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Hepatobiliary excretion and enterohepatic circulation of colchicine in rats

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Abstract

This study investigated the pharmacokinetics of unbound colchicine in rat blood, liver and bile, and its interaction with cyclosporin A (CsA; P-glycoprotein inhibitor) and proadifen (non-specific cytochrome P450 inhibitor) by using a microdialysis and liquid chromatographic system. The pharmacokinetics of colchicine in rat blood showed elimination in a nonlinear manner within the dosage ranges of 1–10 mg/kg. Twenty minutes after administration, colchicine reached maximum concentration in the liver and bile. The liver-to-blood distribution ratios (AUC_{liver}/AUC_{blood}) were 1.8 ± 0.6, 1.0 ± 0.2 and 0.8 ± 0.1, and the bile-to-blood distribution ratios (AUC_{bile}/AUC_{blood}) were 121.6 ± 24.7, 102.2 ± 13.4 and 116.5 ± 18.4 at dosages of 1, 3 and 10 mg/kg, respectively. The high hepatobiliary excretion of colchicine may lead to increased toxicity in normal tissues and indicates that colchicine undergoes hepatobiliary excretion against the concentration gradient from bile-to-blood. The area under the curse (AUC) of colchicine in the liver increased in the proadifen-treated groups, suggesting that metabolism of colchicine may involve cytochrome P450. CsA pretreatment caused an increase in the AUC of colchicine in the blood, a decreased AUC in the bile, and a profound decline in the bile-to-blood distribution ratio. Furthermore, the acute diarrhea and body weight loss caused by colchicine were delayed by pretreatment with CsA. These results indicate that the hepatobiliary excretion of colchicine was regulated by P-glycoprotein (P-gp) and the related acute diarrhea could be modulated by CsA. By using a paired rats model, the enterohepatic circulation of colchicine was also observed.

Keywords: Colchicine; Cytochrome P450; Enterohepatic circulation; Hepatobiliary excretion; P-glycoprotein

1. Introduction

Colchicine is an alkaloid derived from the plant *Colchicum autumnale* which possesses anti-inflammatory and antimitotic characteristics and is most often used to treat symptoms of gout with rheumatic and nonrheumatic conditions. The anti-inflammatory action mechanism of colchicine may inhibit neutrophil chemotaxis, thereby decreasing the inflammatory process (Ben-Chetrit and Levy, 1998). Colchicine inhibits the function of polymorphonuclear leukocytes, and dermatoses with a strong presence of these cells may benefit the most from the administration of this medication (Sullivan et al., 1998).

The use of colchicine in the treatment of liver cirrhosis and primary biliary cirrhosis was reported (Sabouraud et al., 1992). We previously demonstrated that colchicine enhances the radiosensitivity of human hepatoma HA22T/VGH cells (Liu et al., 2005). However, detailed colchicine levels in the liver tissue and bile were not measured. The influence of hepatic diseases on colchicine disposition should be investigated in order to define the most appropriate therapeutic dosing. Pharmacokinetic studies have been relatively limited and their results somewhat contradictory, with mean terminal elimination half-lives of 19 min to 9 h being reported (Levy et al., 1991). Some of these differences may be attributed to assay difficulties. Radioimmunoassay has been used to study post-mortem tissue concentrations and the toxicokinetics of colchicine in cases of acute human poisoning (Rochdi et al., 1992). [Ring C-methoxy-^{3}H]colchicine was used to study the pharmacokinetics of protein unbound colchicine with a limit of detection of 0.15 ng/ml (Desrayaud et al., 1997). In contrast to radioisotope labeling,

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liquid chromatography has a limit of detection of 1 ng/ml for colchicine in serum (Ko et al., 1990).

P-gp is normally located on the apical surface of enterocytes, the biliary canalicular membrane of hepatocytes, and the apical surface of endothelial cells in brain capillaries to protect the organism against xenobiotics by excreting them into the body (Thiebaut et al., 1987). Colchicine is a substrate for the multidrug resistance transporter, P-gp (Riordan and Ling, 1979). Co-administration of SDZ PSC 833 (P-gp inhibitor) increased the brain penetration of colchicine (Desrayaud et al., 1997).

Hepatic transformation, biliary excretion and enterohepatic circulation may have significant effects on the pharmacokinetics of a number of drugs. According to classical pharmacokinetics principles, several factors, including chemical properties and membrane-penetration capacities, can influence distribution or excretion. In addition, hepatic blood flow, protein binding and hepatic intrinsic clearance also play important role. The present study develops a microdialysis coupled to liquid chromatographic system to investigate the mechanism of hepatobiliary excretion and enterohepatic circulation of colchicine which may be regulated by P-gp. Besides, severe diarrhea of colchicine is delayed by pretreatment with P-gp inhibitor.

2. Methods and materials

2.1. Animal experimentation

All experimental protocols involving animals were reviewed and approved by the institutional animal experimentation committee of the National Yang-Ming University and Mackay Memorial Hospital. All animal care and husbandry were conducted in accordance with the Guide for the Care and Use of Laboratory Animal in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Male specific pathogen-free Sprague–Dawley rats weighing 250–300 g were obtained from the Laboratory Animal Center of National Yang-Ming University or the National Laboratory Animal Center, Taipei, Taiwan. The rats were initially anesthetized with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.), and remained anesthetized throughout the experimental period. The femoral vein was exposed for drug administration. The rat's body temperature was maintained at 37 °C with a heating pad during the experiment. In the colchicine-induced diarrhea experiments, rats were housed on wire-bottom cages with paper underneath. Rats received standard rodent chow (Laboratory Autoclavable Rodent Diet 5001, LabDiet, St. Louis, MO, USA) and water *ad libitum* during the experiments.

2.2. Chemicals and reagents

Colchicine and cyclosporin A (CsA) (Sandimmun) were purchased from Sigma Chemicals (St. Louis, MO, USA) and Novartis Pharma (Basle, Switzerland), respectively. The solvents and reagents for chromatography were purchased from BDH (Poole, UK). Triply de-ionized water from Millipore (Bedford, MA, USA) was used for all preparations. Phosphate buffered saline (PBS) was used to dissolve colchicine and CsA for tail-vein injection. The pH value after preparation to the target concentration was 7.0.

2.3. Liquid chromatography

Liquid chromatographic grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). The HPLC system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an off-line fraction collector (CMA 140, Stockholm, Sweden) equipped with a 20 μl sample loop, and an ultraviolet detector (Varian, Walnut Creek, CA, USA). Colchicine in the dialysate was resolved using an Agilent Zorbax extend-C18 column (4.6 mm × 150 mm i.d., particle size 5 μm) maintained at room temperature of 23–25 °C. The mobile phase was comprised of acetonitrile–1 mM octanesulfonic acid (28:72, v/v, pH 3.0 adjusted with orthophosphoric acid) and the flow rate of the mobile phase was 1 ml/min. The mobile phase was filtered through a Millipore 0.45 μm filter and degassed prior to use. The optimal UV detection for colchicine was set at a wavelength of 245 nm. Output data from the detector were integrated via an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.4. Method validation

For assessment of intra-assay and inter-assay variabilities, colchicine was assayed (six replicates) at concentrations of 0.01, 0.05, 0.1, 0.50, 1.00, 5.00, 10.00, 50.00 and 100.00 μg/ml on the same day and on six sequential days, respectively. The accuracy (%Bias) was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentration (C_{obs}) as follows: Bias (%) = $[(C_{obs} - C_{nom})/C_{nom}] \times 100$. The precision relative standard deviation (R.S.D.) was calculated from the observed concentrations as follows: %R.S.D. = [standard deviation (S.D.)/C_{obs}] × 100. Accuracy (%Bias) and precision (%R.S.D.) values of the limit of the quantification were predefined as within ±15% (Bressolle et al., 1996). The lowest concentration of the calibration curve served as the limit of quantification.

2.5. Microdialysis experiment

Blood, liver and bile microdialysis systems consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes. The dialysis probes for blood and liver (1 cm in length) (Tsai, 2003) were made of silica capillaries in a concentric design with the tips covered by a dialysis membrane (Spectrum, 150 μm outer diameter with a cut-off at a nominal molecular mass of 13,000 Da, Laguna Hills, CA, USA). The blood and liver microdialysis probes were positioned within the jugular vein/right atrium and the median lobe of the liver and then perfused with anticoagulant citrate dextrose (ACD) solution (citric acid 3.5 mM; sodium citrate 7.5 mM; dextrose 13.6 mM) at a flow-rate of 2.4 μl/min. The bile duct microdialysis probes were developed in our laboratory (Tsai, 2001;

Tsai et al., 2001; Tsai et al., 2002). A 7 cm dialysis membrane was inserted into a polyethylene tube (PE-60; 0.76 mm i.d.; 1.22 mm o.d., Clay-Adams, NJ, USA). The ends of the dialysis membrane and PE-60 were inserted into silica tubing (40 μm i.d; 140 μm o.d., SGE, Australia) and PE-10 (0.28 mm i.d.; 0.61 mm o.d.), respectively. Both the ends of the tubing and the union were cemented with epoxy and allowed to dry for a period of 24 h. Shunt microdialysis probe implantation was connected to the PE-60 tubing. The inlet of microdialysis probe was connected from anterior region of the proximal part of bile duct. The outlet of microdialysis probe was inserted back to the posterior region of distal part of the bile duct. After bile juice flowed through the microdialysis probe and colchicine penetrate the dialysis membrane into the dialysate according to the concentration gradient, outlet of the shunt probe was connected to the distal portion of bile duct which allowing bile to flow back the duodenum. Following bile duct cannulation, the probe was perfused with Ringer's solution at a flow rate of 2.4 μl/min. All dialysates collected from rat blood, liver and bile were analyzed by the liquid chromatographic system. Multiple microdialysis probes for blood, liver and bile microdialysis sampling were carried out on the same animals. All animals were used only once. The in vivo probe recovery was determined by estimating the loss (the extraction ratio) of colchicine, which was calculated from the concentration in the dialysate (C_{out}) relative to the concentration of colchicine in the perfusate (C_{in}). Recovery (R_{dial}) was expressed using the following equation: $R_{dial} = 1 - (C_{out}/C_{in})$. The in vivo methods for the recovery rat blood microdialysates and bile (Tsai, 2003; Tsai and Tsai, 2004) using microdialysis probes have been described in previous reports.

2.6. Pharmacokinetic analysis

The concentrations of colchicine in rat dialysates were determined from the calibration curves. The midpoint of the 10 min periods was used as the sampling time for the construction of blood and bile colchicine microdialysate concentration–time profiles. After a 2 h post-surgical stabilization period, colchicine (1, 3 or 10 mg/kg, i.v.) was administered to the control group ($n = 6$). For CsA treatment, 20 mg/kg of CsA was administered via the left femoral vein 10 min prior to colchicine injection. For proadifen treatment, 10 mg/kg of proadifen was injected via the left femoral vein 10 min prior to colchicine injection. The volume of each injection was 1 ml/kg. Blood, liver and bile dialysates were assayed by liquid chromatography on the same experimental day. Colchicine concentrations in blood, liver and bile were corrected by the estimated in vivo recoveries from the respective microdialysis probes. Microdialysate concentrations (C_m) of colchicine were converted to unbound concentrations (C_u) as follows: $C_u = C_m/R_{dial}$. The microdialysate recovery and concentration calculations were performed according to the method in our previous report (Tsai and Tsai, 2004). Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic calculation software WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC, USA) by the noncompartmental method.

2.7. Model of colchicine-induced acute diarrhea

To establish a model of colchicine-induced acute diarrhea, groups of rats ($n = 2$–5) were treated with various doses of colchicine by injection via the tail vein. The Sprague–Dawley male rats injected with doses equal to or greater than 0.9 mg/kg body weight colchicine (0.9, 1, 10 mg/kg) all died within 1 h after injection without diarrhea. According to these preliminary experiments, we found that a dose of colchicine of 0.7 mg/kg consistently caused severe acute diarrhea within 1 day without mortality and the diarrhea subsided after 4–6 days. To test the effect of CsA, experiments were performed using this dose of colchicine for generating acute diarrhea. Scoring of the diarrhea was conducted twice a day 30 min prior to injection and for 6 consecutive days thereafter. Body weight was monitored on a daily basis throughout the experiment. The severity of the diarrhea was scored by a modified scale described by others (Kurita et al., 2000): 0 (normal; normal stool or absent); 1 (slight; slightly wet and soft stool); 2 (moderate; unformed liquid stool mixed with some small stool particles); 3 (severe; unformed liquid stool without visible particles and with severe perianal staining of the coat). Changes in the diarrhea score and body weight were used to evaluate the severity of diarrhea for each animal.

2.8. Administration of colchicine and CsA

Totally three groups of rats ($n = 5$ in each group) were treat with solvent (PBS), colchicine (0.7 mg/kg of body weight) alone or CsA pretreatment (20 mg/kg of body weight, by injection via tail vein for 2 consecutive days) plus colchicine (10 min after the second dose of CsA).

2.9. Enterohepatic circulation

The paired rats model for the enterohepatic circulation was designed as follows: a donor rat (for drug given) and a recipient rat (no drug administration), age and weight (280–320 g) matched, were initially anesthetized with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.). The rats remained anesthetized throughout the experiment period with body temperatures maintained at 37 °C with a heating pad. The bile duct of the donor rat was cannulated proximal to the liver with a 20 cm section of PE 10 tubing (i.d. 0.28 mm o.d. 0.61 mm). The other end of the tubing was inserted through the bile duct into the duodenum of the recipient rat. To balance the fluid losses and gains in the donor and recipient rats, the bile duct of the recipient rat was also cannulated to channel bile back to the donor rat (Tsai et al., 2000). After the surgical procedures, colchicine (10 mg/kg) and CsA (20 mg/kg) were intravenously injected into the femoral vein of the donor rat. The dialysates were collected from the jugular vein of the donor and recipient rats for additional assay by liquid chromatography.

2.10. Statistics

The results are represented as mean ± standard error of the mean. Statistical analyses were performed with SPSS version

10.0 (SPSS Inc. Chicago, IL, USA). One-way ANOVA was followed by a Dunnett's post hoc test comparison between the control (colchicine treated alone), CsA, and proadifen treated groups. All statistical tests were performed at the two-tailed 5% level of significance.

3. Results

3.1. Method validation

Typical chromatograms of colchicine in rat blood, liver and bile dialysis are shown in Figs. 1–3 with a retention time of 3.8 min. Fig. 1A shows a standard injection of colchicine (1.0 μg/ml), and Fig. 1B shows the chromatogram of a blank blood dialysate from pre-drug administration. Fig. 1C shows the chromatogram of a blood dialysate sample containing colchicine (0.47 μg/ml) collected 10 min after colchicine administration (3 mg/kg).

Fig. 2A shows the chromatogram of a standard injection of colchicine (1.0 μg/ml), and Fig. 2B shows the chromatogram of a blank liver dialysate. Fig. 2C shows the chromatogram of a liver dialysate sample containing colchicine (0.35 μg/ml) collected 20 min after colchicine administration (3 mg/kg).

Fig. 3A shows the chromatogram of a standard injection of colchicine (10.0 μg/ml), and Fig. 3B shows the chromatogram of a blank bile dialysate. Fig. 3C shows the chromatogram of a bile dialysate sample containing colchicine (8.93 μg/ml) collected 80 min after colchicine administration (3 mg/kg).

The intra-assay and inter-assay precision and accuracy of colchicine were well within the predefined limits of acceptability (Table 1). The average microdialysate recoveries of colchicine (1, 5 and 10 μg/ml) for blood, liver and bile were 26.1 ± 0.9, 19.8 ± 0.7 and 53.3 ± 1.6%, respectively. This method is sufficiently sensitive to allow measurement of colchicine in rat blood, liver and bile for pharmacokinetic study.

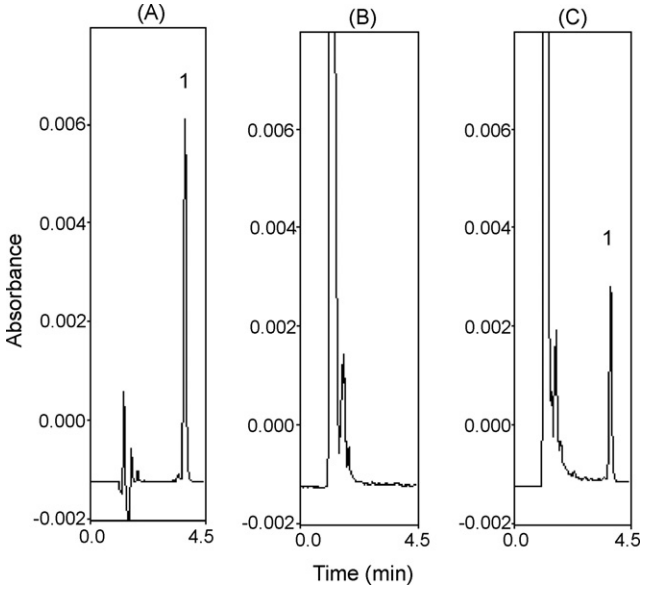

Fig. 1. (A) Standard injection of colchicine (1.0 μg/ml), and (B) chromatogram of a blank blood dialysate from the pre-drug administration. None of the observed peaks interfered with the analyte. (C) Chromatogram of a blood dialysate sample containing colchicine (0.47 μg/ml) collected 10 min after colchicine administration (3 mg/kg). (1) Colchicine.

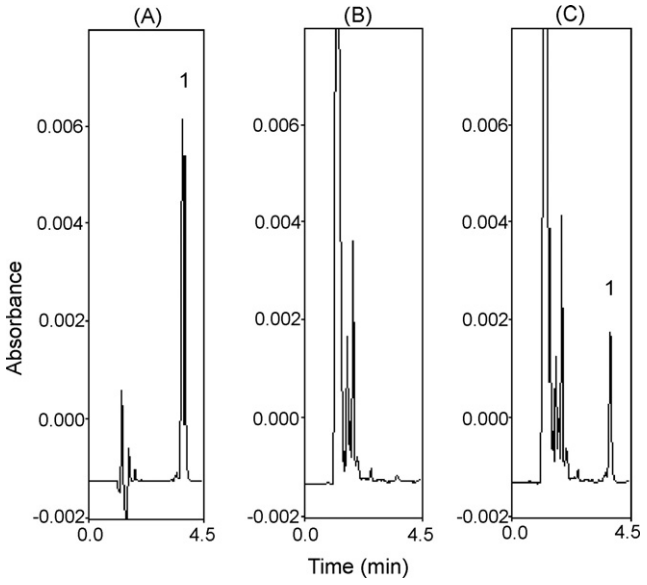

Fig. 2. (A) Chromatogram of a standard colchicine (1.0 μg/ml), and (B) chromatogram of a blank bile dialysate. None of the observed peaks interfered with the analyte. (C) Chromatogram of bile dialysate sample containing colchicine (0.35 μg/ml) collected 20 min after colchicine administration (3 mg/kg). (1) Colchicine.

3.2. Pharmacokinetics of colchicine in blood, liver and bile in dose-dependent

Microdialysis, which excludes large molecules and thus simplifies the sample cleaning procedures often necessary preceding

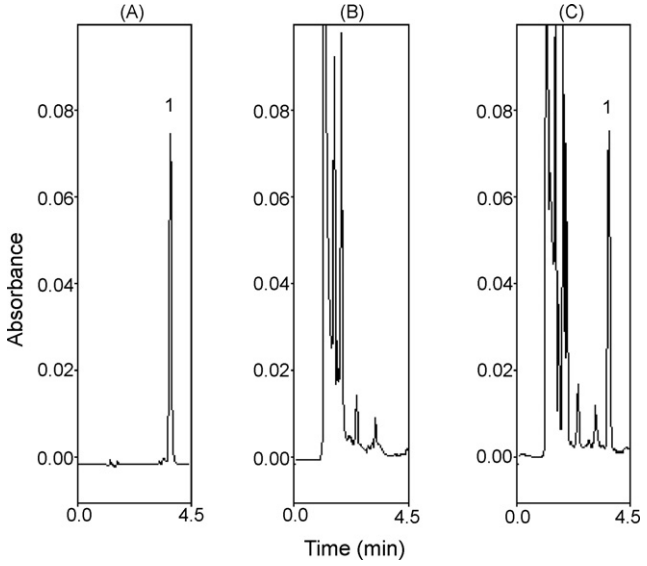

Fig. 3. (A) Chromatogram of a standard colchicine (10.0 μg/ml), and (B) chromatogram of a blank bile dialysate. None of the observed peaks interfered with the analyte. (C) Chromatogram of bile dialysate sample containing colchicine (8.93 μg/ml) collected 80 min after colchicine administration (3 mg/kg). (1) Colchicine.

Table 1
Intra-assay and inter-assay precision (R.S.D.) and accuracy (Bias) of the HPLC method for the determination of colchicine

Nominal concentration (μg/ml)	Observed concentration (μg/ml)	R.S.D. (%)	Bias (%)
Intra-assay			
0.01	0.011 ± 0.001	9.1	10.0
1	1.002 ± 0.003	0.3	0.2
100	99.91 ± 0.30	0.3	−0.09
Inter-assay			
0.01	0.009 ± 0.001	11.1	−10.0
1	0.999 ± 0.001	0.1	−0.1
100	100.05 ± 0.43	0.4	0.05

Data expressed as means ± S.D. ($n = 6$).

liquid chromatographic analysis, provides an excellent means by which unbound drugs in multiple sites in biological fluids and tissues can be concurrently monitored (De Lange et al., 2000). The dialysates were quickly and adequately resolved. Figs. 1–3 show typical chromatograms of colchicine standard, dialysates prior to intravenous administration and those from a rat sample following intravenous administration of colchicine in blood, liver and bile.

Mean colchicine blood, liver and bile concentrations versus time profiles at doses of 1, 3 and 10 mg/kg are presented in Figs. 4–6. The profiles suggest that the pharmacokinetics of colchicine in rat blood, liver and bile exhibited dose dependence in the 1–10 mg/kg range. The concentration of colchicine in the liver and bile gradually increased, reaching a peak concentration in about 20–30 min. The concentration of colchicine in bile was significantly higher than that in blood, suggesting active excretion of colchicine from bile-to-blood at doses of 1–10 mg/kg (Tables 2–4). The hepatobiliary excretion of colchicine was defined as the liver-to-blood

Table 2
Pharmacokinetic data of colchicine (1 mg/kg) in rat blood, liver and bile, both with and without treated with CsA (20 mg/kg) or proadifen (10 mg/kg)

Drug treatment	Colchicine (1 mg/kg)		
	Colchicine alone	With CsA	With proadifen
Blood			
AUC (min μg/ml)	14.8 ± 1.7	24.7 ± 2.2*	16.2 ± 2.3
C_{max} (μg/ml)	1.3 ± 0.4	0.9 ± 0.2	1.0 ± 0.2
Cl (ml/(kg min))	72.9 ± 9.9	42.2 ± 4.3*	67.1 ± 7.8
MRT (min)	16.0 ± 3.9	38.7 ± 3.5*	21.0 ± 1.6
Liver			
AUC (min μg/ml)	23.1 ± 6.8	14.1 ± 1.3	75.9 ± 15.6*
C_{max} (μg/ml)	0.5 ± 0.2	0.4 ± 0.1	1.7 ± 0.4*
MRT (min)	47.2 ± 3.9	45.3 ± 3.8	51.4 ± 3.5
Bile			
AUC (min μg/ml)	1756 ± 441	315 ± 53*	1716 ± 94
C_{max} (μg/ml)	48.5 ± 9.1	5.4 ± 0.8*	43.7 ± 2.5
MRT (min)	72.5 ± 4.1	111.9 ± 6.2*	77.2 ± 5.0
AUC_{liver}/AUC_{blood}	1.8 ± 0.6	0.6 ± 0.05	5.0 ± 1.0*
AUC_{bile}/AUC_{blood}	121.6 ± 24.7	12.9 ± 1.8*	115.2 ± 15.2

Data are expressed as mean ± S.E. mean ($n = 6$). *$p < 0.05$ significantly different from the colchicine alone group.

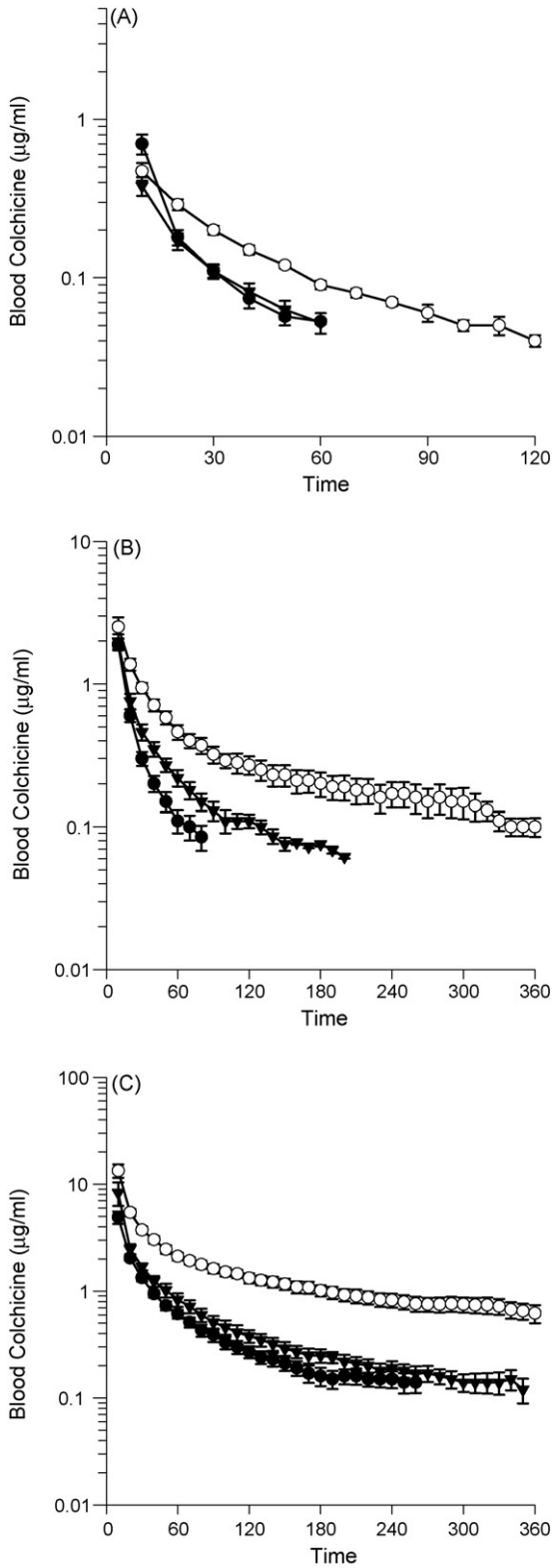

Fig. 4. Concentration–time profiles for colchicine in blood after intravenous administration of (A) 1 mg/kg colchicine, (B) 3 mg/kg colchicine and (C) 10 mg/kg colchicine alone (closed circle), combined with CsA (20 mg/kg) (open circle) and combined with proadifen (10 mg/kg) (closed triangle). Each group of data is represented as mean ± S.E. mean from six individual microdialysis experiments.

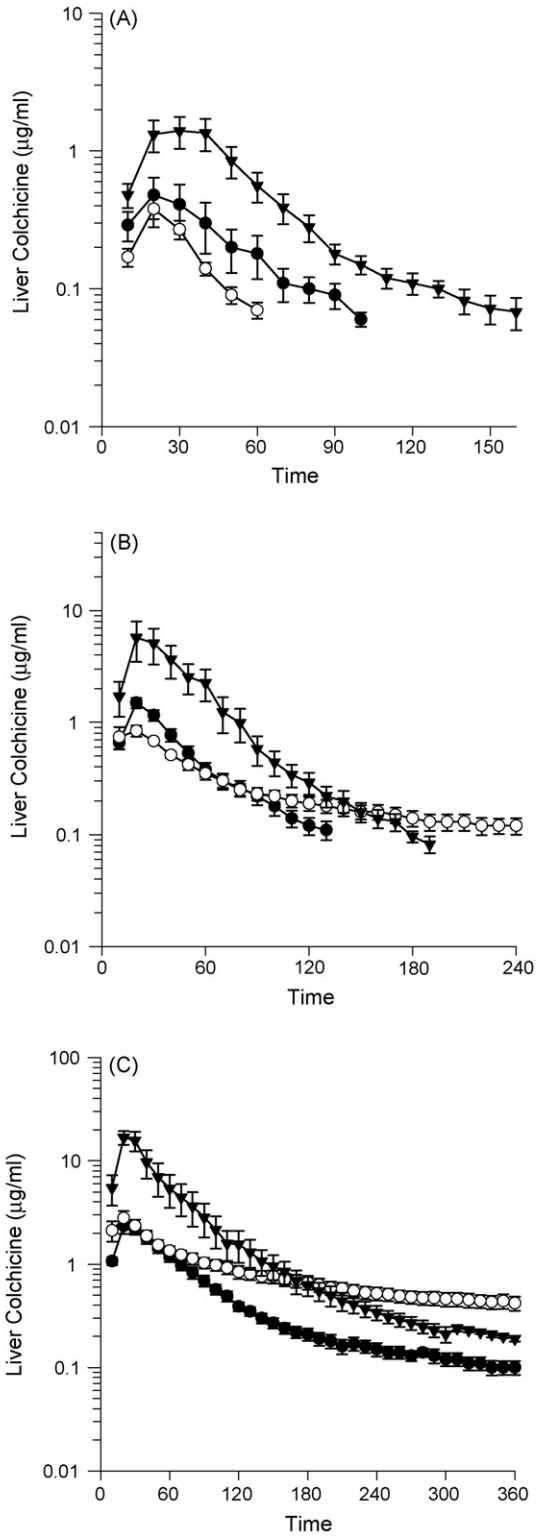

Fig. 5. Concentration–time profiles for colchicine in liver after intravenous administration of (A) 1 mg/kg colchicine, (B) 3 mg/kg colchicine and (C) 10 mg/kg colchicine alone (closed circle), combined with CsA (20 mg/kg) (open circle) and combined with proadifen (10 mg/kg) (closed triangle). Each group of data is represented as mean ± S.E. mean from six individual microdialysis experiments.

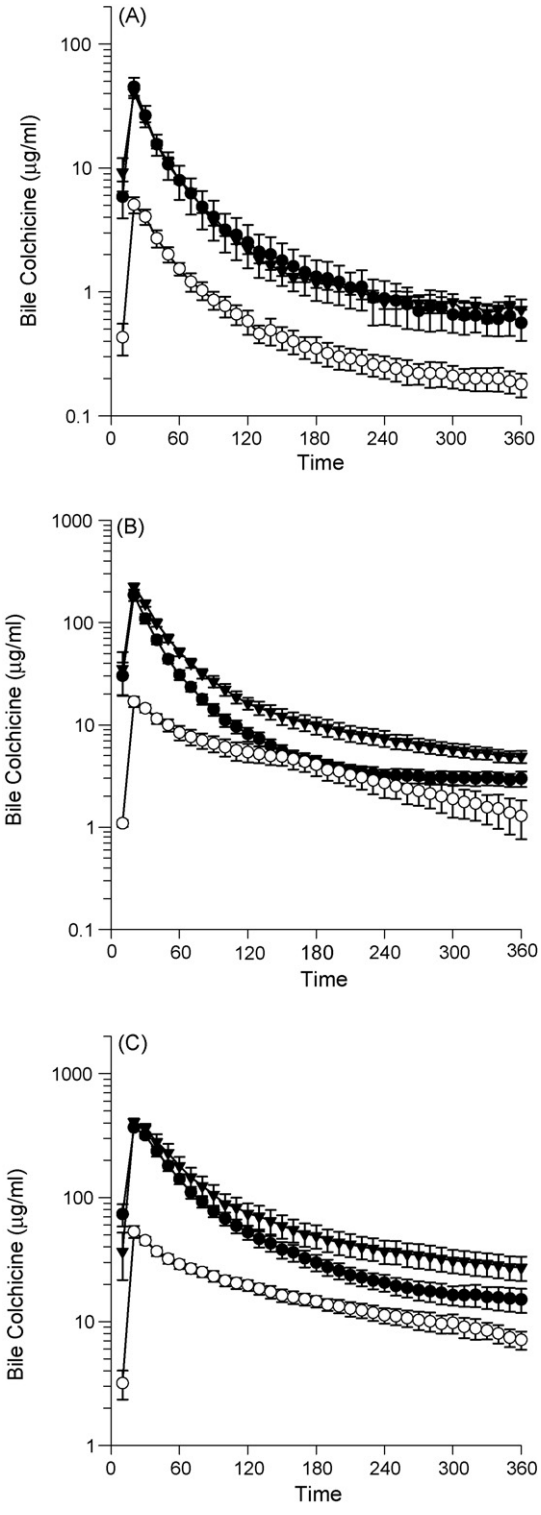

Fig. 6. Concentration–time profiles for colchicine in bile after intravenous administration of (A) 1 mg/kg colchicine, (B) 3 mg/kg colchicine and (C) 10 mg/kg colchicine alone (closed circle), combined with CsA (20 mg/kg) (open circle) and combined with proadifen (10 mg/kg) (closed triangle). Each group of data is represented as mean ± S.E. mean from six individual microdialysis experiments.

Table 3
Pharmacokinetic data of colchicine (3 mg/kg) in rat blood, liver and bile, both with and without treated with CsA (20 mg/kg) or proadifen (10 mg/kg)

Drug treatment	Colchicine (3 mg/kg)		
	Colchicine alone	With CsA	With proadifen
Blood			
AUC (min μg/ml)	68.6 ± 7.8	162.3 ± 21.8*	81.6 ± 10.2
C_{max} (μg/ml)	6.4 ± 1.1	4.8 ± 1.1	5.3 ± 0.8
Cl (ml/(kg min))	47.3 ± 6.6	20.6 ± 3.1*	40.7 ± 6.6
MRT (min)	15.4 ± 2.5	118.2 ± 12.0*	35.0 ± 4.2
Liver			
AUC (min μg/ml)	67.4 ± 7.1	92.5 ± 10.2	263.0 ± 77.4*
C_{max} (μg/ml)	1.5 ± 0.2	0.9 ± 0.1	5.8 ± 2.2*
MRT (min)	51.4 ± 4.2	172.7 ± 24.4*	55.1 ± 5.2
Bile			
AUC (min μg/ml)	6572 ± 640	1963 ± 391*	10173 ± 914*
C_{max} (μg/ml)	185.6 ± 23.0	5.4 ± 0.8*	225.7 ± 16.0
MRT (min)	75.4 ± 6.6	155.1 ± 17.6*	90.7 ± 6.6
AUC_{liver}/AUC_{blood}	1.0 ± 0.2	0.6 ± 0.06	3.3 ± 1.0*
AUC_{bile}/AUC_{blood}	102.2 ± 13.4	12.4 ± 1.8*	134.2 ± 17.8

Data are expressed as mean ± S.E. mean ($n = 6$). *$p < 0.05$ significantly different from the colchicine alone group.

and bile-to-blood distribution (k value), which was calculated by dividing the colchicine AUC in liver or bile by that in blood ($k_{liver} = AUC_{liver}/AUC_{blood}$) ($k_{bile} = AUC_{bile}/AUC_{blood}$) (De Lange et al., 2000). The liver-to-blood distributions were 1.79 ± 0.57, 1.04 ± 0.15 and 0.84 ± 0.07; the bile-to-blood distributions were 121.55 ± 24.65, 102.15 ± 13.43 and 116.46 ± 18.40 after colchicine injection at doses of 1, 3 and 10 mg/kg, respectively (Table 5). These results indicate that colchicine goes through hepatobiliary excretion and against the concentration gradient from a lower blood level excreted to a higher level in bile.

Table 4
Pharmacokinetic data of colchicine (10 mg/kg) in rat blood, liver and bile, both with and without treated with CsA (20 mg/kg) or proadifen (10 mg/kg)

Drug treatment	Colchicine (10 mg/kg)		
	Colchicine alone	With CsA	With proadifen
Blood			
AUC (min μg/ml)	221.6 ± 15.4	874.3 ± 93.0*	393.6 ± 95.9
C_{max} (μg/ml)	12.6 ± 2.8	36.2 ± 9.8*	30.3 ± 12.4
Cl (ml/(kg min))	46.2 ± 3.3	12.2 ± 1.5*	30.5 ± 4.5
MRT (min)	54.2 ± 12.0	128.7 ± 18.0*	57.8 ± 7.3
Liver			
AUC (min μg/ml)	183.7 ± 15.9	391.6 ± 49.6	1018.3 ± 221.5*
C_{max} (μg/ml)	2.5 ± 0.4	2.8 ± 0.5	17.7 ± 2.5*
MRT (min)	98.0 ± 9.5	219.9 ± 30.2*	80.5 ± 14.1
Bile			
AUC (min μg/ml)	24743 ± 2416	8850 ± 1208*	35948 ± 5632*
C_{max} (μg/ml)	36704 ± 14.8	54.8 ± 4.9*	406.2 ± 28.3
MRT (min)	105.7 ± 7.6	292.1 ± 54.7*	164.1 ± 39.7
AUC_{liver}/AUC_{blood}	0.8 ± 0.07	0.5 ± 0.04	2.9 ± 0.7*
AUC_{bile}/AUC_{blood}	116.5 ± 18.4	10.2 ± 1.2*	100.8 ± 14.7

Data are expressed as mean ± S.E. mean ($n = 6$). *$p < 0.05$ significantly different from the colchicine alone group.

Table 5
Unbound colchicine AUC in rat blood following the paired-rat model for the experiment of enterohepatic circulation after colchicine (10 mg/kg, iv) and CsA (20 mg/kg, iv) co-administration into donor rat

Parameter	AUC (min μg/ml)
Donor rat	847.7 ± 141.0
Recipient rat	55.5 ± 29.2*

Data are expressed as mean ± S.E. mean ($n = 4$). Significant difference was observed between the group of donor rat and recipient rat ($p < 0.05$).

3.3. Effect of cytochrome P450 inhibition on colchicine in blood, liver and bile

After co-administration of colchicine (1–10 mg/kg) and proadifen (a cytochrome P450 inhibitor) at doses of 10 mg/kg each, the AUC in blood was not significantly altered. However, the colchicine levels in liver and bile increased (Figs. 4–6). Comparing the liver-to-blood and bile-to-blood of colchicine distribution ratios, the liver-to-blood ratio was significantly increased from 1.8 ± 0.6, 1.0 ± 0.2 and 0.8 ± 0.07 to 5.0 ± 1.0, 3.3 ± 1.0 and 2.9 ± 0.7 at the doses of 1, 3 and 10 mg/kg, respectively, with proadifen co-administration (Tables 2–4). The results indicated that the metabolism of colchicine in the liver was clearly affected by proadifen, a cytochrome P450 inhibitor.

3.4. Effects of CsA co-administration on colchicine in blood

CsA co-administration significantly altered the blood profile of colchicine concentrations in the dose ranges of 1–10 mg/kg (Figs. 4–6). At a lower dose of 1 mg/kg, the blood AUC of colchicine alone and the CsA treated group were 14.8 ± 1.7 and 24.7 ± 2.2 min μg/ml. The blood AUC of colchicine alone and the CsA treated group were 68.6 ± 7.8 and 162.3 ± 21.8 min μg/ml, respectively, at a dose of 3 mg/kg colchicine. At a higher dose of 10 mg/kg, the blood AUC of colchicine increased from 221.6 ± 15.4 to 874.3 ± 93.0 min μg/ml with CsA co-administration. The results indicate that the AUC and the mean resident time (MRT) were significantly increased but the clearance (Cl) was decreased when CsA was co-administered (Tables 2–4).

3.5. Effects of CsA co-administration on colchicine in the liver and bile

Since there were no significant differences between the colchicine alone and colchicine co-administration groups, it is suggested that CsA could not alter the AUC of colchicine in the liver at doses of 1–10 mg/kg. However, the colchicine level in the bile decreased dramatically after it was co-administered with CsA at doses of 1, 3 and 10 mg/kg (Figs. 4–6). The AUC of colchicine alone and with CsA co-administered in bile were 1756 ± 441 and 315 ± 53 min μg/ml,

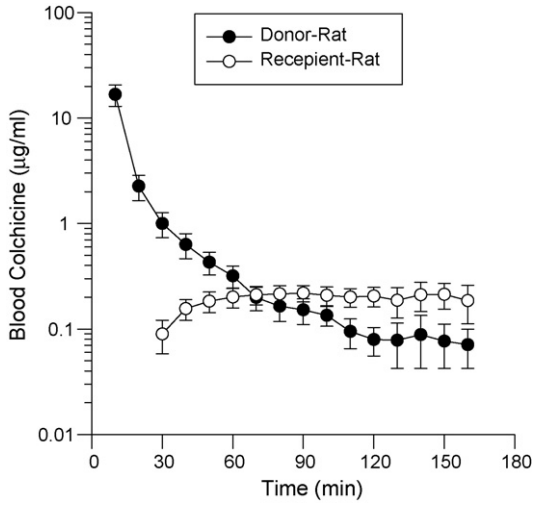

Fig. 7. The concentration–time profiles of colchicine in blood of the donor and recipient rats after colchicine (10 mg/kg) and CsA (20 mg/kg) co-administration ($n = 4$).

6572 ± 640 and 1963 ± 391 min μg/ml, and 24743 ± 2416 and 8850 ± 1208 min μg/ml, respectively, at colchicine doses of 1, 3 and 10 mg/kg (Tables 2–4). Since the liver-to-blood distribution ratio showed no difference but the bile-to-blood ratio was significantly reduced at each dose after co-administration with CsA, it is suggested that the bile efflux transport system of colchicine may be markedly affected by treatment with CsA, a P-gp inhibitor.

3.6. Enterohepatic circulation of colchicine

The high hepatobiliary excretion rate of colchicine suggests the drug potential to undergo enterohepatic circulation. The colchicine concentration in the blood declined in the donor rat, however, the colchicine level increased in the recipient rat after colchicine (10 mg/kg) and CsA (20 mg/kg) intravenous injection into the femoral vein of the donor rat (Fig. 7). The results show that the AUC of colchicine was 847.7 ± 141.0 and 55.5 ± 29.2 min μg/ml in the donor and recipient rats, respectively (Table 5).

3.7. Effects of CsA pretreatment on colchicine-induced diarrhea and body weight loss

Animals in the control group, which were only injected with PBS, had no diarrhea and their total scores remained 0 throughout the experiment course. After injection of 0.7 mg/kg colchicine via tail vein, all rats experienced severe diarrhea within 1 day. The highest diarrhea score was seen on day 1 and it persisted until day 4. Severe diarrhea gradually resolved on days 5–6 and completely resolved on day 7. Pretreatment with CsA delayed the development of severe diarrhea induced by colchicine for 1 day. Rats in this group experienced only mild diarrhea on day 1 and the same severity of diarrhea as the colchicine group from day 2 (Fig. 8). As shown in Fig. 9, a similar pattern of changes was observed in the body weight record. On day 0, there were no obvious differences between these three

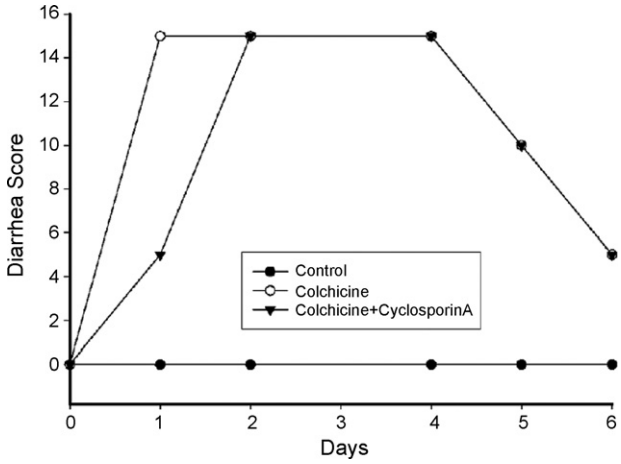

Fig. 8. Diarrhea scores of rats which received various treatments. Control: injected with PBS; colchicine alone: 0.7 mg/kg of body weight; colchicine plus CsA: CsA pretreatment (20 mg/kg of body weight by injection via tail vein for 2 consecutive days) plus colchicine (10 min after the second dose of CsA).

groups. The body weight rapidly decreased on day 1 after treatment with colchicine alone. Pretreatment with CsA delayed the rapid loss of body weight to day 2.

4. Discussion

From literature review, the protein binding of colchicine was weak. The protein-binding rate was ranged from 23 to 38.9% (Lannoy et al., 1994; Sabouraud et al., 1994). Most colchicine was unbound and active form in vivo. The liquid chromatographic method described here has been applied to the determination of unbound colchicine in rat blood, liver and bile with a quantification limit of 10 ng/ml. However, it may not be sufficiently sensitive to detect unbound colchicine in brain extracellular fluid. More sensitive techniques such as radioimmunoassay using [^{3}H]colchicine were employed for the direct measurement of colchicine levels without extrac-

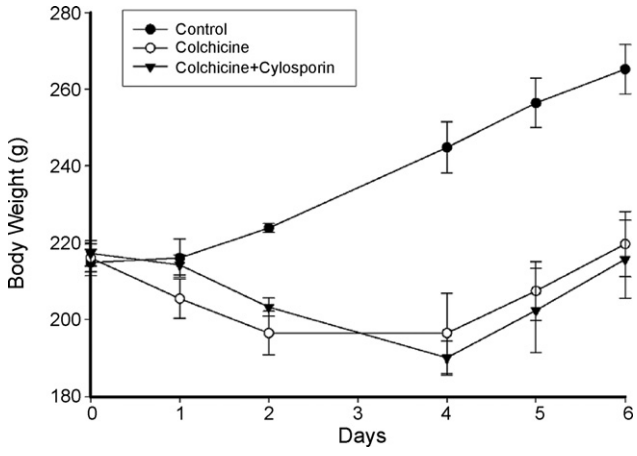

Fig. 9. Body weights of rats which received various treatments. Control: injected with PBS; colchicine alone: 0.7 mg/kg of body weight; colchicine plus CsA: CsA pretreatment (20 mg/kg of body weight by injection via tail vein for 2 consecutive days) plus colchicine (10 min after the second dose of CsA).

tion with a detection limit of 0.15 ng/ml (Scherrmann et al., 1980).

The pharmacokinetics of colchicine measured with radioimmunoassay has been well-established after single oral doses and its elimination half-life was 20 h (Dabouraud et al., 1992). The brain distribution ratio of protein unbound colchicine from brain-to-blood calculated by the formula AUC_{brain}/AUC_{blood} was 0.04 ± 0.01 in the frontal cortex of freely moving rats. The addition of SDZ PSC 833 (a P-gp inhibitor), resulted in a significant increase in the AUC ratio to 0.15 ± 0.06 which reveals that the blood–brain barrier penetration of colchicine is regulated by P-gp (Desrayaud et al., 1997).

In a case report, ingestion of *Colchicum autumnale*, which was confused with wild garlic (*Allium ursinum*), resulted in an episode of nausea, vomiting and watery diarrhea in one person, and death from multi-organ system derangement 48 h after ingestion of the colchicum leaves in another. At autopsy, hemorrhagic lung oedema, hypocellular bonemarrow, centrilobular fatty necrosis of the liver and necrosis of the proximal convoluted tubuli of the kidneys were seen. No colchicine was detected in the post-mortem blood but a colchicine concentration of 7.5 μg/ml was found in the bile (Klintschar et al., 1999). This finding is consistent with our results showing that a high concentration of colchicine in bile was due to the active transport process of hepatobiliary excretion. The colchicine concentrations in bile were approximately 100-fold higher than those in blood. In addition, the high hepatobiliary excretion of colchicine may lead to increased toxicity in normal tissues. The excretion ratios of colchicine from bile-to-blood (AUC_{bile}/AUC_{blood}) were 121.6 ± 24.7, 102.2 ± 13.4 and 116.5 ± 18.4 for doses of 1, 3 and 10 mg/kg, respectively (Tables 2–4). Additionally, the high excretion ratios of colchicine were reduced about 10-fold when co-administered with CsA.

A recent report indicates that colchicine has been used for treatment in symptomatic patients with primary biliary cirrhosis who respond incompletely to ursodeoxycholic acid treatment alone or in combination with methotrexate (Lee and Kaplan, 2003). The combination therapy resulted in a small but significant reduction in disease progress (Almasio et al., 2000). After intravenous administration, 68% of colchicine was excreted in the feces within 48 h, suggesting bile might be the major route of excretion for colchicine (Hunter and Klaassen, 1975). Our results support the idea that the therapeutic effects for patients with primary biliary cirrhosis may due to the active biliary excretion pathway.

As seen in Fig. 5, the concentrations of colchicine in the bile were meaningfully higher than those in the blood at the same period of time, indicating that colchicine was concentrated in the bile by an active transport mechanism. With a dose of 20 mg/kg CsA administered intravenously, hepatobiliary excretion of colchicine was reduced significantly ($p < 0.05$).

Many studies have shown that P-gp plays a transport role in hepatobiliary excretion of certain drugs (Meijer et al., 1997). Furthermore, in several liver diseases biliary transport is disturbed, resulting in jaundice and cholestasis. Many of these symptoms can be attributed to altered regulation of hepatic transporters (Roelofsen et al., 1997). The fact that intravenous co-administration of a single dose of CsA significantly affected the pharmacokinetics of colchicine emphasizes the role of P-gp in drug disposition. The observations were consistent with previous reports that major pharmacokinetic interactions resulted from co-administration of CsA with multidrug resistance-related anticancer agents (e.g., doxorubicin, daunorubicin, etoposide, paclitaxel, and vinblastine) (Sikic et al., 1997).

Severe acute diarrhea is the most common and lethal adverse effect of colchicine. It may correlate to the high throughput of biliary excretion and subsequent intestinal exsorption noted in the present study. By using a microdialysis-based pharmacokinetic model, we demonstrated that the P-gp inhibitor CsA significantly lowered the biliary excretion of colchicine. To verify the involvement of P-gp noted by the pharmacokinetic data, we tried to modulate the pharmacological effects of colchicine by pretreatment with CsA and revealed that CsA delayed the development of severe diarrhea and loss of body weight. The optimal dose of colchicine for inducing acute diarrhea without mortality in SD rats is 0.7 mg/kg. This dose is close to the 1.0 mg/kg used in pharmacokinetic experiments. This indicates that the biliary excretion of colchicine may be mediated by P-gp in vivo and the severe diarrhea caused by colchicine could be modulated by pretreatment with CsA.

Inhibition of cytochrome P450 activity by CsA (Nakamura et al., 1994) may also have contributed to the higher AUC for unbound colchicine in the blood at all three doses (1, 3 and 10 mg/kg). Effects resulting from protein-binding competition between colchicine and CsA cannot be excluded.

The microdialysis method determined concentrations of unbound colchicine. The bile flow rate is liable to be affected by the anesthesia condition of rat. Although bile flow of anesthetized rat could be affected by different medication (the bile composition and flow rate were different between rats anesthetized by pentobarbitone and urethane), however, all rats accepted the same anesthesia protocol avoiding this confounding factor (Butishauser and Stone, 1975). P-glycoprotein also plays an important role in bile flow modulation. In an isolated rat liver perfused model, after cyclosporine A (P-glycoprotein inhibitor) treatment, the basal bile flow significant decreased (Monache et al., 1999). The design of the present experiments, which enabled the preservation of bile and the bulk of its contents, did not allow for the estimation of enterohepatic circulation, enhancement of which by CsA co-administration was unlikely. Taken together, our findings suggest that CsA plays a major role in enhancing the bioavailability and, likely, the effects of colchicine, presumably through inhibition of P-gp-regulated active hepatobiliary excretion and perhaps P450 inhibition. The effects of a P-gp inhibitor such as CsA on the pharmacokinetics of co-administered drugs should be taken into consideration when designing drug treatment regimens.

Data presented in this report demonstrate that proadifen increased colchicine AUC in both rat liver and bile without significant alterations in the bile-to-blood distribution ratio (AUC_{bile}/AUC_{blood}). This observation may be explained by

decreased metabolism in which cytochrome P450 might be involved. Colchicine was mainly demethylated via cytochrome P450 3A4 in liver microsomes (Tateishi et al., 1997). Compared AUC_{liver} with pretreatment 10 mg/kg proadifen and treatment only with various doses colchicine (1, 3 10 mg/kg), the percentage were 30.4 (23.1/75.9), 25.6 (67.4/263.0) and 18.0 (183.7/1018.3), respectively (Tables 2–4). Proadifen was a non-specific cytochrome P450 inhibitor. From our experiment, the estimated percentage of administered colchicines was metabolized by cytochrome P450 ranged from 69.6 to 82% in the dose range from 1 to 10 mg/kg.

In conclusion, our data indicate that the disposition of colchicine might undergo phase I biotransformation and active hepatobiliary excretion in which P-gp might play an important role.

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