JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 27–35

Received April 1, 2005
Accepted September 22, 2005
DOI 10.1211/jpp.58.1.0004
ISSN 0022-3573

Pharmacokinetics of 7-carboxymethyloxy-3′,4′,5-trimethoxy flavone (DA-6034), a derivative of flavonoid, in mouse and rat models of chemically-induced inflammatory bowel disease

Eun J. Kim, Mi Y. Chung, Hye J. Chung, Mi W. Son, Jong W. Kwon, Moohi Yoo and Myung G. Lee

Abstract

The pharmacokinetics (including distribution in the gastrointestinal tract) of 7-carboxymethyloxy-3′,4′,5-trimethoxy flavone (DA-6034) has been investigated in several mouse and rat models of chemically-induced inflammatory bowel disease (IBD). In the female ICR mouse model, IBD was induced by dextran sulfate and the mice administered 30 mg kg^{-1} DA-6034 intravenously or orally. In the male SJL mouse model of IBD induced by oxazolone, 30 mg kg^{-1} DA-6034 was administered orally. In the male Sprague–Dawley rat model of IBD induced by trinitrobenzene sulfonic acid (TNBS), 10 mg kg^{-1} DA-6034 was administered intravenously and orally. After intravenous administration, the total area under the plasma concentration–time curve from time zero to the last measured time, t, in plasma (AUC_{0-t}) values were comparable between control and dextran sulfate-induced IBD mice, and between control and TNBS-induced rats. This suggested that the disposition of DA-6034 was not affected considerably by dextran sulfate in mice and TNBS in rats. However, after oral administration in mice and rats with IBD, the AUC_{0-t} values were greater compared with the respective controls. This could have been due to an increase (slow) in the gastrointestinal transit time (in IBD mice and rats, the percentages of the oral dose recovered from the rinsing fluid of the small intestine and large intestine as unchanged drug were greater and smaller, respectively), and an increase in intestinal permeability.

Research Laboratory, Dong-A Pharmaceutical Company, Ltd., 47 Sanggal-Ri, Kiheung-Up, Yongin, Kyunggi-Do 449-900, Korea

Eun J. Kim, Mi Y. Chung, Mi W. Son, Jong W. Kwon, Moohi Yoo

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Korea

Hye J. Chung, Myung G. Lee

Correspondence: M. G. Lee, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Korea. E-mail: leemg@snu.ac.kr

Funding: This study was supported in part by a grant from the Korea Ministry of Health & Welfare (01-PJ2-PG4-J201PT01-0007), 2003–2005.

Introduction

Flavonoids are known to have potentially useful anti-inflammatory activity (Havesteen 1983; Middleton & Kandaswami 1993; You et al 1999). Eupatilin (5,7-dihydroxy-3′,4′,6-trimethoxyflavone), a derivative of flavonoid, is the main component of the extract of *Artemisiae* species, a species that has been used in the treatment of chronic diarrhoea in Korean folk medicine (Wu 1985; Ahn et al 1997). 7-Carboxymethyloxy-3′,4′,5-trimethoxy flavone (DA-6034) is a new synthetic derivative of eupatilin (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, Korea). Oral DA-6034 is being evaluated in phase II clinical trials for the treatment of ulcerative colitis in Korea.

Dextran sulfate, 2,4,6-trinitrobenzene sulfonic acid (TNBS), oxazolone, acetic acid, dinitrochlorobenzene, indometacin, or carrageenan are usually used to induce inflammatory bowel disease (IBD) in animal models. It was reported that oral DA-6034 was effective in three experimental rat models of IBD: acute chemical colitis induced by intracolonic instillation of 1.2 mL 4% acetic acid solution (Kim et al 1999), chronic chemical colitis induced by intracolonic instillation of 30 mg TNBS (Chang et al 1998; Kim et al 1999), and human leukocytic antigen (HLA)-B27 transgenic rats (Kim et al 1999). Oral DA-6034 (3 mg kg^{-1}) showed a more potent effect than oral prednisolone (1 mg kg^{-1}) or sulfasalazine (100 mg kg^{-1}) based on the macroscopic lesion score (Kim et al 1999). The therapeutic effect of oral DA-6034 could be due to the local effect on the intestinal mucosa.

We have reported the pharmacokinetics of DA-6034 after intravenous or oral administration in control Institute of Cancer Research (ICR) mice, Swiss Jim Lambert (SJL) mice and Sprague–Dawley rats, and dextran sulfate-, oxazolone-, or TNBS-induced mouse or rat models of IBD.

Materials and Methods

Chemicals

7-Carboxymethyloxy-3′,4′,5-trimethoxy flavone (DA-6034) and 7-carboxymethyloxy-3′,4′,5,6-tetramethoxyflavone (DA-6017; an internal standard for high-performance liquid chromatographic (HPLC) analysis of DA-6034) were synthesized by the Research Laboratory of Dong-A Pharmaceutical Company. TNBS, oxazolone, and dextran sulfate were purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). Other chemicals were of reagent grade or HPLC grade.

Animals

Female ICR mice seven-weeks old (20–30 g), male SJL mice eight-weeks-old (20–30 g), and male Sprague–Dawley rats seven-weeks-old (240–270 g) were purchased from Charles River Company Korea (Orient, Seoul, Korea). Animals were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea) at a temperature between 23 ± 2°C with a 12-h light/dark cycle, lights (an illumination intensity of 150–300 lux) on 0700 h and lights off 1900 h. The relative humidity was 55 ± 15% with air ventilation frequencies of 15–20 times h^{-1}. Animals were housed in metabolic cages under the supply of filtered pathogen-free air with food and water freely available. The protocol of the animal study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Induction of IBD by dextran sulfate or oxazolone in mice and by TNBS in rats

The 5% dextran sulfate (dissolved in tap water) was supplied as drinking water for five days (Okayasu et al 1990; Kojouharoff et al 1997) in female ICR mice. Tap water was supplied for the control female ICR mice. The dextran sulfate-induced IBD mice were further divided into two groups for oral study: moderate and severe groups.

Oxazolone (dissolved in 50% ethanol) at a dose of 6 mg was instilled intracolonically (total volume of 0.15 mL) using a 3.5 French catheter (the catheter was inserted up to 4 cm) in 48 h-fasted male SJL mice under light ether anaesthesia (Boirivant et al 1998). The same volume of 50% ethanol was administered in control male SJL mice.

TNBS (dissolved in 50% ethanol) at a dose of 30 mg was instilled intracolonically (total volume of 0.5 mL) with an 8-cm tube in 48 h-fasted male Sprague–Dawley rats under light ether anaesthesia (Morris et al 1989). The same volume of 50% ethanol was administered in control male Sprague–Dawley rats.

Intravenous and oral studies

In the early morning on the sixth day after drinking 5% dextran sulfate or tap water (Okayasu et al 1990; Kojouharoff et al 1997), DA-6034 (dissolved in isotonic 10 mM potassium phosphate buffer of pH 7) at a dose of 30 mg kg^{-1} was administered via the tail vein (total injection volume of 10 mL kg^{-1}) of female control ICR mice and dextran sulfate-induced IBD mice. Each mouse was restrained in a cage during the injection of DA-6034. Blood samples were collected directly from the heart into a heparinized tube at 0 (to serve as a control), 1, 5, 15, 30, 60, 120, 240, 360, 480, and 720 min after intravenous administration of DA-6034 (n = 3–4 at each blood sampling time). After centrifugation of the blood sample at 10 000 *g* for 3 min, a 0.1-mL plasma sample was stored in a − 70°C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA) until HPLC analysis of DA-6034. Other procedures were similar to the methods of Bae et al (2003, 2004, 2005a).

DA-6034 (the same solution as used in the intravenous study) at a dose of 30 mg kg^{-1} was administered orally (total oral volume of 5 mL kg^{-1}) using a feeding tube in the overnight-fasted control and moderate and severe dextran sulfate-induced IBD mice. Blood samples were taken at 0, 15, 30, 60, 120, 180, 240, 360, 480, 960, and 1440 min after oral administration of DA-6034 (n = 5–7 at each blood sampling time). Urine and faeces were collected at 0–480, 0–960, and 0–1440 min. Approximately 5 mL buffer was used to rinse the metabolic cage. This was combined with the urine sample. After measuring the exact volume of the combined urine sample, a 100-μL sample of the combined urine was stored in a − 70°C freezer until HPLC analysis of DA-6034. Approximately 4 mL buffer was added to 100 mg faeces. The mixture was homogenized and centrifuged after standing at room temperature for 10 min. A 100-μL sample of the supernatant was stored in a − 70 °C freezer until HPLC analysis of DA-6034. The blood, urine, and faeces samples were handled similarly to the methods of Bae et al (2003, 2004, 2005a).

At 2, 4, 8, 16, or 24 h after oral administration of DA-6034, mice were exsanguinated and killed via cervical dislocation, and then the abdomen was opened (n = 6–8 at each time). The whole stomach, small intestine, and large intestine (including caecum) were excised and the contents of each tissue were collected by flushing with buffer using a syringe. These washings were stored in a − 70 °C freezer for the measurement of unchanged DA-6034 remaining in the contents (the amounts of DA-6034 unabsorbed including not absorbed from the gastrointestinal tract after biliary excretion). After washing, each tissue was cut into small pieces using scissors and each tissue was homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) with 4 vol buffer (Bae et al 2004). After centrifugation, the supernatant was collected for the measurement of DA-6034. Other procedures were similar to the method of Yoon et al (1998).

In the early morning on the third day after intracolonic instillation of oxazolone, DA-6034 (30 mg DA-6034 was dissolved in 10 mL 0.9% NaCl-injectable solution and 0.2 mL 1 M NaOH) at a dose of 30 mg kg^{-1} was administered orally (total oral volume of 10 mL kg^{-1}) using a feeding tube in overnight-fasted male control SJL mice and oxazolone-induced male IBD mice. Blood samples were taken at 0, 30, 60, 120, 240, 360, 480, 720, 960, and 1800 min after oral administration of DA-6034 (n = 5–7 at each blood sampling time). At 2, 4, or 8 h after oral administration of DA-6034, mice were exsanguinated and killed via cervical dislocation. The abdomen was opened (n = 5 at each time) and the whole

stomach, small intestine, and large intestine (including caecum) were excised. The amount of unchanged DA-6034 unabsorbed in the washings and recovered from the tissues (mainly absorbed) was measured. Other procedures were similar to the dextran sulfate-induced IBD mice.

In the early morning on the fourth day after intracolonic instillation of TNBS or 50% ethanol, the carotid artery (for blood sampling) and the jugular vein (for drug administration; for intravenous study only) of overnight-fasted rats were cannulated under the light ether anaesthesia. Other procedures were similar to the methods of Bae et al (2005b) and Shim et al (2004). DA-6034 (dissolved in 5 mL 0.9% NaCl-injectable solution and 0.2 mL 1 M NaOH) at a dose of 10 mg kg^{-1} was administered over 1 min via the jugular vein (total infusion volume of 2 mL kg^{-1}) of control rats (n = 4) and TNBS-induced IBD rats (n = 5). An approximately 0.22-mL blood sample was collected at 0, 1, 5, 15, 30, 60, 120, 180, 240, 360, and 480 min via the carotid artery after intravenous administration of DA-6034. Urine was collected at 0–24 h. The plasma and urine samples were handled similarly to the reported methods (Bae et al 2005b, Shim et al 2004). DA-6034 (the same solution as used in the intravenous study) at a dose of 10 mg kg^{-1} was administered orally (total oral volume of 2 mL kg^{-1}) using a feeding tube to overnight-fasted control rats (n = 5) and TNBS-induced IBD rats (n = 9). Blood samples were taken at 0, 15, 30, 60, 120, 180, 240, 360, 480 and 1440 min after oral administration of DA-6034. Urine was collected at 0–24 h. The blood and urine samples were handled similarly to the methods of Bae et al (2005b) and Shim et al (2004). At 24 h after oral administration of DA-6034, each rat was exsanguinated and killed via cervical dislocation, and the abdomen was opened. The stomach, small intestine, and large intestine (including caecum) were collected and cut into small pieces using scissors. After washing with isotonic Sørensen's phosphate buffer (pH 7.4), each tissue was homogenized with 4 vol buffer (Bae et al 2004). Other procedures were similar to the above mice studies.

HPLC analysis of DA-6034

Concentrations of DA-6034 in the above plasma samples were analysed by an HPLC method using a fluorescence detector developed from our laboratories. To a 100-μL plasma sample was added 100 μL 0.15 M barium hydroxide, 100 μL 0.2 M zinc sulfate, and 100 μL acetonitrile (containing 300 ng mL^{-1} DA-6017 as internal standard). After vortex-mixing and centrifugation, 50 μL supernatant was injected directly onto the reversed-phase column. The mobile phase, 20 mM KH_2PO_4:acetonitrile (75:25; v/v; adjusted pH to 2.5 with 85% phosphoric acid) was run at a flow rate of 1.0 mL min^{-1}. A fluorescence detector set at an excitation wavelength of 336 nm and an emission wavelength of 440 nm monitored the column effluent. The retention times of DA-6034 and DA-6017 (internal standard) were approximately 6.6 and 9.0 min, respectively. The detection limit of DA-6034 in rat plasma was 0.5 ng mL^{-1}. The coefficients of variation of the assay (within- and between-day) were below 10.0%.

The HPLC system consisted of a model ASI-100 auto-injector (Dionex, München, Germany), a model P580 pump (Dionex), a reversed-phase Nova-Pak C_{18} column (150 mm × 3.9 mm, i.d.; particle size, 5 μm; Waters Corporation, Milford, MA, USA), a model RF-551 fluorescence detector (Shimazu, Kyoto, Japan), and a model D-2500 integrator (Hitachi, Tokyo, Japan).

DA-6034 concentration in the above urine, faeces, washings of gastrointestinal tract, and gastrointestinal homogenate samples were analysed by an HPLC method using an UV detector reported from our laboratories (Lee et al 1998). To a 100-μL sample was added 100 μL 0.1 M HCl, 100 μL acetonitrile (containing 1 μg mL^{-1} DA-6017), and 600 μL ethylacetate. After vortex-mixing for 1 min and centrifugation, a 500-μL organic layer sample was collected and dried under nitrogen gas. Mobile phase (200 μL) was added to reconstitute the residue and a 100-μL sample was injected directly onto the reversed-phase column. The mobile phase, 5 mM sodium methanesulfonate (pH 2.5)/10 mM KH_2PO_4:acetonitrile (70:30; v/v) was run at a flow rate of 1.0 mL min^{-1}. An UV detector set at a wavelength of 334 nm monitored the column effluent. The retention times of DA-6034 and DA-6017 were approximately 5.0 and 6.7 min, respectively.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to the last measured time, t, in plasma, AUC_{0-t}, was calculated by the trapezoidal rule method (Kim et al 1993); 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 maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were read directly from the experimental data.

Statistical analysis

A P value of less than 0.05 was considered to be statistically significant using a two-way multivariate analysis of variance for comparing the means of the two or more groups for more than one dependent variable, a two-way repeated measures analysis of variance followed by Tukey's post-hoc test for comparing the repeatedly measured data between the two or more groups, or an unpaired t-test for comparing the means of the two groups by Statistical Research Institute, College of Natural Sciences, Seoul National University. All results were expressed as mean ± s.d. except median for T_{max}.

Results

Pharmacokinetics of DA-6034 after intravenous and oral administration in female ICR mice with dextran sulfate-induced IBD

The mean plasma concentration–time profiles of 30 mg kg^{-1} DA-6034 after intravenous administration in control female ICR mice and dextran sulfate-induced IBD mice are shown in Figure 1A. The plasma concentration declined rapidly up to 4 h and declined slowly thereafter up to 12 h for both groups of mice. The mean concentration was somewhat higher at 8 h

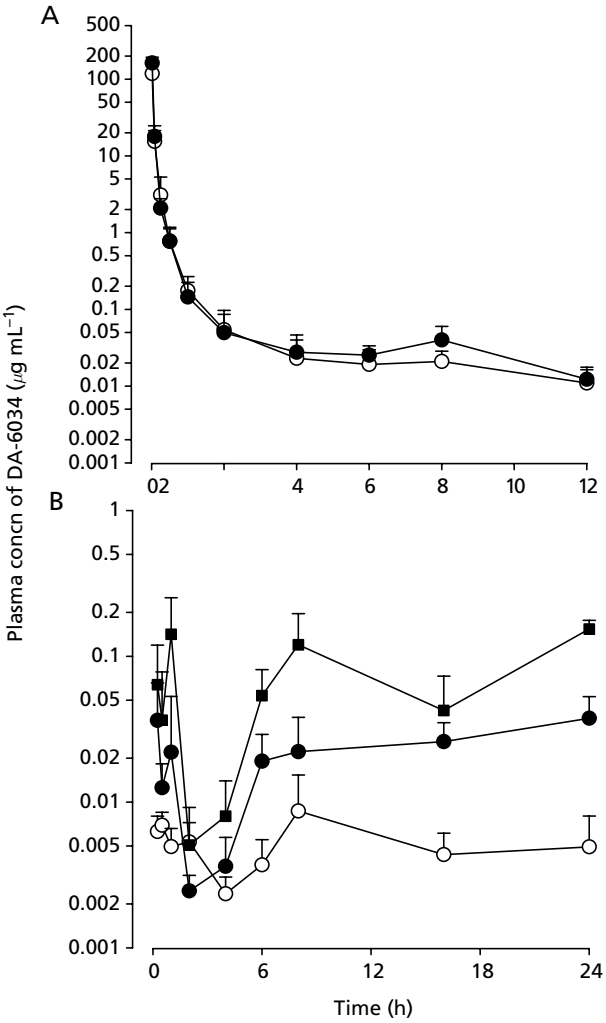

Figure 1 Mean plasma concentration–time profiles of DA-6034 after intravenous injection (A) at a dose of 30 mg kg^{-1} to control female ICR mice (○) and dextran sulfate-induced IBD (●) mice (n = 3–4 at each blood sampling time), and oral administration (B) at a dose of 30 mg kg^{-1} to control (○) and dextran sulfate-induced moderate (●) and severe (■) IBD mice (n = 5–7 at each blood sampling time). Vertical bars represent s.d. Two-way analysis of variance was employed. In A, the dextran sulfate-induced IBD mice group was significantly different from control ($P < 0.05$). In B, after Tukey's post-hoc test, three groups were significantly different from each other ($P < 0.05$).

in dextran sulfate-induced IBD mice due to one outlier having 2.3-times higher concentration. The plasma concentration was similar for both groups of mice and this resulted in comparable $AUC_{0-12\,h}$ values between both groups of mice (6.57 and 7.84 μg h mL^{-1} for control and dextran sulfate-induced IBD mice, respectively).

The dextran sulfate-induced IBD mice were further divided into two groups for oral study: moderate and severe groups. The moderate group passed loose stools and had localized hyperaemia in a small site of the colon. The severe group passed watery diarrhoea and bloody stools, and bled in the upper region of the intestinal to distal colon. In the severe group, body weight decreased by more than 10%.

The mean plasma concentration–time profiles of 30 mg kg^{-1} DA-6034 after oral administration in control, and moderate and severe dextran sulfate-induced IBD mice are shown in Figure 1B. The plasma concentration of DA-6034 fluctuated up to 24 h. The plasma concentrations in the three groups of mice were significantly different from each other. The plasma concentration in severe IBD mice was higher compared with control and moderate IBD groups, and in moderate IBD mice the plasma concentration was significantly higher compared with the control group. This resulted in considerably greater $AUC_{0-24\,h}$ values in IBD mice (0.124, 0.544, and 1.74 μg h mL^{-1} for control, moderate and severe IBD mice, respectively).

The percentage of dose recovered as unchanged DA-6034 from the washings of the gastrointestinal tract, urine, and faeces after oral administration of 30 mg kg^{-1} DA-6034 in control and severe IBD mice are listed in Table 1. The urinary excretion of DA-6034 was almost negligible; less than 2.61% of oral dose was excreted in 24-h urine for both groups of mice. However, the values in faeces were considerable; the 24-h values were 46.4 and 29.5% for control and severe IBD mice, respectively. The values from the stomach washings were less than 15.2% for both groups of mice. The values from the small intestine in severe IBD mice were significantly greater at 2 h (222% increase), 4 h (582% increase), 8 h (98.3% increase), and 16 h (83.6% increase); the values generally decreased with time for both groups of mice. On the other hand, the values from the large intestine in IBD mice were significantly smaller at 2 h (82.5% decrease) and 4 h (57.8% decrease).

The amounts of unchanged DA-6034 recovered from the gastrointestinal tract at 2, 4, 8, 16, and 24 h after oral administration of 30 mg kg^{-1} DA-6034 in control and severe IBD mice are listed in Table 1. The values in stomach were not significantly different between the two groups of mice, with the exception that it was significantly smaller (43.3% decrease) in severe IBD mice at 8 h. The values in small intestine in severe IBD mice were significantly greater at 2 h (171% increase), 4 h (380% increase), and 16 h (140% increase). The values in large intestine were not significantly different between the two groups of mice, with the exception that it was significantly smaller in IBD mice at 2 h (83.4% decrease). The tissue-to-plasma (T/P) ratios of DA-6034 in the gastrointestinal tract ranged from 198 to 9550 for both groups of mice.

Pharmacokinetics of DA-6034 after oral administration in male SJL mice with oxazolone-induced IBD

The mean plasma concentration–time profiles of DA-6034 (30 mg kg^{-1}, p.o.) in control male SJL mice and oxazolone-induced IBD mice are shown in Figure 2. The plasma concentration fluctuated considerably up to 16 h in oxazolone-induced IBD mice. The plasma concentration and C_{max} values (0.00555 ± 0.00505 and 0.409 ± 0.675 μg mL^{-1} for control and IBD mice, respectively) were higher in oxazolone-induced IBD mice compared with control mice. This resulted in considerably greater $AUC_{0-30\,h}$ (0.0534 and 3.69 μg h mL^{-1} for control and IBD mice, respectively).

Table 1 Percentage of DA-6034 recovered as unchanged drug from the washings of the gastrointestinal tract, urine, and faeces, and amounts (μg (g tissue)$^{-1}$) recovered from the gastrointestinal tract at 2, 4, 8, 16, or 24 h after administration of DA-6034 (30 mg kg^{-1}, p.o.) to control female ICR mice and dextran sulfate-induced severe IBD mice. The value in parenthesis represents the mean value of tissue-to-plasma (T/P) ratio

Sample	2 h		4 h		8 h		16 h		24 h	
	Control	Severe IBD	Control	Severe IBD	Control	Severe IBD	Control	Severe IBD	Control	Severe IBD
Washings[a]										
Stomach	8.30 ± 5.15	15.2 ± 15.8	9.14 ± 5.43	11.2 ± 10.9	15.1 ± 5.17	6.61 ± 4.46	8.93 ± 7.62	11.4 ± 4.06	4.60 ± 4.51	4.22 ± 4.46
Small intestine	17.7 ± 5.05	57.0 ± 18.3	5.44 ± 3.03	37.1 ± 22.2	9.33 ± 4.18	18.5 ± 8.00	7.68 ± 2.66	14.1 ± 4.26	4.34 ± 4.13	7.35 ± 4.93
Large intestine	47.5 ± 8.47	8.31 ± 12.8	58.1 ± 6.60	24.5 ± 22.2	36.6 ± 7.32	34.3 ± 12.3	34.0 ± 8.69	42.6 ± 7.10	20.7 ± 11.1	21.2 ± 10.2
Tissues[b]										
Stomach	10.1 ± 5.02 (2110)	21.3 ± 17.3 (3160)	8.26 ± 5.65 (3970)	10.5 ± 17.4 (243)	16.9 ± 6.74 (2820)	9.58 ± 5.09 (198)	16.3 ± 10.1 (4390)	17.4 ± 7.71 (691)	13.5 ± 11.8 (2830)	8.82 ± 4.00 (1690)
Small intestine	14.4 ± 8.63 (2640)	39.0 ± 16.8 (5860)	6.21 ± 4.95 (2410)	29.8 ± 16.2 (2170)	10.2 ± 5.98 (2070)	16.7 ± 11.4 (326)	7.87 ± 3.89 (2010)	18.9 ± 7.34 (520)	9.22 ± 7.56 (1910)	7.81 ± 4.04 (1290)
Large intestine	18.3 ± 11.3 (3480)	5.62 ± 7.15 (496)	20.3 ± 8.19 (9550)	23.8 ± 22.3 (815)	14.1 ± 9.31 (1970)	16.1 ± 6.93 (587)	13.8 ± 6.95 (3200)	21.7 ± 7.37 (668)	11.7 ± 5.18 (2820)	10.6 ± 6.24 (2550)
Urine	NM	NM	NM	NM	0.299 ± 0.102	0.949 ± 0.903	0.641 ± 0.194	1.87 ± 1.25	0.974 ± 0.336	2.61 ± 1.62
Faeces	NM	NM	NM	NM	4.12 ± 7.66	14.8 ± 10.9	18.1 ± 19.2	8.66 ± 5.29	46.4 ± 26.5	29.5 ± 23.1

Values are mean ± s.d., n = 6–8 at each time. A two-way multivariate analysis of variance was performed. NM, not measured. [a]For all tests (Pillai's trace, Wilks' Lamda, Hotelling-Lawley Trace, Roy's Greatest Root) the severe IBD group was significantly different from control ($P < 0.05$). The univariate F test revealed more detailed aspects. Group effect for small intestine, large intestine responses were significant at the $P < 0.001$ level, but stomach was not. [b]For all tests (Pillai's trace, Wilks' Lamda, Hotelling-Lawley Trace, Roy's Greatest Root) the severe IBD group was significantly different from control ($P < 0.05$). After the univariate F test, group effect for small intestine response was significant at the $P < 0.05$ level.

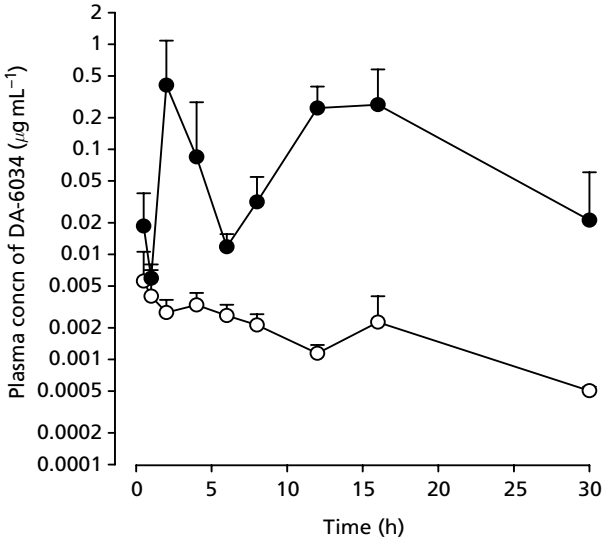

Figure 2 Mean plasma concentration–time profiles of DA-6034 after oral administration of 30 mg kg^{-1} to control male SJL mice (○) and oxazolone-induced IBD (●) mice (n = 5–7 at each blood sampling time). Vertical bars represent s.d. Two-way analysis of variance was employed. The oxazolone-induced IBD mice group was significantly different from control ($P < 0.05$).

The percentages of dose recovered from the washing fluid of the gastrointestinal tract as unchanged DA-6034 after oral administration of 30 mg kg^{-1} in control and IBD mice are listed in Table 2. The values from the stomach were 4.85–30.7% of oral dose up to 8 h for both groups of mice; the values were not significantly different between two groups of mice at each time. The values in the small intestine in IBD mice were significantly greater at 2 h (147% increase), 4 h (179% increase), and 8 h (503% increase). The values decreased with times for both groups of mice. On the other hand, the values in the large intestine in IBD mice were significantly smaller at 2 h (98.5% decrease) and 4 h (95.3% decrease).

The amounts of unchanged DA-6034 recovered from the gastrointestinal tract at 2, 4, and 8 h after oral administration of the drug in SJL mice and oxazolone-induced IBD mice are listed in Table 2. The values in the stomach were not significantly different between the two groups of mice. The values in the small intestine in IBD mice were significantly greater at 2 h (345% increase), 4 h (189% increase), and 8 h (335% increase). The values in large intestine were not significantly different between the two groups of mice, with the exception that it was significantly smaller in IBD mice at 2 h (83.4% decrease). The T/P ratios of DA-6034 in the gastrointestinal tract ranged from 3.19 to 4180 for both groups of mice.

Pharmacokinetics of DA-6034 after intravenous and oral administration in male Sprague–Dawley rats with TNBS-induced IBD

The mean arterial plasma concentration–time profiles of DA-6034 (10 mg kg^{-1}) after intravenous administration in control Sprague–Dawley rats and TNBS-induced IBD rats are shown in Figure 3A. The plasma concentration declined rapidly up to 4 h and declined slowly thereafter up to 8 h for both groups of rats. The concentration was somewhat higher at 6 h in TNBS-induced rats due to one outlier having approximately a 7.0-times higher concentration. The $AUC_{0-6\,h}$ values (2.97 ± 0.775 and 2.84 ± 0.344 μg h mL^{-1} for control and IBD rats, respectively) and percentage of intravenous dose excreted in 24-h urine as unchanged drug (48.2 ± 3.70 and 50.4 ± 16.0% for control and IBD rats, respectively) were not significantly different between the two groups of rats.

The mean arterial plasma concentration–time profiles of DA-6034 (10 mg kg^{-1}) after oral administration in control and IBD rats are shown in Figure 3B, and some pharmacokinetic parameters are listed in Table 3. The plasma concentration declined up to 1–2 h for both groups of rats and fluctuated thereafter up to 6 h in IBD rats. In IBD rats, the $AUC_{0-24\,h}$ values were significantly greater compared with control rats (210% increase). Hence the *F* value was greater

Table 2 Percentage of DA-6034 recovered as unchanged drug from the washings of the gastrointestinal tract, and amounts (μg (g tissue)$^{-1}$) recovered from the gastrointestinal tract at 2, 4, and 8 h after DA-6034 administration (30 mg kg^{-1}, p.o.) to control male SJL mice and oxazolone-induced IBD mice. The values in parentheses represent the mean value of tissue-to-plasma (T/P) ratio

Sample	2h		4h		8h	
	Control	IBD	Control	IBD	Control	IBD
Washings[a]						
Stomach	30.7 ± 14.5	17.3 ± 16.7	20.3 ± 11.3	28.8 ± 18.6	4.85 ± 3.91	15.9 ± 20.0
Small intestine	25.0 ± 13.5	61.7 ± 12.1	21.3 ± 11.8	59.4 ± 18.5	5.89 ± 2.60	35.5 ± 22.5
Large intestine	23.7 ± 8.76	0.282 ± 0.142	20.3 ± 14.6	0.954 ± 1.08	22.0 ± 6.39	19.4 ± 10.3
Tissues[b]						
Stomach	1.88 ± 0.549 (735)	3.27 ± 1.59 (42.6)	1.96 ± 2.18 (536)	1.62 ± 1.13 (100)	1.26 ± 0.291 (615)	2.17 ± 1.12 (93.5)
Small intestine	10.7 ± 3.95 (4180)	47.6 ± 24.1 (543)	10.0 ± 5.92 (3140)	28.9 ± 10.3(1410)	5.89 ± 5.76 (2650)	25.6 ± 13.5 (1120)
Large intestine	2.31 ± 0.962 (878)	0.382 ± 0.331(3.19)	2.13 ± 1.94 (668)	0.787 ± 1.29 (55.9)	1.65 ± 0.683 (800)	4.52 ± 4.48 (227)

Values are mean ± s.d., n = 5 at each time. A two-way multivariate analysis of variance was performed. [a]For all tests (Pillai's trace, Wilks' Lamda, Hotelling-Lawley Trace, Roy's Greatest Root) the IBD group was significantly different from control ($P < 0.05$). After the univariate *F* test, group effect for small intestine, large intestine responses were significant at the $P < 0.05$ level, but stomach was not. [b]For all tests (Pillai's trace, Wilks' Lamda, Hotelling-Lawley Trace, Roy's Greatest Root) the IBD group was significantly different from control ($P < 0.05$). After the univariate *F* test, group effect for small intestine response was significant at the $P < 0.05$ level.

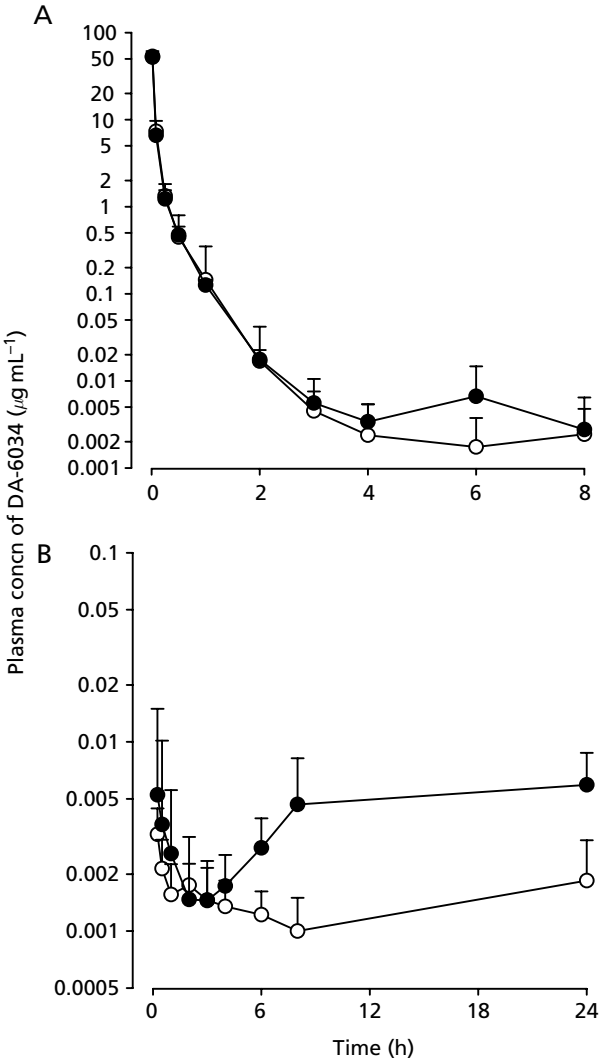

Figure 3 Mean arterial plasma concentration–time profiles of DA-6034 after 1-min intravenous infusion (A) at a dose of 10 mg kg^{-1} to control male Sprague–Dawley rats (○; n = 4) and TNBS-induced IBD rats (●; n = 5), and oral administration (B) at a dose of 10 mg kg^{-1} to control rats (○; n = 5) and TNBS-induced IBD rats (●; n = 9). Vertical bars represent s.d. Two-way repeated measures analysis of variance was employed. For A and B the two groups were not significantly different.

in IBD rats (387% increase). However, the $AUC_{0-6\,h}$ values were comparable between the two groups of rats.

The T/P ratios of DA-6034 after oral administration at a dose of 10 mg kg^{-1} in control and IBD rats are listed in Table 4. In both groups of rats, the T/P ratios in the gastrointestinal tract ranged from 454 to 18 200. In TNBS-induced IBD rats, the T/P ratios were significantly smaller compared with control rats; the ratios were 90.6, 89.8, and 81.3% decreased in the stomach, small intestine, and large intestine, respectively.

Discussion

After intravenous administration, the plasma concentration of DA-6034 rapidly declined (Figures 1 and 3). After oral administration, the concentration again declined rapidly and fluctuated considerably thereafter (Figures 1–3). Hence, to obtain more accurate pharmacokinetic parameters of DA-6034, doses of 10 and 30 mg kg^{-1} were chosen arbitrarily for this study after preliminary work with various doses of DA-6034.

After intravenous administration of 30 mg kg^{-1} DA-6034 in female ICR mice with dextran sulfate-induced IBD, the $AUC_{0-12\,h}$ was comparable with that in control mice. This suggested that the disposition of DA-6034 was not affected considerably by dextran sulfate after intravenous administration in mice. After intravenous administration of 10 mg kg^{-1} DA-6034 in male Sprague–Dawley rats with TNBS-induced IBD, the $AUC_{0-8\,h}$ was comparable with control rats. Again, this suggested that the disposition of DA-6034 was not affected considerably by TNBS after intravenous administration in rats.

After oral administration of DA-6034 to mice and rats, the absorption was rapid. For example, DA-6034 was detected in plasma from the first blood sampling time, 15 min, and rapidly reached the first T_{max} at 30, 15, and 15 min for control, and moderate and severe dextran sulfate-induced IBD mice, respectively (Figure 1B). DA-6034 was detected in plasma from the first blood sampling time, 30 min, and reached the first T_{max} at 30 and 120 min for control and severe oxazolone-induced IBD mice, respectively (Figure 2).

Table 3 Pharmacokinetic parameters of DA-6034 (10 mg kg^{-1}, p.o.) in control male Sprague–Dawley rats and TNBS-induced IBD rats

Parameter	Control	IBD
First peak T_{max} (h)[a]	0.25 (0.25)	0.25 (0.25–1)
First peak C_{max} (μg mL^{-1})	0.00325 ± 0.00122 (0.00127–0.00421)	0.00585 ± 0.00953 (0.00104–0.00605)
$AUC_{0-6\,h}$ (μg h mL^{-1})	0.00916 ± 0.00350 (0.00597–0.0146)	0.0126 ± 0.00601 (0.00501–0.0254)
$AUC_{0-24\,h}$ (μg h mL^{-1})	0.0336 ± 0.00500 (0.0262–0.0396)	0.104 ± 0.0506* (0.0549–0.212)
$Ae_{0-24\,h}$ (% of dose)	0.435 ± 0.0994 (0.298–0.570)	2.67 ± 2.55 (0.297–8.95)
F (%)	0.308	1.50

Values are mean ± s.d. (ranges), n = 5 for control rats and n = 9 for TNBS-induced IBD rats. [a]Median (ranges). Unpaired *t*-test was performed; *$P < 0.05$ compared with control.

Table 4 Plasma concentration (μg mL^{-1}) and tissue-to-plasma (T/P) ratios of DA-6034 at 24 h after administration (10 mg kg^{-1}, p.o.) in control male Sprague–Dawley rats and TNBS-induced IBD rats

Tissue	Control	IBD
Plasma	0.00185 ± 0.0012	0.00593 ± 0.0028
Stomach	8360 ± 8380	790 ± 696*
Small intestine	4450 ± 2680	454 ± 489**
Caecum	18200 ± 25800	1650 ± 1600
Large intestine	13300 ± 13800	2490 ± 2380*

Values are mean ± s.d., n = 5 for control rats and n = 9 for TNBS-induced IBD rats. Unpaired *t*-test was performed; *$P < 0.05$ and **$P < 0.001$ compared with control.

DA-6034 was detected in plasma from the first blood sampling time, 15 min, and reached the first T_{max} at 15 min for control rats and rats with TNBS-induced IBD (Figure 3B).

After oral administration of DA-6034 to mice and rats, the plasma concentration fluctuated considerably (Figures 1B, 2, 3B). Irregular (or slow) partition of drugs between plasma and blood cells (Lee et al 1981), fast uptake of drugs in tissues and slow release of drugs from tissues to blood, enterohepatic recycling of drugs, and/or gastric emptying pattern (Lee et al 1994; Lee & Lee 1996) were reasons for the appearance of multiple (second) peak(s) of drugs in the plasma concentration after oral administration of drugs.

After oral administration of DA-6034 to moderate and severe dextran sulfate-induced IBD mice, oxazolone-induced IBD mice, and TNBS-induced IBD rats, the AUC_{0-t} values were considerably greater compared with the respective controls. This could have been due to delay in intestinal transit time and increased intestinal permeability in IBD. Increased (delayed) small intestinal transit time was reported in ulcerative colitis patients (Nugent et al 2001). Colonic transit was delayed in TNBS-induced colitis rats (Cho et al 2004), and distal colitis increased retention of the ^{51}Cr-marker in the caecum and proximal colon, and retention of the marker in the distal colon decreased in acetic acid-induced colitis rats (Myers et al 1997). Delayed small intestinal transit time could also be supported by the percentage of oral dose recovered from the rinsings of the gastrointestinal tract as unchanged DA-6034 (Tables 1 and 3); in control mice, most of the DA-6034 remained in the large intestine up to 24 h (Table 1) and 8 h (Table 2), whereas it remained in the small intestine up to 4 h (Table 1) and 8 h (Table 2) in severe IBD mice. Pantzar et al (1994) reported that absorption of [^{51}Cr]EDTA increased in oxazolone-induced colitis due to increased intestinal transit time and permeability in the distal small intestine. The myoelectrical change in the ileum of rats with TNBS-induced colitis has been reported. Distal colitis is associated with abnormal myoelectrical activity in the non-inflamed ileum of rats. Neither acetylcholine nor prostaglandins and nitric oxide seem to be involved (Aube et al 1999). It was reported that inflammation in animal models of IBD altered the neurochemical content of some functional classes of enteric neurons (Lomax et al 2005).

The tissue-to-plasma ratios of DA-6034 in the gastrointestinal tract were considerably greater than unity in mice (Tables 2 and 4) and rats (Table 3). This suggested that mice and rat gastrointestinal tracts had a high affinity for DA-6034.

The dextran sulfate-induced IBD mouse model resembles ulcerative colitis in man. However, in this study, inflammation was not localized to the colon. In the severe case, inflammation was observed from the upper small intestine, which is not in the case in man. This was due to supplying the dextran sulfate as drinking water, not by intracolonic instillation. Inflammation in the small intestine was reported also (Ohtsuka & Sanderson 2003) after oral administration (drinking water) of dextran sulfate in mice. Further studies are needed to examine the pharmacokinetics of DA-6034 in IBD patients.

References

Ahn, B. O., Ryu, B. K., Ko, J. I., Oh, T. Y., Kim, S. H., Kim, W. B., Yang, J., Lee, E. B., Hahm, K. G. (1997) Beneficial effect of DA-9601, an extract of *Artemisiae herba*, on animal models of inflammatory bowel disease. *Kor. J. Appl. Pharmacol*. **5**: 165–173

Aube, A. C., Cherbut, C., Barbier, M., Xing, J. H., Roze, C., Galmiche, J. P. (1999) Altered myoelectrical activity in noninflamed ileum of rats with colitis induced by trinitrobenzene sulphonic acid. *Neurogastroenterol. Motil*. **11**: 55–62

Bae, S. K., Kim, E. J., Chung, S. J., Kim, S. G., Lee, M. G. (2003) Pharmacokinetic interaction between oltipraz and dimethyl-4, 4′-dimethyoxy-5,6,5′6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB) after single intravenous and oral administration to rats. *J. Pharm. Pharmacol*. **55**: 1241–1249

Bae, S. K., Kim, E. J., Kwon, J. W., Kim, W. B., Lee, I., Lee, M.G. (2004) Effects of protein–calorie malnutrition on the pharmacokinetics of DA-7867, a new oxazolidinone, in rats. *J. Pharm. Pharmacol*. **56**: 635–642

Bae, S. K., Lee S. J., Kim, Y. H., Kim, T., Lee, M. G. (2005a) Effect of enzyme inducers and inhibitors on the pharmacokinetics of oltipraz in rats. *J. Pharm. Pharmacol*. **57**: 443–452

Bae, S. K., Kim, E. J., Kwon, J. W., Kim, W. B., Lee, I., Lee, M.G. (2005b) Excretion and metabolism of DA-7867, a new oxazolidinone, in rats. *Biopharm. Drug Dispos*. **26**: 67–75

Boirivant, M., Fuss, I. J., Chu, A., Strober, W. (1998) Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *J. Exp. Med*. **188**: 1929–1939

Chang, D. K., Chin, Y. J., Jung, H. C., Song, I. S., Son, M., Yoo, M., Kim, C. Y. (1998) The effect of DA-6034, eupatilin derivatives of flavonoids, on the rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis. *Kor. J. Med*. **55**: 302–309

Chiou, W. L. (1978) Critical evaluation of the potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J. Pharmacokinet. Biopharm*. **6**: 539–546

Cho, S. H., Park, H. J., Chung, J. P., Lee, Y. H., Ji, S. W., No, T. W., Lee, S.I. (2004) Altered colonic transit in TNBS-induced experimental colitis in guinea pig and distribution of nitric oxide synthase in the colonic wall. *Kor. J. Gastroenterol*. **44**: 308–313

Havesteen, B. (1983) Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol*. **32**: 1141–1148

Kim, S. H., Choi, Y. M., Lee, M. G. (1993) Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J. Pharmacokinet. Biopharm*. **21**: 1–17

Kim, Y. S., Son, M., Ko, J. I., Cho, H., Yoo, M., Kim, W. B., Song, I. S., Kim, C. Y. (1999) Effect of DA-6034, a derivative of flavonoid, on experimental animal models of inflammatory bowel disease. *Arch. Pharm. Res*. **22**: 354–360

Kojouharoff, G., Hans, W., Obermeier, F., Mannel, D. N., Andus, T., Scholmerich, J., Gross, V., Falk, W. (1997) Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin. Exp. Immunol*. **107**: 353–358

Lee, S. H., Lee, M. G. (1996) Pharmacokinetics and pharmacodynamics of azosemide after intravenous and oral administration to rats: absorption from various GI segments. *J. Pharmacokinet. Biopharm*. **24**: 551–568

Lee, M. G., Chen, M.-L., Huang, S.-M., Chiou, W. L. (1981) Pharmacokinetics of drugs in blood I. Unusual distribution of gentamicin. *Biopharm. Drug Dispos*. **2**: 89–97

Lee, S. H., Lee, M. G., Kim, N. D. (1994) Pharmacokinetics and pharmacodynamics of bumetanide after intravenous and oral administration to rats: absorption from various GI segments. *J. Pharmacokinet. Biopharm*. **22**: 1–17

Lee, J. J., Son, M. W., Yoo, M. H., Jang, M. S., Kim, W. B., Lee, K. C. (1998) Analysis of DA-6034, a new flavonoid derivative in biological fluids by HPLC. *Yakhak Hoeji* **42**: 149–152

Lomax, A. E., Fernández, E., Sharkey, K. A. (2005) Plasticity of the enteric nervous system during intestinal inflammation. *Neurogastroenterol. Motil*. **17**: 4–15

Middleton, E., Kandaswami, G. (1993) Plant flavonoid modulation of immune and inflammatory cell functions. In: Klurfeld, D. M. (ed.) *Human nutrition. A comprehensive treatise: nutrition and immunology*. Plenum Press, New York, pp 239–266

Morris, G. P., Beck, P. L., Herridge, M. S., Szewczuk, M., Depew, W., Wallace, J. L. (1989) A hapten-induced model for chronic inflammation and ulceration in the rat colon. *Gastroenterology* **96**: 795–803

Myers, B. S., Dempsey, D. T., Yasar, S., Martin, J. S., Parkman, H. P., Ryan, J. P. (1997) Acute experimental distal colitis alters colonic transit in rats. *J. Surg. Res*. **69**: 107–112

Nugent, S. G., Kumar, D., Rampton, D. S., Evans, D. F. (2001) Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. *Gut* **48**: 571–577

Ohtsuka, Y., Sanderson, I. R. (2003) Dextran sulfate sodium-induced inflammation is enhanced by intestinal epithelial cell chemokine expression in mice. *Pediatr Res* **53**: 143–147

Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y., Nakaya, R. (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* **98**: 694–702

Pantzar, N., Ekstrom, G. M., Wang, Q., Westrom, B. R. (1994) Mechanisms of increased intestinal [^{51}Cr]EDTA absorption during experimental colitis in the rat. *Dig. Dis. Sci*. **39**: 2327–2333

Shim, H. J., Kim, Y. C., Lee, J. H., Ahn, B. O., Kwon, J. W., Kim, W. B., Lee, I., Lee, M. G. (2004) Pharmacokinetics of intravenous and oral DA-8159, a new erectogenic, in rats with protein–calorie malnutrition. *J. Pharm. Pharmacol*. **56**: 1543–1550

Wu, C. M. (1985) The chemical constituents of *Artemisiae* species (III). Isolation and identification of the lipophilic constituents from *Artemisiae argyi*. *Chung Yao Tung Pao* **10**: 31–32

Yoon, W. H., Yoo, J. K., Lee, J. W., Shim, C. K., Lee, M. G. (1998) Species differences in pharmacokinetics of a hepatoprotective agent, YH439, and its metabolites, M4, M5, and M7, after intravenous and oral administration to rats, rabbits, and dogs. *Drug Metab. Dispos*. **26**: 152–163

You, K. M., Jong, H. G., Kim, H. P. (1999) Inhibition of cyclooxygenase/lipoxygenase from human platelets by polyhydroxylated/methoxylated flavonoids isolated from medicinal plants. *Arch. Pharm. Res*. **22**: 18–24