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Metabolism of 5'deoxy-5'[^{35}S]-isobutyl-thio-adenosine (SIBA) in rats and mice

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Numerous structural analogues of *S*-adenosyl-homocysteine (SAH)* have been synthetized as potential antiviral and anticancer drugs [1–6]. One of them, 5'-deoxy-5'-*S*-isobutyl-thio-adenosine (SIBA) [7] has been reported to inhibit cell transformation induced by oncogenic RNA or DNA viruses [1, 8, 9], the growth of transformed mouse mammary cells [10] and the mitogen-stimulated blastogenesis of lymphocytes [11]. SIBA has also an antimalarial activity against *Plasmodium falciparum* in culture [12]. When tested in mice *in vivo*, SIBA is effective against Friend's leukemia virus, 1 mg SIBA prolongs survival time by about 42 per cent (J. C. Chermann, in preparation). Since there is an increasing interest in the possible therapeutic use of this compound and analogues, some of the pharmacokinetic properties (tissue distribution and elimination) have been studied in mice and rats. *In vitro* only three metabolic products of SIBA had been identified: 5'-deoxy-5'-*S*-isobutyl-thio-inosine (SIBI), 5'-deoxy-5'-*S*-isobutyl-thio-ribose and adenine, SIBI being a weaker inhibitor of focus formation than SIBA [13].

Compounds

5'-deoxy-5'[^{35}S]-isobutyl-thioadenosine (8 mCi/mmole) was obtained from the Commissariat à l'Energie Atomique (Saclay, France.) All chemicals used were of the highest purity available and came from the following sources: salts and 2-mercaptoethanol, Merck; thin layer silica gel plates, Schleicher & Schüll F1500 LS 254; Kodirex films, Eastman Kodak; Protosol and Econofluor, New England Nuclear Corp.

Animals

Female Wistar rats weighing approximately 250 ± 20 g and 6-week-old male DBA_2 mice were used in these experiments.

Drug administration and collection of samples. In rats, single doses of [^{35}S]-SIBA dissolved in 0.9% NaCl (125 μmoles 0.3 mCi) were administered under ether anaesthesia in the jugular vein. The animals were killed 15, 45, 90 and 120 min after drug administration, and blood and internal organs were collected and frozen. Urine and feces were collected in the usual metabolic cages for rats killed 90 and 120 min after drug administration.

In mice, intraperitoneal injections were performed (21 μmoles 0.13 mCi) and animals were killed after 2, 4, 6, 8 and 10 hr. Internal organs were collected and frozen until use.

Protein concentration. This was determined by the method of Lowry *et al.* [14] using crystalline bovine serum albumin as standard.

Measurement of radioactivity. Radioactivity was measured in Econofluor solution with an Intertechnic liquid scintillation spectrophotometer model SL 30.

Specific radioactivity. Tissue samples were weighed, minced in a counting vial, and solubilized with Protosol overnight at 55°. After cooling, 10 ml of Econofluor were added; samples were equilibrated 1 hr in the counter before counting. The specific radioactivity is expressed as c.p.m./g wet wt.

Cell-free extracts and thin layer chromatography. Extraction was carried out at 4°. Tissue samples were homogenized in a Dounce homogenizer in 20 mM potassium phosphate,

*Abbreviations used: SIBA, 5'-deoxy-5'-*S*-isobutyl-thioadenosine; SIBI, 5'-deoxy-5'-*S*-isobutyl-thioinosine; IBR, 5'-deoxy-5-*S*-isobutyl ribose; SAH, *S*-adenosyl homocysteine; Iso-SIBA, 5'-deoxy-5'-*S*-(1 methyl propyl)-thioadenosine.

Table 1. Specific radioactivity of various rat organs as a function of time after injection of [^{35}S-SIBA]*

Organs	Time (min) 15	45	90	120
Bladder	—	—	480 ± 125	275 ± 23
Kidney	866 ± 208	608 ± 8	433 ± 15	245 ± 54
Liver	688 ± 177	366 ± 17	227 ± 8	122 ± 13
Intestine	594 ± 19	558 ± 9	432 ± 4	298 ± 6
Lungs	439 ± 130	668 ± 7	398 ± 9	221 ± 18
Spleen	355 ± 27	250 ± 14	112 ± 2	80 ± 5
Muscle	327 ± 55	201 ± 10	240 ± 4	145 ± 5
Aorta	324 ± 9	468 ± 3	185 ± 1	159 ± 8
Heart	295 ± 85	248 ± 2	170 ± 2	98 ± 3
Stomach	249 ± 35	118 ± 20	51 ± 8	128 ± 16
Skin	214 ± 17	790 ± 39	239 ± 7	173 ± 6
Ovary	180 ± 34	166 ± 3	119 ± 4	108 ± 9
Bone	108 ± 9	66 ± 1	69 ± 29	54 ± 5
Brain	82 ± 6	68 ± 2	51 ± 1	46 ± 7
Feces	—	—	142 ± 62	45 ± 36
Blood†	265 ± 27	217 ± 14	158 ± 5	150 ± 8
Urine†	—	—	—	38,462 ± 1524

*Results are expressed in 10^3 c.p.m./g wet wt, except for blood and urine (†) which are expressed as 10^3 c.p.m./ml. [^{35}S]SIBA was administered intravenously as described in the text.

pH 7.2, containing 10 mM 2-mercaptoethanol. The homogenate was deproteinized with ethylacetate methanol (9 : 1, v/v). The resulting solution was evaporated to dryness, taken up in water and spotted on silica gel thin layers. The chromatograms were developed with ethylacetate–methanol (9:1). Radioactivity was located by autoradiography (exposure time 2–10 weeks). The radioactive spots were scraped off and radioactivity was counted.

Results and discussion

Tissue distribution. Specific radioactivity of the various rat organs at 15, 45, 90 and 120 min after [^{35}S]-SIBA administration is shown in Table 1. The drug levels found in kidney, liver, intestine, lungs and bladder are significantly higher than those in the brain and bone. The ratio between the highest and the lowest specific activities is 10 at 15, 45 and 90 min after injection. After 120 min this ratio is lowered to 6. Similar results were obtained when specific activities were expressed in c.p.m./mg of protein in a crude extract. The specific radioactivity decreases rapidly with time: at 120 min the values are 18–81 per cent of those obtained at 15 min, depending on the organs; liver spleen and kidney having a higher elimination rate (72–82 per cent loss) than brain (38 per cent loss) and skin (20 per cent loss). The specific radioactivity in urine (c.p.m./ml) is about 250 times more elevated than that of the blood after 2 hr.

In mice, the distribution of the radioactivity is similar to that observed in the rat at 2 hr after injection (Table 2). As in the rat the ratio between the highest (kidney) and the lowest (brain) specific activity is 5 after 2 hr, and falls to 2.7 after 10 hr.

These results clearly show that the drug is rapidly and widely distributed; it can cross the blood–brain barrier, and is eliminated at different rates according to the organs, the half life varying between 40 and 150 min (in rats).

Metabolism of SIBA. Analysis of radioactive materials after chromatography of rat extracts on thin layer silica gel plate showed 6–10 distinct radioactive spots in the solvent system used, according to the tissue sample considered and the time studied (Table 3).

In rat urine, 120 min after administration of SIBA 9 spots could be detected: 29 per cent of the radioactivity was in spot I, 62 per cent in spot II a and b, 3.4 per cent in spot III and 2.9 per cent in spot IV. Only traces of radioactivity (0.3 per cent) were excreted as the unchanged drug (spot IV).

In blood, four main radioactive products were found, spots I, II a, b and VI, representing 16–32 per cent, 10–40 per cent and 11–23 per cent of the radioactivity, respectively, according to the time. SIBA (spot IV) represented 4–8 per cent of the total radioactivity.

In tissue samples 6–11, radioactive spots could be detected after autoradiography. Product(s) I represents 3–55 per cent of the radioactivity according to the tissue; the highest percentage was found in kidney and liver (50–55 per cent) and the lowest in brain (3–20 per cent). There was a great variation of this ratio with the time. Products II a and b represented 20–70 per cent of the material according to the tissues. For most of the organs studied, product III was relatively constant as a function of time, except in brain and lungs where the percentage decreased with time, and in the kidneys and liver where the ratio was increased with time. Product IV represented less than 5 per cent of the radioactivity at all the times studied and for all the organs tested. Product V varied according to the tissue and as a function of time, all the organs tested presenting a peak between 45 and 90 min. Percentage of peak VI represented 1–5 per cent of the tissue radioactivity. In mice, the same metabolites were obtained.

Identification of some metabolites. R_f values, colorimetric analyses of functional groups and u.v. absorbancy of spot III, IV and VI were similar to those reported in our previous work [13] for 5′-deoxy-5′-*S*-isobutyl-thioinosine (SIBI), SIBA and 5′-deoxy-5′-*S*-isobutyl-thioribose, respectively. Products I, II a and b are under investigation. Product V was ultraviolet negative, aniline phtalate negative and oxidizable by permanganate. The fact that it is produced when SIBA is incubated with purified 5′adenylic acid deaminase and purine nucleotide phosphorylase in the presence of 0.2 *M* potassium phosphate suggest that product V is the 5′-*S*-isobutyl ribose 1 phosphate.

In conclusion, after intravenous administration of SIBA, the radioactivity was widely distributed in rats and mice. SIBA crosses the blood–brain barrier in rats and mice. This is in accordance with observations by Pacheco *et al.* (unpublished results) of the effect of SAH and isoSIBA on the central nervous system of rabbits.

The drug was rapidly metabolized since 15 min after injection the percentage of unchanged drug was very small: 2–5 per cent in all tissues tested, and 0.3 per cent in the urine 2 hr after injection.

It is interesting that the percentage of the various metabolites in rat organs are very different from those found in chick embryo fibroblasts *in vitro*. In these cells two major metabolic pathways for SIBA degradation have been observed [9]: an oxidative deamination to 5′-deoxy-5′-*S*-isobutylthioinosine (the main product) and hydrolysis to 5′-deoxy-5′-*S*-isobutyl-thioribose and adenine. Only traces of other metabolites were found. In contrast in rats SIBI and 5′-deoxy-5′-*S*-isobutyl-thioribose were found in small amounts and products II and V were the main metabolites. These differences in metabolism are related to *in vitro–in vivo* systems and not to species differences, since, when

Table 2. Specific radioactivities of different mouse organs after intraperitoneal injection of [^{35}S]-SIBA*

Organs	Time (hr) 2	4	6	8	10
Kidney	450 ± 30	479 ± 15	191 ± 17	110 ± 4	112 ± 22
Lung	394 ± 27	437 ± 4	240 ± 4	83 ± 3	74 ± 10
Liver	439 ± 21	359 ± 29	196 ± 16	104 ± 13	71 ± 8
Spleen	213 ± 19	235 ± 5	182 ± 2	83 ± 3	39 ± 9
Heart	204 ± 17	200 ± 10	134 ± 12	56 ± 8	42 ± 5
Brain	98 ± 7	115 ± 4	102 ± 9	97 ±	45 ± 3
Blood†	—	—	—	16 ± 3	—
Urine†	66,660 ± 1877	32,050 ± 953			

* Results are expressed in 10^3 c.p.m./g wet, except for blood and urine (†) which are expressed as 10^3 c.p.m./ml. [^{35}S]SIBA was administered intraperitoneally (0.17 mCi/0.2 ml) as described in the text.

Table 3. Radioactive metabolites in various rat tissues after drug administration*

Product	R_f	Tissue sample: Kidney		Liver		Muscle		Brain		Blood		Urine
		15 min	120 min	15 min	120 min	15 min	120 min	15 min	120 min	15 min	120 min	120 min
I	0.00	55.4	28.4	49.0	25.0	52.3	14.4	24.5	11.1	24.5	31.8	29.0
II a	0.01	34.0	53.0	41.4	54.8	22.6	40.6	49.0	72.0	14.5	36.5	38.0
b	0.09	—	—	—	—	—	—	—	—	9.6	6.4	24.0
III = SIBI	0.12	5.3	10.9	3.0	8.4	21.7	28.9	16.0	1.8	8.5	2.8	3.4
IV = SIBA	0.21	2.3	4.2	1.9	4.7	1.3	1.9	3.1	0.8	5.9	3.7	0.3
V =	0.31	0.7	0.8	0.5	2.8	0.9	12.0	4.9	14.2	5.7	3.8	0.4
VI = IBR	0.43	0.9	2.7	0.6	3.8	0.6	2.0	2.4	0.1	21.4	10.2	2.9
VII	0.60	—	—	0.3	—	—	—	—	—	7.2	4.4	0.5
VIII	0.80	—	—	0.7	—	—	—	—	—	—	—	0.3
IX	Front	0.4	—	1.0	—	0.5	—	2.0	—	—	—	—

* Results are expressed as percentage (± 0.3 per cent) of the total radioactivity in a given tissue sample.

mice and chick cells were incubated *in vitro* with SIBA the first main product to appear was SIBI.

Thus, in summary, the tissue distribution and metabolism of [^{35}S-]-labelled 5′-deoxy-5′-*S*-isobutyl-thioadenosine (SIBA), a synthetic analogue of *S*-adenosyl-homocysteine, was studied in female rats and mice. After a single injection, the radioactivity is rapidly and widely distributed, It is uneven: at each time studied the highest specific radioactivity was found in kidney, liver, intestine, lungs and bladder, and the lowest in brain and bone. The elimination rate of the drug varies with the organs; it is rather rapid, except in brain.

SIBA is quickly metabolized; 15 min after its administration, the percentage of intact drug was less than 5 per cent in all the tissues tested.

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