Pharmacokinetics of Salazosulfapyridine
(Sulfasalazine, SASP) (I):
Plasma kinetics and plasma metabolites in the rat
after a single intravenous or oral administration

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metabolites, sex difference, pharmacokinetics

Summary
Salazosulfapyridine (Sulfasalazine, SASP, 2-hydroxy-5-[4-[(2-pyridinylamino)
sulfonyl] phenyl] azo] benzoic acid) labelled with 14C in the carboxyl group
and with 3H in chemically as well as metabolically stable positions in the
benzenesulfonoyl ring was administered either intravenously or orally to male
and female rats in order to study the plasma pharmacokinetics of SASP and
the plasma profiles of the metabolites. After intravenous administration SASP
was eliminated rapidly from plasma with a half-life (t1/2) of 8min. The volume
of distribution (Vdss) of SASP was 0.21/l/kg and total clearance (CLtot) was
18ml/min x kg. After oral administration, SASP was present in plasma mainly
during the first 4 hours. The time for maximal concentration (Tmax) varied
for SASP between 1~3 hours, for 14C, corresponding to 5ASA metabolites,
between 3~10 hours and for 3H, corresponding to sulfapyridine metabolites,
between 4~12 hours. The inter-individual variation was considerable. The bioavai-
lability of SASP was 9% and independent of the dose. The maximal concen-
tration (Cmax) and area under the curve (AUC) increased proportionally with
the dose. The absorption of SASP was not influenced by fasting overnight.

There was a clear sex difference in the metabolism of SASP. The plasma
concentration of sulfapyridine metabolites in female rats was twice that in
male rats. No hydroxylated metabolites were found in female rat plasma,
whereas in the male rats, the hydroxylated sulfapyridine metabolites were the
major metabolites.
INTRODUCTION

SASP was first introduced by N. Svartz in 1942 primarily as an antirheumatic drug. Its efficacy in inflammatory bowel diseases such as colitis and Crohn's disease, has vindicated its clinical use for more than 40 years. SASP has also been shown to possess immunomodulating properties and it has recently been reevaluated as an antirheumatic drug. Thus today a lot of clinical studies have been performed for different indications. Review articles on the human pharmacokinetics were published by Das and Dubin (1976) and by Klotz (1985) in Occidentals. An investigation in Japanese healthy subjects by Uchida et al (1990) was recently published as well as a comparison between the pharmacokinetics of SASP in patients with rheumatoid arthritis and inflammatory bowel disease. However, there is a lack of preclinical studies on the pharmacokinetics. At the time when SASP was first introduced into clinical use requirements for preclinical testing was not demanded for a known sulfonamide like sulfapyridine coupled with an azolinkage to salicylic acid. Many animal models for immunological function and chronic inflammatory diseases are in use to study the efficacy of SASP and toxicological studies have been performed on rats and dogs. However, the knowledge about the pharmacokinetics of SASP in animals is limited. The distribution of SASP in mice was studied by autoradiography in 1963 by Hanngren et al. and 11 metabolites were tentatively identified in rat urine by Schröder and Gustavsson (1973). In this paper, the plasma kinetics and plasma metabolites of SASP after a single intravenous or oral administration were studied in the rat. To be able to follow the drug itself as well as metabolites known to be formed by a reductive azo-cleavage it was decided to incorporate carbon-14 in the carboxylic group and tritium in the phenyl ring in the sulfonamide moiety of SASP.

MATERIALS AND METHODS

Materials

$^{14}$C-SASP was prepared from $^{14}$C-labelled salicylic acid according to the synthetic route shown in scheme 1. The salicylic acid was esterified with methanol and coupled to the diazonium salt obtained from sulfanilic acid. The thus formed compound was after protection of the phenolic group activated to give the sulfonyl chloride. The sulfonamide was prepared by coupling of the sulfonyl halide with aminopyridine. Deprotection of the salicylic acid moiety by double ester hydrolysis under basic conditions and precipitation with acetic acid gave $^{14}$C-SASP (details of this method will be published elsewhere). The specific activities of $^{14}$C-SASP for 3 different batches were $76$ MBq/mmol (2.05 mCi/mmol), $162$ MBq/mmol (4.37 mCi/mmol) and $183$ MBq/mmol (4.94 mCi/mmol). The radiochemical purity judged by thin layer and liquid chromatography was $>97%$.

$^{3}$H-SASP was prepared in the following way. 4-amino-3,5-dibromo-N-2-pyridinylbenzenesulfonamide was reduced under basic conditions in a water–dioxane mixture, with palladium on charcoal and sodium borotritide (see scheme 2). The tritiated sulfapyridine was condensed with 2-methanesulfonyloxy-5-nitroso methyl benzoate. Deprotection of the condensation product followed by precipitation with acid gave tritium labelled sulfasalazine (details of this method will be published elsewhere). The specific activities for 2 batches
were 1.09GBq/mmol (29.5mCi/mmol) and 2.87GBq/mmol (77.5mCi/mmol). The radiochemical purity was >97% according to thin layer and liquid chromatography.

SASP, sulfapyridine (SP), N-acetyl sulfapyridine (AcSP), 5-hydroxysulfapyridine (SPOH), N-acetyl-5-hydroxysulfapyridine (AcSPOH), 5-aminosalicylic acid (5ASA) and 5-acetamidosalicylic acid (Ac5ASA) were obtained from Department of Organic Chemistry, Pharmacia AB, Uppsala, Sweden.

Other chemicals and solvents were of analytical grade and were purchased from various suppliers.
Animals and sampling

Male and female Sprague Dawley rats were purchased from Alab, Sollentuna, Sweden. The animals were 90~120 days old and weighed 215~330g. The rats were housed in temperature and humidity controlled rooms (17~22°C and 31~60% humidity) with 12 hours light-dark cycles for at least one week before the experiment. The animals were fed laboratory chow (EWOS R32 Södertälje, Sweden) ad libitum or starved overnight before dosing. Each group consisted of three or five animals. The intravenous dose was 5mg/kg with 0.2MBq of [14C] and 0.4MBq of [3H]SASP per kg body weight given as a solution of [3H,14C]SASP 5mg/ml, in 15mM TRIS buffer, in the tail vein. The oral doses were 50, 200 and 500mg/kg with 2MBq/kg of [14C] and 4MBq/kg of [3H]SASP, and were given by gavage as a suspension of 50 or 100mg SASP per ml in distilled water to rats starved overnight. In parallel an oral dose of 50mg/kg was given to rats having food ad libitum.

Blood was collected in heparinized test tubes during light ether anaesthesia from the orbital plexus or by heart puncture. After intravenous administration blood was sampled after 5, 10, 20, 30, 45, 60, 75 and 90 min, and after 2, 4, 8 and 16 hours. The sampling times after oral administration were 20, 40 min, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48 and 96 hours.
The plasma samples were stored at \(-20^\circ C\) until analysis.

**Measurements of radioactivity**

Radioactivity was determined by liquid scintillation counting with quench correction (Beckman LS 2800) after prior combustion to \(^{14}\)CO\(_2\) and \(^3\)H\(_2\)O with Combustaid (Packard) in a Sample Oxidizer, TRI-CARB Model 306 (Packard Instrument). The tritiated water was condensed in Monophase S (Packard). The \(^{14}\)CO\(_2\) was absorbed to an amine in Carbo Sorb and Permafluor V (Packard) was added. Blank values obtained from blank combustion were subtracted.

The recovery of radioactivity from spiked biological samples was quantitative with a coefficient of variation of \(\pm 0.3\%\).

**Analysis of sulfasalazine**

Plasma samples were mixed with two volumes of methanol to precipitate proteins. The samples were placed at \(-20^\circ C\) for 10 minutes followed by centrifugation and 50\(\mu\)l of the supernatant was injected into the reversed phase column (Nucleosil C\(_{18}\), 10\(\mu\)m Mackerey-Nagd & Co, Düren, FRG) protected with a precolumn (Perisorb C\(_{18}\), 40\(\mu\)m, Merck Darmstadt, FRG). The mobile phase consisted of a methanolic phosphate buffer, 0.025 M, pH2.5 (64 : 36, v : v). The column was kept at 37\(^\circ\)C and the flow rate was 1ml/min.

For detection, a UV detector (Kratos Spectroflow 783) at 360nm was used.

The detection limit in plasma was 1.3\(\mu\)mol/l. The coefficient of variation was \(\pm 3\%\) at 75\(\mu\)mol/l and \(\pm 9\%\) at 1.5\(\mu\)mol/l. The accuracy was 100\% and 99\% and the relative recovery 101 and 110\% at the two concentration levels, respectively.

**Analysis of metabolites**

To the plasma samples (100\(\mu\)l), 10\(\mu\)l 1M acetate buffer and 5\(\mu\)l \(\beta\)-glucuronidase/aryl-sulfatase were added and the mixture was incubated at 37\(^\circ\)C for 3 hours. The incubation was stopped by addition of 400\(\mu\)l of methanol and the samples were allowed to stand for 15 minutes, centrifuged for 15 min at 3000g and 20\(\mu\)l of the supernatant was then injected into a high performance liquid chromatography system with a precolumn (Perisorb RP-18, 30~40 \(\mu\)m) and a reversed phase (Nucleosil C\(_{18}\), 7\(\mu\)m) column. The mobile phase consisted of aqueous 5.3M hexanesulphonic acid and 10mM phosphoric acid, methanol and triethylamine (920 : 120 : 0.3 v/v/v). The flow rate was set to 1ml/min. A UV-detector (254 nm) 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 in plasma were for 5ASA 9.3\(\mu\)mol/l, Ac5ASA 3.3\(\mu\)mol/l, SP 5.1\(\mu\)mol/l, SPOH 5.6\(\mu\)mol/l, AcSP 21.9\(\mu\)mol/l and for AcSPOH 14.2\(\mu\)mol/l.

The coefficient of variation was 13.6\% for 5ASA and 5.6\% for AcSP and below 5\% for the other metabolites at a concentration level of 100\(\mu\)M.

**Pharmacokinetic analysis**

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

RESULTS

The plasma elimination profiles for $^{14}$C and $^3$H respectively, following the intravenous administration of $^{[14]}$C, $^3$H]SASP, were similar and fitted to a two compartment model (Fig. 1). The $\alpha$-phase half-life in plasma was 0.4 hour (about 25 min). The $\beta$-phase showed a half-life of about 3 hours (Table 1). After 16 hours only trace amounts of radioactivity remained in plasma. SASP was detectable in plasma by liquid chromatography only during the first 30 min after intravenous administration. The half-life was only 8 min for SASP (Fig. 1). The plasma clearance for SASP was 17.9 ml/min x kg, which was

![Fig. 1 Plasma concentration of SASP, $^{14}$C and $^3$H after an intravenous injection of $^{14}$C, $^3$H]SASP 5 mg/kg to male rats. Mean ± SEM, n=3.](image)

Table 1  The pharmacokinetic parameters obtained after a single intravenous administration of $^{[14]}$C, $^3$H] SASP to the rat

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dose (mg/kg)</th>
<th>$t_{1/2\alpha}$ (min)</th>
<th>$t_{1/2\beta}$ (hrs)</th>
<th>$V_{ds}$ (l/kg)</th>
<th>$CL_{tot}$ (ml/min x kg)</th>
<th>AUC (h x $\mu$Meq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>SASP 5</td>
<td>8</td>
<td>—</td>
<td>0.2</td>
<td>17.9</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C 0.4hrs</td>
<td>3.1hrs</td>
<td>0.4</td>
<td>1.7</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^3$H 0.4hrs</td>
<td>3.4hrs</td>
<td>0.4</td>
<td>1.4</td>
<td>148</td>
<td></td>
</tr>
</tbody>
</table>

Mean, n=3
10 times faster than the clearance of the radioactivity. SASP thus represented only 9.6% and 7.9% of the plasma radioactivity measured as area under the curve (AUC) of $^{14}\text{C}$ and $^3\text{H}$ respectively. The volume of distribution ($V_{dss}$) for SASP was 0.21/l/kg (Table I). No metabolites of sulfapyridine or 5-aminosalicylic acid were found in the plasma after the i.v. dose.

After oral administration of 50, 200 and 500mg/kg the individual variations in the plasma levels of both SASP and the isotopes were large. After the lowest dose, the concentrations of unchanged SASP in the plasma were not measurable or close to the limit of detection. At the two higher doses, 200 and 500mg/kg b.w., the mean peak plasma concentration of SASP was recorded 1~3 hours after administration and the mean maximum concentrations were 12μM and 25μM respectively (Table II), indicating that the small intestine is the major absorption site. The mean $C_{max}$ as well as the values for AUC increased with the dose. The bioavailability was found to be about 9% independent of the dose.

The concentrations of both $^3\text{H}$ and $^{14}\text{C}$ was higher than the SASP concentration. $C_{max}$ was about 3-fold for $^3\text{H}$ compared with that for $^{14}\text{C}$. The peak for $^3\text{H}$ appeared after 4~12 hours, while the peak for $^{14}\text{C}$ appeared slightly earlier, varying between 3~10 hours. The AUC for $^3\text{H}$ was about 50 times and for $^{14}\text{C}$ about 10 times higher than that of SASP. In female rats the AUC for $^3\text{H}$ was twice as high as that in male rats. (Table II and Fig. 2).

The rats having food ad libitum showed a similar plasma profile to rats starved overnight with similar $C_{max}$, $T_{max}$ and AUC (Table II). In these rats both the blood and the plasma concentrations of radioactivity were determined (Fig. 3). The concentration in blood followed a similar profile to that in plasma. The plasma level was initially twice

<table>
<thead>
<tr>
<th>Table II</th>
<th>The pharmacokinetic parameters calculated after a single oral administration of [$^{14}\text{C}$, $^3\text{H}$] SASP to the rat fasted overnight.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Dose mg/kg</td>
</tr>
<tr>
<td>Female</td>
<td>SASP 50</td>
</tr>
<tr>
<td></td>
<td>$^{14}\text{C}$ 3.4</td>
</tr>
<tr>
<td></td>
<td>$^3\text{H}$ 127.4</td>
</tr>
<tr>
<td>Male</td>
<td>SASP 50</td>
</tr>
<tr>
<td></td>
<td>$^{14}\text{C}$ 5.4</td>
</tr>
<tr>
<td></td>
<td>$^3\text{H}$ 43.0</td>
</tr>
<tr>
<td></td>
<td>SASP 200</td>
</tr>
<tr>
<td></td>
<td>$^{14}\text{C}$ 56.5</td>
</tr>
<tr>
<td></td>
<td>$^3\text{H}$ 168.2</td>
</tr>
<tr>
<td></td>
<td>SASP 500</td>
</tr>
<tr>
<td></td>
<td>$^{14}\text{C}$ 130.2</td>
</tr>
<tr>
<td></td>
<td>$^3\text{H}$ 362.5</td>
</tr>
<tr>
<td>Male*</td>
<td>SASP 50</td>
</tr>
<tr>
<td></td>
<td>$^{14}\text{C}$ 4.1</td>
</tr>
<tr>
<td></td>
<td>$^3\text{H}$ 54.2</td>
</tr>
</tbody>
</table>

Mean, n = 5

*: Nonfasted rat. ND: not determined.
Fig. 2 Plasma concentration of SASP, $^{14}$C and $^3$H after a single oral administration of $^{14}$C, $^3$H/SASP 200 and 500mg/kg to male rats. Mean, n=5.

- $\cdots \cdots$ SASP 200mg/kg
- $\diamond$ SASP 500mg/kg
- $\square$ $^{14}$C 500mg/kg
- $\circ$ $^3$H 500mg/kg

Fig. 3 Radioactivity in blood and plasma after an oral dose of 50mg/kg SASP to non-fasted male rats. Mean ± SEM, n=5.

- $\circ$ $^3$H blood
- $\cdots \cdots$ $^3$H plasma
- $\square$ $^{14}$C blood
- $\cdots \cdots$ $^{14}$C plasma
Table III  The ratio of radioactivity in blood/plasma after a single oral dose of [14C, 3H] SASP 50mg/kg in nonfasted male rats.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>^3H blood/plasma</th>
<th>^14C blood/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67</td>
<td>0.76</td>
<td>0.55</td>
</tr>
<tr>
<td>1.0</td>
<td>0.65</td>
<td>0.67</td>
</tr>
<tr>
<td>2.0</td>
<td>0.70</td>
<td>0.59</td>
</tr>
<tr>
<td>4.0</td>
<td>0.68</td>
<td>0.53</td>
</tr>
<tr>
<td>6.0</td>
<td>0.71</td>
<td>0.58</td>
</tr>
<tr>
<td>10.0</td>
<td>0.85</td>
<td>0.63</td>
</tr>
<tr>
<td>12.0</td>
<td>0.92</td>
<td>0.63</td>
</tr>
<tr>
<td>24.0</td>
<td>1.14</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Mean of 5 animais.

Fig. 4  Metabolites of SASP in plasma after an oral dose of 50mg/kg in female and male rats. Mean ± SEM, n=5.

- SP male
- SP female
- AcSP female
- SPOH male
- AcSPOH male
that in blood but the ratio decreased with time indicating some distribution of the metabolites to the erythrocytes (Table III).

The metabolites SP, AcSP, SPOH, AcSPOH, 5ASA and Ac5ASA were quantitatively analysed. The metabolic pattern was found to be dependent on sex. The main metabolites in plasma of male rats were SP, SPOH and AcSPOH. In the female rats no hydroxylated metabolites were found in plasma. The major plasma metabolites in female rats were SP and AcSP (Fig. 4). The total amount of sulfapyridine metabolites in plasma was higher in female rats than in male rats. The $T_{\text{max}}$ for total sulfapyridine metabolites was 8 hours in both sexes and the $C_{\text{max}}$ was 95 $\mu$M for female rats and 41 $\mu$M for male rats after the 50mg dose (Fig. 5). Since $^{14}$C radioactivity in plasma was low, 5ASA was not possible to quantitate, but after the two highest doses (200 and 500mg/kg) Ac5ASA could be quantitated in male rats with $T_{\text{max}}$ at 10−12 hours. The maximal plasma concentrations, $C_{\text{max}}$, were 5.5 and 13.5$p$M respectively.

![Graph showing plasma concentration of [3H] and total SP-metabolites after an oral dose of 50 mg/kg of $^{14}$C, [3H]SASP to the rat. Mean, n=5.](image)
DISCUSSION

Several pharmacokinetic studies on SASP have been performed in man\(^5,13\textsuperscript{--}15\). The knowledge from these studies is that SASP is poorly absorbed (2\textsuperscript{--}20\%) after oral ingestion and very low amounts are excreted in urine (about 2\%). The major part of SASP undergoes azo reduction in the gastro-intestinal tract and thus forms sulfapyridine and 5-aminosalicylic acid. Sulfapyridine has an almost complete absorption, while about 30\% of 5ASA is found in the urine. One aim in our investigations was to label SASP with double isotopes in such a way that it became possible to follow the parent drug as well as its major metabolites. Therefore SASP was labelled in the 5ASA carboxylic group with carbon-14 and with tritium in the sulfophenyl ring. This labelling was found to be chemically as well as metabolically stable. The dose range was chosen to include a dose corresponding to a high therapeutic dose (50mg/kg) up to a high but subtoxic one, 500mg/kg.

After intravenous administration, plasma elimination of SASP in the rat was rapid with a half-life of 8 minutes which is far more rapid compared to man. In healthy volunteers, the plasma half life of SASP after an i.v. injection was estimated to about 7.5 hours (personal communication, Lundqvist et al.). The total clearance was in the rat 18 ml/min x kg, while in human it is about 0.25ml/min x kg. However, the elimination of both \(^1\)C- and \(^3\)H-radioactivity after an intravenous dose was considerably slower than SASP and both isotopes disappeared in parallel (Fig. 1). The total clearance of SASP was almost 10 times that of the radioactivity indicating formation of a primary metabolite of SASP which has a slower elimination than SASP itself. From the chromatographic data and preliminary mass spectrometric data, the unidentified metabolite was tentatively identified as hydroxylated SASP. This compound was not detected in plasma after oral administration.

After an oral dose, both \(C_{\text{max}}\) and AUC increased proportionally with the dose. However, after the lowest dose, 50mg/kg b.w., which corresponds to a high therapeutic dose, the plasma levels of SASP did not reach the limit of detection, 1.3\(\mu\)mol/l. Plasma concentrations of SASP comparable with those obtained in clinical use varying between about 10 \textsuperscript{--}100 \(\mu\) mol/l were reached in the rat at the highest dose, i.e. \(>10\) times the therapeutic dose, due to the higher clearance of SASP in the rat compared to man.

The bioavailability of SASP was around 9\%, which compares well with the values reported in man, 2\textsuperscript{--}13\% relative bioavailability\(^4\) or 5\textsuperscript{--}22\% when comparing with an i.v. dose (unpublished data). The plasma levels fluctuated with time and varied greatly between animals as have also been observed in man. This is probably due to varying absorption. The absorption of SASP can be affected by gastric emptying, pH and solubility as well as of enterohepatic cycling.

The dominating metabolites in the circulation after oral administration were sulfapyridine and its metabolites. The amount of 5ASA metabolites in the circulation calculated from the AUC of \(^1\)C-radioactivity amounted to about 20\% of the amount of SP-metabolites measured as \(^3\)H-radioactivity. It is known that 5-aminosalicylic acid is not as well absorbed as sulfapyridine. The plasma profiles of \(^3\)H-radioactivity and total SP-metabolites were similar and excluded the presence of major unknown SP-metabolites.

There were distinct sex-dependent differences in the metabolism; male rats hydroxylated
sulfapyridine, female rats did not. As shown elsewhere the hydroxylated SP metabolite was further conjugated and rapidly eliminated resulting in lower SP--metabolite levels in plasma in male rats compared to female rats. It is not uncommon with sex differences in rats depending either on sex hormones or a higher oxidating capacity for cytochrome P-450 in male compared to female rats. No sex difference was observed in dogs, neither have any been reported in man. The metabolic pattern found in male rats was similar to that in man while the dog had a simpler metabolic profile.

Thus in conclusion, SASP has a much shorter half--life in the circulation in rats compared to man. An unidentified metabolite of SASP with a slower elimination than the drug itself was found after intravenous administration. The bioavailability was similar to man, around 9%. There was a sex-dependent difference in the SP--metabolite pattern in the rat depending on an ability of male rats to hydroxylate SP.

References


