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Review

Effects of cysteine on the pharmacokinetics of intravenous 2-(allylthio)pyrazine, a new chemoprotective agent, in rats with protein-calorie malnutrition

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Abstract

The effects of cysteine on the pharmacokinetics of 2-(allylthio)pyrazine (2-AP) were investigated after intravenous administration of the drug (50 mg/kg) to control (Sprague–Dawley) rats (4-week fed on 23% casein diet), and rats with protein-calorie malnutrition (PCM, 4-week fed on 5% casein diet) and PCMC (PCM with 250 mg/kg of oral cysteine, twice daily starting from the fourth week). In rats with PCM, the area under the plasma concentration–time curve from time zero to time infinity (AUC) of 2-AP was significantly smaller than that in control rats. However, in rats with PCMC, the AUC of 2-AP was significantly greater than that in control rats and rats with PCM. This could be due to significantly greater formation of M4 in rats with PCM and significantly smaller formation of M4 in rats with PCMC than that in control rats. In rats with PCMC, some pharmacokinetic parameters of 2-AP restored fully or more than the levels of control rats. For example, in rats with PCMC, the apparent volume of distribution at steady state of 2-AP (7290, 16,600, and 7050 ml/kg for control rats, and rats with PCM and PCMC, respectively), the percentage of dose excreted in 24-h urine as unchanged 2-AP (0.242, 0.727, and 0.130%), and 'the amount' excreted in 24-h urine as M4 (100, 228, and 51%) were comparable to those in control rats. However, the AUC (739, 434, and 1240 μg/min/ml) and total body clearance (67.7, 115, and 40.2 ml/min/kg) of 2-AP were significantly greater and slower, respectively, than those in control rats. This could be at least partly due to increase in *S*-methyltransferase activity (to form M4) in rats with PCM and greater restoration of its activity (decrease in its activity) in rats with PCMC.

Keywords: 2-(Allylthio)pyrazine; Protein-calorie malnutrition with or without oral cysteine; Pharmacokinetics; Rats

Abbreviations: PCM, protein-calorie malnutrition; CL_{NR}, time-averaged nonrenal clearance; 2-AP, 2-(allylthio)pyrazine; AUC, area under the plasma concentration–time curve from time zero to time infinity; HPLC, high-performance liquid chromatography; PCMC, protein-calorie malnutrition with oral cysteine; CL, time-averaged total body clearance; AUMC, area under the first moment of the plasma concentration–time curve; MRT, mean residence time; V_{SS}, apparent volume of distribution at steady state

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1. Introduction

Protein-calorie malnutrition (PCM) is considered to be a global problem, especially for children, infants, and institutional elderly who are more susceptible to PCM (Denke and Wilson, 1998). A number of diseases including cancer, digestive disorders, and AIDS are also associated with PCM (Wykes et al., 1996; Denke and Wilson, 1998). The rate of drug metabolism may be influenced by various physiological, genetic, and environmental factors. Nevertheless, nutritional status was not usually investigated as a factor which may affect the pharmacokinetics of drugs, and hence, the pharmacodynamics of drugs. The changes in drug metabolism and pharmacokinetics in malnutrition were reviewed (Buchanan, 1978; Krishnaswamy, 1978).

The following results have been obtained recently from our laboratories (Cho et al., 1999). Western blot analysis revealed that rats with protein restriction (5% casein diet, PCM) for 4 weeks exhibited 60 and 80% suppression in the hepatic cytochrome P450 1A2 and 2C11 levels, respectively, and P450 2E1 and 3A1/2 levels decreased 40–50%, as compared to control (23% casein diet). Northern blot analysis showed that hepatic cytochrome P450 1A2, 2E1, 2C11, and 3A1/2 mRNAs also decreased in the state of PCM. Interestingly, the altered P450 expression by PCM completely or partly returned to the level of control by oral cysteine supplementation for 1 week (250 mg/kg, twice daily starting from the fourth week). Cysteine supplementation also prevented decreases in P450 1A2, 2E1, 2C11, and 3A1/2 mRNA levels by PCM. The metabolic rate of ethoxyresorufin dealkylase monitored in the hepatic microsomes produced from rats with PCM was consistent with changes in the expression of P450 1A2 apoprotein and mRNA.

The following results have also been obtained from our laboratories. After intravenous administration of azosemide, a loop diuretic, primarily metabolized by cytochrome P450 1A in rats (Lee and Lee, 1997), the time-averaged nonrenal clearance (CL_{NR}) of azosemide was significantly slower in rat with PCM (Kim et al., 2001a). Interestingly, cysteine supplementation (rats with PCMC) significantly reduced the plasma concentrations of the drug and concomitantly restored CL_{NR} to control levels (Kim et al., 2001a). After intravenous administration of adriamycin, primarily metabolized by cytochrome P450 3A in rats (Lee and Lee, 1999), the 24-h urinary excretion of M3, an aglycone metabolite of adriamycin, decreased in rats with PCM (Kim et al., 2000). However, by cysteine supplementation (rats with PCMC), the 24-h urinary excretion of M3 was significantly greater than that in rats with PCM (Kim et al., 2000). Above data indicated that cysteine was efficient in restoring cytochrome P450 expression and metabolic activities.

2-(Allylthio)pyrazine (2-AP, Fig. 1), a new chemoprotective agent, has been synthesized since diallylsulfide has chemoprotective activity and pyrazine has binding affinity to hepatic cytochrome P450 2E1 (inhibits CYP2E1 activity). The hepatoprotective, radioprotective, and chemoprotective effects of 2-AP have been reported in rats (Kim et al., 1996, 1997, 1998; Surh et al., 1997). It has been reported (Bu et al., 2000) that the plasma concentrations of 2-AP were significantly lower and the area under the plasma concentration–time curve from time zero to time infinity (AUC) was significantly smaller after intravenous administration of 2-AP to rats pretreated with dexamethasone (an inducer of CYP3A in rats; Komori and Oda, 1994), phenobarbital (an inducer of CYP 2B1/2, 2C6, 2C7, and 3A1/2 in rats; Correia, 1995; Kawamura et al., 1999), and 3-methylcholanthrene (an inducer of CYP 1A1/2 and 2E1 in rats; Correia, 1995; Spatzenegger et al., 2000). Hence, it was expected that the pharmacokinetics of 2-AP could be changed in rats with PCM and PCMC. The purpose of this paper is to report the pharmacokinetic changes of 2-AP after intravenous administration of the drug at a dose of 50 mg/kg to control rats and rats with PCM and PCMC.

2. Materials and methods

2.1. Chemicals

2-AP was donated by Dr. Young. H. Jung (College of Pharmacy, Sungkyunkwan University, Suwon, South Korea). Cysteine was purchased from Sigma Chemical Company (St. Louis, MO). Other chemicals were of reagent grade or high-performance liquid chromatographic (HPLC) grade, and therefore, were used without further purification.

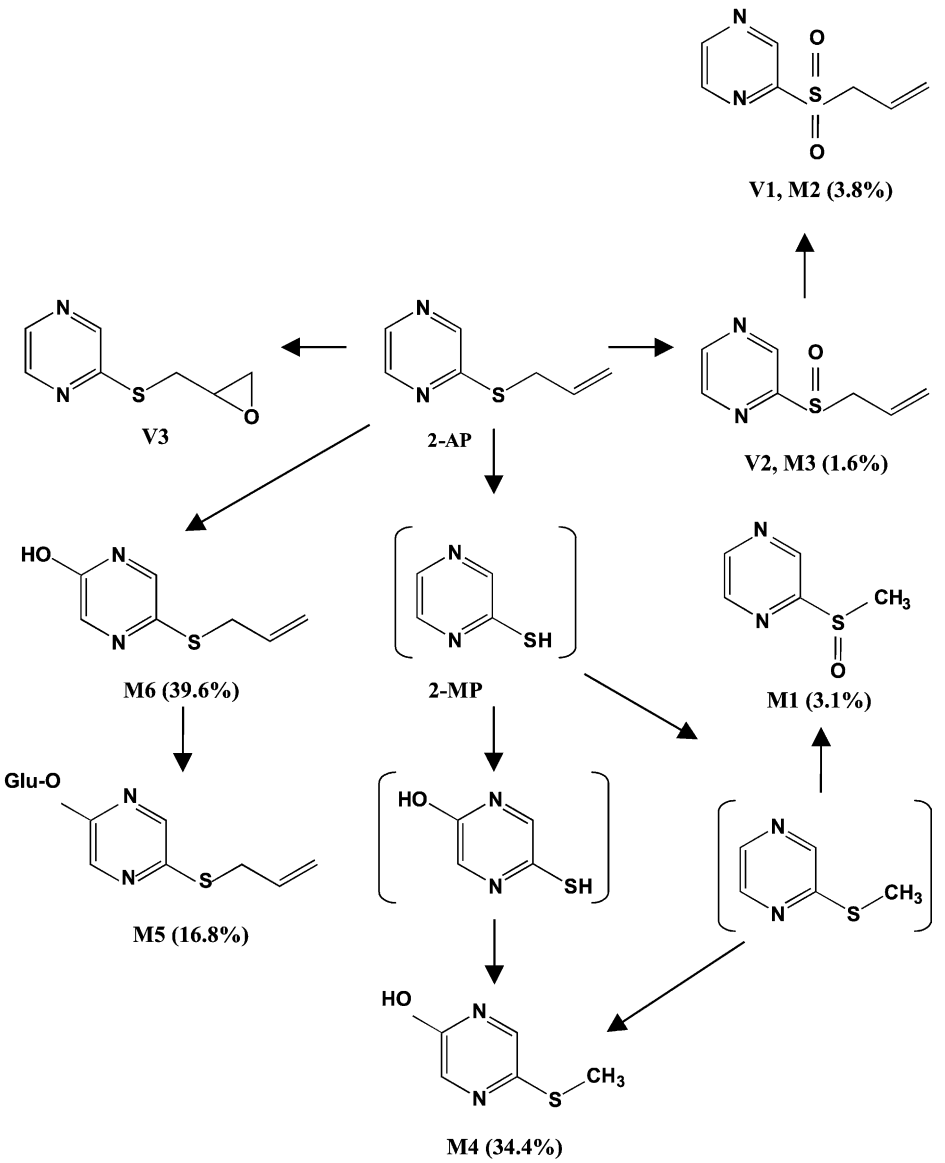

Fig. 1. Proposed metabolic pathways of 2-AP in rats; M1–M6 were identified in rat urine after oral administration of 2-AP, 50 mg/kg, to fasted rats. The numbers in parentheses represent the ratios of area of each peak to total area of all peaks based on HPLC chromatograms of rat urine. V1–V3 were identified after incubation of 2-AP with rat hepatic microsomal fraction. The chemical names of M1–M6 and V1–V3 are as follows: M1, 2-(*S*-methylthio)pyrazine sulfoxide; M2 (V1), 2-(allylthio)pyrazine sulfone; M3 (V2), 2-(allylthio)pyrazine sulfoxide; M4, 2-(*S*-methylthio)-*x*-hydroxylpyrazine; M5, 2-(allylthio)-*x*-hydroxylpyrazine-*O*-glucuronide; M6, 2-(allylthio)-*x*-hydroxylpyrazine; V3, 2-(allylthio)pyrazine epoxide; 2-MP, 2-mercaptopyrazine.

2.2. Rats and diets

Male Sprague–Dawley rats (weight 155–170 g) were purchased from Charles River Company (Atsugi, Japan). The rats were assigned randomly to one of two diets containing either 23% (control rats) or 5% (rats with PCM) casein. Both diets were isocaloric and the compositions of the diets were listed (Cho et al.,

1999). All rats were provided with food and water ad libitum and maintained on each diet for a 4-week period (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). From the start of the fourth week, rats with PCM were further divided randomly into two groups. One group (rats with PCMC) was treated with 250 mg/kg oral cysteine twice daily (cysteine was dissolved in tap water to make 100 mg/ml) and other group (rats with PCM) was treated with the same volume of tap water (without cysteine). Food intake and body weight were recorded at least once a week to assess the influence of the low protein diet.

2.3. Pretreatment of rats

In the early morning after a 4-week fed on each diet, the jugular vein and the carotid artery were catheterized with polyethylene tubing (Clay Adams, Parsippany, NJ) under light ether anesthesia. Both cannulae were exteriorized to the dorsal side of the neck where each cannula terminated with long Silastic tubing (Dow Corning, Midland, MI). Both Silastic tubings were covered with a wire coil to allow free movement of the rats. After the exposed areas were surgically sutured, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from the anesthesia for 4–5 h before the study began. They were not restrained at any time during the whole experimental period.

2.4. Intravenous study

2-AP (the oily 2-AP was suspended with 40% polyethylene glycol) at a dose of 50 mg/kg was administered over 1-min via the jugular vein of control rats ($n = 11$), and rats with PCM ($n = 10$) and PCMC ($n = 7$). Total injection volume was approximately 1 ml. Approximately 0.18 ml of blood was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 600 min after intravenous dosing and two 40-μl aliquots of each plasma sample were stored in a −70 °C freezer (Revco ULT 1490 D-N-S, Western Mednics, CA) until HPLC analysis of 2-AP (Han et al., 1998a). Urine was collected between 0 and 24 h, and an aliquot of the urine was stored in a −70 °C freezer until analysis of 2-AP (Han et al., 1998a). Other procedures were similar to those reported previously (Kim et al., 1993). At the end of experiment (24 h), as much blood as possible was collected via the carotid artery, and the plasma sample was stored in a refrigerator (SR-2307, Samsung Electronics, Seoul, South Korea) for the measurement of plasma protein binding. At the same time (24 h), the weight of whole liver, kidney, or stomach was recorded, and the whole gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker containing 50 ml of methanol (to facilitate extraction of 2-AP) and then cut into small pieces using scissors. After shaking manually and stirring with a glass rod for 10 min, two 100-μl aliquots of the supernatant were collected from the beaker and stored in a −70 °C freezer until HPLC analysis of 2-AP (Han et al., 1998a).

2.5. Tissue distribution study

2-AP (the same suspension as used in the intravenous study) at a dose of 50 mg/kg was intravenously administered over 1-min via the jugular vein of control rats, and rats with PCM and PCMC ($n = 5$, each). Thirty minutes after intravenous administration, as much blood as possible was collected via the carotid artery in a heparinized tube and each rat was sacrificed by cervical dislocation. After centrifugation of blood samples, each plasma was added with 4 vol. of 0.9% NaCl-injectable solution, vortex-mixed, and centrifuged. Approximately 1 g of each liver, lung, brain, heart, kidney, spleen, stomach, small intestine, large intestine, mesentery, fat, and muscle was quickly removed, rinsed or perfused with cold 0.9% NaCl-injectable solution, blotted, minced, homogenized with 4 vol. of 0.9% NaCl-injectable solution in a tissue homogenizer (Ultra-Turrax T25, Janke & Kunkel, IKA Labortechnik, Staufen, Germany), and centrifuged immediately. Two 100-μl aliquots of plasma or the supernatant of each tissue homogenate were stored in a −70 °C freezer until HPLC analysis of 2-AP (Han et al., 1998a).

2.6. Measurement of plasma protein binding

The plasma protein binding of 2-AP in control rats and rats with PCM and PCMC after intravenous

administration of 2-AP was measured using an equilibrium dialysis technique as reported earlier (Han et al., 1998b). The concentration of 2-AP spiked into the plasma side was 50 μg/ml. After 24 h incubation, two 100-μl aliquots were removed from each compartment and stored in a −70 °C freezer until HPLC analysis of 2-AP (Han et al., 1998a).

2.7. HPLC analysis

The concentrations (or amounts in tissue) of 2-AP in the above biological fluids were analyzed by the HPLC method reported from our laboratories (Han et al., 1998a). Acetonitrile (2.5 vol.) containing 1 μg/ml of diazepam (an internal standard) was added to deproteinize the biological samples. After vortex-mixing and centrifugation, a 50-μl aliquot of supernatant was injected directly onto the HPLC column. The mobile phase, acetonitrile:water (55:45, v/v), was run at a flow rate of 1.5 ml/min and the column effluent was monitored by a UV detector set at 330 nm. Retention times for 2-AP and diazepam were approximately 4.0 and 5.1 min, respectively. The detection limits of 2-AP in plasma, urine, and tissue homogenate were 20, 20, and 50 ng/ml, respectively. The coefficients of variation of the assay (within- and between-day) were generally low (below 6.12%).

The analysis of 2-AP metabolites (Fig. 1) in urine was achieved (Kim, 1999) on a Partisil ODS (4.6 mm, i.d. × 250 mm, ℓ; particle size, 4 μm; Beckman, Fullerton, CA) using a linear gradient (Waters, Milford, MA) from 10% methanol in 10 mM ammonium acetate buffer (pH 5.0) to 70% methanol in 17 min and followed by 8 min isocratic elution, at a flow rate of 1.0 ml/min. The eluant was monitored at 320 nm (Waters) and full UV spectrum of peaks was monitored using photodiode array detector (Waters). For spectral analysis of metabolites, each peak eluted from HPLC column was collected and lyophilized. EI mass spectra were obtained by direct injecting samples to a cross-linked Ultra-2 capillary column (0.22 μm; 17 m; Hewlett-Packard, Palo Alto, CA) installed in GC/MSD (HP5972/HP5890, Hewlett-Packard) and ^{1}H-NMR spectra were obtained using 600 MHz NMR (Bruker, Billerica, MA). ES-mass spectra were taken by direct injection into ES/LC/MS.

2.8. Pharmacokinetic analysis

The AUC was calculated by the trapezoidal rule-extrapolation method; this method utilized the logarithmic trapezoidal rule (Chiou, 1978) for the calculation of the area during the declining plasma level phase, and the linear trapezoidal rule for the rising plasma level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by terminal rate constant.

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters after intravenous administration; the time-averaged total body clearance (CL), the area under the first moment of the plasma concentration–time curve (AUMC), the mean residence time (MRT), and the apparent volume of distribution at steady state (V_{SS}) (Kim et al., 1993).

$$\mathrm{CL} = \frac{\mathrm{dose}}{\mathrm{AUC}} \tag{1}$$

$$\mathrm{AUMC} = \int_0^\infty t \times C_\mathrm{p}\, \mathrm{d}t \tag{2}$$

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \tag{3}$$

$$V_{SS} = \mathrm{CL} \times \mathrm{MRT} \tag{4}$$

where C_p is the plasma concentration of 2-AP at time t.

The mean values of each clearance (Chiou, 1980), V_{SS} (Chiou, 1979), and terminal half-life (Eatman et al., 1977) were calculated by the harmonic mean method.

2.9. Statistical analysis

All results are expressed as mean ± S.D. A $P < 0.05$ was considered to be statistically significant using the Duncan's multiple range test of SPSS posteriori analysis of variance among the three means for unpaired data.

3. Results

3.1. Effects of cysteine on body weight gain, food intake, and organ weight in rats with PCM

The effects of dietary protein on body weight gain, food, protein and calorie intake, and liver, kidney and

Table 1
Body weight, food, protein and calorie intake, and liver, kidney and stomach weight after 1-min intravenous infusion of 2-AP (50 mg/kg) to control rats, and rats with PCM and PCMC[a]

Parameter	Control ($n = 11$)	PCM ($n = 10$)	PCMC ($n = 7$)
Initial body weight (g)	156 ± 3.76	160 ± 7.37	163 ± 5.49
Final body weight (g)	332 ± 21.6[b]	160 ± 9.26	176 ± 14.6
Food intake (g/day per rat)	17.4 ± 1.36[c]	8.71 ± 0.775	9.64 ± 0.619
Protein intake (g/day per rat)	4.01 ± 0.31[c]	0.435 ± 0.0388	0.482 ± 0.0309
Calorie intake (kcal/day per rat)	70.5 ± 5.48[c]	35.2 ± 3.13	39.0 ± 2.50
Liver weight (g)	13.6 ± 1.35[c]	6.61 ± 0.825	7.17 ± 0.878
Liver weight (% of body weight)	4.08 ± 0.306	4.14 ± 0.483	4.33 ± 0.326
Kidney weight (g)	2.66 ± 0.217[c]	1.37 ± 0.161	1.51 ± 0.0590
Kidney weight (% of body weight)	0.803 ± 0.0701	0.876 ± 0.111	0.915 ± 0.0748
Stomach weight (g)	1.47 ± 0.153[c]	0.936 ± 0.114	1.01 ± 0.0721
Stomach weight (% of body weight)	0.442 ± 0.0443[c]	0.589 ± 0.0516	0.615 ± 0.0606

[a] Each value represents the mean ± S.D.
[b] Each group was significantly different ($P < 0.05$).
[c] Control group was significantly different ($P < 0.05$) from PCM and PCMC groups.

stomach weight in the present intravenous studies are listed in Table 1. Protein deprivation for 4 weeks (5% casein diet, PCM) caused a significant decrease in body weight gain and food consumption. For example, the body weight gain decreased significantly in rats with PCM (from 160 to 160 g) than that in control rats (from 156 to 332 g), and rats on 5% protein diet (rats with PCM) consumed approximately 49.9% less food than that rats on 23% protein diet (control rats), despite ad libitum supply of food. As a result, their protein and calorie intake decreased significantly by 89.2 and 50.1%, respectively, in rats with PCM. Since the protein and calorie intake decreased significantly in rats with PCM, it is important to realize that rats with PCM suffered from both protein and calorie deficiencies. Therefore, any changes in the pharmacokinetics of 2-AP in rats with PCM should be attributed to PCM and not solely to protein deficiency. The absolute liver, kidney and stomach weight also decreased significantly in rats with PCM and this could be due to significant decrease in body weight gain. However, the relative (% of body weight) weight of liver and kidney was not significantly different between control rats and rats with PCM. Note that cysteine supplementation (rats with PCMC) did not restore the above mentioned parameters to control levels. Similar results were also reported with other rat studies (Kim et al., 2000, 2001a, 2001b).

3.2. Pharmacokinetics after intravenous administration

The mean arterial plasma concentration–time profiles of 2-AP after intravenous administration of drug at a dose of 50 mg/kg to control rats ($n = 11$), and rats with PCM ($n = 10$) and PCMC ($n = 7$) are shown in Fig. 2, and relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration, the plasma concentrations of 2-AP declined in a polyexponential fashion for all three groups of rats (Fig. 2) with mean terminal half-lives of 115, 146, and 217 min for control rats, and rats with PCM and PCMC, respectively (Table 2); each value was significantly different. The plasma concentrations of 2-AP were lower in rats with PCM and higher in rats with PCMC than those in control rats (Fig. 2). As a result, the AUC was significantly smaller (41.3% decrease) in rats with PCM and significantly greater (67.8% increase) in rats with PCMC than that in control rats (Table 2); these differences were found to be statistically significant. This could be due to significantly faster (69.9% increase) CL of 2-AP in rats with PCM and significantly slower (40.6% decrease) CL of 2-AP in rats with PCMC than that in control rats (Table 2); these differences were found to be statistically significant. The MRTs in rats with PCM (36.3% increase) and PCMC (55.8% increase) were significantly longer than that in control rats (Table 2). This suggested that 2-AP resides longer

Table 2
Pharmacokinetic parameters of 2-AP after 1-min intravenous infusion of 2-AP (50 mg/kg) to control rats, and rats with PCM and PCMC[a]

Parameter	Control ($n = 11$)	PCM ($n = 10$)	PCMC ($n = 7$)
Final body weight (g)	332 ± 21.6[b]	160 ± 9.26	166 ± 14.6
2-AP			
AUC (μg/min/ml)	739 ± 228[c]	434 ± 109	1240 ± 454
MRT (min)	113 ± 25.4[b]	154 ± 45.8	176 ± 43.8
Terminal half-life (min)	115 ± 30.7[c]	146 ± 46.1	217 ± 84.3
V_{SS} (ml/kg)	7290 ± 2970[d]	16600 ± 7120	7050 ± 2000
CL (ml/min/kg)	67.7 ± 19.0[c]	115 ± 29.1	40.2 ± 14.6
Amount excreted in 24-h urine (% of dose)	0.242 ± 0.220[d]	0.727 ± 0.418	0.130 ± 0.162
Amount recovered from gastrointestinal tract at 24 h (% of dose)	BD[e]	BD	BD
Plasma protein binding (%)	54.9 ± 7.19	44.1 ± 11.0	44.6 ± 11.6
Metabolites excreted in urine[f]			
Amount excreted in 24-h urine as M4 (relative percent to total area in chromatogram)	100 ± 56.6[d]	228 ± 76.7	51.0 ± 18.5
Amount excreted in 24-h urine as M5 (relative percent to total area in chromatogram)	100 ± 32.1	130 ± 33.8	142 ± 33.8
Amount excreted in 24-h urine as M6 (relative percent to total area in chromatogram)	100 ± 28.8	118 ± 32.2	115 ± 33.9

[a] Each value represents the mean ± S.D.
[b] Control group was significantly different ($P < 0.05$) from PCM and PCMC groups.
[c] Each group was significantly different ($P < 0.05$).
[d] PCM group was significantly different ($P < 0.05$) from control and PCMC groups.
[e] Below detection limit.
[f] Relative value compared to control rats (the mean value in control rats was 100%).

in rats with PCM and PCMC than that in control rats. The V_{SS} was significantly larger in rats with PCM than those in control rats (56.1% decrease) and rats with PCMC (57.5% decrease) (Table 2). This could be due to increase in unbound fraction of 2-AP in plasma of rats with PCM; the unbound fraction in rats with PCM was 24% greater than that in control rats (Table 2). Interestingly, cysteine supplementation (rats with PCMC) reduced the V_{SS} of 2-AP in rats with PCM although the unbound fractions were very similar between rats with PCM and PCMC (Table 2).

The total amount of 24-h urinary excretion of unchanged 2-AP in rats with PCM were significantly greater than those in control rats (66.7% decrease) and in rats with PCMC (82.1% decrease) (Table 2). However, the values were less than 0.727% of intravenous dose for all three groups of rats (Table 2), suggesting that intravenous dose of 2-AP is essentially completely metabolized in rats and similar results were also obtained from other rat studies (Han and Lee, 1999). Note that the total amount of unchanged M4 excreted in 24-h urine ('the amount' of M4, expressed in terms of the relative percentage of area of M4 to total area of all peaks in HPLC chromatogram) in rats with PCM was significantly greater than those in control rats (56.1% decrease) and rats with PCMC (77.6% decrease) (Table 2). However, 'the amounts' of M5 and M6 were not significantly different among three groups of rats (Table 2). The total amount of unchanged 2-AP recovered from gastrointestinal tract at 24 h was below detection limit for all three groups of rats (Table 2) suggesting that gastrointestinal (including biliary) excretion was almost negligible and similar results were also obtained from other rat studies (Han and Lee, 1999). The above data (the negligible renal clearance of 2-AP and negligible contribution of biliary excretion of unchanged 2-AP to nonrenal clearance of 2-AP) suggested that the CLs listed in Table 2 could represent metabolic clearances of 2-AP in rats.

3.3. Tissue distribution study

The tissue distribution of 2-AP 30 min after intravenous administration of the drug at a dose of

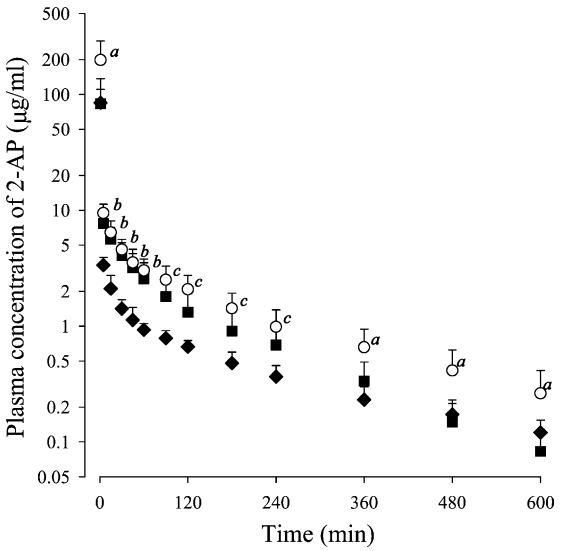

Fig. 2. Mean arterial plasma concentration–time profiles of 2-AP after 1-min intravenous infusion of 2-AP (50 mg/kg) to control rats (■, $n = 11$), and rats with PCM (◆, $n = 10$) and PCMC (○, $n = 7$). Vertical bars represent S.D. (a) PCMC group was significantly different ($P < 0.05$) from control and PCM groups. (b) PCM group was significantly different ($P < 0.05$) from control and PCMC groups. (c) Each group was significantly different ($P < 0.05$).

50 mg/kg to control rats, and rats with PCM and PCMC ($n = 5$, each) are listed in Table 3. In control rats, rat tissues studied had a good affinity to 2-AP; the tissue-to-plasma (T/P) ratios were greater-than-unity in all the tissues studied (Table 3), and this could be supported by considerably large value of V_{SS} of 2-AP, 7290 ml/kg (Table 2). The T/P ratios (affinity) of 2-AP were not significantly different among three groups of rats at 30 min except in heart and kidney in rats with PCM (Table 3).

4. Discussion

It has been reported (Bu et al., 2000) that the AUC of 2-AP was significantly smaller after intravenous administration of 2-AP to rats pretreated with dexamethasone (an inducer of CYP3A), phenobarbital (an inducer of CYP 2B1, 2C6, and 3A1/2) and 3-methylcholantheren (an inducer of CYP 1A1/2 and 2E1). It has also been reported (Cho et al., 1999) that the expression and mRNA levels of CYP 1A2, 2E1, 2C11, and 3A1/2 decreased in rats with PCM. Therefore, it was expected that the plasma concentrations of 2-AP were higher and the resultant AUC of 2-AP was

Table 3
Amount of 2-AP obtained from each gram of tissue (μg/ml for plasma or μg/g for other tissues) 30 min after 1-min intravenous infusion of 2-AP (50 mg/kg) to control rats and rats with PCM and PCMC[a]

Tissue	Control ($n = 5$)	PCM ($n = 5$)	PCMC ($n = 5$)
Plasma	11.3 ± 1.30	7.19 ± 0.994	10.6 ± 1.45
Brain	28.5 ± 5.78 (2.58 ± 0.744)	18.4 ± 2.68[b] (2.56 ± 0.194)	27.4 ± 4.34 (2.59 ± 0.272)
Large intestine	50.8 ± 7.67 (4.56 ± 0.894)	43.8 ± 14.8 (6.22 ± 2.51)	53.4 ± 9.59 (5.09 ± 1.08)
Small intestine	36.7 ± 11.5 (3.29 ± 1.10)	32.9 ± 7.58 (4.56 ± 0.643)	42.2 ± 9.67 (4.01 ± 0.933)
Fat	26.7 ± 6.29 (2.40 ± 0.650)	15.6 ± 7.18[b] (2.18 ± 0.908)	29.8 ± 7.94 (2.82 ± 0.740)
Muscle	22.1 ± 7.35 (2.03 ± 0.859)	18.0 ± 1.26 (2.53 ± 0.239)	23.4 ± 4.20 (2.22 ± 0.382)
Stomach	15.6 ± 15.3 (1.30 ± 1.16)	5.26 ± 6.09[c] (0.828 ± 1.00)	29.2 ± 22.6 (2.74 ± 2.04)
Mesentery	67.5 ± 12.6 (6.01 ± 1.09)	47.0 ± 14.3[c] (6.67 ± 2.37)	73.2 ± 18.3 (6.88 ± 1.32)
Lung	32.8 ± 13.1 (2.94 ± 1.21)	30.9 ± 6.41 (4.33 ± 0.885)	30.6 ± 13.6 (2.91 ± 1.21)
Heart	25.9 ± 6.16 (2.31 ± 0.549)	22.3 ± 4.37 (3.10 ± 0.462)[b]	24.5 ± 6.62 (2.28 ± 0.445)
Liver	66.9 ± 13.2 (6.05 ± 1.66)	39.7 ± 3.49[b] (5.59 ± 0.860)	74.5 ± 22.2 (7.02 ± 1.85)
Kidney	28.2 ± 6.55 (2.54 ± 0.703)	46.5 ± 5.39[b] (6.63 ± 1.52)[b]	32.7 ± 8.30 (3.09 ± 0.770)
Spleen	31.8 ± 7.18 (2.89 ± 0.831)	21.7 ± 2.02[d] (3.03 ± 0.192)	28.1 ± 6.42 (2.66 ± 0.577)

The numbers in parentheses represent tissue-to-plasma (T/P) ratios.
[a] Each value represents the mean ± S.D.
[b] PCM group was significantly different ($P < 0.05$) from control and PCMC groups.
[c] PCM group was significantly different ($P < 0.05$) from PCMC group.
[d] PCM group was significantly different ($P < 0.05$) from control group.

significantly greater in rats with PCM than those in control rats. However, the results were opposite in the present study; the metabolism of 2-AP rather increased in rats with PCM than that in control rats. In order to find the reason for the unexpected result, 'the amounts' of 2-AP metabolites excreted in 24-h urine were analyzed (Table 2). 'The amount' of M4 was significantly greater (128% increase) in rats with PCM than that in control rats (Table 2). Although the values in parentheses in Fig. 1 were obtained based on the ratio of area of each peak to total area of all peaks in HPLC chromatogram (not quantitative data), the area ratio of M4 to total area was 34.4% (Fig. 1), therefore, M4 seemed to be one of main metabolites of 2-AP in rats.

Note that the decrease in metabolism of M4 (restoration of metabolism of M4 to the control level) in rats with PCM by cysteine supplementation (rats with PCMC) was observed. 'The amount' of unchanged M4 excreted in 24-h urine in rats with PCMC was not significantly different compared with that in control rat, however, the value was significantly smaller than that in rats with PCM (77.6% decrease) (Table 2). Therefore, the AUC of 2-AP in rats with PCMC was significantly greater than that in control rats (67.8% increase) and rats with PCM (186% increase) (Table 2).

It has been reported (Kim, 1999) that three major metabolites of 2-AP (Fig. 1), 2-AP sulfone (V1), 2-AP sulfoxide (V2), and 2-AP epoxide (V3) were found after incubation of 2-AP with rat liver microsomes in the presence of NADPH-generating system. After oral administration of 2-AP (Kim, 1999), however, only trace amounts of V1 and V2 were detected in rat urine and major metabolites of 2-AP in rat urine were M4, M5, and M6 (Fig. 1).

The exact reason why the formation of M4 increased after intravenous administration of 2-AP in rats with PCM is not clear, but some factors could be suggested to explain the above phenomena. First, increased expression of *S*-methyltransferase in rats with PCM could be one factor. The common metabolic pathway to form M4, M5, and M6 is pyrazine hydroxylation (Fig. 1) and 'the amounts' of M5 and M6 excreted in 24-h urine were not significantly different between control rats and rats with PCM. This suggested that increase in *S*-methyltransferase (phase II enzymes) activities to form M4 (after deallylation of 2-AP and hydroxylation, Fig. 1) could cause metabolic difference between control rats and rats with PCM. It has been reported (Kim, 1999) that *S*-methylation of 2-MP (Fig. 1) was mediated both by microsome and cytosol; 2-MP methyltransferase activities showed biphasic kinetics and microsomal activity was 10 times greater than that of cytosolic activity. Inhibition studies demonstrated that the *S*-methylation of 2-MP could be mediated by microsomal thiol methyltransferase and cytosolic thiol thiopurine methyltransferase (Kim, 1999). Decreased metabolism of intravenous 2-AP in rats pretreated with SKF-525A (Bu et al., 2000) which also used as an inhibitor of potent cytosolic thiopurine methyltransferase and microsomal thiol methyltransferase (Otterness et al., 1986), might be at least partially due to decrease in the *S*-methyltransferase activities to form M4 by pretreatment with SKF-525A. It has been reported that the phase II metabolism of compounds, such as glucuronide conjugates of acetaminophen (Jung, 1985), benzoic acid (Thabrew et al., 1980) and *p*-nitrophenol (Ericksson et al., 1975), and glycine conjugate of salicylic acid (Yue and Varma, 1982) increased in rats with PCM. Second, increased expression of some glutathione-*S*-transferase (GST) in rats with PCM could be another factor. It has been reported (Cho et al., 2000) that some GST levels increased in rats with PCM, which may be caused by increase in certain GST mRNAs with the antioxidant response element (ARE) activation. Similar results have also been reported from malnourished children (Ramdath and Golden, 1993). It has also been suggested (Kim, 1999) that most of metabolites of 2-AP excreted in rat bile after oral administration of 2-AP (15 metabolites were found) might be glutathione conjugates (e.g. glutathione conjugates of V3, Fig. 1), most of which were not extracted by organic solvents. The disappearance of V3 after incubation with cytosol and glutathione was confirmed (Kim, 1999).

5. Conclusions

After intravenous administration of 2-AP at a dose of 50 mg/kg to rats with PCM, the AUC of 2-AP was significantly smaller than that in control rats. This seemed to be due to increase in phase II metabolism, *S*-methylation (to form M4). However, in rats with PCMC, the AUC was significantly greater than that

in control rats, and this could be due to significantly decreased formation of M4 in rats with PCMC. Some pharmacokinetic parameters of intravenous 2-AP in rats with PCMC restored fully (the percentage of dose excreted in 24-h urine as unchanged 2-AP, the amount excreted in 24-h urine as M4, and V_{SS}) or more than (AUC and CL) the control levels (control rats).

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