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Effects of CYP inducers and inhibitors on the pharmacokinetics of intravenous theophylline in rats: involvement of CYP1A1/2 in the formation of 1,3-DMU

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Abstract

The types of hepatic cytochrome P450 (CYP) isozymes responsible for the metabolism of theophylline and for the formation of 1,3-dimethyluric acid (1,3-DMU) in rats in-vivo does not seem to have been studied at the dose ranges of dose-independent metabolic disposition of theophylline in rats (up to 10 mg kg^{-1}). Therefore, theophylline (5 mg kg^{-1}) was administered i.v. to male Sprague–Dawley rats pretreated with various inducers and inhibitors of CYP isozymes. In rats pretreated with 3-methylcholanthrene (3-MC), orphenadrine or dexamethasone (main inducers of CYP1A1/2, CYP2B1/2 and CYP3A1/2, respectively, in rats), the time-averaged non-renal clearance (CL_{NR}) of theophylline was significantly faster than in their respective controls (1260, 42.7 and 69.0% increases, respectively). However, in rats pretreated with troleandomycin (a major inhibitor of CYP3A1/2 in rats), CL_{NR} was significantly slower than in the controls (50.7% decrease). The 24 h urinary excretion of 1,3-DMU was increased significantly only in rats pretreated with 3-MC. The ratio of area under the curve for 1,3-DMU and theophylline ($AUC_{1,3\text{-}DMU}/AUC_{theophylline}$) was increased significantly in rats pretreated with 3-MC (160% increase) and decreased significantly in rats pretreated with troleandomycin (50.1% decrease); however, the ratio was not increased in rats pretreated with dexamethasone. These data suggest that theophylline is primarily metabolized via CYP1A1/2, CYP2B1/2, and CYP3A1/2, and that 1,3-DMU is primarily formed via CYP1A1/2, and possibly CYP3A1/2, in rats.

Introduction

McManus et al (1988) reported that theophylline (1,3-dimethylxanthine), a potent bronchodilator, is metabolized to 1-methylxanthine (1-MX), 3-methylxanthine (3-MX) and 1,3-dimethyluric acid (1,3-DMU); 1-MX is further metabolized to 1-methyluric acid (1-MU) via xanthine oxidase in rats. There have been a few reports on the metabolism of theophylline and on the formation of 1,3-DMU in-vivo in male Sprague–Dawley rats. For example, the following results have been reported after i.v. administration of theophylline at a dose of 38 mg kg^{-1} to rats pretreated with 3-methylcholanthrene (3-MC) and phenobarbital – inducers of hepatic microsomal cytochrome P450 (CYP) 1A1/2 and CYP2B1/2, respectively (Correia 1995) – (Williams et al 1979). The total area under the plasma concentration–time curve from time zero to infinity (AUC) of theophylline was estimated to be lower, and time-averaged non-renal clearance (CL_{NR}) faster. The 24 h urinary excretion of theophylline was significantly lower, whilst that of 1,3-DMU was significantly higher than in the controls. The following results have been reported after i.v. administration of theophylline at a dose of 15 mg kg^{-1} to rats pretreated with various inducers of hepatic CYP isozymes, such as β-naphthoflavone (CYP1A1), phenobarbital (CYP2B1/2), isoniazid (CYP2E1), clotrimazole (CYP3A1) and clofibrate (CYP4A1) in rats (Bachmann et al 1993; Correia 1995), and various inhibitors of hepatic CYP isozymes, such as α-naphthoflavone (CYP1A1/2), orphenadrine (CYP2B/C), diallyl sulfide (CYP2E1), ajmaline (CYP2D6), troleandomycin (CYP3A1/2) and 10-undecynoic acid (CYP4A1) (Bachmann et al 1993; Correia 1995). The time-averaged total body clearance (CL) of theophylline was significantly faster in rats pretreated with β-naphthoflavone or phenobarbital, and significantly slower in rats pretreated with troleandomycin (Bachmann et al 1993). They concluded that: CYP1A is principally responsible for the overall oxidation of theophylline; CYP2B/C probably also mediates some theophylline oxidation;

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the involvement of CYP2D, 2E, 3A and 4A subfamilies is relatively trivial. Metabolism of theophylline via CYP1A2 (Ueng et al 2005) and CYP1A2 and CYP3A2 (Nosaka et al 2006) at i.v. doses of 3 and 10 mg kg^{-1}, respectively, has also been reported in male Sprague–Dawley rats.

Many investigators have reported dose-dependent metabolic disposition of theophylline in humans (Lesko 1979; Birket et al 1984; Massey et al 1984) and in rats (Teunissen et al 1985). Metabolic disposition of theophylline in the rat has been reported to be independent of dose up to 10 mg kg^{-1} (Teunissen et al 1985). Although metabolism of theophylline and/or formation of 1,3-DMU in rats in-vivo has been reported (Williams et al 1979; Bachmann et al 1993), the i.v. doses of theophylline used in these studies (38 mg kg^{-1} and 15 mg kg^{-1} respectively), were in the range of dose-dependent metabolism. To our knowledge, there have been no studies on the metabolic disposition of theophylline and 1,3-DMU in the dose range of dose-independent metabolism in rats (up to 10 mg kg^{-1}). Thus, the present study in rats was performed at an i.v. theophylline dose of 5 mg kg^{-1}.

Among several metabolites of theophylline, 1,3-DMU was chosen in this study because it is the major metabolite of theophylline. McManus et al (1988) reported that formation of 1, 3-DMU was the major metabolite formed in liver microsomes from male hooded Wistar rats, and 1-MX and 3-MX were minor pathways, accounting for approximately 2.30 and 2.90%, respectively, of the total metabolites. In our preliminary study levels of 1-MX, 3-MX and 1-MU were below the detection limit. The purpose of this paper is to report involvement of hepatic CYP1A1/2, CYP2B1/2 and CYP3A1/2 in the disappearance (primarily metabolism) of theophylline, and of hepatic CYP1A1/2 and possibly CYP3A1/2 in the formation of 1,3-DMU after i.v. administration of theophylline at a dose of 5 mg kg^{-1} to male Sprague–Dawley rats pretreated with various inducers and inhibitors of hepatic CYP isozymes.

Materials and Methods

Chemicals

Intravenous aminophylline solution (10 mL ampoule; 25 mg mL^{-1} as aminophyllline) was a product from Daewon Pharmaceutical Company (Seoul, South Korea). 1,3-DMU, β-hydroxyethyltheophylline (internal standard for the HPLC analysis of theophylline), 3-MC, orphenadrine citrate (a main inducer of CYP2B1/2 in rats (Murray et al 2003)), isoniazid, dexamethasone phosphate (a main inducer of CYP3A1/2 in rats (Correia 1995)), SKF-525A (a non-specific inhibitor of CYP isozymes (Correia 1995)), sulfaphenazole (a main inhibitor of CYP2C11 in rats (Ogiso et al 1999)), quinine hydrochloride (a main inhibitor of CYP2D1 in rats (Tomkins et al 1997)), and troleandomycin were purchased from Sigma-Aldrich (St Louis, MO, USA). Other chemicals were of reagent or HPLC grade.

Animals

The protocol for this animal study was approved by the Animal Care and Use Committee of the College of Pharmacy of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats, 6–8 weeks old and weighing 255–305 g, were purchased from the Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea). They were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 22 ± 2°C and a 12-h light (0700–1900) / dark (1900–0700) cycle and a relative humidity of 55 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered pathogen-free air, and with food (Samyang Company, Pyuingtaek, South Korea) and water available ad libitum.

Pretreatment of rats with inducers and inhibitors of CYP isozymes

Rats in each group received one of the following treatments (which were dissolved in 0.9% injectable NaCl solution unless stated otherwise): sulfaphenazole 80 mg (2 mL) kg^{-1} i.v. (dissolved in distilled water with a minimum amount of 10 N NaOH to make a pH of approximately 8.0 (Ogiso et al 1999)); SKF-525A 50 mg (3.3 mL) kg^{-1} i.p. (Conney 1971); troleandomycin 500 mg (5 mL) kg^{-1} i.p. (dissolved in 0.9% NaCl acidified to pH 4.0 with HCl (Sinclair et al 2000)); quinine hydrochloride 20 mg (5 mL) kg^{-1} i.p. (Tomkins et al 1997); dexamethasone phosphate 50 mg (5 mL) kg^{-1} i.p. daily for 3 days (Arlotto et al 1987; Ross et al 1993); isoniazid 150 mg (3 mL) kg^{-1} i.p. (Ryan et al 1985); orphenadrine citrate 60 mg (5 mL) kg^{-1} i.p. (Murray et al 2003); 3-MC 20 mg (3.3 mL) kg^{-1} i.p. daily for 4 days (dissolved in corn oil (Williams et al 1979; Choi et al 1991)). Control rats received an i.p. or i.v. injection of 5 mL kg^{-1} 0.9% NaCl, except for the 3-MC controls, which received 3.3 mL kg^{-1} corn oil. During the pretreatment, the rats had free access to food and water.

Intravenous study

In the early morning, the jugular vein (for drug administration) and the carotid artery (for blood sampling) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ, USA) was under light ether anaesthesia (Kim et al 1993). Both cannulae were exteriorized to the dorsal side of the neck, where each was terminated with a long silastic tube (Dow Corning, Midland, MI, USA). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Then, each rat was housed individually in a metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from anaesthesia for 4–5 h before beginning the experiment. The rats were not restrained during the study.

Experiments were performed just after injection for the sulfaphenazole control and treatment groups (Ogiso et al 1999; Bae et al 2005), during the first hour for the SKF-525A (Conney 1971) and quinine groups (Tomkins et al 1997), after 2 h for the troleandomycin groups (Wrighton et al 1985; Arlotto et al 1987; Sinclair et al 2000), on the fourth day for the dexamethasone, isoniazid and orphenadine groups (Ryan et al 1985; Arlotto et al 1987; Ross et al 1993; Sinclair et al 2000; Murray et al 2003), and on the fifth day for the 3-MC groups (Williams et al 1979; Choi et al 1991).

Theophylline (aminophylline injectable solution diluted in injectable 0.9% NaCl) at a dose of 5 mg kg^{-1} was administered

i.v. over 1 min via the jugular vein of control groups (n = 7 for all groups except those for 3-MC and sulfaphenzole (n = 8) and SKF-525A (n = 9)), and pretreatment groups (n = 6 for 3-MC group; n = 7 for dexamethasone, quinine and sulfaphenzole groups; n = 8 for isoniazid, orphenadrine and troleandomycin groups; n = 9 for SKF-525A group). The total injection volume was 2 mL kg^{-1}. A blood sample (approximate 0.12 mL) was taken via the carotid artery at 0 (control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after the start of the theophylline infusion. After taking each sample, the cannula was flushed with 0.25 mL heparinized injectable 0.9% NaCl (20 U mL^{-1}) to prevent blood clotting. Blood samples were immediately centrifuged (16000 *g*, 5 min) and a 50 μL aliquot of each plasma sample was stored at −70°C for later HPLC analysis of theophylline and 1,3-DMU (Kwaskatsu et al 1989). At the end of 24 h, each metabolic cage was rinsed with 20 mL distilled water, and the rinsed material was combined with the 24 h urine sample. The volume of the combined urine sample was measured, then two 100 μL aliquots were taken and stored at −70°C for later HPLC analysis of theophylline (Kwaskatsu et al 1989). At the same time (24 h), each rat was exsanguinated and asphyxiated by cervical dislocation.

Measurement of rat plasma protein binding of theophylline using equilibrium dialysis

Protein binding of theophylline to fresh rat plasma from the orphenadrine- and sulfaphenazole-pretreated and control rats was determined using equilibrium dialysis (Shim et al 2000) at theophylline concentrations of 2 and 10 μg mL^{-1}. Plasma (1 mL) was dialysed against 1 mL buffer (isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran) in a 1 mL dialysis cell (Spectrum Medical Industries, Los Angeles, CA, USA) using a Spectra/Por 4 membrane (MW cut-off 12000–14000 Dalton; Spectrum Medical Industries). After 24 h incubation at 37°C, two 50 μL aliquots were collected from the buffer and plasma compartments and stored at −70°C for later HPLC analysis of theophylline (Kwaskatsu et al 1989).

HPLC analysis of theophylline and 1,3-DMU

Concentrations of theophylline and 1,3-DMU were determined using a previously reported HPLC method (Kwaskatsu et al 1989). Briefly, a 0.3 mL aliquot of acetonitrile containing 2 μg mL^{-1} β-hydroxyethyltheophylline (internal standard) was added to a 50 μL aliquot of sample. After vortex mixing and centrifugation, the supernatant was evaporated (Dry Thermo Bath MG-2001, Eyela, Tokyo, Japan) under a gentle stream of nitrogen gas at 60°C. The residue was reconstituted in 0.1 mL mobile phase and a 0.05 mL aliquot of the supernatant was injected directly onto a reverse-phase (C_{18}) HPLC column. The mobile phases were 10 mM acetate buffer (pH 5.0):acetonitrile:tetrahydrofuran at a ratio of 94:5:1 (v/v/v) for the rat plasma samples and 97:2:1 (v/v/v) for the rat urine samples, delivered at a flow rate of 1.0 mL min^{-1}. The column eluent was monitored at 280 nm at room temperature.

The retention times of theophylline, 1,3-DMU and β-hydroxyethyltheophylline (internal standard) were approximately 11.1, 6.3 and 13.2 min, respectively, in rat plasma samples and approximately 18.7, 9.9 and 23.5 min, respectively, in rat urine samples. The quantitation limits in rat plasma and urine samples were 0.1 and 0.5 μg mL^{-1}, respectively, for theophylline and 0.05 and 0.5 μg mL^{-1}, respectively for 1,3-DMU. The inter- and intra-day coefficients of variation of theophylline were below 6.81% for rat plasma in the concentration range 0.1–100 μg mL^{-1}, and 5.61% for urine samples in the concentration range 0.5–20 μg mL^{-1}. Corresponding values of 1,3-DMU were below 8.89% for rat plasma in the concentration ranges of 0.05–100 μg mL^{-1} and below 8.76% in the concentration range 0.5–20 μg mL^{-1} for rat urine samples.

Pharmacokinetic analysis

The AUC was calculated using the trapezoidal rule with extrapolation to infinity (Chiou 1978). The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant. Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters using a non-compartment model (WinNonlin; professional edition version 2.1, Pharsight, Mountain View, CA, USA): the CL, the CL_{NR}, the time-averaged renal clearance (CL_R), the terminal half-life, the first moment of AUC, the mean residence time (MRT) and the apparent volume of distribution at steady state (V_{ss}) (Kim et al 1993).

Statistical analysis

A *P* value below 0.05 was deemed to be statistically significant using an unpaired *t*-test. All the results are expressed as mean ± s.d.

Results

Pharmacokinetics of theophylline and 1,3-DMU after intravenous administration of theophylline to rats pretreated with CYP inducers

The mean arterial plasma concentration–time profiles of theophylline and 1,3-DMU following 1 min i.v. infusion of theophylline, 5 mg kg^{-1}, to control rats and rats pretreated with 3-MC, orphenadrine, isoniazid and dexamethasone are shown in Figure 1, and relevant pharmacokinetic parameters are listed in Table 1. After i.v. infusion of theophylline, the plasma concentrations of theophylline declined in a polyexponential fashion in all groups of rat.

In 3-MC treated rats, changes in pharmacokinetic parameters of theophylline compared with their controls were as follows: the AUC was significantly lower (90.9% decrease), terminal half-life and MRT were significantly shorter (90.8 and 93.6% decrease, respectively), CL and CL_{NR} were significantly faster (926 and 1260% increase, respectively), and percentage of the i.v. dose of theophylline excreted in the

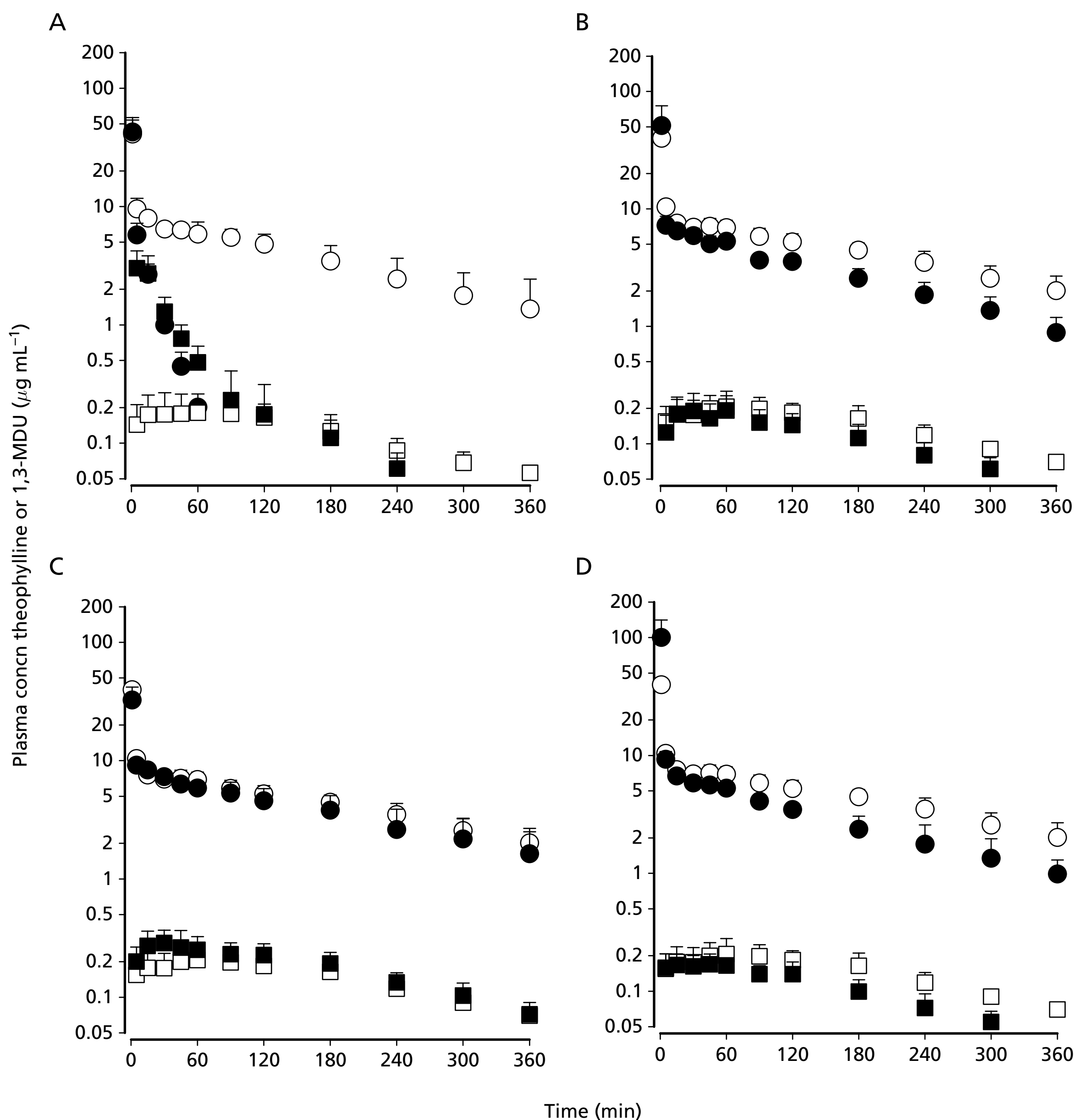

Figure 1 Mean arterial plasma concentration–time profiles of theophylline (circles) and 1,3-DMU (squares) after 1 min i.v. infusion of theophylline at a dose of 5 mg kg^{-1} to rats pretreated with enzyme inducers (closed symbols), 3-methylcholanthrene (A), orphenadrine citrate (B), isoniazid (C) or dexamethasone phosphate (D), and their respective control rats (open symbols). Vertical bars represent s.d.

24-h urine as unchanged theophylline ($Ae_{0-24\,h}$) was significantly lower (88.4% decrease). Changes in the pharmacokinetic parameters of 1,3-DMU compared with controls were as follows: the AUC was significantly higher (82.0% increase), terminal half-life was significantly shorter (88.8% decrease), the maximum plasma concentration was significantly higher (1750% increase), the time of C_{max} was significantly shorter (90.5% decrease), and $Ae_{0-24\,h}$ (expressed in terms of percentage of the i.v. dose of theophylline) was significantly greater (116% increase).

In orphenadrine-treated rats, changes in pharmacokinetic parameters of theophylline compared with the control rats were as follows: the AUC was significantly smaller (41.1% decrease), terminal half-life and MRT were significantly shorter (24.5 and 28.4% decrease, respectively), V_{ss} was significantly larger (19.7% increase) and CL, CL_R and CL_{NR} were significantly faster (65.8, 124, and 42.7% increase, respectively). Pharmacokinetic parameters of 1,3-DMU were not significantly different between orpheandrine-treated and control rats.

In isonizid-treated rats, pharmacokinetic parameters of both theophylline and 1,3-DMU were not significantly different from those in control rats.

In dexamethasone-treated rats, changes in pharmacokinetic parameters of theophylline compared with the control

Table 1 Pharmacokinetic parameters of theophylline and 1,3-DMU after 1 min i.v. infusion of theophylline, 5 mg kg^{-1}, to rats pretreated with 3-methylcholanthrene (3-MC; inducer of CYP1A1/2), orphenadrine (OP; inducer of CYP2B1/2), isoniazid (IN; inducer of CYP2E1) or dexamethasone (DX; inducer of CYP3A1/2), and their respective control rats; T suffix indicates pretreated rats; C suffix indicates control group

Parameter	3-MC-C (n = 8)	MC-T (n = 6)	OP-C, IN-C, DX-C (n = 7)	OP-T (n = 8)	IN-T (n = 8)	DX-T (n = 7)
Body weight (g)						
Initial	261 ± 3.74	263 ± 6.43	285 ± 7.07	288 ± 6.67	288 ± 14.2	283 ± 7.07
Final	275 ± 9.26	270 ± 6.32	299 ± 18.6	271 ± 12.9**	279 ± 14.0*	239 ± 12.1***
Theophylline						
AUC (μg min mL^{-1})	1810 ± 727	164 ± 33.6***	2190 ± 494	1290 ± 182***	1900 ± 618	1480 ± 292**
$t_{1/2}$ (min)	129 ± 58.5	11.9 ± 1.09**	155 ± 39.0	117 ± 21.2*	145 ± 51.9	146 ± 41.7
MRT (min)	184 ± 84.0	11.7 ± 3.27***	229 ± 53.9	164 ± 33.3*	209 ± 70.8	170 ± 27.3*
V_{ss} (mL kg^{-1})	502 ± 39.8	384 ± 189	522 ± 40.9	625 ± 61.9**	551 ± 41.9	635 ± 142
CL (mL min^{-1} kg^{-1})	3.10 ± 0.967	31.8 ± 7.42***	2.37 ± 0.497	3.93 ± 0.607***	3.01 ± 1.39	3.48 ± 0.611*
CL_R (mL min^{-1} kg^{-1})	0.833 ± 0.242	1.06 ± 0.427	0.666 ± 0.0858	1.49 ± 0.656**	0.624 ± 0.172	0.589 ± 0.245
CL_{NR} (mL min^{-1} kg^{-1})	2.26 ± 0.975	30.7 ± 7.20***	1.71 ± 0.502	2.44 ± 0.503*	2.24 ± 1.31	2.89 ± 0.415***
$Ae_{0–24\,h}$ (%)	29.1 ± 11.3	3.37 ± 1.08***	29.1 ± 6.39	37.2 ± 13.0	23.9 ± 8.19	16.3 ± 5.52**
1,3-DMU						
AUC (μg min mL^{-1})	59.9 ± 11.6	109 ± 20.8***	63.3 ± 17.3	49.5 ± 8.04	77.9 ± 8.46	49.7 ± 5.58
$t_{1/2}$ (min)	143 ± 17.8	16.0 ± 2.04***	169 ± 38.0	152 ± 52.0	145 ± 44.3	173 ± 48.9
C_{max} (μg mL^{-1})	0.208 ± 0.00801	3.84 ± 0.372**	0.231 ± 0.0409	0.208 ± 0.0652	0.305 ± 0.0839	0.187 ± 0.0373
T_{max} (min)	52.5 (1–20)	5 (5 – 15)*	60 (60 –120)	30 (15–60)	30 (15–120)	60 (5–120)
$Ae_{0–24\,h}$ (%)	21.2 ± 5.00	45.7 ± 17.9**	29.1 ± 6.13	33.4 ± 9.44	32.0 ± 11.0	16.2 ± 6.06**
$AUC_{1,3\text{-}DMU}$ /$AUC_{theophylline}$ (%)	3.83 ± 1.63	67.8 ± 10.5***	3.08 ± 1.32	3.83 ± 0.883	4.58 ± 1.67	3.45 ± 0.615

AUC, area under the plasma concentration–time curve; $t_{1/2}$, terminal elimination half-life; MRT, mean residence time; V_{ss}, volume of distribution at steady state; CL, total body clearance; CL_R, renal clearance; CL_{NR}, non-renal clearance; $Ae_{0–24\,h}$, percentage of the i.v. dose of theophylline excreted in the 24-h urine as unchanged theophylline (given as % of theophylline dose).
Values are mean ± s.d., *$P < 0.05$,**$P < 0.01$ and ***$P < 0.001$ compared with control.

rats were as follows: the AUC was significantly smaller (32.4% decrease), MRT was significantly shorter (25.8% decrease), CL and CL_{NR} were significantly faster (46.8 and 69.0% increase, respectively) and $Ae_{0–24\,h}$ was significantly lower (44.0% decrease). Pharmacokinetic parameters of 1,3-DMU were similar in the treatment and control groups, except for a significantly smaller $Ae_{0–24\,h}$ in the treatment group compared with controls (44.3% decrease; expressed in terms of percentage of the i.v. dose of theophylline).

The $AUC_{1,3\text{-}DMU}/AUC_{theophylline}$ ratio increased significantly (1670% increase vs controls) only in 3-MC treated rats.

Final body weight (body weight gain) was significantly lower in orphenadrine-, isoniazid- and dexamethasone-treated rats compared with their respective controls, as reported in other studies (Bae et al 2005; Choi & Lee 2006; Lee et al 2006).

Pharmacokinetics of theophylline and 1,3-DMU after intravenous administration of theophylline to rats pretreated with CYP inhibitors

Mean arterial plasma concentration–time profiles of theophylline and 1,3-DMU for the 1 min i.v. infusion of theophylline, 5 mg kg^{-1}, to rats pretreated with SKF-525A, sulfaphenzole, quinine and troleandomycin and their respective controls are shown in Figure 2, and relevant pharmacokinetic parameters are listed in Table 2. After 1 min i.v. infusion, the plasma concentrations of theophylline declined in a polyexponential fashion in all groups of rat.

In SKF-525A-treated rats, changes in pharmacokinetic parameters of theophylline compared with controls were as follows: AUC was significantly higher (55.9% increase), terminal half-life and MRT were significantly longer (52.3 and 54.1% increase, respectively) and CL, CL_R and CL_{NR} were significantly slower (32.1, 29.3 and 30.6% decrease, respectively) than in the controls. Pharmacokinetic parameters of 1,3-DMU were not significantly different between treated and control rats.

Pharmacokinetic parameters of both theophylline and 1,3-DMU were not significantly different in quinine-treated rats compared with controls.

In troleandomycin-treated rats, changes in pharmacokinetic parameters of theophylline compared with the controls were as follows: the AUC was significantly larger (93.4% increase), terminal half-life and MRT were significantly increased (103% and 97.0% increase, respectively), and CL, CL_R and CL_{NR} were significantly slower (47.3, 37.0 and 50.7% decrease, respectively) than in the controls.

Pharmacokinetic parameters of 1,3-DMU were not significantly different between troleandomycin-treated and control rats.

Pharmacokinetic parameters of both theophylline and 1,3-DMU were not significantly different in sulfaphenazole-treated rats compared with controls, except the V_{ss} of theophylline was significantly higher than in the controls (44.0% increase).

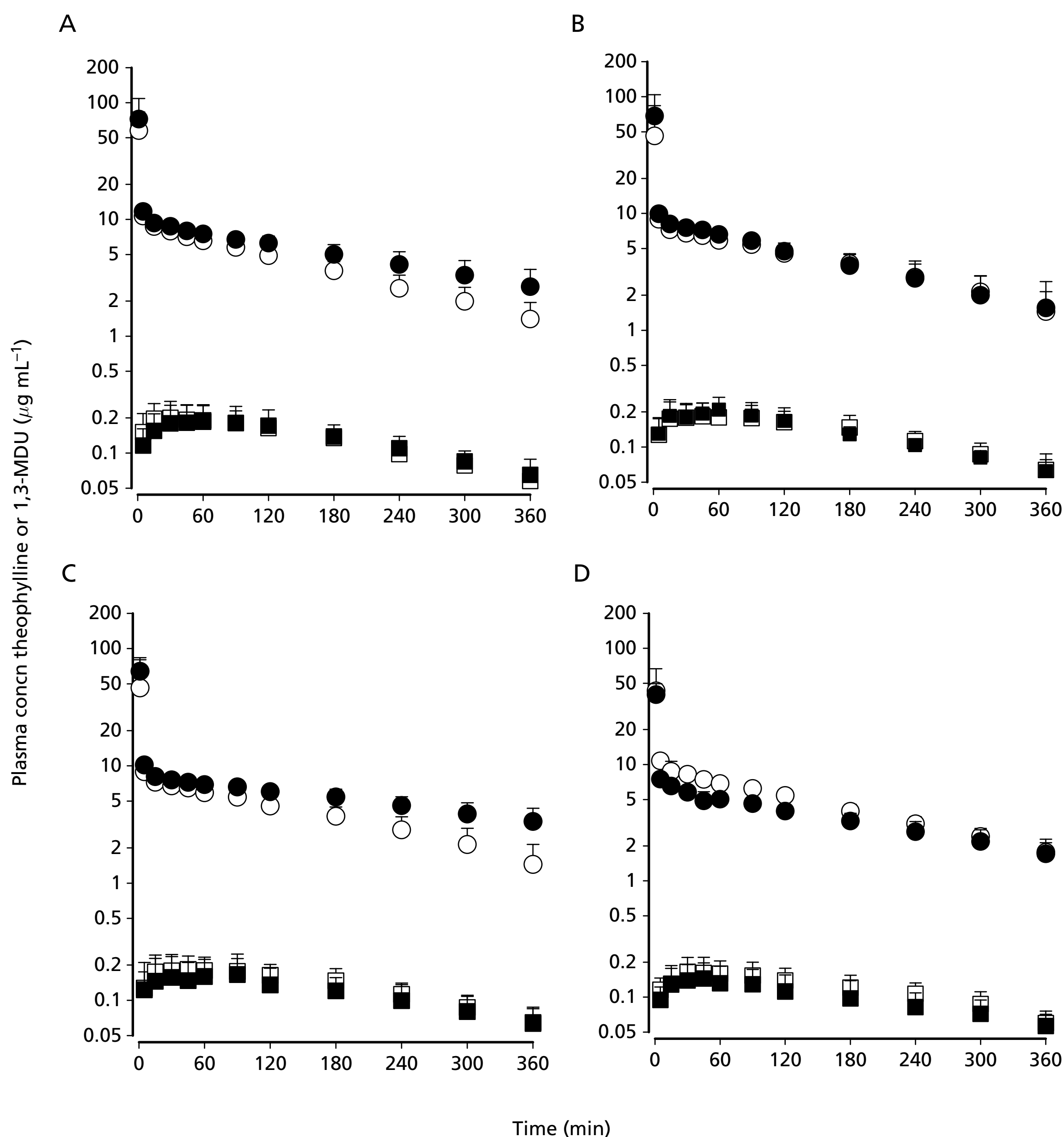

Figure 2 Mean arterial plasma concentration–time profiles of theophylline (circles) and 1,3-DMU (squares) after 1 min i.v. infusion of theophylline at a dose of 5 mg kg^{-1} to rats pretreated with enzyme inhibitors (closed symbols), SKF-525A (A), quinine hydrochloride (B), troleandomycin (C) or sulfaphenazole (D), and their respective control rats (open symbols). Vertical bars represent s.d.

The $AUC_{1,3\text{-DMU}}/AUC_{\text{theophylline}}$ ratios in SKF-525A- and troleandomycin-treated rats were significantly lower than in their respective controls (29.7% and 50.1% decrease, respectively).

Rat plasma protein binding of theophylline

In the preliminary study, equilibrium of theophylline between plasma and the buffer compartments was established after 8 h incubation, and the binding value was not influenced for up to 36 h incubation. Therefore, 24-h incubation was used. After 24-h incubation, no visible volume shift or bacterial growth were observed. The dialysis cells were washed with soap and water, and then soaked in 70% ethanol overnight to minimize bacterial growth. The Spectra/Por 2 membrane was pretreated by boiling and rinsing with distilled water and the buffer solution before use. To minimize volume shift, dextran was spiked into the buffer side to yield a final concentration of 3% (Boudinot & Jusko 1984).

Plasma protein binding (bound fraction) values of theophylline at 2 $\mu g\ mL^{-1}$ in orphenadrine-treated and control rats were 9.04 ± 5.70% and 13.3 ± 2.10%, respectively, and in

Table 2 Pharmacokinetic parameters of theophylline and 1,3-DMU after 1 min i.v. infusion of theophylline, 5 mg kg^{-1},to rats pretreated with SKF-525-A (SK; non-specific inhibitor of CYP isozymes), quinine (QN; inhibitor of CYP2D1), troleandomycin (TM; inhibitor of CYP3A1/2), or sulfaphenazole (SP; inhibitor of CYP2C11), and their respective controls. T suffix indicates pretreated rats; C suffix indicates control group

Parameter	SK-C (n = 9)	SK-T (n = 9)	QN-C, TM-C (n = 7)	QN-T (n = 7)	TM-T (n = 8)	SP-C (n = 8)	SP-T (n = 7)
Final body weight (g)	263 ± 7.12	264 ± 12.9	287 ± 11.5	294 ± 8.02	283 ± 9.61	278 ± 11.0	281 ± 6.26
Theophylline							
AUC (μg min mL^{-1})	1880 ± 391	2930 ± 899**	1820 ± 554	2070 ± 886	3520 ± 1060**	2100 ± 331	1860 ± 478
$t_{1/2}$ (min)	132 ± 31.5	201 ± 78.7*	137 ± 43.7	154 ± 89.3	278 ± 103**	152 ± 23.5	197 ± 56.1
MRT (min)	181 ± 39.1	279 ± 108*	199 ± 55.9	211 ± 118	392 ± 147**	210 ± 25.8	272 ± 77.9
V_{ss} (mL kg^{-1})	483 ± 35.9	463 ± 31.0	551 ± 85.7	495 ± 65.9	549 ± 56.3	504 ± 48.1	726 ± 71.7***
CL (mL min^{-1} kg^{-1})	2.77 ± 0.579	1.88 ± 0.647**	2.92 ± 0.679	2.74 ± 0.898	1.54 ± 0.461***	2.42 ± 0.334	2.81 ± 0.598
CL_R (mL min^{-1} kg^{-1})	0.808 ± 0.161	0.571 ± 0.202**	0.768 ± 0.247	0.655 ± 0.185	0.484 ± 0.177*	0.832 ± 0.246	0.851 ± 0.258
CL_{NR} (mL min^{-1} kg^{-1})	1.96 ± 0.526	1.36 ± 0.506*	2.15 ± 0.475	2.08 ± 0.762	1.06 ± 0.347***	1.59 ± 0.331	1.96 ± 0.468
$Ae_{0-24\,h}$ (%)	29.8 ± 6.33	27.9 ± 6.76	25.9 ± 5.32	25.0 ± 5.50	31.8 ± 7.56	34.4 ± 9.30	30.5 ± 6.91
1,3-DMU							
AUC (μg min mL^{-1})	61.3 ± 10.3	64.6 ± 23.3	66.1 ± 19.6	66.1 ± 10.6	58.3 ± 20.6	60.0 ± 15.8	57.4 ± 14.0
$t_{1/2}$ (min)	169 ± 47.0	174 ± 73.3	175 ± 82.5	199 ± 84.8	186 ± 47.4	203 ± 78.6	288 ± 119
C_{max} (μg mL^{-1})	0.211 ± 0.0686	0.200 ± 0.0748	0.211 ± 0.0523	0.225 ± 0.0596	0.193 ± 0.0952	0.178 ± 0.0577	0.146 ± 0.0491
T_{max} (min)	30 (15–90)	60 (30–180)	45 (30–120)	60 (15–90)	37.5 (15–90)	30 (15–60)	30 (5–120)
$Ae_{0-24\,h}$ (%)	21.9 ± 3.11	18.9 ± 6.45	22.1 ± 6.25	19.1 ± 3.32	16.9 ± 4.03	26.1 ± 4.47	23.4 ± 4.04
$AUC_{1,3\text{-}DMU}/AUC_{theophylline}$ (%)	3.40 ± 0.891	2.39 ± 0.944*	3.71 ± 0.870	3.56 ± 1.08	1.85 ± 0.885*	2.90 ± 0.739	3.16 ± 0.864

AUC, area under the plasma concentration–time curve; $t_{1/2}$, terminal elimination half-life; MRT, mean residence time; V_{ss}, volume of distribution at steady state; CL, total body clearance; CL_R, renal clearance; CL_{NR}, non-renal clearance; $Ae_{0-24\,h}$, percentage of the i.v. dose of theophylline excreted in the 24-h urine as unchanged theophylline (given as % of theophylline dose).
Values are mean ± s.d., *$P < 0.05$,**$P < 0.01$ and ***$P < 0.001$ compared with control.

sulfaphenzaole-treated and control rats were 9.17 ± 4.56% and 14.8 ± 4.55%, respectively (not significantly different). The corresponding values at 10 μg mL^{-1} were 8.71 ± 4.38%, 11.1 ± 4.02%, 12.5 ± 3.14% and 11.4 ± 2.09%, respectively (not significantly different).

Discussion

Many investigators have reported dose-dependent metabolic disposition of theophylline in humans (Lesko 1979; Birket et al 1984; Massey et al 1984) and in rats (Teunissen et al 1985). Therefore, theophylline at a dose of 5 mg kg^{-1}, which has been reported to be in the range of dose-independent metabolic disposition of theophylline in rats (up to 10 mg kg^{-1}) (Teunissen et al 1985), was administered i.v. to rats.

After i.v. administration of theophylline, contribution of biliary excretion of unchanged drug to the CL_{NR} of theophylline is negligible: the percentage of the i.v. dose of theophylline excreted in the 8 h bile sample after bile duct cannulation in four rats was 2.29 ± 0.292% (Kim et al 2003). Thus, the CL_{NR} values for theophylline listed in Tables 1 and 2 represents the metabolic clearance of the drug, and changes in CL_{NR} of theophylline represent changes in the metabolism of the drug in rats.

SKF-525A (a non-specific inhibitor of hepatic CYP isozymes in rats) was administered to rats to find out whether hepatic CYP isozymes are involved in the metabolism of theophylline. Studies in male and female Sprague–Dawley rat tissue slices have shown that metabolism of theophylline is localized only to the liver (in the microsome fraction and not in the mitochondria or cytosol), since heart, lung, intestine, brain, adrenal, kidney and spleen did not metabolize theophylline (Lohmann & Miech 1976). In SKF-525A-treated rats, the CL_{NR} of theophylline was significantly slower than in the controls (Table 2), indicating that theophylline is metabolized by hepatic CYP isozymes in rats. Thus, various inducers (Table 1) and inhibitors (Table 2) of hepatic CYP isozymes were administered to rats to find out which types of hepatic CYP isozymes are involved in the metabolism of theophylline and in the formation of 1,3-DMU in rats.

In 3-MC-, orphenadrine- and dexamethasone-treated rats (main inducers of CYP1A1/2, CYP2B1/2 and CYP3A1/2 in rats, respectively), the CL_{NR} of theophylline was significantly faster than in their respective controls (Table 1). Among these, changes in CL_{NR} of theophylline were greatest in the 3-MC-treated rats (Table 1). In contrast, in troleandomycin-treated rats (a main inhibitor of CYP3A1/2 in rats), the CL_{NR} of theophylline was significantly slower than in the controls (Table 2). These data suggest that hepatic CYP1A1/2, CYP2B1/2 and CYP3A1/2 could contribute to the metabolism of theophylline in rats. The metabolism of theophylline at a dose of 3 mg kg^{-1} via CYP1A2 (Ueng et al 2005) and at a dose of 10 mg kg^{-1} via CYP1A2 and CYP3A2 (Nosaka et al 2006) have also been reported in the dose range of dose-independent metabolic disposition of theophylline in rats. Note that orphenadrine citrate is an inducer of CYP2B1/2 in rats after 3 days' administration (Murray et al 2003), while it is an inhibitor of CYP2B/C in rats after a single administration (Bachmann et al 1993).

In the present study, the 24 h urinary excretion of 1,3-DMU was increased significantly only in 3-MC-treated rats (Table 1). The $AUC_{1,3\text{-}DMU}/AUC_{theophylline}$ ratio was significantly higher in 3-MC-treated rats and significantly lower in troleandomycin-treated rats (Tables 1 and 2). Although the exact reason is not clear, the ratio was not increased in dexamethasone-treated rats (Tables 1 and 2). These data suggest that 1,3-DMU could be formed mainly by CYP1A1/2 and possibly by CYP3A1/2 in rats.

Note that in 3-MC-treated rats, the terminal half-life of 1,3-DMU was significantly shorter (16.0 min) than that in other groups of rat (Tables 1 and 2). This could have been due to faster formation of 1,3-DMU in 3-MC-treated rats compared with other groups of rats (Figures 1 and 2). After i.v. administration of theophylline to other groups of rat, the plasma concentrations of 1,3-DMU were almost constant during the blood sampling times (Figures 1 and 2) and mean terminal half-lives of 1,3-DMU were 143–288 min (Tables 1 and 2). These data indicate that in most groups of rat, 1,3-DMU was continuously formed from theophylline whereas in 3-MC-treated rats, the pattern of decay of plasma concentrations of 1,3-DMU was similar to that following i.v. administration of 1,3-DMU (Kim et al 2003). Kim et al (2003) reported similar terminal half-life of 1,3-DMU, 10.5 ± 3.83 min, after i.v. administration of 1,3-DMU at a dose of 5 mg kg^{-1} to five control rats.

After i.v. administration of theophylline to orphenadrine- and sulfaphenzole-treated rats, the V_{ss} of theophylline was significantly larger than in their respective controls (Tables 1 and 2). However, this could not have been due mainly to a significantly increased free fraction of theophylline in plasma (unbound to plasma protein) in the orphenadrine- and sulfaphenzole-treated rats. As mentioned above, plasma protein binding values of theophylline were similar between orphenadrine-treated and control rats, and between sulfaphenzole-treated and control rats at both theophylline concentrations. Although the exact reason is not clear, the larger V_{ss} of theophylline in orphenadrine- and sulfaphenzole-treated rats could reflect increased affinity of rat tissues to theophylline by orphenadrine and sulfaphenazole. A significantly increased V_{ss} for ipriflavone in sulfaphenazole-treated rats have also been reported even though the plasma protein binding was not changed (Chung et al 2006).

Note that the various inducers and inhibitors of CYP isozymes used in this study could have effects on various isozymes (Correia 1975), but only the CYP isozyme that significantly changed was mentioned in this study. Thus, these results are confined to the main CYP isozymes changed by the various inducers and inhibitors of CYP isozymes.

Conclusion

Theophylline was primarily metabolized via CYP1A1/2, CYP2B1/2, and CYP3A1/2, and 1,3-DMU was primarily formed via CYP1A1/2 and possibly via CYP3A1/2 in rats.

Drug interactions between theophylline and many drugs have been reported (Upton 1991a,b). The present results could help to explain the possible drug interactions between

theophylline and other drugs (primarily metabolized via CYP1A1/2, CYP2B1/2 and/or CYP3A1/2).

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