Metabolism, Excretion, and Pharmacokinetics of S- Allyl-L-Cysteine in Rats and Dogs

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ABSTRACT

The metabolism, excretion, and pharmacokinetics of S-allyl-L-cysteine (SAC), an active key component of garlic supplements, were examined in rats and dogs. A single dose of SAC was administered orally or i.v. to rats (5 mg/kg) and dogs (2 mg/kg). SAC was well absorbed (bioavailability >90%) and its four metabolites—N-acetyl-S-allyl-L-cysteine (NAc-SAC), N-acetyl-S-allyl-L-cysteine sulfoxide (NAc-SACS), S-allyl-L-cysteine sulfoxide (SACS), and L-γ-glutamyl-S-allyl-L-cysteine—were identified in the plasma and/or urine. Renal clearance values (<0.01 l/h/kg) of SAC indicated its extensive renal reabsorption, which contributed to the long elimination half-life of SAC, especially in dogs (12 hours). The metabolism of SAC to NAc-SAC, principal metabolite of SAC, was studied in vitro and in vivo. Liver and kidney S9 fractions of rats and dogs catalyzed both N-acetylation of SAC and deacetylation of NAc-SAC. After i.v. administration of NAc-SAC, SAC appeared in the plasma and its concentration declined in parallel with that of NAc-SAC. These results suggest that the rate and extent of the formation of NAc-SAC are determined by the N-acetylation and deacetylation activities of liver and kidney. Also, NAc-SACS was detected in the plasma after i.v. administration of either NAc-SAC or SACS, suggesting that NAc-SACS could be formed via both N-acetylation of SACS and S-oxidation of NAc-SAC. In conclusion, this study demonstrated that the pharmacokinetics of SAC in rats and dogs is characterized by its high oral bioavailability, N-acetylation and S-oxidation metabolism, and extensive renal reabsorption, indicating the critical roles of liver and kidney in the elimination of SAC.

Introduction

Garlic (Allium sativum) has been widely recognized as a health-promoting and disease-preventing food, and it is used traditionally as a complementary medicine in the treatment of several diseases (Rivlin, 2001). For this reason, different types of garlic preparations have been developed and are commercially available, including garlic powders, garlic oil, and aged garlic extract (AGE). A number of clinical trials have demonstrated the beneficial pharmacological effects of AGE (Steiner et al., 1996; Budoff et al., 2004, 2009; Ried et al., 2010; Nantz et al., 2012). AGE is a unique garlic product that is manufactured from garlic clove through a long extraction process lasting more than 10 months. This extraction process can eliminate or modify the odorous, harsh, and irritating compounds included in fresh garlic and leads to the enrichment of water-soluble sulfur-containing compounds (Amagase et al., 2001). Indeed, AGE contains a variety of water-soluble organosulfur compounds such as S-allyl-L-cysteine (SAC), S-1-propenyl-L-cysteine, and S-allylmercaptocysteine. Among them, SAC has been recognized as an active key component of AGE (Amagase et al., 2001). The biological and pharmacological activities of SAC have been reported, including an antioxidant effect (Imai et al., 1994), anticancer effect (Chu et al., 2007), and cardioprotective and renal-protective effects (Chuah et al., 2007; Cruz et al., 2007).

The pharmacological activities of AGE are dependent on the pharmacokinetic properties of its active ingredients, such as SAC. To date, the pharmacokinetics of SAC has been studied in a few animal studies. SAC was well absorbed in mice, rats, and dogs; oral bioavailabilities were high (87%–103%) (Nagae et al., 1994; Yan and Zeng, 2005). An excretion study of SAC has identified its three metabolites, N-acetyl-S-allyl-L-cysteine (NAc-SAC), N-acetyl-S-allyl-L-cysteine sulfoxide (NAc-SACS), and S-allyl-L-cysteine sulfoxide (SACS), in the urine of rats (Krause et al., 2002). Although previous studies have reported several findings on the pharmacokinetics of SAC, the overall pharmacokinetic profile of SAC has not been fully understood. In particular, its metabolism, including the entire metabolic pathway, the presence of other metabolite(s), and the primary organ(s) responsible for the metabolism, has not been clarified.

The primary objective of the present study was to investigate the metabolism of SAC and clarify its metabolic pathway. For this purpose, we conducted excretion and pharmacokinetic studies of SAC in rats and dogs. In vitro metabolism studies using subcellular fractions of liver and kidney were also carried out to examine the N-acetylation metabolism of SAC and SACS to NAc-SAC and NAc-SACS, respectively. The results of the current study would be useful in designing and conducting future human pharmacokinetic studies of SAC.

Materials and Methods

General Chemicals. Formic acid of liquid chromatography-mass spectrometry (LC-MS) grade was purchased from Wako Pure Chemical Industries (Osaka, Japan). Acetonitrile of LC-MS grade was purchased from Kanto Chemical (Tokyo, Japan). Acetyl-CoA and pentadecanoylfluoroacetonic acid of LC-MS grade were purchased from Tokyo Chemical Industry (Tokyo, Japan). Liver and kidney S9 fractions of rats, dogs, and humans were purchased from XenoTech (Lenexa, KS).

ABBREVIATIONS: AGE, aged garlic extract; AUC, area under the plasma concentration versus time curve; CL, plasma clearance; CLint, intrinsic clearance; CLr, renal clearance; GFR, glomerular filtration rate; GSAC, L-γ-glutamyl-S-allyl-L-cysteine; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NAc-SAC, N-acetyl-S-allyl-L-cysteine; NAc-SACS, N-acetyl-S-allyl-L-cysteine sulfoxide; SAC, S-allyl-L-cysteine; SACS, S-allyl-L-cysteine sulfoxide; SBC, S-1-butynyl-L-cysteine; t½, half-life.
Chemicals. SAC was purchased from Tokyo Chemical Industry. The authentic standards of NAc-SAC, NAc-SACS (mixture of two diastereomers), (+)-SACS and L-γ-glutamyl-L-α-cysteine (GSAC), and the internal standard of S-1-butenyl-L-cysteine (SBC) were synthesized in the Drug Discovery Laboratory of Wakanuga Pharmaceutical (Hiroshima, Japan).

Animals and Dosing Solutions. The animal studies were performed according to the protocols approved by the Wakanuga Pharmaceutical Company Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (230–280 g; Charles River, Japan) and male beagle dogs (10–14 kg; Kitayama Labes, Nagano, Japan) were fasted overnight before the studies. All compounds of NAc-SAC, NAc-SACS, SAC, and SACS were dissolved in distilled water for oral administration and in saline for i.v. injection.

Excretion Study in Rats. Male rats were administered a single i.v. or oral dose (5 mg/kg) of SAC, NAc-SAC, NAc-SACS, and SACS. Urine samples were collected from rats individually housed in a metabolic cage (Natsume Seiskusho, Tokyo, Japan) for up to 24 hours after dosing. Bile samples were collected from bile duct-cannulated rats individually kept in a Bollmann cage (Natsume Seiskusho) for 24 hours after dosing. The urine and bile samples were stored at −30°C until analysis. The extent of urinary and biliary excretion (percent of dose) was calculated on a molar basis by dividing the amount of analytes excreted in biological fluids by the amount of compound administered.

Pharmacokinetic Studies in Rats and Dogs. Male rats and dogs were administered a single i.v. or oral dose (5 mg/kg in rats and 2 mg/kg in dogs) of SAC, NAc-SAC, NAc-SACS, and SACS. In the rat study, blood samples were collected through an indwelling catheter inserted in the left femoral vein at 0.083, 0.25, 0.5, 1, 2, 3, 4, and 6 hours after dosing. In the dog study, blood samples were collected by venipuncture of the cephalic vein at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours after dosing. Urine of dogs was also collected for 0–24 and 24–48 hours after dosing. The blood sampling times in the rat and dog studies were determined based on the reported half-life (t1/2) values (Nagae et al., 1994). Blood samples were rapidly cooled on ice and centrifuged at 1000 × g for 10 minutes at 4°C to obtain the plasma. Plasma and urine samples were stored at −30°C until analysis.

Quantitative Analysis of Plasma, Urine, and Bile. An aliquot (80–100 μl) of plasma, urine, and bile samples was mixed with 1 ml of methanol and spiked with SBC (internal standard). After centrifugation, a portion of the supernatants was evaporated to dryness. The residues were dissolved in 0.3 ml of solvent A with SBC (internal standard). After centrifugation, a portion of the supernatants of plasma, urine, and bile samples was mixed with 1 ml of methanol and spiked with SBC (internal standard). After centrifugation, a portion of the supernatants was evaporated to dryness. The residues were dissolved in 0.3 ml of solvent A (0.1% pentadecafluorooctanoic acid, 99.5% water/0.5% acetonitrile with 0.1% formic acid) and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

LC-MS/MS Analysis. LC-MS/MS analysis was performed using a Waters LC-MS/MS system (Waters, Milford, MA). Chromatographic separation was achieved on an ACQUITY UPLC C18 BEH column (2.1 × 50 mm, 1.7 μm). Waters) kept at 45°C using a linear gradient at 0.8 ml/min with solvents of A and B (0.1% pentadecafluorooctanoic acid, 100% water/90% acetonitrile with 0.1% formic acid). The gradient was started at 99.9% A, and solvent B was linearly increased from 0%–1% (0–0.5 minutes), 2%–20% (0.5–2.0 minutes), 20%–40% (2.0–4.0 minutes), 40%–99.9% (4.0–4.5 minutes), and maintained at 0.1% (4.5–10 minutes). The mass spectrometer was operated in the positive ion mode with electrospray ionization. The ionization source parameters were capillary voltage 0.5 kV, source temperature 150°C, and desolvation gas temperature 400°C, with desolvation gas and cone gas flow rates of 1000 and 50 l/h, respectively. The MSMS transitions (m/z) monitored were used for analysis: m/z 162.1 > 145.1 for SAC, 204.1 > 162.1 for NAc-SAC, 220.1 > 130.1 for NAc-SACS, 178.1 > 88.1 for SACS, 291.1 > 162.1 for GSAC, and 176.1 > 159.1 for SBC at cone and collision energies of 15 and 10 V, respectively.

Pharmacokinetic Analysis. Pharmacokinetic parameters were determined by noncompartmental method using a WinNonlin software (version 2.1, Pharsight, Mountain View, CA). Maximum plasma concentration and time to reach the maximum concentration were determined directly from the experimental data. The area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal rule. Bioavailability was calculated by the ratio of AUC values after oral and i.v. administration of SAC.

In Vitro Drug Metabolism Study. The drug metabolism study was carried out using liver and kidney S9 fractions of rats (male), dogs (male), and humans (mix of male and female). In N-acetylation metabolism studies of SAC and SACS, the reactionmixtures (100 μl) containing 0.1 M phosphate buffer (pH7.4), 5 mM SAC or SACS, 2 mM acetyl-CoA, and each S9 fraction (0.25–1.0 mg protein/ml) were incubated for 10–240 minutes at 37°C. In deacetylation metabolism studies of NAc-SAC and NAc-SACS, the reaction mixtures (100 μl) containing 0.1 M phosphate buffer (pH7.4), 5 mM NAc-SAC or NAc-SACS, and each S9 fraction (0.1–1 mg protein/ml) were incubated for 10–120 minutes at 37°C. All reaction mixtures were added with 1 ml of methanol to terminate the reactions and spiked with SBC. After centrifugation, a portion of the supernatants was evaporated to dryness. The residues were dissolved in solvent A and analyzed with LC-MS/MS.

Determination of Intrinsic Clearance. Intrinsic clearance for deacetylation (CLint, deacetylation) of NAc-SAC and NAc-SACS in each S9 fraction was determined by in vitro half-life method (Obach, 1999). The intrinsic clearance for N-acetylation (CLint, acetylation) of SAC and SACS was calculated using the following formula by nonlinear curve regression fitting (Kyperotis K., Keyence, Osaka, Japan): CI = A/N × (1 − exp−a) × P × (CLint, acetylation + CLint, deacetylation). Where P, S, and CI are the protein concentration, initial concentration of SAC and SACS, and the concentration of their N-acetylated metabolites at the incubation time, t, respectively.

Results

Metabolism, Excretion, and Pharmacokinetics in Rats. After oral or i.v. administration of SAC (5 mg/kg) to rats, the amount of SAC and its metabolites excreted in urine and bile was quantified and expressed as percentage of the dose given (Tables 1 and 2). SAC was excreted little in both urine and bile in the unchanged form. Almost the entire SAC dose administered was excreted as its N-acetylated metabolites, NAc-SAC and NAc-SACS, in the urine; the urinary excretion of SAC, NAc-SAC, SACS, and NAc-SACS was 0.95% (i.v.) and 1.6% (oral), 84% and 83%, 0.01% and 0.01% and, 11% and 11% of the SAC dose, respectively. The total recovery of SAC and its three metabolites in the urine was 96% and 96% of the oral and i.v. dose, respectively.

The pharmacokinetic profile of SAC in rats (5 mg/kg, oral and i.v.) is summarized in Table 3. SAC was well absorbed, with the bioavailability of 92.1%. The pharmacokinetic parameters of t1/2, plasma clearance (CL),

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urinary Excretion (% of Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAC</td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>1.6 ± 0.92</td>
</tr>
<tr>
<td>NAc-SAC</td>
<td>1.8 ± 0.53</td>
</tr>
<tr>
<td>NAc-SACS</td>
<td>0.72 ± 0.60</td>
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</table>

TABLE 1

Urinary excretion of SAC, NAc-SAC, SACS, and NAc-SACS in rats after oral and i.v. administration

Data represent mean ± S.D. of three rats.

n.d., not detected.

A single intraperitoneal injection (5 mg/kg) of SAC, NAc-SAC, SACS, and NAc-SACS was administered to rats.

Amount (% of dose, on a molar basis) of SAC, NAc-SAC, SACS, and NAc-SACS excreted in urine for 24 hours postdose.
and volume of distribution were 1.1−1.2 hours, 0.91 l/h/kg, and 1.0 l/kg, respectively. The renal clearance (Cl_r, 0.0086 l/h/kg) of SAC was much smaller than glomerular filtration rate (GFR, approximately 0.3 l/h/kg) of rats (Davies and Morris, 1993), indicating that SAC undergoes extensive renal reabsorption. The mean plasma concentration-time curves of SAC are shown in Fig. 1, A (oral) and B (i.v.). After the administration of SAC, its three urinary metabolites, NAc-SAC, SACS, and NAc-SACS, were detected in the plasma. LC-MS/MS analysis also identified GSAC as a novel metabolite of SAC. The plasma concentration of SAC was considerably higher than those of the metabolites at all the time points analyzed.

The metabolism, excretion, and pharmacokinetics of NAc-SAC, SACS, and NAc-SACS (5 mg/kg, i.v.) were also studied. A large portion of NAc-SAC administered was excreted in the urine as the unchanged form; the urinary excretion of NAc-SAC, SAC, SACS, and NAc-SACS was 84%, 1.8%, 0.02%, and 12% of the NAc-SAC dose, respectively (Table 1). Almost the entire SAC dose administered was excreted into the urine as NAc-SACS; the urinary excretion of SAC and NAc-SACS was 0.10% and 85% of the SAC dose, respectively. Almost the entire NAc-SACS dose administered was recovered in the urine in the unchanged form (96% of the dose).

The pharmacokinetic parameters of NAc-SAC, SAC, and NAc-SACS (5 mg/kg, i.v.) are summarized in Table 3. The Cl_r values of NAc-SAC (1.5 l/h/kg) and NAc-SACS (1.2 l/h/kg) were higher than the GFR of rats (Davies and Morris, 1993), suggesting that the N-acetylated metabolites of SAC were eliminated by active renal secretion. Similar to SAC, SACS had extremely lower Cl_r value (0.0014 l/h/kg). The mean plasma concentration-time curves of NAc-SAC and SAC (5 mg/kg, i.v.) are shown in Fig. 1, C and D, respectively. After the administration of NAc-SAC, SAC, NAc-SACS, and GSAC were detected in the plasma. The plasma concentration of SAC exceeded that of NAc-SAC in 30 minutes or later and declined in parallel with that of NAc-SAC (Fig. 1C). After the administration of NAc-SAC, its plasma level rapidly decreased along with the appearance of SAC and NAc-SACS.

**Table 2**

<table>
<thead>
<tr>
<th>Compounda</th>
<th>Bilary Excretion (% of Dose)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SAC</td>
</tr>
<tr>
<td>SAC</td>
<td>0.45±0.1</td>
</tr>
<tr>
<td>NAc-SAC</td>
<td>0.49±0.2</td>
</tr>
<tr>
<td>SACS</td>
<td>0.50±0.1</td>
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**Table 3**

<table>
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<tr>
<th>Parameters</th>
<th>SACa</th>
<th>NAc-SACa</th>
<th>SACa</th>
<th>NAc-SACa</th>
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</thead>
<tbody>
<tr>
<td>Cl_r (l/h/kg)</td>
<td>6.2±0.56</td>
<td>3.9±0.64</td>
<td>6.2±0.56</td>
<td>3.9±0.64</td>
</tr>
<tr>
<td>t_max (h)</td>
<td>1.1±0.27</td>
<td>1.2±0.19</td>
<td>1.0±0.25</td>
<td>1.0±0.25</td>
</tr>
<tr>
<td>CL (l/h/kg)</td>
<td>0.91±0.035</td>
<td>1.8±0.34</td>
<td>1.5±0.13</td>
<td>1.3±0.19</td>
</tr>
<tr>
<td>Vss (l/kg)</td>
<td>0.0086</td>
<td>1.5</td>
<td>0.0014</td>
<td>1.2</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>92.1</td>
<td>70.3</td>
<td>91.9</td>
<td>89.1</td>
</tr>
<tr>
<td>AUC (mg/l/h)</td>
<td>5.0±0.21</td>
<td>5.07±0.58</td>
<td>1.87±0.33</td>
<td>n.d.</td>
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<tr>
<td>NAc-SAC</td>
<td>1.80±0.19</td>
<td>1.96±0.39</td>
<td>2.89±0.66</td>
<td>n.d.</td>
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<tr>
<td>SAC</td>
<td>0.57±0.013</td>
<td>0.62±0.014</td>
<td>3.47±0.32</td>
<td>n.d.</td>
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<tr>
<td>NAc-SACS</td>
<td>0.11±0.025</td>
<td>0.11±0.013</td>
<td>0.13±0.073</td>
<td>1.29±0.090</td>
</tr>
</tbody>
</table>

n.d., not detected; t_max, time to reach Cmax; Vss, distribution volume at steady state.

"A single oral or i.v. dose (5 mg/kg) of SAC, NAc-SAC, and SAC was administered to bile duct-cannulated rats.

The pharmacokinetic parameters of SAC, NAc-SAC, SAC, and NAc-SACS in rats after oral and i.v. administration are summarized in Tables 4 and 5, respectively. SAC was also well absorbed in dogs, with the bioavailability of 92.0%. Unlike in rats, NAc-SAC was excreted little in the urine of dogs; the urinary excretion of SAC, NAc-SAC, NAc-SACS, and NAc-SACS was 0.32% (i.v.) and 0.34% (oral), 0.89% and 0.91%, 1.6% and 1.7%, and 12% and 10% of the SAC dose, respectively. The Cl value (0.048 l/h/kg) of SAC in dogs was significantly lower than that (0.91 l/h/kg) in rats, which contributed to the long t1/2 value (12 hours) in dogs. As observed in rats, the Cl value (0.0002 l/h/kg) of SAC was much lower than the GFR of dogs (Davies and Morris, 1993), indicating extensive renal reabsorption of SAC. All metabolites of SAC identified in the rat plasma, NAc-SAC, SAC, NAc-SACS, and GSAC, were also detected in the dog plasma after oral and i.v. administration of SAC (Fig. 2, A and B).

The urinary excretion and pharmacokinetics of NAc-SAC, SACS, and NAc-SACS (2 mg/kg, i.v.) were also studied. In addition to NAc-SAC, all the SAC, SACS, NAc-SACS, and GSAC were detected in the plasma of dogs given NAc-SAC (Fig. 2C); the urinary excretion rates of SAC, NAc-SAC, SACS, and NAc-SACS was 0.49%, 1.8%,...
1.8%, and 12% of the NAc-SAC dose, respectively (Table 4). The small CLr value (0.0019 l/h/kg) of NAc-SAC suggested that it apparently underwent renal reabsorption (Table 5). After administration of SACS, NAc-SACS was detected in the plasma and excreted in the urine (Fig. 2D; Table 4); the urinary excretion of SACS and NAc-SACS was 5.6% and 22% of the SACS dose, respectively. Unlike in rats, SACS was detected in the plasma of dogs given NAc-SACS (Fig. 2E); the urinary excretion of NAc-SACS and SACS was 82% and 2.5% of the NAc-SACS dose, respectively (Table 4).

**In Vitro Drug Metabolism.** In vitro metabolism study was carried out using liver and kidney S9 fractions of rats, dogs, and humans. The CLint values for SAC N-acetylation and NAc-SAC deacetylation in each S9 fraction were determined and compared (Table 6). The CLint values for SAC N-acetylation were five to seven times greater than those for NAc-SAC deacetylation in rat liver and kidney S9 fractions. The CLint values for SAC N-acetylation and NAc-SAC deacetylation were similar in dog liver S9 fraction; however, the CLint value (460 μl/min/mg protein) for NAc-SAC deacetylation was approximately 80 times greater than that (5.9 μl/min/mg protein) for SAC N-acetylation in dog kidney S9 fraction. In human liver and kidney S9 fractions, the CLint values for NAc-SAC deacetylation were more than 10 times greater than those for SAC N-acetylation.

The CLint values for SACS N-acetylation and NAc-SACS deacetylation were also determined (Table 6). In liver and kidney S9 fractions of rats, dogs, and humans, the CLint values for SACS N-acetylation were one or two magnitudes lower than those for SAC N-acetylation, whereas all S9 fractions had little activity for NAc-SACS deacetylation except for dog kidney S9 fraction.

**Discussion**

To date, the metabolism of SAC in animals and humans has not been well studied. Nagae et al. (1994) have reported that SAC was excreted in the unchanged form in rats and in the urine of dogs only to a small extent. Krause et al. (2002) have identified three metabolites of SAC (i.e., NAc-SAC, SACS, and NAc-SACS) in the urine of rats administered SAC. In that study, the total recovery of SAC and its three metabolites in the urine was 40%–50% of the SAC dose; however, the residual 50%–60% was uncertain. In the present study, we aimed to clarify the metabolism of SAC in rats and dogs by

**TABLE 4**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urinary Excretion (% of Dose)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAC</td>
<td>NAc-SAC</td>
</tr>
<tr>
<td>i.v.</td>
<td>0.32 ± 0.07</td>
<td>0.89 ± 0.18</td>
</tr>
<tr>
<td>Oral</td>
<td>0.34 ± 0.19</td>
<td>0.91 ± 0.44</td>
</tr>
<tr>
<td>i.v.</td>
<td>0.49 ± 0.06</td>
<td>1.8 ± 0.54</td>
</tr>
<tr>
<td>NAc-SAC</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>i.v.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d., not detected.

* A single oral or i.v. dose (2 mg/kg) of SAC, NAc-SAC, SACS, and NAc-SACS was administered to dogs.

* Amount (% of dose, on a molar basis) of SAC, NAc-SAC, SACS, and NAc-SACS excreted in urine for 48 hours postdose.
conducting the excretion and pharmacokinetic studies in vivo and metabolism study in vitro.

Initially, as reported previously (Nagae et al., 1994; Chang-Kai and Yan and Zeng, 2005), our studies confirmed the high oral absorption of SAC in rats and dogs with its bioavailability values of 92.1% and 92.0%, respectively.

In rats, after administration of SAC (5 mg/kg, oral and i.v.), SAC was excreted only to a small degree in the unchanged form, but it was excreted as its \(N\)-acetylated metabolites, NAc-SAC and NAc-SACS.

The urinary excretion of SAC, NAc-SAC, SACS, and NAc-SACS was 0.95%, 84%, 0.01%, and 11% of the i.v. dose of SAC, respectively (Table 1). We also identified GSAC in the plasma as a novel metabolite of SAC in addition to its three urinary metabolites, NAc-SAC, SACS, and NAc-SACS (Fig. 1, A and B). The plasma concentration of SAC was considerably greater than that of NAc-SAC and NAc-SACS, despite the fact that almost the entire SAC dose administered was excreted as its \(N\)-acetylated metabolites. It is well established that amino acids are actively reabsorbed from the urine via several types of transporter in the kidney (Silbernagl et al., 1975; Bröer, 2008). The CL\(_r\) values of SAC (0.0086 l/h/kg) and its \(N\)-acetylated metabolites (\(>1.2\) l/h/kg) indicated that SAC undergoes extensive renal reabsorption, whereas NAc-SAC and NAc-SACS are eliminated by active renal secretion (Table 3). These results clearly explained why SAC was eliminated as its \(N\)-acetylated metabolites; SAC was ultimately metabolized to NAc-SAC and NAc-SACS, both of which were actively excreted in the urine, through the cycle of its urinary excretion, renal

### Table 5

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SAC</th>
<th>NAc-SAC</th>
<th>SACS</th>
<th>NAc-SACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(_{\text{max}}) (mg/l)</td>
<td>3.2 ± 0.025</td>
<td>2.1 ± 0.14</td>
<td>9.7 ± 2.1</td>
<td>12 ± 3.3</td>
</tr>
<tr>
<td>t(_{\text{max}}) (h)</td>
<td>—</td>
<td>1.3 ± 0.58</td>
<td>—</td>
<td>11 ± 1.0</td>
</tr>
<tr>
<td>t(_{1/2}) (h)</td>
<td>12 ± 0.39</td>
<td>12 ± 1.2</td>
<td>11 ± 1.5</td>
<td>6.0 ± 0.28</td>
</tr>
<tr>
<td>CL (l/h/kg)</td>
<td>0.048 ± 0.006</td>
<td>0.11 ± 0.031</td>
<td>0.074 ± 0.010</td>
<td>0.20 ± 0.009</td>
</tr>
<tr>
<td>CL(_r) (l/h/kg)</td>
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<td>0.0019</td>
<td>0.0042</td>
<td>0.17</td>
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<tr>
<td>V(_{\text{ss}}) (l/kg)</td>
<td>0.83 ± 0.10</td>
<td>—</td>
<td>1.3 ± 0.10</td>
<td>0.51 ± 0.059</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>—</td>
<td>92.0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data represent mean ± S.D. of three dogs.

n.d., not detected; t\(_{\text{max}}\), time to reach C\(_{\text{max}}\); V\(_{\text{ss}}\), distribution volume at steady state.

*A single oral or i.v. dose (2 mg/kg) of SAC, NAc-SAC, SACS, and NAc-SACS was administered to dogs.

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**Fig. 2.** Plasma concentration-time profiles of SAC, NAc-SAC, SACS, NAc-SACS, and GSAC in dogs after administration of a single dose (2 mg/kg) of SAC (A, oral; B, i.v.), NAc-SAC (C, i.v.), SACS (D, i.v.), and NAc-SACS (E, i.v.). Each point represents mean ± S.D. of three dogs.
Our excretion and pharmacokinetic studies in rats and dogs demonstrated that NAc-SAC was also metabolized back to SAC. After i.v. administration of NAc-SAC, SAC rapidly appeared in the plasma, and its plasma concentration declined in parallel with that of NAc-SAC (Figs. 1C and 2C). These results suggested that the formation of NAc-SAC from SAC was dependent on the metabolic activities of organs responsible for SAC N-acetylation and NAc-SAC deacetylation. SAC is one of cysteine S-conjugates that are formed in a series of drug metabolism of glutathione S-conjugates and furthermore undergo N-acetylation metabolism in the liver and kidney of mammalian species (Dekant et al., 1988; Hinchman and Ballatori, 1994). Based on these works, we carried out a metabolism study in vitro to examine both the metabolism of SAC N-acetylation and NAc-SAC deacetylation using liver and kidney S9 fractions of rats, dogs, and humans. In our preliminary experiments, the enzymatic activities of the S9 fractions for SAC N-acetylation and NAc-SAC deacetylation were present in different subcellular fractions, microsomes and cytosol, respectively (data not shown). Therefore, we used the S9 fractions for direct comparison of both metabolisms. Our study demonstrated that liver and kidney S9 fractions of rats, dogs, and humans had activities for both SAC N-acetylation and NAc-SAC deacetylation, although a large species difference was observed (Table 6). In rat liver and kidney S9 fractions, the CLint values for SAC N-acetylation were five to seven times greater than those for NAc-SAC deacetylation, suggesting that NAc-SAC could be formed in the liver and kidney of rats. On the other hand, both S9 fractions had little activity for NAc-SAC deacetylation. This result was consistent with our finding in vivo that SAC was not detected in the plasma after the administration of NAc-SAC to rats (Table 3). In the study in dogs, it was of special note that the kidney S9 fraction had an extremely high activity for NAc-SAC deacetylation; the CLint value (460 μl/min/mg protein) for NAc-SAC deacetylation was approximately 80-fold greater than that for SAC N-acetylation. The metabolism of SAC to NAc-SAC seems to be the primary elimination pathway of SAC in rats and dogs, based on the AUC ratios (1:2–1:3) of NAc-SAC to SAC. However, nonrenal CL value (0.048 l/h/kg) of SAC in dogs was significantly smaller than that in rats (0.90 l/h/kg). Together with little urinary excretion of NAc-SAC in dogs, these results suggested that NAc-SAC was converted to SAC before its urinary excretion by the strong renal activity for NAc-SAC deacetylation and excreted in the urine as SAC. As a result, it was estimated that almost all the SAC administered circulated repeatedly as the unchanged form through its renal reabsorption and was ultimately excreted in bile. This conclusion could explain why SAC had a long t1/2 value in dogs, but the biliary excretion of SAC should be confirmed by future study. Among all the S9 fractions tested, only dog kidney S9 fraction had activity for NAc-SAC deacetylation, which was consistent with the in vivo result that SAC was detected in the plasma of dogs given NAc-SAC (Fig. 2E). In human S9 fractions, the CLint values for NAc-SAC deacetylation were more than 10 times greater than those for SAC N-acetylation, suggesting a short residence time for NAc-SAC in humans.

In this study, the metabolic pathway of SAC was investigated both in vitro and in vivo. The S-oxidation metabolism of SAC in vitro by flavin-containing monoxygenases of mammalian species has been reported (Ripp et al., 1999; Krause et al., 2003). Our in vitro studies demonstrated that N-acetylation of SAC and SACS was catalyzed by liver and kidney S9 fractions of rats and dogs. The pharmacokinetic studies of SAC and NAc-SAC suggested that NAc-SACS was formed via both N-acetylation of SAC and S-oxidation of NAc-SAC in vivo. Collectively, the entire metabolic pathways of SAC in rats and dogs are shown in Fig. 3.
dogs are postulated in Fig. 3, displaying each metabolite formation as the novel metabolite of SAC for the first time (Fig. 1, A and B; Fig. 2, A and B). γ-Glutamyl transpeptidase catalyzes the transfer of γ-glutamyl group from glutathione to peptides, amino acids, and water (Griffith et al., 1979). The metabolism of SAC to GSAC by this enzyme will be investigated in our future study.

In conclusion, this study demonstrated that the pharmacokinetics of SAC in rats and dogs was characterized by high oral bioavailability, N-acetylation and S-oxidation metabolism, and extensive renal reabsorption. This study also emphasizes the critical roles of the liver and kidney in the elimination of SAC: the metabolism of SAC to its N-acetylated metabolites in the liver and kidney facilitates the elimination of SAC, whereas renal reabsorption of SAC delays its elimination.

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Participated in research design: Amano.
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