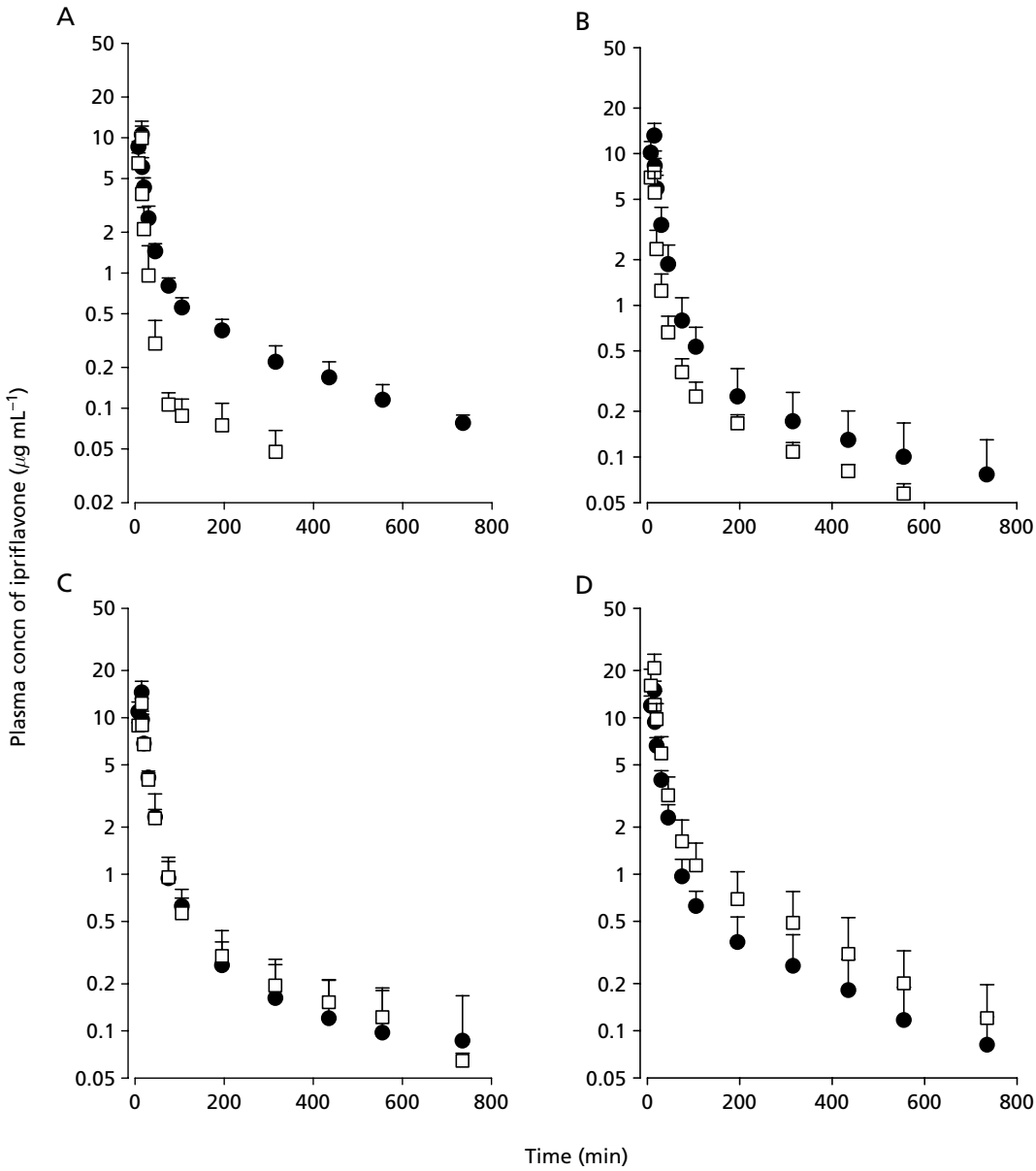

Figure 1 Mean arterial plasma concentration–time profiles of ipriflavone after 15 min of intravenous infusion at a dose of 20 mg kg^{-1} in rats pretreated with enzyme inducers (□), 3-methaylcholanthrene (A), phenobarbital (B), isoniazid (C) and dexamethasone (D), and their respective control rats (●). Vertical bars represent s.d.

were not significantly different. The plasma concentrations of ipriflavone (Figure 2C) and the pharmacokinetic parameters of ipriflavone listed in Table 3 were not significantly different between the QNC and QNT groups. Similar results were also obtained for the TMC and TMT groups (Figure 2D and Table 3).

Discussion

It has been reported (Kim & Lee 2002) that the AUC values of ipriflavone are dose-proportional after intravenous administration at doses of 5–40 mg kg^{-1} in rats. Hence, an intravenous dose of 20 mg kg^{-1} was arbitrarily chosen in the present study.

After intravenous administration, the contribution of CL_R (time-averaged renal clearance) to CL of ipriflavone was almost negligible; the percentages of intravenous dose of ipriflavone excreted in 24-h urine as unchanged drug were smaller than 0.0976% for all rats studied (data not shown). The contribution of gastrointestinal (including biliary) excretion of unchanged ipriflavone to non-renal clearance of ipriflavone was also negligible; ipriflavone was below the detection limit in the entire gastrointestinal tract at 24 h as unchanged drug for all rats studied; this was not due to chemical and/or enzymatic degradation of

Table 2 Pharmacokinetic parameters of ipriflavone after 15 min of intravenous infusion at a dose of 20 mg kg^{-1} in rats pretreated with enzyme inducers, 3-methylcholanthrene (MCT), phenobarbital (PBT), isoniazid (INT), and dexamethasone (DXT), and their respective control rats (MCC, PBC, INC, DXC)

Parameter	MCC (n=6)	MCT (n=6)	PBC, INC (n=10)	PBT (n=8)
Initial body weight (g)	258 ± 6.67	263 ± 6.99	260 ± 16.2	236 ± 9.16*
Final body weight (g)	286 ± 24.6	278 ± 13.2	281 ± 6.45	266 ± 6.94*
AUC (μg min mL^{-1})	413 ± 25.5	167 ± 30.3***	487 ± 86.4	291 ± 44.7***
Terminal half-life (min)	301 ± 129	211 ± 92.5	270 ± 119	251 ± 119
MRT (min)	208 ± 58.3	71.9 ± 39.9***	167 ± 44.4	184 ± 110
CL (mL min^{-1} kg^{-1})	47.9 ± 3.37	121 ± 25.0***	41.1 ± 7.14	68.7 ± 11.2***
V_{ss} (mL kg^{-1})	10300 ± 3520	9870 ± 4910	5930 ± 2310	10500 ± 11900

Parameter	INT (n=5)	DXC (n=13)	DXT (n=18)
Initial body weight (g)	267 ± 8.16	250 ± 24.9	260 ± 20.9
Final body weight (g)	263 ± 9.08**	257 ± 33.5	246 ± 8.19
AUC (μg min mL^{-1})	488 ± 64.1	530 ± 93.5	847 ± 263***
Terminal half-life (min)	309 ± 130	231 ± 126	215 ± 177
MRT (min)	163 ± 56.3	162 ± 77.7	196 ± 108
CL (mL min^{-1} kg^{-1})	41.0 ± 5.41	37.8 ± 7.29	23.6 ± 9.27***
V_{ss} (mL kg^{-1})	7540 ± 3350	8750 ± 74700	6390 ± 145000

Values are mean ± s.d., *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with control.

ipriflavone in gastrointestinal tract. For example, ipriflavone was stable for up to 24-h of incubation in various buffer solutions having pHs ranging from 1 to 13 except at pH 8 and an ipriflavone concentration of 10 μg mL^{-1} (Kim et al 1999). Ipriflavone was also stable for up to 3 h of incubation in five human gastric juices having pHs of 1.54, 1.84, 3.81, 2.03 and 1.16, at an ipriflavone concentration of 5 μg mL^{-1} (Kim et al 1999). The above data suggest that ipriflavone is almost completely metabolized after intravenous administration in rats, and the CL values could represent the metabolic clearance values in rats. Hence, the changes in CL values of ipriflavone by pretreatment with enzyme inducers (Table 2) or inhibitors (Table 3) could indicate changes in the metabolism of ipriflavone in rats.

In order to find out whether or not CYP isozymes are involved in the metabolism of ipriflavone in rats, SKF 525-A (a non-specific inhibitor of CYP isozymes in rats; Correia 1995) was pretreated in rats. In rats pretreated with SKF 525-A (SKT), the CL of ipriflavone was significantly slower than that in control rats (Table 3), suggesting that ipriflavone is metabolized via CYP isozymes in rats. Hence, various CYP enzyme inducers and inhibitors were pretreated to find out what types of CYP isozymes are involved in the metabolism of ipriflavone in rats. In rats pretreated with MCT, a main inducer of CYP1A1/2 (Spatzenegger et al 2000) in rats, the CL of ipriflavone was significantly faster than that in control rats (Table 2). Moreover, the in-vitro CL_{int} for the disappearance of ipriflavone was significantly slower on addition of furafylline, an inhibitor of CYP1A2 in rats (Correia 1995), than that without addition of furafylline (Table 1). The above data suggest that CYP1A1/2 could contribute to the metabolism of ipriflavone in rats. In rats pretreated with PBT, a main inducer of CYP2B1/2 in rats (Kawamura et al 1999), the CL of ipriflavone was significantly faster than that in control rats (Table 2). However, in rats pretreated with orphenadrine (OPT), a selective inducer of CYP2B1/2 in rats (Murray et al 2003), the CL of ipriflavone was comparable to controls (data not shown). The above data suggested that contribution of CYP2B1/2 to the metabolism of ipriflavone is questionable in rats. In rats pretreated with sulfaphenazole (SPT), a main inhibitor of CYP2C11 in rats (Ogiso et al 1999), the CL of ipriflavone was significantly slower than that in control rats (Table 3). This suggests that CYP2C11 could contribute to the metabolism of ipriflavone in rats. In rats pretreated with quinine (QNT), an inhibitor of CYP2D1 in rats (Tomkins et al 1997), the CL values of ipriflavone were comparable between the QNC and QNT groups (Table 3). This suggests that the contribution of CYP2D1 to the metabolism of ipriflavone is not considerable. In rats pretreated with isoniazid (INT), an inducer of CYP2E1 in rats (Correia 1995), the CL values were not significantly different between the INC and INT groups (Table 2). This suggests that CYP2E1 may not contribute considerably to the metabolism of ipriflavone in rats. Unexpectedly, in rats pretreated with dexamethasone, an inducer of CYP3A23/2 in rats (Halpert 1988), the CL was significantly slower than that in control rats (Table 2). Although the exact reason is not clear, this may be due to a decrease in CYP2C11 by dexamethasone; it has been reported (Levin et al 1987) that dexamethasone decreased CYP2C11 protein or activity levels in adult male rats, and ipriflavone was metabolized via CYP2C11 in rats as mentioned earlier. In rats pretreated with troleandomycin (TMT), an inhibitor of CYP3A23/2 (Wrighton et al 1985), the CL of ipriflavone was not significantly different compared with that in control rats (Table 3). This suggests

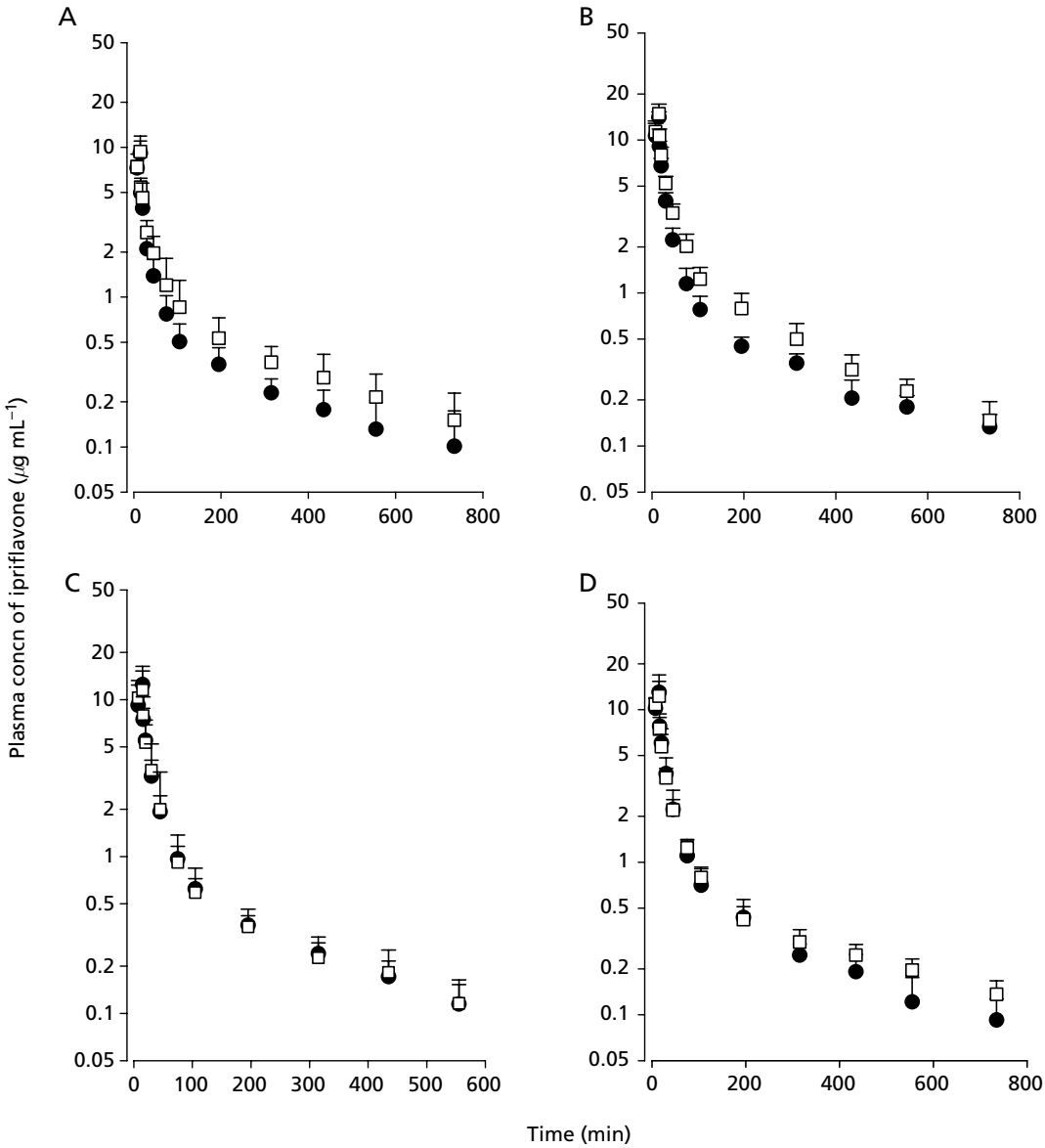

Figure 2 Mean arterial plasma concentration–time profiles of ipriflavone after 15 min of intravenous infusion at a dose of 20 mg kg^{-1} (15 mg kg^{-1} for SKC and SKT groups) in rats pretreated with enzyme inhibitors (□), SKF525-A (A), sulfaphenazole (B), quinine (C), and troleandomycin (D), and their respective control rats (●). Vertical bars represent s.d.

that the contribution of CYP3A1/2 to the metabolism of ipriflavone is not considerable in rats. Note that the various enzyme inducers and inhibitors studied had activities to various CYP isozymes; however, only the main CYP isozyme was mentioned in this study. Hence, the results are confined to the main CYP isozymes. More studies are required to further evaluate what other types of CYP isozymes are more involved in the metabolism of ipriflavone in rats.

The present results could help to explain the possible pharmacokinetic changes of ipriflavone in rats with protein–calorie malnutrition (PCM; the expressions and mRNA levels of CYP1A2 and 2C11 decreased in rats with PCM; Cho et al 1999), acute renal failure induced by uranyl nitrate (U-ARF; the expression and mRNA level of CYP2C11 decreased while those of CYP1A2 were not changed in rats with U-ARF; Moon et al 2003), analbuminaemia (mutant Nagase analbuminaemic rats; the expression and mRNA level of CYP1A2 increased in analbuminaemic rats; Kim et al 2003) and diabetes mellitus induced by alloxan or streptozotocin (the expression and mRNA level of CYP1A2 increased whereas those 2C11 decreased in the diabetic rats; Kim et al 2005) where the CYP isozymes are changed.

Conclusion

Ipriflavone is metabolized mainly via CYP1A1/2 and 2C11 in male rats. If the present rat data could be

Table 3 Pharmacokinetic parameters of ipriflavone after 15 min of intravenous infusion at a dose of 20 mg kg^{-1} (15 mg kg^{-1} for SKC and SKT groups) to rats pretreated with enzyme inhibitors, SKF-525-A (SKT), sulfaphenazole (SPT), quinine (QNT), and troleandomycin (TMT), and their respective control rats (SKC, SPC, QNC, TMC)

Parameter	SKC (n=8)	SKT (n=10)	SPC (n=7)	SPT (n=8)
Body weight (g)	306 ± 15.1	319 ± 23.8	296 ± 6.27	291 ± 3.54
AUC (μg min mL^{-1})	368 ± 55.3	558 ± 142*	615 ± 54.7	794 ± 96.7**
Terminal half-life (min)	239 ± 98.5	284 ± 141	382 ± 157	257 ± 153
MRT (min)	193 ± 44.6	305 ± 163	291 ± 90.7	234 ± 118
CL (mL min^{-1} kg^{-1})	40.8 ± 6.80	28.6 ± 7.49*	32.5 ± 2.90	25.2 ± 3.37**
V_{ss} (mL kg^{-1})	7220 ± 1470	7080 ± 3150	8790 ± 3320	5210 ± 2660***
Parameter	**QNC (n=8)**	**QNT (n=8)**	**TMC (n=8)**	**TMT (n=8)**
Body weight (g)	283 ± 8.43	293 ± 5.94	289 ± 14.6	283 ± 9.26
AUC (μg min mL^{-1})	463 ± 88.4	457 ± 160	518 ± 87.4	606 ± 95.9
Terminal half-life (min)	224 ± 90.1	211 ± 42.2	218 ± 97.0	360 ± 217
MRT (min)	177 ± 64.6	150 ± 18.9	174 ± 81.0	326 ± 161
CL (mL min^{-1} kg^{-1})	43.2 ± 8.82	43.8 ± 15.3	38.6 ± 7.72	33.8 ± 5.54
V_{ss} (mL kg^{-1})	6920 ± 2860	6580 ± 2250	5880 ± 3490	9390 ± 4050

Values are mean ± s.d., *$P < 0.001$, **$P < 0.05$ and ***$P < 0.01$ compared with control.

extrapolated to humans, modification of the dosage regimen of ipriflavone may be required in patients who are concurrently taking drugs (Parkinson 1996) or foods (Fontana et al 1999; Nishikawa et al 2004) that can induce or inhibit CYP1A1 and/or 2C9. Human CYP1A1, 1A2 and 2C9, and rat CYP1A1, 1A2 and 2C11 have 78, 70 and 77% homology, respectively (Lewis 1996).

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