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

Pharmacokinetics of SASP in the rat following consecutive oral doses.

Birgitta SJÖQUIST, Nils-Otto AHNFELT, Stig ANDERSSON, Roland d'ARGY, Masanori HATSUOKA*,
Tetsuya SUGA**, Takafumi WATANABE** and Ingrid LJUNGSTEDT-PÅHLMAN

Chemical and Biomedical Research, Pharmacia Medical Products,
S-751 82 Uppsala, Sweden

*Therapeutics Group, Pharmacia Kabushiki Kaisha, Honda Denki Bldg. 5-37
Kami-Osaki 4-chome, Shinagawa-ku, Tokyo 141, Japan

**Department of Clinical Biochemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi,
Hachioji, Tokyo 192-03, Japan

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Summary

Repeated daily oral doses of [^{14}C , ^3H]salazosulfapyridine 50mg/kg b.w. was given to male and female rats during 21 days to investigate the absorption, tissue distribution and elimination of SASP and metabolites. The peak plasma level of radioactivity was slightly increased for both ^3H and ^{14}C during repeated administration although the increase was not statistically significant. The elimination rate from plasma was similar after the last 21st dose as after a single dose. Thus the 24 hour plasma level was not increased. The concentration of unchanged SASP in plasma was too low to be measurable by liquid chromatography. There was no significant binding of radioactivity to the erythrocytes after the first dose, but after repeated doses some distribution of ^{14}C to the blood cells was seen.

The dominating picture of the tissue distribution was that most of the radioactivity remained in the gastrointestinal tract. Even 96 hours after the last dose, when only trace amounts of radioactivity remained, the highest radioactivity was found in caecum.

Except for the gastrointestinal tract, the concentration of radioactivity was initially lower in the tissues than in plasma and was dominated by ^3H . The concentration of ^3H in tissues in relation to the plasma concentration was not changed during the repeated administration. However, the ^{14}C concentration increased.

The tissue distribution was also studied by autoradiography and image analysis after the same pretreatment as above and with a final high dose of [^3H]SASP. The time points studied were 4, 16, and 96 hours after the last given dose. The highest uptake was noticed in the connective tissues, the lung, the kidney, the epididymis, the penis and the pituitary gland.

With whole body autoradiography the tissue distribution was also studied 5 min, 1 hour and 16 hours after an *i.v.* injection of ^{14}C - or ^3H -labelled SASP in pregnant rats, which had been pretreated orally in 10 days with unlabelled SASP. The aim of this study was to investigate whether such a pretreatment caused an altered distribution pattern, compared to that after an *i.v.* single dose, in the fetal and placental tissues. The fetal concentration did not rise significantly. However, the maternal tissues indicated a slight increase for most tissues.

No changes were found in the excretion of radioactivity or in the metabolite pattern in urine and faeces during the administration. Neither did a consecutive administration of SASP cause any induction of hepatic drug metabolizing enzymes, such as cytochrome P-450, cytochrome b5, aminopyrine demethylase and aniline hydroxylase.

INTRODUCTION

The pharmacokinetics of SASP in rat as shown in preceding papers^{1~3)} was similar to that in man⁴⁾, with respect to absorption and metabolism, but the plasma clearance was far more rapid in the rat. In human studies, a high daily dose of SASP (4 g) to healthy volunteers resulted in steady state concentrations of SASP in serum ranging from 4.7 to 45 $\mu\text{g/ml}$ ⁵⁾. Similar serum concentrations of SASP was also found in Japanese healthy volunteers⁶⁾ and in patients with ulcerative colitis⁷⁾ after repeated dosing. Information about pharmacokinetics in animals after multiple doses of SASP is scarce. Thus the present studies were designed to evaluate the distribution, metabolism and elimination of [³H, ¹⁴C]SASP after repeated daily administration to the rat. Separate enzyme induction studies were performed with unlabelled SASP.

MATERIALS AND METHODS

Materials

[¹⁴C] and [³H]SASP was prepared as described earlier¹⁾ with ¹⁴C in the 5-aminosalicylic acid moiety and ³H in the phenyl ring in the sulfonamide moiety of SASP. The specific activity of [¹⁴C]SASP thus obtained was 183 MBq/mmol (4.94 mCi/mmol). The radiochemical purity judged by thin layer and liquid chromatography was >97%. The specific activity of [³H]SASP was 2.87 GBq/mmol (77.5 mCi/mmol) and the radiochemical purity was >97% according to thin layer and liquid chromatography.

SASP was obtained from Pharmacia AB, Uppsala, Sweden and metabolites of SASP were synthesized in the department of Organic Chemistry, Pharmacia AB, Uppsala. Other chemicals used were of analytical grade obtained from different suppliers.

Animals and dosage

Male and female Sprague Dawley rats were purchased from Alab AB, Sollentuna, Sweden. The animals were 90~120 days old and weighed 230~412 g. The rats were housed in temperature and humidity controlled rooms (17~22°C and 31~60% respectively) with 12 hours light-dark cycle for at least one week before the experiment. The animals were fed laboratory chow (EWOS R32 Södertälje, Sweden) *ad libitum*, and were starved overnight before the last dose was given. Three animals were used in each group. The daily oral doses were 50 mg/kg [³H, ¹⁴C]SASP with 2 and 4 MBq/kg of ¹⁴C and ³H respectively in a volume of 1 ml. The drug was administered by gavage once daily during 21 days. In the autoradiographic study the last dose contained 334 MBq/kg of ³H radioactivity and no ¹⁴C radioactivity. In another autoradiographic study pregnant rats gestational day 9 at the start of the experiment were administered 50 mg/kg of unlabelled SASP orally for 10 days and thereafter an *i.v.* dose of either ¹⁴C or ³H labelled SASP (25 MBq/kg or 35 MBq/kg respectively) was given.

In an enzyme induction study male Wistar rats were used. These animals were supplied from Tokyo Laboratory Animals Co. Ltd., Japan. At the start of the experiment they were 4 weeks old. SASP was administered orally as a suspension in water at daily doses of 50 and 500 mg/kg b.w., respectively. Phenobarbital-NA (analytical grade, Wako

Pure Chemicals Co. Ltd., Japan) was administered intraperitoneally at 25mg/kg b.w. After 14 days of treatment half of the group including a control group were examined one day after the final dose. The other animals were examined one week after the end of the treatment.

Tissue distribution study by dissection

The rats were housed in individual metabolism cages for urine and faeces collection during 24 hours after the first, 7th, 14th and 21st dose. In one of these groups the urine and faeces collection continued for further 3 days after the last dose.

Blood was sampled from the orbital plexus under ether anaesthesia at time 0, 4, 8 and 24 hours after day and dose number 1, 7, 14 and 21. From each animal, blood samples were taken at 3 occasions during the period. Blood was collected by heart puncture under ether anaesthesia at 4, 8, 12, 24 and 96 hours after the last dose. The animals were then sacrificed and different tissues were dissected from the male rats. All gastrointestinal tissues were collected together with its content.

Three groups of male rats were sacrificed 4 hours after the dose on day 1, 7 and 14. Blood and tissues were collected as described above.

The faeces was homogenized with methanol (3 times by weight). The homogenized samples as well as the urine and tissues were kept at -20°C until analysed.

The blood was collected in heparinized tubes. One part of the blood was directly analysed for radioactivity, the rest was centrifuged and the plasma was stored at -20°C for analyses.

Whole body autoradiography

The technique of whole body autoradiography has earlier been described^{8,9)}. Briefly; the animals were killed by CO_2 inhalation at the intended times. They were rapidly frozen in hexan cooled on dry ice (-70°C) and then mounted for sectioning in a gel of carboxymethyl cellulose (CMC). From each rat, $20\mu\text{m}$ thick sagittal sections were cut at different levels with a cryomicrotome (-20°C), (LKB 2250 PMV Cryomicrotome). The obtained sections were caught on tape (Minnesota Mining & Manufacturing Co., No 810). After being freeze-dried at -20°C the sections containing ^3H were apposed against Ultrofilm ^3H (LKB) for about four to six weeks.

The qualitative autoradiographic findings were complemented with quantitative data as measured by computerized image analysis¹⁰⁾ and as earlier has been described²⁾. The densitometric readings obtained from ^3H autoradiograms were converted to radioactivity using radio standards (^3H Microscales, Amersham International, UK).

Enzyme induction study

At day one after the final 14th dose and after the recovery period of one week, the rat livers were perfused with saline under light anaesthesia using diethylether. Then, the liver was quickly removed, weighed, homogenized in ice-cold 1.15% KCl 1 mM EDTA-10mM phosphate buffer (pH 7.4) in a Potter-Elvehjem homogenizer with a Teflon pestle, and the obtained homogenate was adjusted to 20% (w/v) with the above solution. A por-

tion of the homogenate was then assayed for peroxisomal β -oxidation (FAOS) activity according to the method by Lazarow and de Duve¹¹⁾. Hepatic protein content was determined by the Lowry method using bovine serum albumin as a standard¹²⁾. The homogenate was centrifuged at $10,000 \times g$ for 15 min at 4°C and divided into two parts. One part of the supernatant was centrifuged at $105,000 \times g$ for 60 min at 4°C and the resulting pellet was suspended in 0.1M phosphate buffer (pH 7.4) (at 0.2g tissue/ml) for assaying cytochrome P-450 and b5 contents by the method of Omura *et al.*¹³⁾ and the protein content. The other part was centrifuged similarly and the resulting pellet was suspended in 1.15% KCl, 1 mM EDTA and 10mM phosphate buffer (pH 7.4) at 0.2g/ml for determination of aniline hydroxylase, aminopyrine demethylase by the method of Mazel¹⁴⁾ and protein.

Analyses

Tissues, blood, plasma, urine and faeces were analysed regarding both ^3H and ^{14}C radioactivity by combustion followed by liquid scintillation counting as earlier has been described²⁾. The recovery of radioactivity added to different biological samples were quantitative for both ^{14}C and ^3H with a coefficient of variation (CV) varying between 0.3 ~5% for the different samples, with the highest CV for faeces. The lowest concentration of detection for labelled SASP was about $0.1\mu\text{M eq}$.

Quantitative analyses of SASP and its metabolites in plasma, urine and faeces were performed by liquid chromatography with UV-detection. A reversed phase C_{18} column with mixtures of methanol and phosphate buffer was used as mobile phase.

The metabolites of SASP were determined by enzymatic hydrolysis of glucuronide and sulphate conjugates followed by reversed phase liquid chromatography on a C_{18} column using an aqueous methanolic eluent containing hexane sulphononic acid and triethylamine. An UV-detector, and a fluorescence detector were coupled in series. The limits of determination were 1.3, 3.4 and $3.8\mu\text{mole/l}$ in plasma, urine and faeces respectively for SASP and for SASP metabolites between 3.3 to $21.9\mu\text{mole/l}$.

It was not possible to determine 5ASA due to interfering peaks from endogenous compounds in the chromatograms obtained with this system.

Calculations and statistics

The measured radioactivity was referred to the wet weight of the tissues and was calculated as equivalent concentrations ($\mu\text{M eq}$). The concentrations of radioactivity were also adjusted for the differences in the individual doses by normalizing the values to the same dose, 50mg/kg.

The arithmetic mean with standard error of the mean (SEM) was calculated for the groups. Statistical significance was evaluated by Student's *t*-test and analysis of variance.

RESULTS

Blood and plasma concentrations of ^{14}C , ^3H , SASP and metabolites

The peak concentrations of both radionuclides in plasma, found 8 hours after the administration, were slightly increased, although not statistically significant, from dose 1 to

Fig. 1a Plasma concentration of ^{14}C in male rats (open symbols) and female rats (filled symbols) rats at 8 hrs (circles) and at 24 hours (triangles) after daily repeated oral doses of $[^3\text{H}, ^{14}\text{C}]$ SASP 50mg/kg $\times$ day. Mean $\pm$ SEM, $n=3$.

Fig. 1b Plasma concentration of ^3H in male rats (open symbols) and female rats (filled symbols) rats at 8 hrs (circles) and at 24 hours (triangles) after daily repeated oral doses of $[^3\text{H}, ^{14}\text{C}]$ SASP 50mg/kg $\times$ day. Mean $\pm$ SEM, $n=3$.

Fig. 2a Mean plasma concentrations vs time profile of ^{14}C in male rats (open symbols) and female rats (filled symbols) after a single oral dose (dotted lines) or repeated, 21 daily doses, (solid line) of $[^3\text{H}, ^{14}\text{C}]$ SASP, 50mg/kg $\times$ day, $n=3$.

Fig. 2b Mean plasma concentrations vs time profile of ^3H in male rats (open symbols) and female rats (filled symbols) after a single oral dose (dotted lines) or repeated, 21 daily doses, (solid line) of $[^3\text{H}, ^{14}\text{C}]$ SASP, 50mg/kg $\times$ day, $n=3$.

dose 21 (Fig. 1 a, b). The ^{14}C concentrations were slightly higher in the males than in the females. The ^3H level was more than two times higher in the female compared to that in the male rats. The concentration of radioactivity at 24 hours was unchanged during the treatment period. The blood concentration was lower than that in plasma. The ratio between the plasma and blood concentrations of ^{14}C was about 2 at peak time (at 8

Fig. 3 Excretion of ^3H (circles) and ^{14}C (squares) in urine collected during 24 hours after repeated daily oral doses of [^3H , ^{14}C] SASP 50mg/kg $\times$ day (in total 21 doses) in male rats (open symbols) and female rats (filled symbols). Mean $\pm$ SEM, $n=3$.

Fig. 4 Excretion of ^3H (circles) and ^{14}C (squares) in faeces collected during 24 hours after repeated daily oral doses of [^3H , ^{14}C] SASP 50mg/kg $\times$ day (in total 21 doses) in male rats (open symbols) and female rats (filled symbols). Mean $\pm$ SEM, $n=3$.

hours) in both male and female rats. The ratio was slightly decreased during the repeated administration, and after the 21st dose, the ratio was at peak time 1.6 and 1.3 in the male and female animals respectively. The ratio between plasma and blood concentration of ^3H was about 1.9 at peak time and was not changed during administration. The elimination rate from plasma was similar after repeated doses as after a single dose (Fig. 2 a, b).

Unchanged SASP could not be detected in plasma by liquid chromatography.

The metabolites that were quantified by HPLC were sulfapyridine (SP), N-acetylsulfapyridine (AcSP), hydroxylated sulfapyridine (SPOH), N-acetyl-hydroxysulfapyridine (AcSPOH) and 5-acetamidosalicylic acid (Ac5ASA). The same metabolites were found in similar proportions after single and repeated administration. There was a clear sex difference. The main metabolites in the male rats were in the order $\text{SP} > \text{AcSP} > \text{SPOH} > \text{AcSPOH}$. In female rats no hydroxylated SP-metabolites were found in plasma. The main metabolites were in the order $\text{SP} > \text{AcSP}$.

Urinary and faecal excretion of ^{14}C , ^3H , SASP and metabolites

No essential changes in the excretion of radioactivity were seen during the repeated administration (Fig. 3, 4). ^{14}C was mainly excreted in the faeces while ^3H was mainly excreted in the urine. The faecal excretion was lower in the females than in male, especially for ^3H .

Unchanged SASP was not detected in urine from the female rats. In the male rats small amounts were detected in some of the animals, preferentially after repeated doses. In the faeces small amounts were detected in some samples from both male and female rats.

The metabolite pattern found in urine was not changed during the repeated administra-

Table 1a Tissue concentration of [^{14}C] nmol eq/g during multiple daily oral dose of [^{14}C , ^3H]SASP 50mg/kg for 21 days to male rats.

Tissue	Dose 1 4h	Dose 7 4h	Dose 14 4h	Dose 21 4h	Dose 21 96h
Plasma	5.9 $\pm$ 1.8 (1.0)	8.1 $\pm$ 0.7 (1.0)	7.9 $\pm$ 0.9 (1.0)	11.8 $\pm$ 2.2 (1.0)	0.7 $\pm$ 0.1 (1.0)
Blood	3.0 $\pm$ 0.7 (0.5)	4.4 $\pm$ 0.4 (0.5)	4.8 $\pm$ 0.4 (0.6)	7.2 $\pm$ 1.3 (0.6)	1.3 $\pm$ 0.1 (1.4)
Liver	2.6 $\pm$ 0.5 (0.4)	6.4 $\pm$ 0.3 (0.8)	8.6 $\pm$ 0.2 (1.1)	10.3 $\pm$ 1.6 (0.9)	3.1 $\pm$ 0.4 (4.4)
Kidney	3.2 $\pm$ 0.8 (0.6)	6.1 $\pm$ 0.1 (0.8)	14.4 $\pm$ 1.4 (1.8)	13.4 $\pm$ 2.8 (1.1)	2.7 $\pm$ 0.3 (3.8)
Lung	1.1 $\pm$ 0.1 (0.2)	3.9 $\pm$ 0.6 (0.5)	8.3 $\pm$ 3.7 (1.0)	6.0 $\pm$ 1.3 (0.5)	1.8 $\pm$ 0.1 (2.6)
Thyroid	1.1 $\pm$ 0.0 (0.2)	7.5 $\pm$ 2.5 (0.9)	9.6 $\pm$ 3.5 (1.2)	12.8 $\pm$ 8.2 (1.1)	1.7 $\pm$ 0.2 (2.5)
Testicles	0.6 $\pm$ 0.4 (0.1)	1.8 $\pm$ 0.1 (0.2)	3.3 $\pm$ 0.6 (0.4)	3.0 $\pm$ 0.5 (0.3)	1.2 $\pm$ 0.1 (1.7)
Thymus	0.7 $\pm$ 0.4 (0.1)	2.0 $\pm$ 0.1 (0.3)	3.4 $\pm$ 0.1 (0.4)	8.0 $\pm$ 1.7 (0.7)	1.8 $\pm$ 0.3 (2.6)
Heart	0.7 $\pm$ 0.2 (0.1)	1.8 $\pm$ 0.1 (0.2)	2.4 $\pm$ 0.0 (0.3)	3.5 $\pm$ 0.6 (0.3)	1.2 $\pm$ 0.2 (1.7)
Spleen	0.6 $\pm$ 0.2 (0.1)	2.4 $\pm$ 0.1 (0.3)	4.3 $\pm$ 0.8 (0.5)	5.1 $\pm$ 1.6 (0.4)	1.9 $\pm$ 0.2 (2.7)
Lymph nodes	1.6 $\pm$ 0.7 (0.3)	4.0 $\pm$ 1.1 (0.5)	3.8 $\pm$ 0.6 (0.5)	6.7 $\pm$ 1.3 (0.6)	2.7 $\pm$ 0.3 (3.9)
Brain	0.2 $\pm$ 0.0 (0)	0.9 $\pm$ 0.1 (0.1)	1.2 $\pm$ 0.1 (0.2)	1.6 $\pm$ 0.1 (0.1)	1.0 $\pm$ 0.1 (1.5)
Urine bladder	38.7 $\pm$ 21.6 (6.6)	27.5 $\pm$ 12.6 (3.4)	30.9 $\pm$ 7.6 (3.9)	37.1 $\pm$ 15.1 (3.2)	2.6 $\pm$ 0.3 (3.7)
Stomach	2240 $\pm$ 785 (379)	2897 $\pm$ 572 (357)	3624 $\pm$ 97.9 (455)	799.6 $\pm$ 99.4 (67.9)	5.6 $\pm$ 2.8 (8.0)
Duodenum+jejunum	394 $\pm$ 129 (66.6)	960 $\pm$ 88.4 (118)	858 $\pm$ 189 (108)	604.3 $\pm$ 167.0 (51.3)	3.3 $\pm$ 1.3 (4.7)
Ileum	1195 $\pm$ 360 (202)	2720 $\pm$ 274 (335)	4031 $\pm$ 1571 (506)	2287 $\pm$ 402 (194)	14.0 $\pm$ 5.6 (20.0)
Caecum	1559.0 $\pm$ 689 (264)	1849 $\pm$ 94.9 (228)	2762 $\pm$ 863 (347)	2763 $\pm$ 827.6 (235)	22.4 $\pm$ 5.2 (32.0)
Colon	761 $\pm$ 379 (129)	516 $\pm$ 121 (63.6)	1622 $\pm$ 809 (204)	1377 $\pm$ 641 (117)	10.9 $\pm$ 3.2 (15.6)
Rectum	4.7 $\pm$ 2.6 (0.8)	139 $\pm$ 133 (17.1)	405 $\pm$ 390 (50.8)	504 $\pm$ 260 (42.8)	12.4 $\pm$ 4.9 (17.7)
Skin	0.7 $\pm$ 0.2 (0.1)	2.3 $\pm$ 0.2 (0.3)	4.1 $\pm$ 0.9 (0.5)	3.6 $\pm$ 0.4 (0.3)	2.1 $\pm$ 0.2 (3.0)
Knee joint	0.7 $\pm$ 0.2 (0.1)	2.1 $\pm$ 0.3 (0.3)	4.1 $\pm$ 0.3 (0.5)	3.7 $\pm$ 0.5 (0.3)	2.0 $\pm$ 0.2 (2.8)

Mean $\pm$ SEM, n=3

() : Tissue/plasma ratio

Table 1b Tissue concentration of [^3H] nmol eq/g during multiple daily oral dose of [^{14}C , ^3H]SASP 50mg/kg for 21 days to male rats.

Tissue	Dose 1 4h	Dose 7 4h	Dose 14 4h	Dose 21 4h	Dose 21 96h
Plasma	40.9 $\pm$ 8.4 (1.0)	47.1 $\pm$ 6.7 (1.0)	60.2 $\pm$ 7.1 (1.0)	60.9 $\pm$ 19.9 (1.0)	0.3 $\pm$ 0.0 (1.0)
Blood	23.6 $\pm$ 4.3 (0.6)	26.4 $\pm$ 3.4 (0.6)	35.8 $\pm$ 4.2 (0.6)	34.2 $\pm$ 10.4 (0.6)	1.6 $\pm$ 0.2 (5.3)
Liver	51.4 $\pm$ 16.3 (1.3)	66.7 $\pm$ 4.7 (1.4)	93.7 $\pm$ 5.2 (1.6)	89.3 $\pm$ 27.3 (1.5)	9.0 $\pm$ 0.5 (29.9)
Kidney	43.2 $\pm$ 14.5 (1.1)	52.3 $\pm$ 4.3 (1.1)	106.3 $\pm$ 14.8 (1.8)	75.3 $\pm$ 21.5 (1.2)	8.6 $\pm$ 0.9 (28.8)
Lung	23.2 $\pm$ 7.8 (0.6)	26.6 $\pm$ 2.3 (0.6)	42.0 $\pm$ 6.9 (0.7)	32.2 $\pm$ 8.2 (0.5)	1.7 $\pm$ 0.1 (5.7)
Thyroid	18.5 $\pm$ 5.2 (0.5)	28.6 $\pm$ 5.3 (0.6)	43.7 $\pm$ 3.6 (0.7)	31.0 $\pm$ 9.9 (0.5)	2.9 $\pm$ 0.2 (9.7)
Testicles	8.7 $\pm$ 4.2 (0.2)	11.4 $\pm$ 1.4 (0.2)	19.4 $\pm$ 3.7 (0.3)	12.9 $\pm$ 2.5 (0.2)	1.3 $\pm$ 0.0 (4.4)
Thymus	11.1 $\pm$ 4.9 (0.3)	12.1 $\pm$ 1.3 (0.3)	18.2 $\pm$ 1.5 (0.3)	19.5 $\pm$ 4.2 (0.3)	2.2 $\pm$ 0.2 (7.5)
Heart	17.7 $\pm$ 7.0 (0.4)	17.4 $\pm$ 1.2 (0.4)	27.3 $\pm$ 2.3 (0.5)	22.7 $\pm$ 6.5 (0.4)	2.4 $\pm$ 0.1 (8.0)
Spleen	11.8 $\pm$ 5.1 (0.3)	15.7 $\pm$ 1.5 (0.3)	27.0 $\pm$ 3.5 (0.5)	20.0 $\pm$ 6.8 (0.3)	2.9 $\pm$ 0.2 (9.8)
Lymph nodes	21.5 $\pm$ 8.1 (0.5)	16.9 $\pm$ 3.7 (0.4)	25.1 $\pm$ 3.7 (0.4)	19.3 $\pm$ 4.2 (0.3)	0.7 $\pm$ 0.3 (2.5)
Brain	7.7 $\pm$ 3.5 (0.2)	9.1 $\pm$ 0.5 (0.2)	13.5 $\pm$ 1.4 (0.2)	12.1 $\pm$ 2.1 (0.2)	3.0 $\pm$ 0.3 (10.0)
Urine bladder	449 $\pm$ 280 (11.0)	247 $\pm$ 125 (5.2)	221 $\pm$ 48.2 (3.7)	198 $\pm$ 77.2 (3.3)	5.1 $\pm$ 1.0 (17.0)
Stomach	2659 $\pm$ 896 (65.0)	3413 $\pm$ 730 (72.5)	4039 $\pm$ 85.2 (67.1)	985 $\pm$ 76.6 (16.2)	8.8 $\pm$ 4.4 (29.4)
Duodenum+jejunum	600 $\pm$ 99 (14.7)	1171 $\pm$ 66.7 (24.9)	1128 $\pm$ 146 (18.7)	975 $\pm$ 193 (16.0)	4.5 $\pm$ 0.9 (14.9)
Ileum	1461 $\pm$ 439 (35.7)	2800 $\pm$ 298 (59.5)	4070 $\pm$ 1642 (67.7)	2820 $\pm$ 325 (46.3)	20.4 $\pm$ 5.3 (68.0)
Caecum	837 $\pm$ 369 (20.5)	1343 $\pm$ 30 (28.5)	1859 $\pm$ 634 (30.9)	2003 $\pm$ 480 (32.9)	30.5 $\pm$ 2.5 (102)
Colon	446 $\pm$ 216 (10.9)	425 $\pm$ 95.5 (9.0)	1007 $\pm$ 438 (16.7)	1022 $\pm$ 431 (16.8)	17.7 $\pm$ 5.4 (58.9)
Rectum	14.1 $\pm$ 3.4 (0.3)	168 $\pm$ 148 (3.6)	244 $\pm$ 195 (4.1)	368 $\pm$ 202 (6.1)	14.8 $\pm$ 5.1 (49.2)
Skin	15.9 $\pm$ 6.1 (0.4)	19.3 $\pm$ 2.1 (0.4)	29.8 $\pm$ 1.4 (0.5)	22.1 $\pm$ 5.5 (0.4)	3.4 $\pm$ 0.2 (11.5)
Knee joint	10.4 $\pm$ 4.2 (0.3)	12.3 $\pm$ 0.6 (0.3)	18.8 $\pm$ 0.7 (0.3)	15.4 $\pm$ 3.8 (0.3)	1.3 $\pm$ 0.1 (4.2)

Mean $\pm$ SEM, n=3

() : Tissue/plasma ratio

tion. However, there was a large sex difference. In the males the amounts of excreted metabolites were in the order $\text{AcSPOH} > \text{SPOH} > \text{Ac5ASA} > \text{AcSP} > \text{SP}$ while in female rats the amount of excreted metabolites were $\text{AcSP} > \text{Ac5ASA} > \text{SP} > \text{SPOH} > \text{AcSPOH}$. The metabolite pattern in faeces was not changed during the repeated administration.

Radioactivity in tissues

The concentrations of ^{14}C and ^3H in tissues at 4 hours after dose number 1, 7, 14, 21 in addition to 96 hours after the 21st dose are shown in Table Ia and b. The major part of the dose remained in the gastrointestinal tract. In other tissues the concentration of radioactivity was lower than the plasma level. The ^3H radionuclide, was found at higher concentrations in the tissues compared to ^{14}C . The concentration of ^3H in tissues was slightly increased during treatment, while the tissue/plasma ratio was not changed. However, the ^{14}C increased more than the increase of the plasma concentration for several tissues.

The highest concentration of ^{14}C 4 hours after administration of the last dose was besides the gastrointestinal tract, found in the kidneys, thyroid, plasma and in the liver, 10 ~ 13 nmole eq/g. The highest increase of the ratio of the concentration tissue/plasma was seen in the thyroid followed by the thymus and the spleen (Table Ia). The highest concentration of ^3H was besides the gastrointestinal tracts, found in the kidneys, liver and plasma, ranging between 61 and 106 nmole eq/g.

The ratio $^3\text{H}/^{14}\text{C}$ reflecting the relation between sulfapyridine and 5ASA metabolites,

Fig. 5 Autoradiograms showing the distribution of ^3H labelled SASP in male rats after oral administration. In (a) the uptake at 4 hours after 21 days daily treatments, and in (b) the uptake at 4 hours after a single oral dose. Note a rather high concentration in the connective tissues, and within the testicles after 21 days of treatment (a).

Fig. 6 Autoradiograms showing the distribution of [^3H] labelled SASP in male rats after oral administration. In (a) the distribution at 16 hours after 21 days daily treatments, and in (b) the distribution at the same time, but after just a single dose. Note a somewhat higher tissue concentration in (a).

Fig. 7 Autoradiograms showing the distribution of [^3H] labelled SASP in male rats 96 hours after oral administration. In (a) after 21 days daily treatments, and in (b) after just a single dose. Note a faint of radioactivity in the intestines after repeated doses, but no radioactivity after a single dose (b).

Fig. 8 Autoradiograms showing the distribution of [^3H] labelled SASP in pregnant rats 5 min after an *i.v.* injection. In (a) after oral pretreatment in 10 days, and in (b) without pretreatment. Note a somewhat higher concentration in the placenta and yolk-sac in Fig. 8a compared to Fig. 8b.

was initially high in all tissues except the intestinal parts with its content. After repeated administration, the ratio decreased in all the tissues having the initial high ratio.

The ^3H radioactivity was eliminated faster than the ^{14}C activity and 96 hours after the last dose almost equivalent and very low concentrations of both radionuclides remained except for those in the gastrointestinal tract where the highest concentration was found in caecum.

In the autoradiographic study the rats had been administered [^{14}C , ^3H]SASP for 20 days before the final dose which had a 100-fold higher specific activity of ^3H . All studied tissues showed gradually decreasing ^3H radioactivity with time after the last dose. The testicles however had its peak concentration at 16 hours. Only faint of radioactivity remained in the liver, the kidney and within the gastrointestinal tract 96 hours after the last administration. Beside the gastrointestinal tract, the highest distribution was found at 4 hours after the final dose in the kidney cortex, the penis, the testicles, the epididymis, the liver, the connective tissues, the pituitary gland, and the lung (Fig.5). At 16 hours the highest uptakes were found in the kidney cortex, the liver and the connective tissues

Table II Hepatic protein and enzyme concentrations after 14 days of treatment with SASP or phenobarbital analysed one day (treated) or one week (recovered) after the final dose.

	Control	SASP 50mg/kg	SASP 500mg/kg	Phenobarbital
Relative liver weight (% of b.w.)				
Treated	4.4±0.4	4.5±0.4	4.2±0.3	4.4±0.2
Recovered	4.7±0.3	4.4±0.2	4.5±0.5	4.3±0.2
Protein (mg/g liver)				
Treated	208±8	207±8	214±5	224±4*
Recovered	207±5	205±7	211±10	211±16
FAOS (nmol/min/mg protein)				
Treated	3.43±0.66	3.68±0.57	3.82±0.59*	3.24±0.42
Recovered	3.29±0.13	4.00±0.73	4.85±0.41*	3.98±0.58
Cytochrome p-450 (nmol/mg protein)				
Treated	0.56±0.04	0.58±0.07	0.62±0.13	1.02±0.23*
Recovered	0.75±0.03	0.58±0.05*	0.60±0.11	0.60±0.10*
Cytochrome b5 (nmol/mg protein)				
Treated	0.71±0.06	0.73±0.05	0.86±0.09*	0.94±0.08*
Recovered	0.80±0.04	0.72±0.07	0.75±0.08	0.86±0.11
Aminopyrine demethylase (nmol/min/mg protein)				
Treated	3.28±0.55	2.79±0.62	2.65±0.65	3.97±0.26
Recovered	3.92±0.45	3.21±0.40*	3.58±0.45	3.97±0.80
Aniline hydroxylase (nmol/min/mg protein)				
Treated	0.58±0.07	0.48±0.06	0.51±0.09	0.43±0.02*
Recovered	0.52±0.03	0.45±0.05	0.48±0.03	0.53±0.12

Mean±SD, n=5.

*p<0.05 vs control

and at 96 hours in the kidney cortex and the liver (Fig. 6, 7).

Within the gastrointestinal tract relatively high radioactivity was seen in the stomach, the small intestines and the colon from 4 hours to 96 hours.

In the autoradiographic study with oral pretreatment of unlabelled SASP for 10 days, the pretreatment did not rise the radioactivity after an *i.v.* injection in fetal tissues compared to after a single *i.v.* dose (Fig. 8). A rise was seen in the placenta and the yolk-sac for both labellings, but was most evident for the ³H-labelling.

Enzyme induction studies

The hepatic protein and enzyme concentrations obtained after 14 days of treatment with SASP or phenobarbital as a reference compound are presented in Table II. A comparison was made between livers from animals analysed one day after the final dose (treated)

and values obtained from livers excised from rats which had a one week drug free period before examination (recovered).

The relative liver weights (% of body weight) in SASP treated animals were not significantly different from the control groups neither in treated or "recovered" groups, showing that SASP does not induce hepatomegaly even at the higher dose of 500mg/kg b.w. The hepatic cytochrome P-450 content showed a 2-fold increase in the phenobarbital-treated group although after withdrawing the drug the content decreased to around the control level. However, the SASP-treated groups did not differ significantly from the control. In one "recovered" group receiving SASP (50mg/kg b.w.), a significant decrease in the cytochrome content was observed. However, the change was not dose-dependent, suggesting that the change might be artefactual and of no biological significance. The phenobarbital-treated and SASP-treated groups (500mg/kg) showed slight increases in hepatic cytochrome b5. A slight decrease in the activities of aminopyrine demethylase and aniline hydroxylase was observed in the "recovered" SASP (50mg/kg) groups. In the phenobarbital-treated group the aminopyrine demethylase activity tended to increase while the aniline hydroxylase activity decreased slightly. FAOS, a hepatic peroxisomal enzyme, was slightly increased in the SASP-treated group, 500mg/kg.

DISCUSSION

The bioavailability of SASP is low, (about 9% in the rat) and the half-life in plasma is short, (8 min) as previously shown¹⁾. Thus, the dose used in this study, (50mg/kg) which is somewhat higher than the therapeutic dose, gave no measurable levels of unchanged SASP in plasma during the period of treatment. However, by using labelled SASP, with ¹⁴C in the 5ASA moiety and ³H in the sulfapyridine part, the drug together with its main metabolites could separately be followed.

It has previously been shown that after an oral dose of SASP to the rat, sulfapyridine metabolites were mainly present in plasma which in this study corresponds to the ³H radioactivity¹⁾. After repeated daily oral doses, the peak level of ³H in plasma slightly but not significantly increased in the male and female rat. The elimination was the same as after a single dose. No change was seen in the excreted amount of radioactivity during administration. Thus, the absorption is not significantly changed during repeated administration. Neither was the metabolic pattern in plasma and urine changed during repeated administration. This was in good agreement with the *in vivo* studies showing no induction of microsomal enzymes in the liver, as cytochrome P-450 and b5, after SASP treatment. Other liver enzymes, as aniline hydroxylase and aminopyrine demethylase did also show unchanged activity except a slight increase of the peroxisomal enzyme, FAOS. It is well known that certain hypolipidemic agents and plasticizers, such as clofibrate and di(2-ethylhexyl)phthalate, induce hepatomegaly, proliferation of hepatic peroxisomes, and induction of various peroxisomal and non-peroxisomal enzymes^{15, 16)}. Furthermore, the co-induction of microsomal lauric acid hydroxylase by these agents was recently reported¹⁷⁾. These agents are classified as non-mutagenic hepatocarcinogens^{18, 19)}. The hepatic peroxisomal enzymes, FAOS, is considered to be the most sensitive biochemical marker for peroxisome proliferation. The slight increase in the activity observed

in the group treated with SASP (500mg/kg b.w.) and the slight dose dependency, were remarkably small compared to the results reported for other peroxisome proliferators.

The metabolism of sulfapyridine was different in male and female rats as previously has been shown¹⁻³). The main difference was a higher oxidative metabolic capacity in male rats resulting in more of hydroxylating sulfapyridine in male compared female rats. A sex difference in metabolism is common in rats and might be linked to either sex hormones²⁰) or a higher content of cytochrome P-450 content of the liver microsomes in male compared to female rats²¹). The hydroxylated sulfapyridine was further conjugated and rapidly excreted in urine or eliminated through the bile resulting in higher faecal excretion and lower plasma levels of tritium in male rats. Such sex differences were seen throughout this study.

The tissue distribution pattern was roughly the same when comparing single and repeated administration⁸). The concentration of radioactivity in the tissues was dominated by ³H, corresponding to SP-metabolites, with an even distribution in the body. There was no accumulation as the concentrations did not change compared to the plasma concentration during the repeated doses. The concentration of ³H in the fetus was also unchanged.

The concentration of ¹⁴C, however, increased and in some tissues, kidneys, liver and thyroid, the tissue concentration exceeded the plasma concentration after 2 weeks of administration. The comparably high concentration in the thyroid measured in dissected tissue was not seen in the autoradiography. A contamination during dissection from the larynx can not be excluded. The ¹⁴C radioactivity was also to some extent distributed to the blood cells after repeated dosing.

It can be concluded that repeated administration of SASP to rat does not induce any changes in absorption, metabolism or elimination and no accumulation in tissues were seen.

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