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

Tissue distribution and excretion of SASP in the rat after a single intravenous or oral administration.

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Summary

The tissue distribution has been studied in rats of both sexes by whole body autoradiography and by dissection of organs after both a single oral and single intravenous administration of radiolabelled salazosulfapyridine (sulfasalazine, SASP). The compound was labelled in two different positions: ^{14}C in the carboxyl group in the salicylic acid part of the molecule and ^3H in the sulfonamide part of the molecule making it possible to follow the metabolites after cleavage of the azobridge.

The results of these investigations showed that following oral administration, the drug remained mainly in the gastro-intestinal tract. At about 8 hours a certain tissue distribution was noticed for both ^{14}C and ^3H . The distribution pattern was very different for the two labellings: the ^{14}C radioactivity was at that time heterogeneously distributed to certain tissues as liver, bone marrow, intestinal mucosa, kidney, lung, connective tissues, ovary, salivary and hardenian gland, while the ^3H radioactivity was evenly distributed to all tissues including the central nervous system. The radioactivity was almost eliminated from the tissues within 24 hours.

Also after *i.v.* injection high concentration of radioactivity was found in the gastrointestinal tract. Already 5 min after administration 25% of the dose was found in the duodenum and 30% in the liver, indicating an extensive biliary excretion. Beside the gastrointestinal tract the highest radioactive concentrations were found in the liver, connective tissues, lung, kidney, and blood. The radioactivity of ^{14}C labelled SASP decreased more rapidly than that of the ^3H . The distribution pattern was roughly the same when comparing males and female rats.

In the fetal tissues the ^3H radioactivity passed the placental barrier at 4 hours after the injection and was distributed evenly in the fetal tissues, while the ^{14}C radioactivity was only found in the fetal intestines in small amounts.

Trace amounts of excreted radioactivity was found in milk. The milk to plasma ratio for ^{14}C was 0.13 ± 0.01 20 min after intravenous administration.

The total recovery of excreted radioactivity in male rats at different doses was 73~89% for ^{14}C and 79~92% for ^3H . ^{14}C radioactivity was mainly found in the faeces while the ^3H activity was found in about the same amount in faeces and in urine. Female rats had less faecal excretion than males. Only trace amounts of radioactivity were found in the expired air.

INTRODUCTION

Salazosulfapyridine, SASP, a drug known to be effective in inflammatory bowel diseases, has also been reevaluated as an antirheumatic drug with immunomodulating properties. The knowledge about the tissue distribution in human is limited. The apparent volume of distribution has only been hypothetically calculated as only oral studies have been published. The *in vitro* binding to human serum proteins is extensive about 99%¹⁾. The drug was shown to be present in the synovial fluid in patients with rheumatoid arthritis, although only in some patients the concentration exceeded the plasma concentration of SASP²⁾. According to whole body autoradiography studies on mice, SASP has an affinity for connective tissue^{3,4)}.

The present study was designed to evaluate the tissue distribution and excretion of SASP in the rat. The SASP molecule was labelled with ³H and ¹⁴C in such a way that the ³H radioactivity corresponded either to intact SASP or sulfapyridine and its metabolites and the ¹⁴C radioactivity corresponded either to intact SASP or 5ASA and its metabolites. Whole body autoradiography with image analysis was used in combination with, and to select organs for the dissection study in which a more precise quantification of the radioactivity was performed.

MATERIALS AND METHODS

Materials

[¹⁴C]SASP was synthesized from ¹⁴C labelled salicylic acid according to a synthetic route shown by Sjöquist *et al.*⁵⁾ in the preceding paper. The specific activities of [¹⁴C]SASP thus obtained were for 2 different batches 76MBq/mmol (2.05 mCi/mmol), and 162 MBq/mmol (4.37 mCi/mmol). The radiochemical purity judged by thin layer and liquid chromatography was >97%.

[³H]SASP was prepared according to Sjöquist *et al.*⁵⁾. The specific activities obtained was 1.09GBq/mmol (29.5 mCi/mmol) and 2.87GBq/mmol (77.5 mCi/mmol). The radiochemical purity was >97% according to thin layer and liquid chromatography.

SASP was obtained from Pharmacia AB, Uppsala, Sweden. Other chemicals and solvents were of analytical grade and were obtained from various suppliers.

Animals and dosage

Male and female Sprague Dawley rats were purchased from Alab, Sollentuna, Sweden. The animals were 90~120 days old and weighed 215~330 g. The rats were housed in temperature and humidity controlled rooms 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* or starved overnight before an oral dose of [¹⁴C, ³H]SASP was given. Three to five animals were used in each group. The intravenous dose was 5mg/kg with 0.2MBq of [¹⁴C] and 0.4MBq of [³H]SASP per kg body weight in the dissection study and the mass balance study while the radioactivity dose was higher in the autoradiography study; about 2.5 and 25MBq of ¹⁴C and ³H respectively. The oral dose was 50mg/kg with 2 and 4MBq/kg of ¹⁴C and ³H respectively in the dissection study,

about 15 and 250MBq/kg of ^{14}C and ^3H respectively in the autoradiography study, and 50, 200 and 500mg/kg with 2 and 4MBq/kg of ^{14}C and ^3H respectively in the mass balance study.

Plasma protein and blood cell binding

In vitro binding studies were performed with blood and plasma from rat, dog and man, or human serum albumin in phosphate buffer (45mg/ml), incubated with [^3H]SASP at 37°C in the concentration range 25~200 μM . The protein binding of the drug in plasma was determined by ultrafiltration measuring the radioactivity. The fraction bound to the blood cells was determined after centrifugation by comparing the radioactivity in whole blood with that in the cell fraction taking the hematocrit value into account. *In vivo* plasma protein and blood cell binding was studied with the same methods after administration of [^3H]SASP intravenously or orally in rat. Blood was withdrawn by heart puncture 5 and 30 min after *i.v.* and 40 min 4 and 8 hours after oral administration and the protein binding of radioactively labelled substance in the plasma was determined as well as the binding to the blood cells.

Whole body autoradiography

Twelve to fourteen animals per administration group including two animals for each time point were given either [^{14}C]SASP or [^3H]SASP. Male and pregnant rats were dosed intravenously and nonpregnant rats were dosed orally. The survival times were selected to 5, 20 min; 1, 4, 8, 16 and 96 hours after the oral administration and 1, 5, 20 min; 1, 4 and 16 hours after the *i.v.* administration. The animals were killed by CO_2 inhalation. They were then mounted for sectioning according to the technique of Ullberg⁶⁾ in a gel of carboxymethyl cellulose (CMC), and rapidly frozen in hexane cooled with dry ice (-70°C). From each rat, 20 μm 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 for 24 hours, the sections containing ^3H were apposed against Ultrofilm ^3H (LKB), a film that is specifically sensitive to the weak radiation of tritium and an X-ray film (Industrex, KODAK) was used to register ^{14}C . The blackening of the films were converted to radioactivity using radio standards (^3H Microscales and ^{14}C Microscales, Amersham International, UK).

Tissue dissection

At intervals between 5 minutes and 16 hours after the single *i.v.* dose and between 20 minutes and 96 hours after the single oral dose, blood was collected in heparinized tubes by heart puncture under light ether anaesthesia, the animals were decapitated and tissues were dissected and weighed before storage at -20°C until analysed for radioactivity.

Mass balance

The animals (n=5) were after a single intravenous (males) or oral dose (males and females) housed individually in metabolism cages. Prior to the administration, the ani-

mals were acclimatized in the cages for 24 hours. Urine and faeces were collected at intervals up to 7 days. The collection vials were protected from light during sampling of urine and faeces. In addition, CO₂ in expired air was collected during 96 hours from rats given [¹⁴C, ³H]SASP 500mg/kg body weight *p.o.* The cages were rinsed with water after completion of the study and the water was analysed regarding radioactive content. All samples were immediately stored at -20°C until analysis. Before storage, methanol (3 times by weight), was added to the faeces samples before homogenization.

Excretion to the milk

After delivery the rats were kept with the pups for 6~12 days before they were placed in metabolic cages. Twenty minutes or 1 hour after *i.v.* administration and 8 hours after an oral dose of 200mg/kg the lactating rats were given oxytocin (9 IE Syntocinon per kg body weight) to facilitate lactation.

Measurement of radioactivity

The radioactivity of all tissue samples was determined by liquid scintillation counting (Beckman LS 2800) after combustion to ¹⁴CO₂ and ³H₂O using Combustaid (Packard Instrument) in a Sample Oxidizer, TRI-CARB Model 306 (Packard Instrument). Tissues with a wet weight >0.5 g were either divided into smaller pieces, or larger tissues as liver and intestines were homogenized prior to combustion. The tritiated water was condensed in Monophase S (Packard Instrument). The ¹⁴CO₂ 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 added to different biological samples were quantitative for both ¹⁴C and ³H with a coefficient of variation (CV) varying between 0.3~5% for the different samples with the highest CV for faeces. The limit of detection for labelled SASP was 0.1μM eq.

The qualitative autoradiographic findings were complemented with quantitative data as measured by computerized image analysis⁷⁾. The densitometric system is built around a Cromemco 2-HD microcomputer equipped with a Z80 8 bit processor and a MC 68000 32 bit processor, 768 Kbytes of main memory, 20 Mbytes of disc memory and SDI graphic system. A black-and-white video camera (Philips LDH 400/61) connected to the computer through a Cromemco SDD video interface, which digitizes light-intensity into 256 levels, was used to measure optical densities. The measuring procedure was first performed without and then with the autoradiogram in the picture field.

The densities of the autoradiogram was calculated in 241×384 picture elements (pixels). The consistency of the system was tested and calibrated with a set of known densities (Kodak Wratten gelatin filters). The resulting picture was displayed on a RGB monitor (Barco CD 233) and-with the aid of a digitizer pad-various regions of interest (ROI) were delineated for calculation of average density, or average concentration when radiostandards were used.

Analysis of SASP by liquid chromatography

Faeces samples were homogenized in methanol (1:3, w:w). The urine samples were

mixed with two volumes of methanol to precipitate proteins. The urine and faeces samples were placed at -20°C for 10 minutes followed by centrifugation at 1500 g. The supernatant ($50\mu\text{l}$) was injected into the reversed phase column (Nucleosil C_{18} , $10\mu\text{m}$ Mackerey-Nagel & Co, Düren, FRG) protected with a precolumn (Perisorb C_{18} , $40\mu\text{m}$, Merck Darmstadt, FRG). The mobile phase consisted of methanol : phosphate buffer 0.025M, pH2.5 (64 : 36, v : v). The column was kept at 37°C and the flow rate was 1 ml/min. For detection a UV detector (Kratos Spectroflow 783) at 360 nm was used. The detection limit in urine and faeces was $3.4\mu\text{mole/l}$ and $3.8\mu\text{mole/l}$ respectively.

Calculations

The tissue concentrations were adjusted for the difference in the individual doses. The concentrations of radioactivity were calculated as nmole equivalent by taking the specific activity of the formulations into account. Means and standard error of the mean (SEM) were estimated.

RESULTS

Protein binding

The protein binding of SASP *in vitro* in plasma from rat, dog and human was very similar in the different species, 99.7~99.9%, and was independent of the concentration of SASP (Table I). Binding studies with purified human serum albumin in a phosphate buffer gave similar result which confirms that albumin is the major binding protein in plasma. No binding of SASP to the blood cells was found. *In vivo* binding studies gave similar result after an *i.v.* injection of [^3H]SASP in rat. However, after an oral dose the free fraction of ^3H radioactivity increased over time indicating the presence of sulfa-pyridine metabolites and that they bind to lesser extent to plasma protein than unchanged SASP (Table II). Also a small fraction of ^3H , probably corresponding to sulfa-pyridine metabolites, was bound to blood cells (Table II).

Whole body autoradiography after oral administration

The tissue levels of radioactivity from 5 min to 96 hours were very low after oral administration and the radioactivity ^{14}C and ^3H remained almost exclusively in the gastrointestinal tract (Fig. 1a and d). However, at 4 and 8 hours a certain radioactivity was registered also in tissues outside the gastrointestinal tract for both labellings. The tissue

Table I Binding [^3H]SASP *in vitro* to plasma proteins after incubation at 37°C in plasma or human serum albumin solution.

SASP conc. μM	%			
	Rat	Dog	Human	HSA
25	99.92 $\pm$ 0.01	99.66 $\pm$ 0.02	99.90 $\pm$ 0.01	99.93
50	99.93 $\pm$ 0.01	99.74 $\pm$ 0.03	99.91 $\pm$ 0.01	99.94
100	99.90 $\pm$ 0.01	99.69 $\pm$ 0.02	99.90 $\pm$ 0.01	99.93
200	99.88 $\pm$ 0.01	99.65 $\pm$ 0.04	99.89 $\pm$ 0.01	99.93

The results are the means $\pm$ SD from 2 individuals, each in duplicate.

Table II Binding of [^3H]SASP *in vivo* to plasma proteins and blood cells after oral and *i.v.* administration of [^3H]SASP in male rats.

Adm	Dose mg/kg	Time	%	
			Plasma proteins	Blood cells
<i>i.v.</i>	5	5 min	99.8 $\pm$ 0.03	0
<i>i.v.</i>	5	30 min	99.8 $\pm$ 0.07	0
<i>p.o.</i>	50	40 min	96.9 $\pm$ 1.4	7.0 $\pm$ 5.6
<i>p.o.</i>	50	4 h	93.3 $\pm$ 1.9	12.3 $\pm$ 3.2
<i>p.o.</i>	50	8 h	90.9 $\pm$ 1.3	19.0 $\pm$ 1.7

Mean $\pm$ SD, n=3.

peak concentrations of the ^{14}C labelling were found at 8 hours. At that time the highest radioactivity was found in the liver and kidney. Relatively high radioactivity was also found in the bone marrow, the intestinal mucosa, the blood, the lung, the connective tissues, the ovary, (not shown) the salivary and the Harderian gland (Fig. 1c).

The distribution pattern of the ^3H labelling was different from that of the ^{14}C pattern at the corresponding time (8 hours) showing a much more even pattern (Fig. 1b). Thus, at that time most tissues showed about the same concentration including the central nervous system. Also in body fluids, such as pleural and synovial fluids a certain ^3H radioactivity was noticed (not shown). However, the highest tissue concentration was observed in the connective tissues (Fig. 1b).

Tissue dissection after oral administration

The quantitative study on tissue concentrations in male rat confirmed that the major fraction of the oral dose remained in the gastrointestinal tract. During the first hours when the peak plasma level of SASP was expected as well as later when sulfapyridine metabolites appear in plasma⁵⁾, about 90% of the dose remained in the gastrointestinal tract. Almost 50% of both ^{14}C and ^3H remained in the stomach at 4 hours after the administration. After 24 hours only 5% of the dose was found in the gastrointestinal tract, distributed mainly in the caecum. In other tissues there was a peak concentration of both ^{14}C and ^3H after 8 hours, coinciding with the plasma peak concentrations. Except these peak concentrations high levels of the isotopes compared with the plasma level were also seen, 20 minutes after the oral dose in some animals, with a wide individual variation (Table III a,b). At eight hours after administration, the kidney was the only tissue of those analysed that showed a concentration of both ^{14}C and ^3H radioactivity higher than the plasma level. The elimination rate in the tissues was similar to the elimination from plasma. After 24 hours low concentrations were seen in the tissues.

Whole body autoradiography after *i.v.* administration

After an *i.v.* administration in male rats the blood concentration of ^{14}C and ^3H labelled SASP or its metabolites showed relatively high levels compared with those in most tis-

Fig. 1 Autoradiograms showing the distribution of SASP in female rats after oral administration : (a) ^{14}C at 1 hour, (b) ^3H at 8 hours, (c) ^{14}C at 8 hours and (d) ^{14}C at 16 hours after administration. No tissue levels are seen in (a) and (d), and the radioactivity remained in the gastrointestinal tracts. In (b) and (c) the different distribution patterns obtained by isotopes are shown. Note a rather evenly distributed radioactivity in (b), but with a certain uptake in connective tissues. In (c) the radioactivity is heterogeneously distributed showing rather high radioactivity in liver, intestines, kidney, bone marrow and Harders gland. (White corresponds to high radioactivity).

Table III a Tissue concentration of ³H radioactivity nmol eq/g after an oral dose of [¹⁴C, ³H]SASP 50mg/kg in male rats.

Tissue	20 min	1.0 h	4.0 h	8.0 h	12.0 h	24.0 h	96.0 h
Plasma	1.2± 0.2 (1.0)	1.6± 0.5 (1.0)	19.0± 4.7 (1.0)	43.0± 5.9 (1.0)	27.7± 8.3 (1.0)	2.4± 0.4 (1.0)	0.1±0.0
Liver	13.0± 8.9 (10.6)	2.5± 0.6 (1.5)	21.7± 5.7 (1.1)	45.7± 5.8 (1.1)	28.5± 8.1 (1.0)	5.4± 1.6 (2.3)	0.9±0.1
Kidney	4.7± 2.0 (3.9)	0.9± 0.3 (0.5)	21.1± 4.9 (1.1)	72.2± 20.2 (1.7)	61.4± 14.1 (2.2)	5.4± 0.8 (2.2)	0.7±0.0
Lung	3.5± 1.4 (2.9)	1.7± 0.8 (1.0)	10.4± 2.3 (0.6)	22.0± 2.6 (0.5)	14.3± 3.8 (0.5)	1.2± 0.1 (0.5)	0.2±0.1
Thyroid	116.0± 50.7 (94.3)	5.5± 3.2 (3.3)	8.6± 1.9 (0.5)	12.6± 1.0 (0.3)	13.3± 4.1 (0.5)	1.4± 0.2 (0.6)	0.2±0.1
Testicles	5.8± 2.9 (4.8)	0.8± 0.4 (0.5)	6.0± 1.6 (0.3)	19.4± 1.1 (0.5)	9.3± 2.2 (0.3)	0.8± 0.2 (0.3)	0.3±0.1
Stomach	9266±177.0 (7533)	2234±629.9 (1354)	1866±471.7 (98.5)	319.4± 80.7 (7.4)	102.8± 48.3 (3.7)	40.9±35.5 (17.1)	0.4±0.3
Duodenum+jejunum	767.9±297.5 (624)	267.9±125.4 (162)	881.8±207.8 (46.5)	510.4± 71.4 (11.9)	315.7± 89.0 (11.4)	9.7± 1.6 (4.1)	0.8±0.1
Ileum	58.1± 40.7 (47.3)	2369±1640 (1436)	1636±696.1 (86.4)	1132±115.5 (26.3)	688.9±160.5 (24.9)	21.4± 3.8 (9.0)	3.2±1.0
Caecum	6.8± 1.4 (5.5)	4.2± 2.2 (2.6)	521.6±159.2 (27.5)	1026.0±127.4 (23.9)	958.7± 50.8 (34.6)	196.4±33.7 (82.2)	3.7±0.8
Colon	14.1± 5.4 (11.5)	0.6± 0.2 (0.4)	457.5±169.9 (24.1)	974.5± 77.6 (22.7)	874.3±128.6 (31.6)	81.3±41.0 (34.0)	2.5±0.4
Rectum	10.6± 5.9 (8.6)	0.8± 0.3 (0.5)	8.0± 2.1 (0.4)	536.8±136.0 (12.5)	618.4±246.7 (22.3)	301.1±94.4 (125.4)	4.0±1.4
Skin	32.4± 24.9 (26.3)	8.7± 6.3 (5.3)	20.6± 12.6 (1.1)	19.6± 1.8 (0.5)	10.9± 2.6 (0.4)	1.9± 0.1 (0.8)	1.3±0.7
Knee joint	5.5± 1.7 (4.1)	0.5± 0.1 (0.3)	5.0± 0.9 (0.3)	11.5± 2.0 (0.3)	7.2± 1.5 (0.3)	1.5± 0.3 (0.6)	0.3±0.1

Mean±SEM, n=5
() : Tissue/plasma ratio

Table III b Tissue concentration of ^{14}C radioactivity nmol eq/g after an oral dose of $[^{14}\text{C}, ^3\text{H}]\text{SASP}$ 50mg/kg in male rats.

Tissue	20 min	1.0 h	4.0 h	8.0 h	12.0 h	24.0 h	96.0 h
Plasma	0.5 $\pm$ 0.1 (1.0)	0.9 $\pm$ 0.3 (1.0)	4.0 $\pm$ 0.7 (1.0)	4.5 $\pm$ 0.3 (1.0)	3.5 $\pm$ 0.3 (1.0)	0.6 $\pm$ 0.1 (1.0)	0.2 $\pm$ 0.1
Liver	11.5 $\pm$ 8.7 (22.6)	1.7 $\pm$ 0.5 (1.9)	2.8 $\pm$ 0.4 (0.7)	4.4 $\pm$ 0.3 (1.0)	4.0 $\pm$ 0.5 (1.1)	3.2 $\pm$ 1.5 (5.3)	0.5 $\pm$ 0.1
Kidney	4.8 $\pm$ 2.4 (9.5)	0.6 $\pm$ 0.1 (0.6)	2.8 $\pm$ 0.6 (0.7)	9.6 $\pm$ 2.1 (2.1)	14.2 $\pm$ 3.6 (4.0)	1.2 $\pm$ 0.2 (2.0)	0.3 $\pm$ 0.0
Lung	2.6 $\pm$ 1.3 (5.2)	2.1 $\pm$ 1.4 (2.3)	1.4 $\pm$ 0.2 (0.3)	2.1 $\pm$ 0.2 (0.5)	1.7 $\pm$ 0.2 (0.5)	0.4 $\pm$ 0.1 (0.7)	0.3 $\pm$ 0.1
Thyroid	147.4 $\pm$ 98.0 (289)	14.0 $\pm$ 10.9 (14.9)	1.9 $\pm$ 0.2 (0.5)	3.2 $\pm$ 0.5 (0.7)	2.1 $\pm$ 0.3 (0.6)	1.0 $\pm$ 0.3 (1.6)	0.1 $\pm$ 0.0
Testicles	2.2 $\pm$ 0.6 (4.3)	0.3 $\pm$ 0.1 (0.3)	0.9 $\pm$ 0.4 (0.2)	2.6 $\pm$ 0.3 (0.6)	1.0 $\pm$ 0.0 (0.3)	0.2 $\pm$ 0.0 (0.3)	0.2 $\pm$ 0.0
Stomach	8875 $\pm$ 318.8 (17402)	2149 $\pm$ 615.3 (2286)	1812 $\pm$ 489.7 (451)	332.9 $\pm$ 93.9 (74.2)	138.3 $\pm$ 80.0 (39.4)	40.2 $\pm$ 36.2 (65.8)	0.8 $\pm$ 0.3
Duodenum+jejunum	1185 $\pm$ 329.0 (2324)	256.7 $\pm$ 105.1 (273)	733.2 $\pm$ 183.5 (182)	210.2 $\pm$ 47.5 (46.8)	181.6 $\pm$ 98.4 (51.8)	4.9 $\pm$ 2.6 (8.1)	0.6 $\pm$ 0.1
Ileum	38.5 $\pm$ 23.8 (75.4)	2600 $\pm$ 1805 (2766)	1673 $\pm$ 741.0 (416)	678.5 $\pm$ 160.7 (151)	471.4 $\pm$ 248.7 (134)	5.8 $\pm$ 1.8 (9.5)	2.9 $\pm$ 1.0
Caecum	7.9 $\pm$ 1.7 (15.4)	6.2 $\pm$ 3.8 (6.6)	850.8 $\pm$ 256.7 (212)	1890 $\pm$ 125.7 (421)	1359 $\pm$ 150.4 (387)	161.4 $\pm$ 24.9 (265)	3.3 $\pm$ 0.9
Colon	15.1 $\pm$ 5.8 (29.6)	0.6 $\pm$ 0.1 (0.6)	723.3 $\pm$ 215.4 (180)	1993 $\pm$ 223.5 (444)	1139 $\pm$ 128.9 (324)	67.4 $\pm$ 32.3 (110)	2.4 $\pm$ 0.3
Rectum	12.7 $\pm$ 7.6 (25.0)	0.8 $\pm$ 0.2 (0.9)	4.0 $\pm$ 1.8 (1.0)	1503 $\pm$ 398.8 (335)	910.9 $\pm$ 376.4 (260)	279.2 $\pm$ 83.2 (458)	3.4 $\pm$ 1.0
Skin	14.1 $\pm$ 8.0 (27.7)	2.7 $\pm$ 1.4 (2.8)	4.6 $\pm$ 3.1 (1.2)	5.1 $\pm$ 0.7 (1.1)	2.1 $\pm$ 0.3 (0.6)	1.0 $\pm$ 0.2 (1.6)	1.5 $\pm$ 0.7
Knee joint	5.5 $\pm$ 2.2 (10.9)	0.6 $\pm$ 0.2 (0.6)	1.1 $\pm$ 0.2 (0.3)	1.7 $\pm$ 0.3 (0.4)	1.4 $\pm$ 0.3 (0.4)	0.5 $\pm$ 0.1 (0.9)	0.2 $\pm$ 0.0

Mean $\pm$ SEM, n=5

() : Tissue/plasma ratio

Table IV Tissue concentration of ^3H radioactivity nmol eq/g after an intravenous injection of [^{14}C , ^3H]SASP 5mg/kg in male rats.

Tissue	5 min	20 min	1.0 h	4.0 h	8.0 h	16.0 h
Plasma	63.6 $\pm$ 6.3 (1.0)	49.3 $\pm$ 6.4 (1.0)	29.1 $\pm$ 1.0 (1.0)	10.4 $\pm$ 1.5 (1.0)	5.9 $\pm$ 0.5 (1.0)	0.7 $\pm$ 0.1 (1.0)
Liver	99.9 $\pm$ 8.2 (1.6)	19.3 $\pm$ 4.2 (0.4)	7.0 $\pm$ 0.3 (0.2)	6.2 $\pm$ 0.6 (0.6)	3.8 $\pm$ 0.5 (0.6)	1.0 $\pm$ 0.4 (1.4)
Kidney	21.7 $\pm$ 1.9 (0.3)	11.7 $\pm$ 3.0 (0.2)	7.3 $\pm$ 0.3 (0.2)	9.1 $\pm$ 0.5 (0.9)	5.5 $\pm$ 0.6 (0.9)	0.9 $\pm$ 0.1 (1.3)
Lung	11.5 $\pm$ 0.9 (0.2)	9.0 $\pm$ 0.5 (0.2)	7.3 $\pm$ 0.4 (0.2)	4.9 $\pm$ 0.5 (0.5)	2.8 $\pm$ 0.3 (0.5)	0.3 $\pm$ 0.1 (0.5)
Thymus	2.5 $\pm$ 0.4 (0.0)	2.2 $\pm$ 0.5 (0.0)	1.6 $\pm$ 0.2 (0.1)	1.6 $\pm$ 0.1 (0.2)	0.9 $\pm$ 0.1 (0.2)	0.1 $\pm$ 0.0 (0.1)
Testicles	0.7 $\pm$ 0.0 (0.0)	1.3 $\pm$ 0.1 (0.0)	2.1 $\pm$ 0.2 (0.1)	2.4 $\pm$ 0.6 (0.2)	1.7 $\pm$ 0.2 (0.3)	0.1 $\pm$ 0.0 (0.2)
Stomach	1.8 $\pm$ 0.5 (0.0)	4.1 $\pm$ 1.0 (0.1)	1.2 $\pm$ 0.3 (0.0)	2.3 $\pm$ 0.4 (0.2)	1.6 $\pm$ 0.3 (0.3)	6.7 $\pm$ 3.5 (9.5)
Duodenum+jejunum	143.1 $\pm$ 7.9 (2.2)	309.9 $\pm$ 26.3 (6.3)	271.3 $\pm$ 35.9 (9.3)	48.0 $\pm$ 5.6 (4.6)	28.9 $\pm$ 2.9 (4.9)	2.6 $\pm$ 0.6 (3.6)
Ileum	3.2 $\pm$ 0.2 (0.0)	3.1 $\pm$ 0.5 (0.1)	104.3 $\pm$ 88.3 (3.6)	114.3 $\pm$ 14.5 (11.0)	114.8 $\pm$ 7.0 (19.3)	7.5 $\pm$ 1.5 (10.5)
Caecum	0.8 $\pm$ 0.0 (0.0)	0.6 $\pm$ 0.0 (0.0)	1.0 $\pm$ 0.2 (0.0)	164.7 $\pm$ 10.2 (15.9)	166.6 $\pm$ 22.3 (28.0)	29.5 $\pm$ 2.2 (41.6)
Colon	1.1 $\pm$ 0.1 (0.0)	1.0 $\pm$ 0.2 (0.0)	1.7 $\pm$ 0.4 (0.1)	64.1 $\pm$ 29.1 (6.2)	163.7 $\pm$ 26.0 (27.6)	18.6 $\pm$ 10.4 (26.2)
Rectum	2.4 $\pm$ 0.6 (0.0)	1.8 $\pm$ 0.6 (0.0)	15.7 $\pm$ 15.1 (0.5)	2.2 $\pm$ 0.8 (0.2)	114.8 $\pm$ 58.3 (19.3)	53.4 $\pm$ 12.6 (75.1)
Skin	2.8 $\pm$ 0.6 (0.0)	3.4 $\pm$ 0.4 (0.1)	3.1 $\pm$ 0.3 (0.1)	2.3 $\pm$ 1.2 (0.2)	3.4 $\pm$ 0.2 (0.6)	0.8 $\pm$ 0.1 (1.1)
Knee joint	4.3 $\pm$ 0.8 (0.1)	2.6 $\pm$ 0.3 (0.0)	2.1 $\pm$ 0.2 (0.1)	1.6 $\pm$ 0.2 (0.1)	0.9 $\pm$ 0.0 (0.2)	0.0 $\pm$ 0.0 (0.0)

Mean $\pm$ SEM, n=3

() : Tissue/plasma ratio

Fig. 2 Autoradiograms showing the distribution of SASP after an intravenous injection : (a) ^3H in a pregnant rat (gestational day 18) at 5 min, (b) ^{14}C in a male rat at 4 hours and (c) ^3H in a pregnant rat (gestational day 18) at 4 hours after the injection. Note the virtual absence of ^3H radioactivity in fetuses at 5 min (a), and the evenly distributed ^3H radioactivity also in fetuses at 4 hours (c). Also note a rather high uptake of [^{14}C] radioactivity in the connective tissues, the testicle and within the synovial fluid (b).

sues (initial data on the male rats are not shown but the distribution was similar as in the female pregnant rats shown in Fig. 2a. The tissue levels of both ^3H and ^{14}C decreased initially rather rapidly with time for most tissues. However, at 4 hours a redistribution of the ^3H labelled substance was obvious, and some tissues as the intestinal mucosa, the muscles, the myocardium and the brain showed from 20 min to 4 hours a gradually increasing concentration. For the connective tissues, the synovial fluid and the testicles both labellings showed a slow increase during that time. High radioactivity in these tissues is illustrated in Fig. 2b.

Very high radioactivity was noticed from 5 min to 16 hours after the administration in the gastrointestinal tract with both of the labellings. The content of the small intestines showed high radioactivity from 5 min to 4 hours, while the contents of colon and rectum showed high levels from 4 to 16 hours after administration. The highest concentrations besides the gastrointestinal tract were found in the liver, the lung and the intestinal mucosa. The concentration of ^{14}C in the intestinal mucosa was higher than that of the ^3H . The uptake in the kidney cortex was consistently higher for the ^3H labelling, which roughly followed that of the blood, than that of the ^{14}C labelling. Within the kidney pelvis and urinary bladder rather high radioactivities were noticed from 5 min to 4 hours for both labellings. However, the excretion of ^{14}C radioactivity into the kidney pelvis was initially high (at 5 min), while the urinary excretion of ^3H radioactivity seemed to be more extensive at later times, 1~4 hours.

Tissue dissection after *i.v.* administration

The tissue concentrations of ^{14}C and ^3H in male rats after *i.v.* administration of [^{14}C , ^3H]SASP were initially similar and only ^3H is presented here (Table V). High concentrations exceeding that of the plasma concentration were found in the liver and duodenum after 5 min indicating a rapid excretion in the bile. About 30% of the dose was found in the liver and about 25% was found in duodenum 5 minutes after injection. After 20 minutes the concentrations of both isotopes were decreased in the liver below the plasma concentration, while the concentration in duodenum increased during that time. After one hour about 55% of the dose was found in the duodenum. The high amount in duodenum was further transported through the intestines during the following 16 hours.

The concentration of ^3H and ^{14}C in the kidneys and the lungs, roughly followed the plasma profile but the level was lower. The concentrations in the lungs, thymus, testicles, skin and knee joint were all low compared with the plasma concentrations.

The ratio $^3\text{H}/^{14}\text{C}$ in the different tissues showed an increase over time from about 0.9 after 5 min to about 4 after 4 hours indicating that the SASP molecules were splitted and there was a distribution of sulfapyridine metabolites at that time. The highest ratio was found in the liver, kidneys, stomach and duodenum. However the $^3\text{H}/^{14}\text{C}$ ratio was below the initial value in caecum, colon and rectum, indicating a higher concentration of aminosalicylic acid (5ASA) metabolites compared to sulfapyridine (SP) metabolites.

Distribution in pregnant rats (autoradiography)

After an *i.v.* dose of [^3H , ^{14}C]SASP to pregnant female rats, the tissues were cleared

very rapidly as was also seen in the male rats. The radioactivity of the ^{14}C labelled substance decreased more rapidly than that of the ^3H . The distribution pattern for female rats was similar to that of the male. Thus a high concentration was noticed in the blood by both labellings and the highest tissue concentrations were seen in the liver, the kidney cortex and the intestinal mucosa. Within the connective tissues rather high concentration were noticed from 5 min to 1 hour after the administration for the ^{14}C labelling and from 5 min to 4 hours for the ^3H labelling.

No ^{14}C radioactivity was ever observed in fetal tissues, except at 4 hours a certain very low amount, compared to that of the mother, was noticed within the fetal intestinal content. For the ^3H labelling no radioactivity was noticed in the fetus until at 4 hours (for a comparison see Fig. 2a and c). At that time (Fig. 2c) the labelled substance passed the placenta barrier, and the distribution pattern of the fetus was equal to that of the mother, which showed a very even distribution of radioactivity. At 16 hours the fetal ^3H radioactivity had decreased considerably, and was at that time hardly noticeable.

The placental concentration was always below that of the blood. A rather high uptake was obtained in the yolk sac at 1 hour after administration for both of the labellings. On gestational day 11 no difference in distribution of radioactivity to fetuses was registered compared to day 18.

Excretion to the milk

The excretion of SASP to breast milk from the rat was very low. The milk to plasma ratio was 0.13 ± 0.01 for ^{14}C and 0.01 ± 0.01 for ^3H 20 minutes after *i.v.* administration. After *p.o.* administration the corresponding ratios were 0.66 ± 0.16 and 0.59 ± 0.03 for ^{14}C and ^3H at 8 hours after administration (Table V). The concentrations of ^{14}C and ^3H were 8.7 and $179 \mu\text{M eq}$ respectively.

Table V Radioactivity in breast milk after administration of [^{14}C , ^3H]SASP as a single oral dose 200mg/kg or an intravenous dose 5mg/kg in the rat. Ratio between the concentration in breast milk and plasma.

Administration	Isotope	Time after dose	Ratio breast milk/plasma	Conc. $\mu\text{M eq}$
<i>i.v.</i>	^{14}C	20 min	0.13 ± 0.01	0.64 ± 0.04
		1 h	0.54 ± 0.06	0.84 ± 0.13
	^3H	20 min	0.01 ± 0.01	0.01 ± 0.02
		1 h	0.18 ± 0.07	0.81 ± 0.43
<i>p.o.</i>	^{14}C	8 h	0.66 ± 0.16	8.7 ± 1.6
	^3H	8 h	0.59 ± 0.03	179 ± 9.6

Mean $\pm$ SEM, n = 5

Urinary and faecal excretion

The total mean recovery after different oral doses varied between 69.2 and 92.4% for ^3H and between 80.0 and 89.8% for ^{14}C (Table VI). After an *i.v.* dose, the total recovery for ^3H and ^{14}C was 79.2 and 73.2% respectively. Most of the radioactivity was recovered

Table V Cumulative excretion of radioactivity during 7 days in % of the dose given intra-venously (*i.v.*) or orally (*p.o.*)

	5 <i>i.v.</i>	50 <i>p.o.</i>	200 <i>p.o.</i>	200 ¹⁾ <i>p.o.</i>	500 <i>p.o.</i>
¹⁴ C					
Urine	19.1±2.6	14.3±0.8	20.8±0.8	16.8±1.2	23.3±5.1
Faeces	53.7±5.0	74.2±7.8	59.3±7.8	72.6±3.4	57.0±6.7
Total ²⁾	73.2±4.7	89.4±7.3	80.0±7.8	89.8±2.5	83.4±6.7
³ H					
Urine	32.6±2.3	42.3±0.7	49.5±2.2	44.7±2.5	40.6±8.5
Faeces	46.2±2.7	48.9±3.1	41.2±4.3	21.0±2.5	47.6±7.5
Total ²⁾	79.2±2.9	92.4±2.7	90.6±2.3	69.2±3.0	91.7±4.3

Mean ± SEM

1) Female rats

2) Including cage rinse

Fig. 3 Accumulated excretion of ³H in urine and faeces from rats administered [¹⁴C, ³H]SASP 200mg/kg body weight *p.o.* Mean, n=5.

.....○..... urine male
----○---- faeces male
——○—— total male
.....●..... urine female
----●---- faeces female
——●—— total female

Fig. 4 Accumulated excretion of ¹⁴C in urine and faeces from rats administered [¹⁴C, ³H]SASP 200mg/kg body weight *p.o.* Mean, n=5.

.....□..... urine male
----□---- faeces male
——□—— total male
.....■..... urine female
----■---- faeces female
——■—— total female

during the first 48 hours after administration according to Fig. 3~6. The greatest portion of ¹⁴C radioactivity was recovered from faeces, independent of the route of administration, about 60% in the male rats and 73% in the female rats. ¹⁴C in urine amounted

Fig. 5 Accumulated excretion of ^3H in urine and faeces from male rats administered 5mg [^{14}C , ^3H] SASP/kg body weight intravenously. Mean, $n=3$.

.....○..... urine
 ---○--- faeces
 —○— total

Fig. 6 Accumulated excretion of ^{14}C in urine and faeces from male rats administered 5mg [^{14}C , ^3H] SASP/kg body weight intravenously. Mean, $n=3$.

.....□..... urine
 ---□--- faeces
 —□— total

Table VII Recovery of SASP in urine or faeces after peroral or intravenous administration of SASP to male and female rats

Recovery % of dose (Mean $\pm$ SEM, $n=5$)		
Dose mg/kg b.w.	urine	faeces
5 ¹⁾	2.00 $\pm$ 0.39	0.65 $\pm$ 0.46
50	0.01 $\pm$ 0.01	0.49 $\pm$ 0.12
200 ²⁾	0.02 $\pm$ 0.01	0.27 $\pm$ 0.07
200	0.10 $\pm$ 0.08	0.12 $\pm$ 0.04
500	0.04 $\pm$ 0.01	2.48 $\pm$ 1.61

1) intravenous administration

2) female rats

to about 20% in males and 17% in female rats. For ^3H however, about equal amount of the radioactivity was excreted in urine and faeces in the male rats, while the female rats showed a lower faecal excretion.

The excretion of ^{14}C in expired air was $0.2 \pm 0.06\%$ of the given dose (500 mg/kg b. w.) (not shown). The radioactive content in the female rat carcasses after completion of the sampling of urine and faeces was $0.1 \pm 0.01\%$ ^{14}C and $0.5 \pm 0.1\%$ ^3H of the administered dose.

The recovery of unchanged SASP in urine and faeces ranged as mean value between 0.01 to 2.48% with the higher values in samples from rats given 500mg/kg b.w. (Table VII).

DISCUSSION

SASP has been shown to be effective in rheumatoid arthritis⁸⁻¹⁰). However, neither the mechanism nor the site of action of the drug is known. Only a small fraction of an oral dose, is systematically available (about 10%). This has been shown in different species; the rat⁵), the dog¹¹) and man¹²). There are several similarities in the pharmacokinetic behaviour of SASP in the rat compared with man. Although the rat has a much more rapid clearance, the absorption is similar and also the metabolism^{5,13}). The extensive binding to serum proteins seen in human (99%)¹¹) was in this study also found in the rat and the dog. There was no binding to the blood cells. Sulfapyridine is present at higher concentrations in plasma after an oral dose compared with SASP, and it binds to a lesser extent to plasma proteins. According to Fischer and Klotz¹⁴) the *in vitro* binding of sulfapyridine was about 50% and for acetylsulfapyridine about 74%. Similar result was also obtained *in vivo* in the rat in this study, as after an oral dose of [³H]SASP, the bound radioactivity decreased over time when sulfapyridine metabolites have been shown to appear in the plasma⁵). A small fraction of the radioactivity was also bound to the blood cells.

The low bioavailability, a small apparent volume of distribution⁵) and the extensive protein binding indicate that the tissue concentrations of SASP can be expected to be low. This was also confirmed in this study. After an *i.v.* injection of radioactively labelled SASP, the tissue concentration of radioactivity, was low compared to the plasma levels throughout the period studied.

However, the high degree of plasma protein binding of SASP did not prevent a rapid hepatic clearance of SASP. Five minutes after the *i.v.* injection more than 50% of the dose was found in the liver and duodenum, indicating both a rapid liver uptake and biliary excretion of intact substance. The tissue distribution pattern obtained after the intravenous injection was in close agreement with previous autoradiographic investigations in mice^{3,4}). The distribution pattern of ¹⁴C and ³H labelled SASP was very similar up to 4 hours. However, it is possible that part of the radioactivity also early after administration may be represented by metabolites of unsplit SASP. In the previous study⁵) a considerably slower elimination of the radioactivity in plasma was obtained compared to that of intact SASP. In the connective tissues, the radioactivity was successively increased to concentrations similar to that in liver and plasma.

Beside the extensive hepato-biliary excretion and an urinary excretion there was a transport through the gastrointestinal wall. This has been shown earlier in mice, by having the common bile duct ligated³).

The distribution of tritium in pregnant rats and fetuses was equal to that obtained after injection of ³⁵S-labelled sulfapyridine³) showing that no SASP penetrates through the placenta. Sulfapyridine or sulfapyridine metabolites were transferred and distributed in the fetus as in the mother. However, negligible amounts of ¹⁴C-radioactivity was detected in the autoradiographic study, indicating 5-aminosalicylic acid and/or metabolites

in the fetal intestine.

After an oral administration of radiolabelled SASP in rat it was shown in the previous paper⁵⁾ that mainly sulfapyridine was systemically available, having a plasma peak after 8 hours. 5-aminosalicylic acid was also absorbed to some extent, with a plasma peak after about 8 hours. The major part of the radioactivity was however found in the gastrointestinal tract. The tissue concentrations of radioactivity followed the plasma profile with maximal levels about 8 hours after the administration. Due to the reductive cleavage of SASP to different metabolites, the ^{14}C and ^3H autoradiograms gave rise to quite different distribution patterns. The characteristics of the ^{14}C pattern, corresponding to metabolites of 5-aminosalicylic acid, were high radioactivity in liver, kidney, connective tissues, blood, bone marrow and ovary. A similar distribution picture has earlier been obtained after *i.v.* injection of ^{14}C labelled 5-amino-salicylic acid³⁾. The distribution pattern also showed some similarities with ^{14}C salicylic acid and ^{14}C acetylsalicylic acid¹⁵⁾, e.g. the concentration in synovial fluids and in connective tissues. The ^3H pattern, corresponding to sulfapyridine metabolites, was characterised by an evenly distributed radioactivity. A certain distribution was noticed in connective tissues which is in contrast to what has earlier been noted after an *i.v.* injection of ^{35}S -labelled sulfapyridine, where no binding to connective tissues was found⁴⁾. This may indicate a slow uptake of SASP into the connective tissues (at 8 hours) after the oral dose.

Unchanged SASP is mainly excreted via the urine in the man and nothing was detectable in faeces¹⁶⁾. In the rat however the main part, more than 90% of the excreted SASP was found in faeces after an oral dose correlating well to the high biliary excretion seen in the rat. The amount of SASP excreted in faeces varied between 0.12~2.48% of the dose.

Sulfapyridine metabolites were found in the faeces to a higher extent in the rat than what has been found in human beings. The urinary excretion of sulfapyridine metabolites in man is 60~80%¹⁶⁾ while in the rat it was 40~50% and equal amounts in the faeces. However, the faecal excretion was found to be lower in female rats than in male rats depending on sex differences in the metabolism^{5,13)}. It is conceivable that the differences between male and female rats were due to a less effective biliary excretion possibly caused by the lower hydroxylation and conjugation rate of SP observed in the female rats.

In conclusion, due to a low bioavailability, low volume of distribution and high biliary excretion, the major part of the dose remains in the gastrointestinal tract after an oral as well as an intravenous dose of SASP. Thus, the tissue concentrations were low, particularly for ^{14}C reflecting the 5ASA metabolites. Higher and more evenly distributed levels were obtained with ^3H corresponding to sulfapyridine metabolites.

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