PHARMACOKINETICS OF A CHEMOPROTECTIVE AGENT, 2-(ALLYLTHIO)PYRAZINE, AFTER INTRAVENOUS AND ORAL ADMINISTRATION TO RATS: HEPATIC AND GASTRIC FIRST-PASS EFFECTS

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ABSTRACT:
Pharmacokinetic parameters of 2-(allylthio)pyrazine (2-AP) were evaluated after i.v. administration of the drug (10, 20, 50, and 100 mg/kg body weight) and oral administration of the drug (10, 50, and 100 mg/kg body weight) to rats. The hepatic, gastric, and intestinal first-pass effects of 2-AP were also measured after i.v., intraportal, intraduodenal, and oral administration of the drug (10 and 50 mg/kg body weight) to rats. After i.v. administration, the pharmacokinetic parameters of 2-AP were dose-independent at the dose ranges studied. However, after oral administration, the dose-normalized total area under the plasma concentration-time curve from time zero to time infinity values (43.5, 125, and 205 μg min/ml, based on 10 mg/kg body weight) increased significantly with increasing doses. The extent of absolute oral bioavailability (F) values also increased with increasing oral doses; the values were 19.6, 56.7, and 93.6% for 10, 50, and 100 mg/kg body weight, respectively. The oral data above could be due to saturable hepatic, gastric, and/or intestinal first-pass effects. This was proved by saturable gastric first-pass effect (the significant area under the plasma concentration-time curve from time zero to time infinity difference between oral and intraduodenal administration of 2-AP); the values were 62.6 and 26.6% at 2-AP doses of 10 and 50 mg/kg body weight, respectively. Approximately 20% of oral dose was eliminated by liver (hepatic first-pass effect) for both oral doses of 10 and 50 mg/kg body weight. However, the first-pass effects of 2-AP in the intestine, heart, and lung were almost negligible. The low F value after oral administration of 2-AP at low dose (10 mg/kg body weight) was mainly due to gastric first-pass effect in rats.

Diallylsulfide (a component of Allium sativum) has chemoprotective activity and pyrazine has binding affinity to cytochrome P-450 2E1 (inhibits CYP2E1 activity). Therefore, pyrazine was attached to the allylsulfide radical of diallylsulfide to form 2-(allylthio)pyrazine (2-AP) Fig. 1 to increase the binding affinity of diallylsulfide to CYP2E1. In rats pretreated with 2-AP, the percentages of i.v. dose of acetaminophen excreted in both 8-h bile and 24-h urine as acetaminophen-glucuronide increased significantly; however, the values in 24-h urine as acetaminophen-glutathione and acetaminophen-cysteine conjugates decreased significantly compared with those in control rats (Kwak et al., 1998). This might be because of an increase in uridine diphosphoglucuronyl transferase activity and suppression of cytosolic glutathione S-transferase A3 (Nam, 1998). 2-AP also showed a chemopreventive effect on vinyl carbamate- or vinyl carbamate epoxide-induced hepatotoxicity, mutagenicity, and tumorigenicity (Surt et al., 1997) and hepatoprotective effect on retinol palmitate- and pyridine-potentiated carbon tetrachloride-induced hepatotoxicity (Kim et al., 1996). 2-AP is under preclinical study as a chemoprotective agent to prevent the development of cancer.

The purposes of the present study were to report the pharmacokinetics of 2-AP after i.v. administration of the drug (10, 20, 50, and 100 mg/kg body weight) and oral administration of the drug (10, 50, and 100 mg/kg body weight) to rats; the hepatic, gastric, and intestinal first-pass effects of 2-AP after i.v., intraportal, intraduodenal, and oral administration of the drug (10 and 50 mg/kg body weight) to rats; and the tissue distribution of 2-AP after i.v. administration of the drug (10 mg/kg body weight) to rats.

Materials and Methods
Chemicals. 2-AP and diazepam [the internal standard of high-performance liquid chromatography (HPLC) assay] were supplied by Central Research Institute of the Bukwang Pharmaceutical Company (Seoul, South Korea) and Hwan In Pharmaceutical Company (Seoul, South Korea), respectively. Reduced forms of nicotinamide adenine dinucleotide phosphate, Tris buffer, uridine diphosphoglucuronic acid, and polyethylene glycol 400 were products of Sigma Chemical Company (St. Louis, MO). Other chemicals were of
reagent grade or HPLC grade and therefore were used without further purification.

Disappearance of 2-AP in Homogenates of Rat Tissues. The procedures were similar (Kim et al., 1995; Lee and Lee, 1995) to those reported previously (Litterst et al., 1975). Approximately 1 g of each liver, kidney, spleen, stomach, small intestine, large intestine, heart, brain, and lung was excised from rats (N = 4) after cervical dislocation, rinsed with 50 mM Tris-HCl buffer (pH 7.4), blotted dry with tissue paper, and weighed. Metabolic activity was initiated by adding 1 ml of plasma or 1 ml of 9000g supernatant of each tissue homogenate to a glass test tube containing 25 μl (50 μg, dissolved in methanol) of 2-AP, 100 μl (1 mM) of reduced form of nicotinamide adenine dinucleotide phosphate, 1.9 ml (100 mM) of Tris-HCl buffer of pH 7.4, and 25 μl (3.3 mM) of uridine diphosphoglucuronic acid. To terminate enzyme activity, 1 ml of 1 N NaOH was added after a 30-min incubation in a water bath shaker kept at 37°C and at a rate of 50 oscillations/min.

Intravenous and Oral Studies. The carotid artery (for blood sampling) and/or the jugular vein (for drug administration) of male Sprague-Dawley rats (weighing 250–350 g; Charles River Company, Atsugi, Japan) were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) under light ether anesthesia. 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 anesthesia for 4 to 5 h before the study began. They were not restrained at any time during the study; heparinized 0.9% NaCl-injectable solution (20 U/ml) were used to flush the catheter immediately after each blood sampling. At the end of 24 h, each metabolic cage was rinsed with 20 ml of distilled water, and the rinses were combined with the 24-h urine. After measuring the exact volume of the combined urine, 0.05-ml aliquots of the plasma and the combined urine samples were kept frozen until HPLC analysis of 2-AP (Han et al., 1998). 2-AP, 50 mg/kg body weight, was also administered i.v. to the additional rats (N = 3) after bile duct cannulation for 8-h bile sampling (Lee and Lee, 1996). Bile duct was cannulated with a polyethylene tube (Clay Adams) under light ether anesthesia, and 2-AP was administered after 4 to 5 h of recovery from anesthesia. For this bile experiment, each rat was held in supine position during the entire experimental period (8 h) by tying four feet on a plate. Each rat was kept warm by illuminating the electrical light. Aliquots of the bile samples were also kept frozen until HPLC analysis of 2-AP (Han et al., 1998).

2-AP (dissolved in 40% polyethylene glycol 400), 10 (N = 15), 50 (N = 12), and 100 (N = 13) mg/kg body weight, was administered orally (total oral volume was approximately 1.5 ml) to rats with a feeding tube after overnight fasting with free access to water. Aliquots of blood samples (0.12 ml) were collected via the carotid artery at 0 (to serve as a control), 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, 720, 840, and 960 min after i.v. administration. Approximately 0.3-ml aliquots of heparinized 0.9% NaCl-injectable solution (20 U/ml) were used to flush the cannula immediately after each blood sampling. At the end of 24 h, each metabolic cage was rinsed with 20 ml of distilled water, and the rinses were combined with the 24-h urine. After measuring the exact volume of the combined urine, 0.05-ml aliquots of the plasma and the combined urine samples were kept frozen until HPLC analysis of 2-AP (Han et al., 1998). 2-AP, 50 mg/kg body weight, was also administered i.v. to the additional rats (N = 3) after bile duct cannulation for 8-h bile sampling (Lee and Lee, 1996). Bile duct was cannulated with a polyethylene tube (Clay Adams) under light ether anesthesia, and 2-AP was administered after 4 to 5 h of recovery from anesthesia. For this bile experiment, each rat was held in supine position during the entire experimental period (8 h) by tying four feet on a plate. Each rat was kept warm by illuminating the electrical light. Aliquots of the bile samples were also kept frozen until HPLC analysis of 2-AP (Han et al., 1998).

Measurement of Hepatic First-Pass Effect of 2-AP. The carotid artery and the jugular vein of each rat were cannulated under light ether anesthesia. At the same time, the portal vein was also cannulated (Kim et al., 1995a) by the modified Suzuki method (Xu et al., 1992). After abdominal incision under light ether anesthesia, the pyloric vein was isolated (to minimize impaired blood flow in the portal vein) and the tapered end of a 27-gauge needle bent at a 45° angle was inserted. Bleeding was prevented by applying epoxy glue (Krazy Glue; Krazy Glue Inc., Itasca, IL). A long polyethylene tube was
attached to the other end of the needle and exteriorized to the dorsal side of the neck. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company). 2-AP (dissolved in 40% polyethylene glycol 400) was infused in 30 min after 4 to 5 h of recovery from anesthesia with the assistance of an infusion pump (model 2400-006; Harvard Instrument, Southnatick, MA) at a dose of 10 and 50 mg/kg body weight for both i.v. and intraportal administration (N = 5 for each group). The total infusion volume was approximately 1.0 ml. At the same time, an equal volume (1.0 ml) of 40% polyethylene glycol 400 solution was also infused in 30 min via the portal vein for i.v. study and via the jugular vein for intraportal study. Approximately 0.12-ml aliquots of blood samples were collected via the carotid artery at 0 (to serve as a control), 15, 30, (at the end of the infusion), 31, 35, 40, 45, 50, 60, 75, 90, 120, 150, 210, 270, 330, 390, 510, 630, and 750 min after the beginning of the infusion. Approximately 0.3-ml aliquots of heparinized 0.9% NaCl-injectable solution (20 U/ml) were used to flush cannula immediately after each blood sampling. Urine samples were collected between 0 and 24 h. Aliquots of the plasma (0.05 ml) or the urine samples were kept frozen until HPLC analysis of 2-AP (Han et al., 1998).

Measurement of Gastric or Intestinal First-Pass Effect of 2-AP. Rats were fasted overnight with free access to water. The carotid artery and the pyloric vein of each rat were cannulated after abdominal incision under light ether anesthesia (Xu et al., 1992; Kim et al., 1997a). For intraportal administration, 40% polyethylene glycol 400 solution (1.5 ml) was administered orally with a feeding tube. Other procedures were similar to those described to measure hepatic first-pass effect of 2-AP.

Tissue Distribution Study. The procedures were similar to those reported previously (Lee and Lee, 1995; Yoon et al., 1998) except that each piece of tissue was homogenized with 4 volumes of cold distilled water. 2-AP (dissolved in 40% polyethylene glycol 400; 10 mg/kg body weight) was administered i.v. in 1 min into the jugular vein of rats (N = 5). At 30 min after i.v. administration, as much blood as possible was collected via the carotid artery in a heparinized tube and each rat was exsanguinated.

HPLC Analysis of 2-AP. The concentrations of 2-AP in the biological fluids above were analyzed by the HPLC method reported from our laboratory (Han et al., 1998). Acetonitrile (2.5 volumes) containing 1 µg/ml of diazepam (the internal standard) was added to deproteinize biological samples. After vortex mixing and centrifugation, 50-µl aliquots of supernatant were 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 UV detection at 330 nm. The detection limits for 2-AP in human plasma and urine as well as rat tissue homogenates 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%).

### Pharmacokinetic Analysis

The area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method (Kim et al., 1993); this method utilized the logarithmic trapezoidal rule (Chiou, 1978) for 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 the terminal rate constant.

The standard method (Gibaldi and Perrier, 1982) was used to calculate the CL, first moment of AUC (AUMC), mean residence time (MRT), and apparent volume of distribution at steady state (Vss) (Kim et al., 1993).

\[
CL = \frac{\text{dose}}{\text{AUC}}
\]

\[
\text{AUMC} = \int_0^\infty t \cdot C_p \, dt
\]

\[
\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} - \frac{T}{2}
\]

\[
V_{ss} = CL \cdot MRT
\]

where \(C_p\) is the plasma concentration of 2-AP at time \(t\) and \(T\) is the infusion time.

The extent of absolute oral bioavailability (F) value after oral administration of 2-AP to rats was estimated by comparing the AUC values after i.v. and oral administration of the same dose of 2-AP to rats because the pharmacokinetic parameters of 2-AP (especially CL value) after i.v. administration to rats were dose-independent (Table 1).

The harmonic mean method was used to calculate the mean values of Vss (Chiou, 1979), terminal half-life (Eatman et al., 1977), and CL (Chiou, 1980).

### Statistical Analysis

A p value of less than .05 was considered to be statistically significant using Duncan’s Multiple Range Test of Statistical Package for the Social Science posteriori analysis of variance program among three or four means for the unpaired data or t test between two means for the unpaired data (Statistical Research Institute, College of Natural Sciences, Seoul National University, Seoul, South Korea). All data were expressed as mean ± SD.

### Results

Disappearance of 2-AP in Homogenates of Rat Tissues. Rat liver, kidney, spleen, and stomach homogenates showed a considerable disappearance of 2-AP among the tissues studied. But other tissues studied showed almost negligible disappearance of 2-AP. The amounts of 2-AP remaining after a 30-min incubation of 50 µg of the drug in plasma and 9000 µg supernatant fraction of liver, kidney, spleen, stomach, small intestine, large intestine, heart, brain, and lung were 50.5 ± 0.308 µg/ml, and 43.2 ± 1.34, 46.2 ± 2.55, 43.4 ± 3.79, 47.7 ± 1.70, 49.6 ± 1.44, 49.9 ± 0.482, 49.0 ± 0.262, 49.2 ± 0.457.
and 49.7 ± 0.707 μg/g tissue, respectively, in the presence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH).

Intravenous Administration of 2-AP to Rats. After a 1-min i.v. infusion of 2-AP (10, 20, 50, and 100 mg/kg body weight) to rats, the mean arterial plasma concentrations of the drug declined in a parallel fashion in all four i.v. doses studied (Fig. 2) with mean terminal half-lives of 540 and 569 min for 50 and 100 mg/kg body weight, respectively (Table 1). Note that all of the pharmacokinetic parameters of 2-AP listed in Table 1 were not significantly different among four different i.v. doses. The percentages of the i.v. dose of 2-AP excreted in 24-h urine as unchanged drug (Ae0–24h) were negligible; the values were 0.0673, 0.526, 0.345, and 0.0926% for i.v. doses of 10, 20, 50, and 100 mg/kg body weight, respectively, to rats (Table 1). The biliary excretion of unchanged 2-AP was also negligible; the percentages of i.v. dose of 2-AP excreted in 8-h bile as unchanged drug after i.v. administration of the drug, 50 mg/kg body weight, to rats (N = 3) were less than 0.0432%. Note that the terminal phase of 2-AP in the plasma concentrations started from 12 h after i.v. administration of the drug (50 mg/kg body weight, to rats (N = 3) were less than 0.0432%. Note that the terminal phase of 2-AP in the plasma concentrations started from 12 h after i.v. administration of the drug (50 mg/kg body weight, to rats (Fig. 2). However, the plasma concentrations of 2-AP at 10 and 20 mg/kg body weight were detected for only up to 6 h (Fig. 2) because of our HPLC assay sensitivity. Therefore, the pharmacokinetic parameters of 2-AP, such as terminal half-life, MRT, and VSS, at the doses of 10 and 20 mg/kg body weight, are listed in parentheses in Table 1, and the values are not included in statistical analysis.

Oral Administration of 2-AP to Rats. After oral administration of 2-AP (10, 50, and 100 mg/kg body weight) to rats, the mean arterial plasma concentrations of the drug reached its peak at the first blood-sampling time (15 min) for all three oral doses studied (Fig. 3), indicating that the absorption of 2-AP was fast. The mean plasma concentrations of 2-AP also declined in a parallel fashion in all three oral doses studied (Fig. 3), with mean terminal half-lives of 103 and 92.5 min for oral doses of 50 and 100 mg/kg body weight, respectively (Table 2). Note that the dose-normalized (based on 10 mg/kg body weight) AUC values of 2-AP increased significantly with increasing oral doses; the values were 43.5 ± 23.2, 125 ± 25.2, and 205 ± 56.1 μg min/ml for 10, 50, and 100 mg/kg body weight, respectively. The F values also increased with increasing oral doses; the values were 19.6, 56.7, and 93.6% for oral doses of 10, 20, and 100 mg/kg body weight, respectively (Table 2). The percentages of oral dose of 2-AP excreted in 24-h urine as unchanged drug were negligible; the values were 0.0306, 0.0492, and 0.00263% for oral doses of 10, 50, and 100 mg/kg body weight, respectively (Table 2). The values recovered from 24-h gastrointestinal tract were under detection limit for all three oral doses studied, indicating that the absorption of 2-AP was essentially complete. For the same reason mentioned in the i.v. study, the terminal half-life of 2-AP at the oral dose of 10 mg/kg body weight is listed in parentheses in Table 2, and that value is not included in statistical analysis.

Measurement of Hepatic First-Pass Effect of 2-AP. After 30-min i.v. and intraportal infusion of 2-AP (10 mg/kg body weight) to rats, the mean arterial plasma concentrations of 2-AP declined in a parallel fashion for both routes of administration, and similar results also were obtained from 50 mg/kg body weight (Fig. 4). The AUC values of 2-AP after intraportal administration of the drug, 10 (113 ± 43.5 versus 231 ± 58.3 μg min/ml) and 50 (976 ± 55.4 versus 1560 ± 108 μg min/ml) mg/kg body weight, were significantly smaller than those after i.v. administration (Fig. 5).

Measurement of Gastric or Intestinal First-Pass Effect of 2-AP.
The AUC values of 2-AP after oral administration of the drug, 10 (47.5 ± 35.5 versus 148 ± 28.9 µg min/ml) and 50 (721 ± 548 versus 1200 ± 376 µg min/ml) mg/kg body weight, were significantly smaller than those after intraportal administration. The corresponding values of 2-AP after oral administration of the drug, 10 (47.5 ± 35.5 versus 127 ± 77.2 µg min/ml) and 50 (721 ± 548 versus 982 ± 50.1 µg min/ml) mg/kg body weight, were also significantly smaller than those after intraduodenal instillation. However, the AUC values of 2-AP after intraportal administration of the drug, 10 (148 ± 28.9 versus 127 ± 77.2 µg min/ml) and 50 (1200 ± 376 versus 982 ± 50.1 µg min/ml) mg/kg body weight, were not significantly different from the values after intraduodenal administration of the same dose of the drug (Fig. 5).

**Table 2**

Mean (± S.D.) pharmacokinetic parameters of 2-AP after oral administration of the drug, 10, 50, and 100 mg/kg body weight, to rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Body Weight</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 mg/kg (N = 15)</td>
</tr>
<tr>
<td>Terminal half-life (min)</td>
<td>(23.3 ± 4.90) b</td>
</tr>
<tr>
<td>AUC* (µg min/ml)</td>
<td>43.5 ± 23.2</td>
</tr>
<tr>
<td>Ae&lt;sub&gt;0–24 h&lt;/sub&gt; (% of oral dose)</td>
<td>0.0306 ± 0.0300</td>
</tr>
<tr>
<td>F (%)</td>
<td>19.6</td>
</tr>
</tbody>
</table>

* Dose-normalized AUC values were significantly different (p < .01) among doses.

The number in parentheses represents terminal half-life of 2-AP at the dose of 10 mg/kg body weight. Because the detection of plasma concentrations of 2-AP at 10 mg/kg body weight was shorter than those at 50 and 100 mg/kg body weight, the value was not included in statistical analysis.

The AUC values of 2-AP after oral administration of the drug, 10 (47.5 ± 35.5 versus 148 ± 28.9 µg min/ml) and 50 (721 ± 548 versus 1200 ± 376 µg min/ml) mg/kg body weight, were significantly smaller than those after intraportal administration. The corresponding values of 2-AP after oral administration of the drug, 10 (47.5 ± 35.5 versus 127 ± 77.2 µg min/ml) and 50 (721 ± 548 versus 982 ± 50.1 µg min/ml) mg/kg body weight, were also significantly smaller than those after intraduodenal instillation. However, the AUC values of 2-AP after intraportal administration of the drug, 10 (148 ± 28.9 versus 127 ± 77.2 µg min/ml) and 50 (1200 ± 376 versus 982 ± 50.1 µg min/ml) mg/kg body weight, were not significantly different from the values after intraduodenal administration of the same dose of the drug (Fig. 5).

**Tissue Distribution of 2-AP after i.v. Administration to Rats.**

The amounts of 2-AP recovered from each gram tissue (µg/ml plasma or µg/g other tissues) and tissue/plasma (T/P) ratio at 30 min after 1-min i.v. infusion of the drug (10 mg/kg body weight) to rats (N = 5) are listed in Table 3. The T/P values of 2-AP were greater than unity in all tissues (or organs) studied except in the lung, indicating that each rat tissue has high affinity to 2-AP except the lung.

**Discussion**

After i.v. administration of 2-AP (10–100 mg/kg body weight, to rats), the CL values (45.6–48.9 ml/min/kg body weight based on plasma data, Table 1) were considerably smaller than the cardiac output in rats (296 ml/min/kg body weight based on blood data; Davies and Morris, 1993), suggesting that the first-pass effect of 2-AP in the lung and heart could be negligible, if any, in rats.

After oral administration of 2-AP (10–100 mg/kg body weight) to rats, the AUC values after dose normalization (based on 10 mg/kg body weight) increased significantly and the F values also increased with increasing oral doses (Table 2). This was not due to the increase in degradation of 2-AP in acidic gastric juice at low oral dose; it has been reported (K.S.W., S-J.W., C.H.K. and M.G.L., in press) that 2-AP was stable for up to 48 h incubation in solutions of pH 1 to 12 and 3 h incubation in five human gastric juices (pH values of 1.16,
1.54, 1.84, 2.03, and 3.81, respectively) in a water-bath shaker kept at 37°C and at a rate of 50 oscillations/min. Because the pharmacokinetic parameters of 2-AP after i.v. administration of the drug were dose-independent (Table 1), the dose-dependent pharmacokinetic parameters of 2-AP, such as AUC and F values, after oral administration of the drug could be due to saturable hepatic, gastric, and/or intestinal first-pass effects. As mentioned in tissue homogenate studies, the liver and stomach showed considerable disappearance of 2-AP. In the present rat study, the F values of 2-AP were 19.6 and 56.7% after oral administration of the drug, 10 and 50 mg/kg body weight, respectively, and based on the gastrointestinal recovery study, absorption of 2-AP was essentially complete. Therefore, 80.4% (100–19.6) and 43.3% (100–56.7) of orally administered 2-AP, 10 and 50 mg/kg body weight, respectively, were lost by gastrointestinal and/or hepatic first-pass effects. After intraportal and intraduodenal administration of 2-AP (10 and 50 mg/kg body weight), there was no significant difference in AUC values for both doses, suggesting that the intestinal first-pass effect of the drug was not considerable in rats. After oral administration of 2-AP (10 and 50 mg/kg body weight), the AUC values of 2-AP were 37.4 and 73.4%, respectively, of those after intraduodenal instillation. The data above indicated that the gastric first-pass effect of 2-AP was approximately 62.6% and 26.6% at doses of 10 and 50 mg/kg body weight, respectively. The gastric first-pass effect seemed to be saturated because 26.6% (at 50 mg/kg body weight) was significantly rated because 26.6% (at 50 mg/kg body weight) was significantly smaller than 62.6% (at 10 mg/kg body weight).

After intraportal administration of 2-AP (10 and 50 mg/kg body weight) to rats, the AUC values of 2-AP were 48.9 and 62.6%, respectively, of those after i.v. administration of 2-AP. The data above indicated that approximately 51.1% and 37.4% of 2-AP were eliminated by liver (hepatic first-pass effect) after intraportal administration of the drug, 10 and 50 mg/kg body weight, respectively. Therefore, approximately 20.0% (100 – 19.6 – 62.6 for 10 mg/kg body weight and 100 – 56.7 – 26.6 for 50 mg/kg body weight) of orally administered 2-AP, 10 and 50 mg/kg body weight, could be eliminated by a hepatic first-pass effect.

Recently, five metabolites of 2-AP were tentatively identified by gas chromatography/mass spectroscopy in rats. Based on the area of each peak on HPLC chromatograms, 2-(methylthio)-4-hydroxyypyrazine (M4) and 2-(allylthio)-4-hydroxy pyrazine (M5) were main metabolites of 2-AP in rats (Fig. 1). More studies are required for metabolites pharmacokinetics of 2-AP in rats.

In conclusion, gastric and hepatic first-pass effects of 2-AP were responsible for the dose-dependent pharmacokinetics of 2-AP after oral administration of the drug to rats, and the gastric first-pass effect was one of the main reasons for the low F of 2-AP at low dose, 10 mg/kg body weight. The considerable gastric first-pass effect of furosemide in rats (Lee and Chiou, 1983), chlorpheniramine in rabbits (Huang et al., 1981), and ethanol in humans (Haber et al., 1996) have been reported.

**References**


**TABLE 3**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>2-AP µg/ml plasma or µg/g tissue</th>
<th>T/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1.64 ± 0.925</td>
<td>1.00</td>
</tr>
<tr>
<td>Liver</td>
<td>7.13 ± 2.69</td>
<td>5.24 ± 2.17</td>
</tr>
<tr>
<td>Lung</td>
<td>0.260 ± 0.146</td>
<td>0.160 ± 0.0353</td>
</tr>
<tr>
<td>Heart</td>
<td>2.82 ± 1.41</td>
<td>1.85 ± 0.381</td>
</tr>
<tr>
<td>Brain</td>
<td>2.95 ± 1.46</td>
<td>1.99 ± 0.575</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.70 ± 4.70</td>
<td>5.67 ± 2.03</td>
</tr>
<tr>
<td>Stomach</td>
<td>3.71 ± 0.995</td>
<td>3.30 ± 2.83</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3.35 ± 1.53</td>
<td>2.25 ± 0.499</td>
</tr>
<tr>
<td>Large intestine</td>
<td>6.23 ± 2.92</td>
<td>4.24 ± 1.39</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.43 ± 1.90</td>
<td>2.03 ± 0.353</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.12 ± 0.998</td>
<td>1.36 ± 0.197</td>
</tr>
<tr>
<td>Fat</td>
<td>4.31 ± 2.45</td>
<td>3.03 ± 0.979</td>
</tr>
</tbody>
</table>