Pharmacokinetics of Salazosulfapyridine (Sulfasalazine, SASP) (V):

Pharmacokinetics of SASP after a single intravenous or oral administration in the dog.

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Summary

Salazosulfapyridine (Sulfasalazine, SASP) labelled with \(^{14}\text{C}\) in the carboxyl group and with \(^{3}\text{H}\) in the benzenesulfonyl ring was administered intravenously or orally to male and female beagle dogs with and without bile fistulas in order to study the pharmacokinetics. After intravenous administration a plasma elimination half life \((t_{1/2p})\) for SASP was calculated to 14 min. The volume of distribution \((V_{d.0})\) was found to be 0.47 l/kg and the total clearance was about 35 ml/min x kg. After oral administration of 50, 250 or 500 mg/kg the time for maximal plasma concentration of SASP was 1-3 hours and the maximal concentration was 2.81±1.42 pM after a dose of 50 mg/kg and 15.08±3.04 pM after 500 mg/kg body weight.

The bioavailability varied between 5.4-30% which is within the same range as reported from studies in man. The renal clearance was between 0.1-0.2 ml/min x kg.

Metabolites of SASP were separated by HPLC and identified by mass spectrometry. 5-aminosalicylic acid (5ASA), sulfapyridine (SP) and its glucuronide were found in plasma and urine. 5ASA, N-acetylated 5ASA (Ac5ASA), SP and unmetabolized SASP were found in faeces.

The cumulative biliary excretion of SASP after i.v. administration was as a mean 95% of the administered dose. Similar figures were obtained for the \(^{14}\text{C}\) and \(^{3}\text{H}\) radioactivity. Thus a quantitative excretion of the unchanged drug was found in the bile during 24 hours after administration and >90% was recovered during the first 2 hours. No metabolites were found in the bile. After oral administration of SASP 18% of the administered dose was recovered as the unchanged drug in bile, 17% as \(^{14}\text{C}\) and 19% as \(^{3}\text{H}\) radioactivity within 48 hours. As after intravenous administration no metabolites were found in bile after the oral administration.

The total recovery of excreted radioactivity after i.v. and oral administration was 69-96%. The tritium radioactivity representing the SP metabolites was mainly found in urine, while carbon-14 representing SASP and 5ASA metabolites was preferentially excreted in faeces. This is in accordance with the higher absorption of SP and its metabolites, compared to 5ASA which is poorly absorbed and thus mainly eliminated via faeces together with a small amount of unchanged SASP.
INTRODUCTION

In the preceding papers the pharmacokinetics of SASP in the rat has been described. As a complement to these studies and since toxicological studies have been performed not only in rats but also in dogs, this paper will deal with the pharmacokinetics of SASP in the dog after a single intravenous or oral administration. The present study was designed to facilitate comparison with data obtained from rats. The elimination of SASP and its metabolites were followed in plasma, urine, faeces and bile and kinetic parameters were calculated. To our knowledge no such data are available in the literature.

MATERIALS AND METHODS

Materials

$[^{14}C]$SASP and $[^{3}H]$SASP were synthesized according to a synthetic route as previously described\(^1\). The specific activity of $[^{14}C]$SASP was 76MBq/mmol (2.05mCi/mmol) and the specific activity of $[^{3}H]$SASP was 1.09GBq/mmol (29.5mCi/mmol). The radiochemical purity was >97% for both $[^{14}C]$SASP and $[^{3}H]$SASP judged by thin layer and liquid chromatography. SASP was obtained from Pharmacia AB, Uppsala, Sweden. Sulfapyridine (SP), N-acetylsulfapyridine (AcSP), 5-acetamidosalicylic acid (Ac5ASA), 5-hydroxysulfapyridine (SPOH), N-acetyl-5-hydroxysulfapyridine (AcSPOH) were synthesized in the department of Organic Chemistry, Pharmacia AB, Uppsala, Sweden. Other chemicals used were of analytical grade obtained from different suppliers.

Animals and dosage

Male and female beagle dogs were obtained from Shamrock Farms, Henfield, Sussex, England. The dogs were 4~8 month old and weighed 9~13kg. The animals were fed twice a day with solid food (Peka Bello, Peter Kölln, Elushow, West Germany) and had free access to tap water. The dogs were housed in an animal room maintaining a room temperature of 18~27°C, a relative humidity of 30~70% and with a 12 hour light cycle. The animals were acclimatized to the laboratory conditions for at least four weeks before commencement of the experiment. During the experiments the dogs were housed individually in metabolism cages for collection of urine and faeces. The animals were allowed 1 day for acclimatization in the cages before administration. Food was withdrawn the evening before administration of the drug until 4 hours after administration. Two or three dogs were used in each group. The intravenous dose was 5mg/kg body weight with 1 and 2MBq/kg of $^{14}C$ and $^{3}H$ respectively. The intravenous dose was administered in a foreleg-vein as a solution containing 5mg SASP/ml TRIS buffer (15μM). The oral doses were 50, 250 and 500mg/kg body weight and was administered as a suspension of 100mg SASP per ml distilled water and given by gavage.

Bile fistula operation

The animals were weighed, anaesthetized with thiopentobarbital 1.5mg/ml (25mg/kg i. v.) and intubated for mechanical ventilation. Halothane was used as an inhalation anaesthetic. The animal was shaved and washed with Jodopax (5% idodine, Ferrosan, Swe-
den) diluted in 70% ethanol and rinsed with saline. The animal was covered with a sterile operation cloth open over the incision area. Laparotomy in linea alba was made from the edge of the breast bone and 25 cm downwards. The gall-bladder duct was ligated and the gall-bladder was emptied. The bladder was excised as carefully as possible leaving the part connected to the liver in place to avoid major bleedings. Surgigel (Absorbable Hemostatic Gauze, Ethicon, Scotland) was put on the wound to control bleeding. The bile duct was ligated 2~3 cm down the stalk from the liver. A hole was cut in the bile duct between the liver and the ligature. The catheter tip (about 3 cm in length) was guided into the bile duct and secured. The pancreatic duct was also ligated. The distal part of the bile duct was ligated into the intestine and the suture used to guide another catheter into the intestine and also used as fixation. The bile duct was cut as mentioned earlier. The catheter was cut to a desired length. Three side holes were cut above the tip. The catheter was then guided through the hole in the bile duct so the 3 holes in the catheter were placed in the intestinal lumen and secured. The proximal and distal catheters were connected with a suitable extension to avoid leakage into the abdominal cavity during the preparation and to control function. A cut was made in the abdominal muscle and skin in a place suitable in relation to the bile duct and convenient and not readily accessible for the dog. The base plate of a specially constructed bile fistula was guided to the outside and fixed with sutures in the abdominal muscle. The edges of the abdominal muscle and the skin were fitted in to milled out slits in the fistula and closely sutured. The catheters were disconnected, guided through tightly fitting holes in a silicon membrane in the center of the fistula and reconnected. The catheter leading to the intestine was labelled. A cover was screwed on. The peritoneum and the abdominal muscle were sutured with silk, the skin with stainless steel suture. After bile fistula operation the animals were allowed to recover during at least one week before administration.

Sample collection

After intravenous administration blood was collected at the following times: 0, 5, 10, 20, 30, 40, 60 and 90 minutes and after: 2, 3, 4, 6 and 8 hours. Urine and faeces were collected during the following time intervals at: 0~24, 24~48, 48~72, 72~96 and 96~120 hours. After oral administration blood was collected at the following times: 0, 0.5, 1, 2, 4, 6, 8, 10, 14, 24, 32, 48, 72 and 96 hours and urine and faeces during: 0~8, 8~24, 24~48, 48~72, 72~96, 96~144 and 144~168 hours. Plasma was prepared from the blood and all samples were stored at -20°C pending analysis.

In operated animals bile was collected continuously during the following time periods after intravenous administration: -10~0, 0~10, 10~20, 20~30, 30~40, 40~60 min and thereafter in 30 minutes periods up to 8 hours after administration. Blood samples were collected in heparinized tubes from a vein in the foreleg, which was not used for administration. Samples were collected according to the following time schedule: 0, 5, 10, 30 minutes, 1, 2, 4, 6 and 8 hours after administration. After oral administration bile was collected continuously in 30 minutes intervals up to 10 hours and then during the following time intervals 10~14, 14~24 and 24~48 hours. Blood samples (minimum 3 ml) were collected in heparinized test tubes from a foreleg vein according to the following time
schedule 0, 0.5, 1, 2, 4, 6, 8, 10, 14, 24 and 48 hours after administration.

**Measurement of radioactivity**

Radioactivity of all samples was determined by liquid scintillation counting (Beckman LS 2800) after prior combustion with Combustaid in a Sample Oxidizer, TRI-CARB Model 306 (Packard Instrument), to $^{14}\text{CO}_2$ and $^3\text{H}_2\text{O}$. The tritiated water was condensed in Monophase S (Packard). The $^{14}\text{CO}_2$ was absorbed to an amine in Carbo Sorb and PermafluorV (Packard) was added. The recovery of radioactivity added to biological samples was quantitative with a coefficient of variation of ±0.3% for plasma, 3.2% for urine and 1.5% for faeces.

**Analysis of SASP**

Plasma, urine or bile samples were mixed with two volumes of methanol to precipitate proteins. The samples were placed at −20°C for 10 minutes followed by centrifugation and 50µl of the supernatant was injected into a high performance liquid chromatography system equipped with a reversed phase column (Nucleosil C$_{18}$, 10µM, Mackerey-Nagel & Co, Düren, FRG) and a precolumn (Perisorb C$_{18}$, 40µm, Merck Darmstadt, FRG). A UV detector (Kratos Spectroflow 783) was used at 360nm. The mobile phase consisted of methanol/phosphate buffer 0.025M, pH 2.5 (64:36, v:v). The column was kept at 37°C and the flow rate was set to 1ml/min.

Faeces samples were homogenized in methanol, 1.5 times its weight and were centrifuged at 1000g for 10 min. An aliquote of 100µl supernatant was diluted with 100µl water and 20µl of the mixture was injected into the chromatography system.

The detection limit of SASP in dog plasma was 1.4µmole/l, in dog urine 4.1µmole/l, in dog faeces 3.6µmole/l and in dog bile 7.9µmole/l. The coefficient of variation was <4.5% at 100µmole/l and <7% at 1.3~5µmole (n =10). The accuracy was 101~107% and the relative recovery 99~101% (n =6) at 100µmole/l.

**Analysis of metabolites**

To the plasma, urine or bile samples (100µl) were added 10µl 1M acetate buffer and 5µl β-glucuronidase/arylsulfatase and the mixture was incubated at 37°C for 3 hours. The faeces samples were homogenized in methanol, 1.5×faeces weight. An aliquote of 150µl of the supernatant obtained after centrifugation was evaporated and redissolved in 150µl of water and 10µl of β-glucuronidase/arylsulfatase. The faeces samples were further treated as the other matrices. The incubations were stopped by addition of 400µl of methanol and the samples were allowed to stand for 15 minutes. The samples were centrifuged and 20µl of the supernatant was injected into a high performance liquid chromatography system with a reversed phase (Nucleosil C$_{18}$, 7µm) column and precolumn (Perisorb RP−18, 30~40µm). The mobile phase was a mixture of water containing 5.3M hexanesulfonic acid and 10mM phosphoric acid, methanol and triethylamine (920:180:0.3). The flow rate was set to 1ml/min. A UV−detector (254nm), a fluorescence detector (315/470 nm) and an electrochemical (+0.6V) detector were connected in series for determination of SP, SPOH (UV) and Ac5ASA, AcSP (fluorescence) and 5ASA (electrochemical).
The detection limits for the different metabolites were around 10~40μmole/l. The recoveries for these metabolites were in the range 94~101% (n=6). The coefficient of variation were in the range of 1.4~13.6% (n=6) at a concentration level of about 100μmole/l. It was not possible to determine 5ASA in urine and faeces due to interfering background in the chromatograms.

**Pharmacokinetic analysis**

Values of \( C_{\text{max}} \) and \( T_{\text{max}} \) were directly read from concentration data. Values of area under the curve were calculated by the trapezoidal method. Pharmacokinetic calculations were performed using the feathering technique with a computer program written in SAS (SAS Inst. Inc., Box 8000 Cary N.C., USA).

**Metabolite identification**

Samples collected from the dogs that received SASP 250mg/kg body weight, p.o., were used for identification of metabolites of SASP. The samples were diluted three times with NH₄Ac buffer, 0.1M, pH 4.8, followed by fractionation of the metabolites by high performance liquid chromatography. A reversed phase column (Nucleosil C₁₈, 5μM, 125×4mm) was used with 0.1M NH₄Ac/MeOH as mobile phase and with gradient elution. (MeOH (%) / time (min) : 0/0, 10/10, 10/14, 40/24, 100/26 and 100/30). Fractions containing radioactivity were collected for further analysis by mass spectrometry. A triple stage quadrupole mass spectrometer (TSQ 70, Finnigan Mat, San Jos’e, USA) equipped with a thermospray interface was used for identification of metabolites in the collected fractions. Aliquots of 50μl were injected directly via a sample injection loop into the mass spectrometer. The flow rate of the mobile stream, MeOH/0.5M NH₄Ac (30 : 70), was set to 1.2 ml/min. Argon (0.5mtorr) was used as collision gas to obtain daughter ion mass spectra. Vaporizer and block temperature as well as repeller potential and collision energy were optimized for each substance.

**RESULTS**

**Plasma kinetics**

The plasma elimination of SASP, \(^{14}\text{C}\) and \(^{3}\text{H}\) during the first hour after intravenous administration is presented in Fig. 1. The plasma concentrations of SASP were only measurable during the first 40 minutes after administration. The concentration of radioactivity showed an elimination rate parallel with that of SASP. The initial plasma half-life \( (t_{1/2\alpha}) \) including the distribution phase was 4 min and the plasma half-life elimination \( t_{1/2\beta} \) was 14±3 min (mean±SEM). The apparent volume of distribution \( (V_{\text{dss}}) \) was 0.47 ±0.06 l/kg and the plasma clearance \( (CL_{\text{tot}}) \) was 34.80±1.79 ml/min \( \times \) kg (mean±SEM). The values calculated for \(^{14}\text{C}\) and \(^{3}\text{H}\) were similar (Table 1). Two to three hours after the i.v. administration the radioactivity rose and peak concentrations were reached after 6 hours reflecting the appearance of splitting products of SASP (Fig. 2). The tritium concentration representing SP and its metabolites was higher than the carbon-14 level representing the 5ASA metabolites.

In the bile fistula operated dogs the plasma half-life \( (t_{1/2\beta}) \) of SASP after i.v. admin-
Table 1  The pharmacokinetic parameters obtained after a single intravenous administration of $^{14}$C, $^{3}$H\text{SASP} to male dogs

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>$t_{1/2\alpha}$ min</th>
<th>$t_{1/2\beta}$ min</th>
<th>$V_{dss}$ l/kg</th>
<th>$CL_{tot}$ ml/min$\times$kg</th>
<th>AUC h$\times$μM eq</th>
</tr>
</thead>
<tbody>
<tr>
<td>SASP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4$^{1)}$</td>
<td>14 ± 3</td>
<td>0.47 ± 0.05</td>
<td>34.80 ± 1.79</td>
<td>6.04 ± 0.31</td>
</tr>
<tr>
<td>5$^{2)}$</td>
<td>5</td>
<td>29 ± 0</td>
<td>0.12 ± 0.04</td>
<td>7.70 ± 2.12</td>
<td>29.38 ± 8.08</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>20 ± 0</td>
<td>0.33 ± 0.01</td>
<td>23.40 ± 0.66</td>
<td>8.95 ± 0.26</td>
</tr>
<tr>
<td>5$^{2)}$</td>
<td>5</td>
<td>17 ± 2</td>
<td>0.09 ± 0.01</td>
<td>9.94 ± 1.71</td>
<td>22.74 ± 3.40</td>
</tr>
<tr>
<td>$^{3}$H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>17 ± 0</td>
<td>0.30 ± 0.02</td>
<td>25.0 ± 0.90</td>
<td>8.28 ± 0.30</td>
</tr>
<tr>
<td>5$^{2)}$</td>
<td>5</td>
<td>20 ± 4</td>
<td>0.10 ± 0.01</td>
<td>10.12 ± 1.64</td>
<td>22.16 ± 3.13</td>
</tr>
</tbody>
</table>

Mean ± SEM, n=3.

1) n=1
2) bile fistula operated dogs

Fig. 1  Plasma elimination of SASP, $^{14}$C and $^{3}$H during the first hour after i.v. administration of $^{14}$C,$^{3}$H\text{SASP} 5mg/kg body weight.
Mean, n=3.

Fig. 2  Plasma concentration of $^{14}$C and $^{3}$H in dogs administered $^{14}$C,$^{3}$H\text{SASP} 5mg/kg body weight intravenously.
Mean ± SEM, n=3.
Table II  The pharmacokinetic parameters of SASP calculated after a single oral administration of \([^{14}C, \, {^3}H\text{-SASP}}\) to the dog

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose mg/kg</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; μM eq</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; h</th>
<th>t&lt;sub&gt;1/2 β&lt;/sub&gt; h</th>
<th>AUC h×μM eq</th>
<th>Bioavailability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50</td>
<td>2.81 ± 1.42</td>
<td>1.5 ± 0.5&lt;sup&gt;1)&lt;/sup&gt;</td>
<td>—</td>
<td>3.26 ± 2.01</td>
<td>5.40 ± 3.32</td>
</tr>
<tr>
<td>Female</td>
<td>250</td>
<td>6.31 ± 0.48</td>
<td>2.33 ± 0.33</td>
<td>4.2 ± 0.9</td>
<td>59.98 ± 7.50</td>
<td>19.86 ± 2.48</td>
</tr>
<tr>
<td>Male</td>
<td>250</td>
<td>11.48 ± 2.26</td>
<td>1.33 ± 0.33</td>
<td>4.1 ± 0.4</td>
<td>90.88 ± 23.16</td>
<td>30.08 ± 7.67</td>
</tr>
<tr>
<td>Male</td>
<td>500</td>
<td>15.08 ± 3.04</td>
<td>1.33 ± 0.33</td>
<td>5.4 ± 2.2</td>
<td>94.10 ± 19.44</td>
<td>15.58 ± 2.39</td>
</tr>
<tr>
<td>Male operated&lt;sup&gt;2)&lt;/sup&gt;</td>
<td>500</td>
<td>141.5 ± 37.1</td>
<td>6.67 ± 0.67</td>
<td>5.7 ± 3.8</td>
<td>1322 ± 364</td>
<td>45.00 ± 12.41</td>
</tr>
</tbody>
</table>

Mean ± SEM, n = 3.
1) n = 2
2) bile fistula operated dogs

Mean ± SEM, n = 3. SEM < 34% of mean.

![Graph](image_url)

**Fig. 3** Plasma concentration of SASP after oral administration of 250mg/kg body weight to male and female dogs. Mean, n = 3. SEM < 34% of mean.

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After single oral administration, SASP was measurable in plasma during eight to ten hours, (Fig. 3, Table II). The time for maximal concentration (T<sub>max</sub>) was between one to three hours and the maximal concentration (C<sub>max</sub>) between 2.81 ± 1.42μM (50mg/kg, p.o.) to 15.08 ± 3.04μM (500mg/kg, p.o.). The C<sub>max</sub> for female and male dogs that received...
250 mg/kg p.o., were 6.31 ± 0.48 µM and 11.48 ± 2.26 µM respectively. There was a pronounced interindividuation variation in the plasma concentrations of SASP. These data indicate that the major absorption site of SASP was in the small intestine. After oral administration to bile fistula operated dogs Tmax varied between 4~10 hours with a Cmax in the range of 86~562 µM SASP. The bioavailability in non operated male dogs were 5.4, 30.1 and 15.6% at the doses of 50, 250 and 500 mg/kg respectively and 19.9% for female dogs receiving 250 mg/kg. The plasma half-life (t1/2β) of SASP was 4.13 ± 0.44 hours, (250 mg/kg, p.o. male) and 5.43 ± 2.2 hours, (500 mg/kg p.o.) The data obtained for the 50 mg p.o. dose were too few for calculation of the elimination half-life.

The radioactivity persisted for much longer time in plasma due to the formation of metabolites by reductive cleavage of SASP. ³H and ¹⁴C corresponding to sulfapyridine and 5ASA respectively with their metabolites gave maximal plasma concentrations varying between 6~24 hours depending on the dose (Fig. 4), with higher dose the Cmax was shifted to a later time.

Biliary excretion

The cumulative biliary excretion of SASP after intravenous administration was 98% (90~106%) and 92% (87~97%) in female and male dogs respectively. The major part was eliminated during the first 2 hours after administration (Fig. 5). Measured as radioactivity the biliary excretion in female and male dogs were for ¹⁴C 89% and 103% and for ³H 89% and 101% respectively. It is evident that SASP is eliminated unchanged via biliary excretion and thus no metabolites were found in bile after i.v. administration.
The mean cumulative biliary excretion of SASP after oral administration was almost completed 48 hours after administration and amounted to 16% (14～19%) for female and 19% (17～22%) for male dogs (Fig. 6). The corresponding figures for $^{14}$C was 17% (12～21%) and for $^3$H 19% (9～28%) for both female and male dogs. As after intravenous administration no metabolites were found in bile.

**Urinary and fecal excretion**

The intravenous as well as the oral doses were excreted quantitatively within 48 hours in urine and faeces. The recoveries from the dogs given $[^3$H, $^{14}$C]SASP 250 or 500mg/kg body weight orally were approximately 90% of the given dose while the recoveries from the dogs given $[^{14}$C, $^3$H]SASP 50mg/kg body weight orally and after the intravenous dose were slightly lower (Figs. 7～10, Table III). The amount of SASP excreted unchanged after oral administration ranged as a mean between 0.16～27.39% of the dose and the main part was found in faeces (Table III). No unchanged SASP was recovered
Table III Recovery of SASP in urine and faeces after peroral administration of SASP to male and female dogs

<table>
<thead>
<tr>
<th>Dose mg/kg bw</th>
<th>Recovery % of dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>urine</td>
</tr>
<tr>
<td>50</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>250</td>
<td>0.10±0.04</td>
</tr>
<tr>
<td>250</td>
<td>0.18±0.07</td>
</tr>
<tr>
<td>500</td>
<td>0.08±0.01</td>
</tr>
</tbody>
</table>

Mean±SEM, n=3.
1) female

Fig. 7 Accumulated excretion of 3H in urine and faeces from dogs administered [14C, 3H]SASP 250mg/kg body weight orally. Mean, n=3.

Fig. 8 Accumulated excretion of 14C in urine and faeces from dogs administered [14C, 3H]SASP 250mg/kg body weight orally. Mean, n=3.

after the intravenous administration. The renal clearance of SASP was ranged between 0.1–0.2ml/min × kg.

Most of the radioactivity excreted represented metabolites. SP and its metabolites represented by the 3H radioactivity, were mainly excreted via urine except for the highest dose and 5ASA and its metabolites, represented by the 14C radioactivity, were mainly
excreted via faeces (Table IV). The sum of SP metabolites and SASP corresponded well to that of the recovered $^3$H in urine and faeces, while the recovery of $^{14}$C was much higher compared to the sum of SASP and Ac5ASA (Table V). This is explained by the fact that 5ASA was not determined in urine and faeces due to methodological problems. 5ASA is however a major metabolite in urine and faeces from dogs as judged by the radioactivity profiles in Fig. 11 and 12.

**Metabolism**

Metabolic profiles were obtained from urine and faeces samples, containing the highest radioactivity, collected during 8~24 hours after administration and plasma samples collected after 14 hours, from male and female dogs given SASP 250mg/kg body weight p.o. Faeces from a female dog contained 5ASA, SP and SASP (Fig. 11a). The metabolite profile of faeces from a male dog contained in addition to 5ASA, SP and SASP also Ac5ASA (Fig. 11b). The concentration of Ac5ASA in faeces from the female dog was too low to give any response in the radioactivity flow detector but a small peak was seen in the UV chromatogram. The urine from male and female dogs also showed similar
Table V  Summary of total recovery during 7 days in % of administered dose of $^{14}$C and $^3$H after intravenous (i.v.) and oral (p.o.) administration of $[^{14}$C, $^3$H]SASP to dogs.

<table>
<thead>
<tr>
<th>Dose, mg/kg b.w.</th>
<th>Urine*</th>
<th>Faeces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 i.v. male</td>
<td>$^{14}$C 27.4±5.8</td>
<td>47.5±6.3</td>
<td>74.9</td>
</tr>
<tr>
<td></td>
<td>$^3$H 62.0±1.9</td>
<td>20.5±3.0</td>
<td>82.5</td>
</tr>
<tr>
<td>50 p.o. male</td>
<td>$^{14}$C 25.7±5.7</td>
<td>43.4±7.4</td>
<td>69.1</td>
</tr>
<tr>
<td></td>
<td>$^3$H 54.4±5.3</td>
<td>17.2±3.8</td>
<td>71.6</td>
</tr>
<tr>
<td>250 p.o. male</td>
<td>$^{14}$C 27.5±7.7</td>
<td>61.2±13.6</td>
<td>88.7</td>
</tr>
<tr>
<td></td>
<td>$^3$H 61.8±10.6</td>
<td>34.1±10.6</td>
<td>95.9</td>
</tr>
<tr>
<td>250 p.o. female</td>
<td>$^{14}$C 38.4±9.7</td>
<td>51.3±9.5</td>
<td>89.7</td>
</tr>
<tr>
<td></td>
<td>$^3$H 65.8±11.6</td>
<td>25.1±7.9</td>
<td>90.9</td>
</tr>
<tr>
<td>500 p.o. male</td>
<td>$^{14}$C 25.5±11.4</td>
<td>59.2±20.1</td>
<td>84.7</td>
</tr>
<tr>
<td></td>
<td>$^3$H 48.8±20.1</td>
<td>46.3±26.8</td>
<td>95.1</td>
</tr>
</tbody>
</table>

Mean = SEM, n = 3
*including cage rinse

Fig. 11 UV, $^3$H and $^{14}$C chromatograms of faeces from a female dog (a) and a male dog (b).

Figures and profiles and contained SP and SPglucuronide together with 5ASA (Fig. 12). Plasma samples contained 5ASA, SPglucuronide and SP (Fig. 13).

Identification of 5ASA, Ac5ASA, SP and SASP was made by comparison of daughter ion mass spectra of collected fractions with those of synthesized standards, as exemplified for SP in Sjöquist et al.\(^7\). SPglucuronide was identified from its fragmentation pattern. Thus, m/z 442 corresponds to the protonated molecular ion of SPglucuronide and was selected for collision induced dissociation to form daughter ions m/z 110, 156,
Fig. 12 UV, $^3$H and $^{14}$C chromatograms of urine from a female dog (a) and a male dog (b).

Fig. 13 UV, $^3$H and $^{14}$C chromatograms of plasma from a female dog (a) and male dog (b).

The latter formed from m/z 442 after the loss of 176 which corresponds to the glucuronide moiety (Fig. 14). The metabolite pathways of SASP in the dog are illustrated in Fig. 15.
Table V Accumulated excretion of sulfasalazine, metabolites, $^{14}$C and $^{3}$H in urine and faeces collected during 7 days from dogs as % of given dose after an oral administration of $[^{14}$C,$^{3}$H] SASP 250mg/kg bw.

<table>
<thead>
<tr>
<th>Animal</th>
<th>SASP (%)</th>
<th>Ac5ASA (%)</th>
<th>SP (%)</th>
<th>SPOH (%)</th>
<th>AcSP (%)</th>
<th>AcSPOH (%)</th>
<th>SASP+ Ac5ASA (%)</th>
<th>$^{14}$C (%)</th>
<th>SASP+ SP-metab (%)</th>
<th>$^{3}$H (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>4.95</td>
<td>8.84</td>
<td>21.97</td>
<td>9.66</td>
<td>0.00</td>
<td>0.84</td>
<td>13.79</td>
<td>51.30</td>
<td>37.42</td>
<td>25.10</td>
</tr>
<tr>
<td>Males</td>
<td>10.09</td>
<td>9.02</td>
<td>33.16</td>
<td>5.21</td>
<td>0.05</td>
<td>0.12</td>
<td>19.11</td>
<td>61.25</td>
<td>48.63</td>
<td>34.10</td>
</tr>
<tr>
<td>Urine:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>0.10</td>
<td>0.66</td>
<td>4.76</td>
<td>62.56</td>
<td>0.00</td>
<td>0.00</td>
<td>0.76</td>
<td>35.86</td>
<td>67.42</td>
<td>63.73</td>
</tr>
<tr>
<td>Males</td>
<td>0.18</td>
<td>0.49</td>
<td>4.87</td>
<td>50.80</td>
<td>0.02</td>
<td>0.00</td>
<td>0.67</td>
<td>23.07</td>
<td>55.87</td>
<td>57.52</td>
</tr>
</tbody>
</table>

Mean, n=3.

**DISCUSSION**

Human pharmacokinetics of SASP has been studied by several investigators and been reviewed$^{2-4}$ as also referred to in Sjöquist et al.$^{1}$ in this series. The absorption of SASP in the dog after oral administration was similar to that found in man and in the rat. A mean of 18% of administered dose was absorbed but with large interindividual differences. The elimination half-life was about 14 min in the dog which is in the same range as for the rat (8 min). In man the half-lifes presented in the literature are based on oral administrations and vary between 2.9~10 hours$^{3,4}$. From in house unpublished data obtained after i.v. administration to healthy volunteers a $t_{1/2}$ of 7.5 hours was obtained, which means the plasma elimination in man is more than 10 times slower than in the
rat and the dog. The Vdss calculated from single dose administration was similar for rats, dogs and man 0.1~0.7l/kg, which is close to the blood volume indicating a low tissue distribution. The total clearance was 34.8 in the dog and in the rat 17.9ml/min x kg, while the clearance in man was 100 fold lower. Thus the dominating difference between man and the animals studied was a lower clearance in man. After oral administration the time for maximal plasma concentration was 1~3 hours in both dogs and rats, while it was longer in man (6 hours).

After intravenous administration intact SASP was rapidly excreted in bile. In the non operated animals the SASP delivered to the intestine viable was reduced by azo reductases and the metabolites were absorbed. The tritium labelled SP metabolites were absorbed to a higher extent than the carbon-14 labelled 5ASA and Ac5ASA as shown in Fig. 2. This is in agreement with what has earlier been shown in man3).

After intravenous administration the plasma elimination half-life of SASP was longer in bile fistula operated dogs than in dogs without bile fistulas t1/2=28 min compared to t1/2=14 min in male dogs and the clearance of SASP was about 4 times faster in the normal dogs compared to the operated dogs. The AUC was increased in the bile fistula dogs, 29h x μM compared to about 6h x μM in normal dogs. The biliary excretion is almost the only elimination route after i.v. administration since within 2 hours a quantitative excretion of intact SASP was observed in the bile. The total clearance can thus be considered as the hepatic clearance. The limiting factor of SASP elimination can be assumed to be the hepatic circulation rate or the biliary flow.

Although 90% of the intravenous dose was recovered in the bile, 60% of the 3H radioactivity given i.v. was found in urine in the mass balance study. This is probably due to the metabolism of SASP which reached the intestines after the biliary elimination. This is also supported by the plasma profile after the i.v. dose, Fig. 2, where a second peak was shown. In the bile study however all the bile was collected and no SASP reached the colon where it is mainly metabolized.

After oral administration the time for maximal concentration of SASP in plasma was
as a mean 7 hours in the bile fistula operated dogs compared to 1~3 hours in non operated dogs. Also the maximal concentration was increased from 15.1µM eq in non operated male dogs to 142µM in bile fistula operated dogs receiving the same oral dose. These differences also indicate that the bile fistula operation might delay the biliary excretion and/or decrease the mobility in the gastrointestinal tract.

As a mean 18% of unchanged SASP was excreted in the bile after oral administration of [14C, 3H]SASP. No metabolites were found in bile and the mean excretion of 14C and 3H radioactivity was also very close to the values obtained from the analysis of SASP.

Compared to the biliary excretion in the rat the data from the dogs in this study were similar after intravenous administration. However, after oral administration the rat excreted less unchanged SASP (<3% of the dose) than the dog. Furthermore in the rats metabolites were found after oral administration. In man quantitative data on biliary excretion are difficult to obtain. However in patients the biliary excretion have been reported to be about 2.5% of the administered dose5. In another study in man 9% of the dose was recovered in bile via a T-tube6.

The metabolite profiles of SASP in urine and faeces from the dog differ from those obtained from the rat in that they contained fewer metabolites7. No sex differences were observed in dogs. The lower hydroxylation capacity found in female rats compared to male rats were not observed in female dogs. Furthermore, the sulphate conjugates of SPOH and AcSPOH found in rats were not found in dogs. The metabolism of SASP in man is more similar to the rat than the dog but no sex differences have been reported in man. A metabolic difference observed in man but not in animals is that there is a genetical difference in the degree of acetylation in man thus producing N-acetyl sulfapyridine to different degrees. In an Occidental population the ratio between slow and rapid acetylator has been reported to be around 50/50 while in the Japanese the corresponding figures were 10/903,4. Since slow acetylators receive higher SP plasma levels compared to fast acetylators the latter are protected from side effects related to high SP concentrations3.

In conclusion, the pharmacokinetics of SASP in the dog was similar to that of the rat regarding a rapid plasma clearance, and an extensive biliary excretion. This is in contrast to man. The bioavailability of an oral dose was in the same range in dogs as in man and in the rat. The metabolism of SASP in dogs was in general less extensive compared to that of the rat and man and no sex differences in the metabolism were observed in the dog.

Bile fistula operated dogs showed a slower elimination of SASP indicating a delay of biliary excretion in the operated animals compared to the intact dog.

The recoveries of radioactivity that were obtained in the present study (72~96% for 3H and 69~90% for 14C) were regarded as quantitative considering the difficulties in sample collection involved in mass balance studies on dogs.
References


