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S-adenosyl-L-methionine *N*-ole-1-oyltaurate: pharmacokinetic of the orally administered salt in rats

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Abstract

A pharmacokinetic study based on the distribution of radioactivity from the double labelled *S*-adenosyl-L-methionine (SAM) has been carried out by oral administration of the liposoluble stable salt [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate to rats. The SAM sulfate *p*-toluensulfonate salt, the only SAM salt at present commercialized as drug, was chosen as reference compound to have a comparative pharmacokinetic analysis. The metabolism of the SAM is characterised by a differential use of the two labelled moieties by the various organs, liver being the most active in metabolizing the sulfonium compound with a preferential uptake of the methyl-^{14}C fragment. The radioactivity detected after the administration of [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate is, in all the organs examined, two times higher than the [methyl-^{14}C, 8-^{3}H]SAM sulfate *p*-toluensulfonate compound, attesting that the liposoluble [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate is provided with better bioavailability.

Keywords: [methyl-^{14}C, 8-^{3}H]SAM; *N*-ole-1-oyltaurate; Pharmacokinetic; Rats

1. Introduction

S-adenosyl-L-methionine (SAM) is the only natural sulfonium compound present in tissues and fluids of mammals. It was discovered by Cantoni (1952, 1953) which, on the basis of biochemical evidences, proposed a chemical structure that was later confirmed by studies of degradation and synthesis. This versatile and ubiquitous molecule acts as the principal biological donor of methyl groups to different nucleophiles such as nitrogen, oxygen, carbon or sulfur of a wide variety of methyl acceptor (Shapiro et al., 1964; Paik et al., 1975).

The other important metabolic pathway in which SAM is involved is the biosynthesis of

Abbreviations: Treated rats, rats which received [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate; Control rats, rats which received [methyl-^{14}C, 8-^{3}H]SAM sulfate *p*-toulensulfonate.

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aliphatic polyamine. Its enzymatic decarboxylation product, *S*-adenosyl-(5′)-3-methyltiopropylamine (deca-SAM), transfers the propylaminic group to putrescine and spermidine with the formation of spermidine and spermine, respectively (Zappia et al., 1980; Giulidori et al., 1984).

The several biochemical roles in which SAM is involved amply justify its therapeutic uses. SAM is employed in depressive syndromes for its capability to increase serotonin and noradrenalin turnover in the brain, in osteoarthritis for its analgesic and anti-inflammatory properties and moreover, it prevents the reduction of phospholipid methylation maintaining the membrane fluidity (Gualano et al., 1983; Cimino et al., 1984; Barcelo et al., 1990). The role of SAM as a therapeutic agent in liver diseases has also been investigated since it protects this organ against the damages caused by deleterious agents (alcohol, CCl_4) (Osman et al., 1993; Varela-Moreiras et al., 1995; Garcia-Ruiz et al., 1995). In addition, its use as an anti-oxidant for storage of organ grafts and as potential therapeutic agent in HIV infections has been reported (May, 1993; Evans et al., 1997).

In our previous study, we reported the design and the synthesis of SAM salts with large size anions in order to increase SAM stability

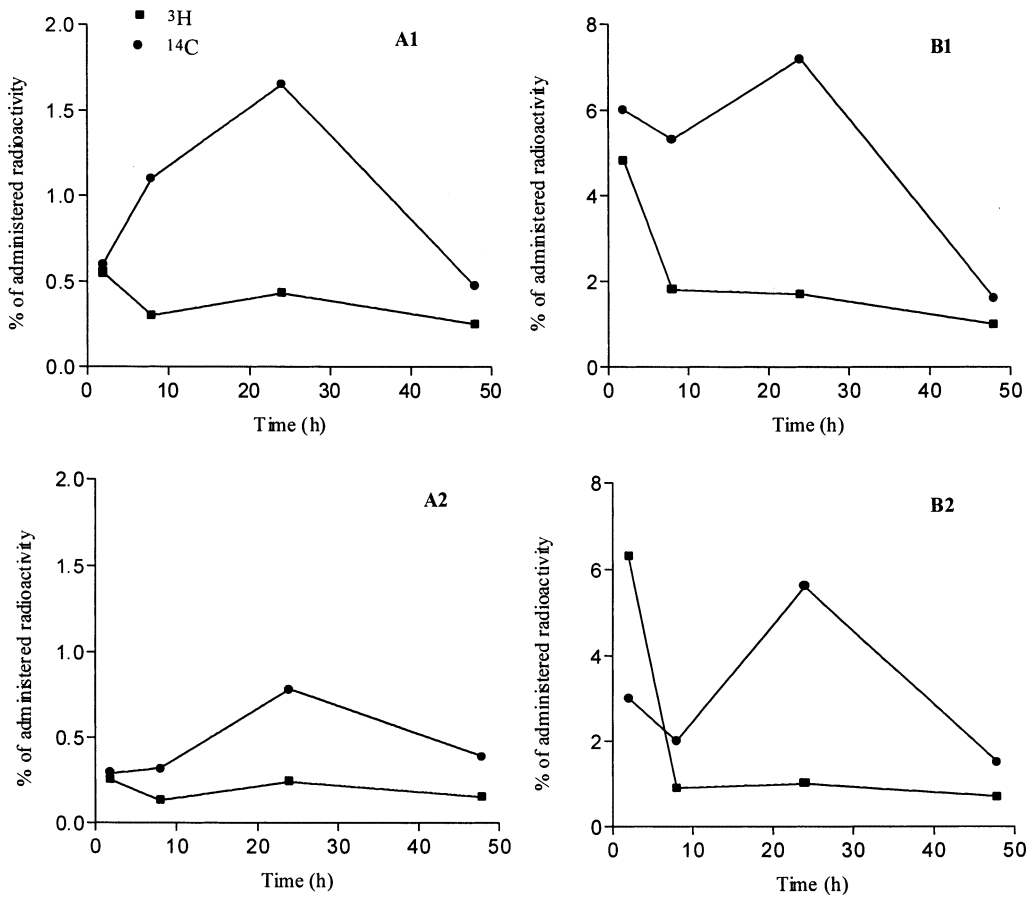

Fig. 1. Radioactivity levels in stomach (A) and small intestine (B) of rats treated with [methyl-^{14}C, 8-^{3}H]SAM. Two milligrams of active principle (^{3}H 20 μCi, ^{14}C 10 μCi) were administered by gavage to rats either as *N*-ole-1-oyltaurate (A1, B1) or as sulfate, *p*-toluensulfonate (A2, B2). At different times the animals were sacrified and the radioactivity levels were evaluated as described in the Section 3. The results are expressed as percentage of the total administered radioactivity. All the values represent the average of the results obtained from five animals.

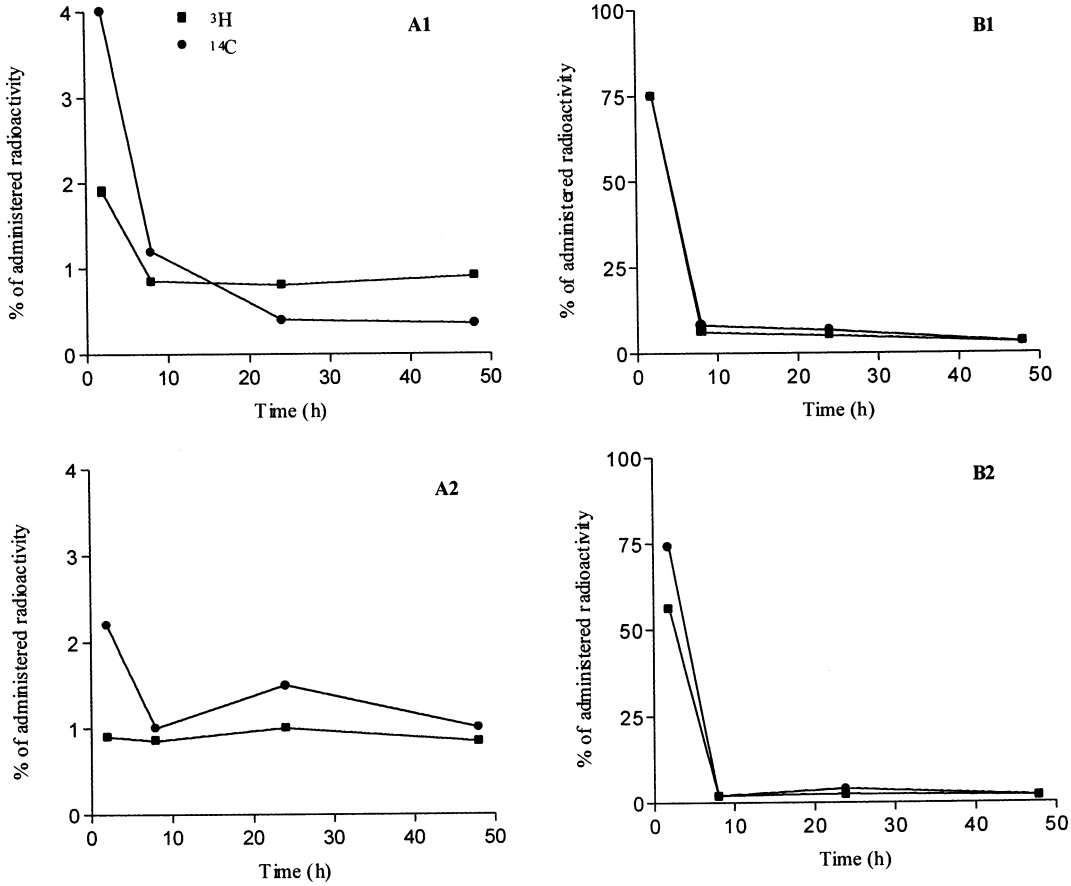

Fig. 2. Radioactivity levels in gastric content (A) and small intestinal content (B) of rats treated with [methyl-^{14}C, 8-^{3}H]SAM. Two milligrams of active principle (^{3}H 20 μCi, ^{14}C 10 μCi) were administered by gavage to rats either as *N*-ole-1-oyltaurate (A1, B1) or as sulfate, *p*-toluensulfonate (A2, B2). At different times the animals were sacrified and the radioactivity levels were evaluated as described in the Section 3. The results are expressed as percentage of the total administered radioactivity. All the values represent the average of the results obtained from five animals.

(Morana et al., 2000). Moreover, these salts are liposoluble, and consequently, they could show good bioavailability when orally administered in the treatment of such pathologies, representing a possible alternative to other salts in the pharmacological therapy.

The aim of the present study is to carry out a comparative pharmacokinetic analysis of the SAM when it is orally administered to rats as liposoluble SAM *N*-ole-1-oyltaurate or as SAM sulfate *p*-toluensulfonate, at present used in therapy.

A double labelled [methyl-^{14}C, 8-^{3}H]SAM, prepared in our laboratory, has been used because it represents an useful tool to test the structural integrity of the molecule during the absorption and distribution processes, and moreover it allows to follow the metabolic fate of the different structural components of the sulfonium compound in the various metabolic compartments.

2. Materials

[8-^{3}H]adenine and [methyl-^{14}C] SAM sulfate were obtained from Amersham Life Science (Milano, Italy). [8-^{3}H]SAM sulfate was prepared from L-methionine-enriched yeast grown in our labora-

tory. Scintillation liquids and tissue solubilizer were from Beckman.

3. Methods

3.1. Preparation of [methyl-^{14}C, 8-^{3}H]SAM sulfate

Because of the absence of commercial sources of [8-^{3}H]SAM sulfate, we prepared it as previously described, using L-methionine-enriched *Saccharomyces cerevisiae* and [8-^{3}H]adenine (50 mCi/l) (Morana et al., 2000). [methyl-^{14}C, 8-^{3}H]SAM sulfate was prepared mixing [8-^{3}H]SAM sulfate with [methyl-^{14}C] SAM sulfate ($^{3}H/^{14}C$ ratio: 2), and diluting with cold SAM sulfate obtained from a parallel experiment. The specific radioactivity of the resulting product was 4 Ci/mol for ^{3}H and 2 Ci/mol for ^{14}C, respectively.

3.2. Preparation of [methyl-^{14}C, 8-^{3}H]SAM N-ole-1-oyltaurate

The preparation of [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate was realized as follows: 567 mg of sodium *N*-ole-1-oyltaurate (1.38 mmol) prepared as previously described were solubilized in 2

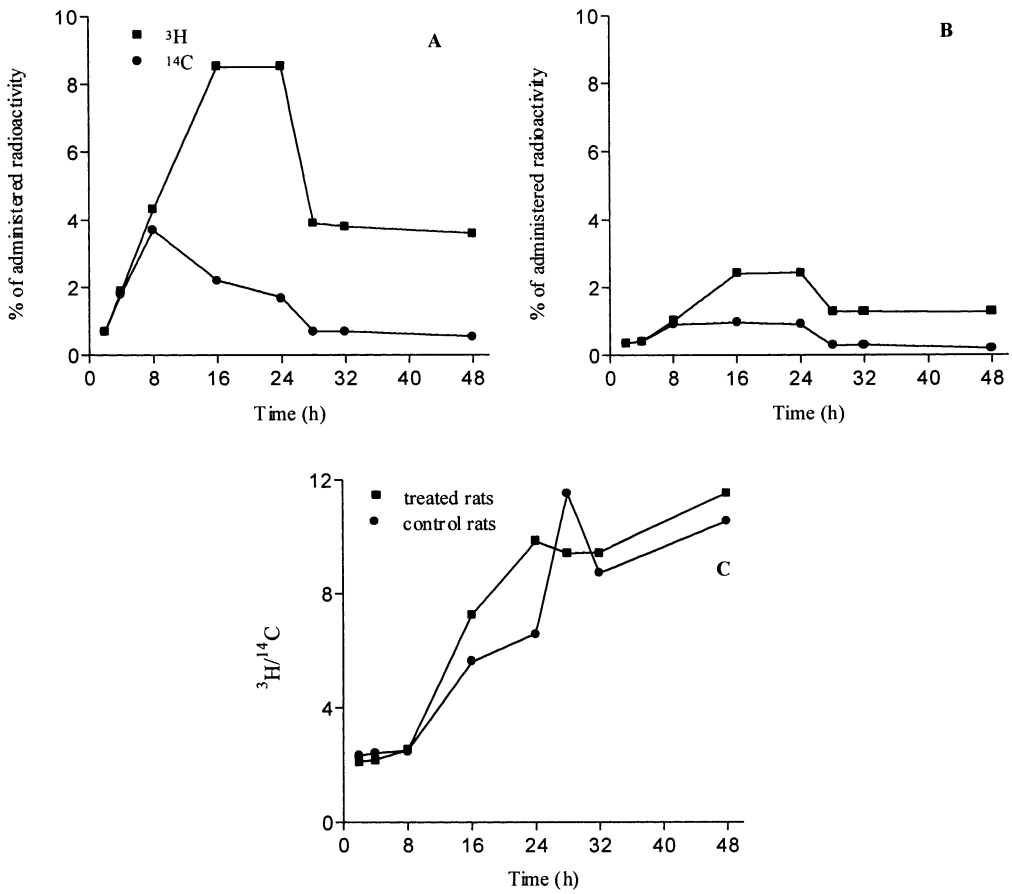

Fig. 3. Analysis of plasma radioactivity in rats orally treated with [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (A) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (B). $^{3}H/^{14}C$ plasma ratio at different times following oral administration of [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (■) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (●) (C). At different times the animals were sacrified and the radioactivity levels were evaluated as described in the Section 3. The results are expressed as percentage of the total administered radioactivity. All the values represent the average of the results obtained from five animals.

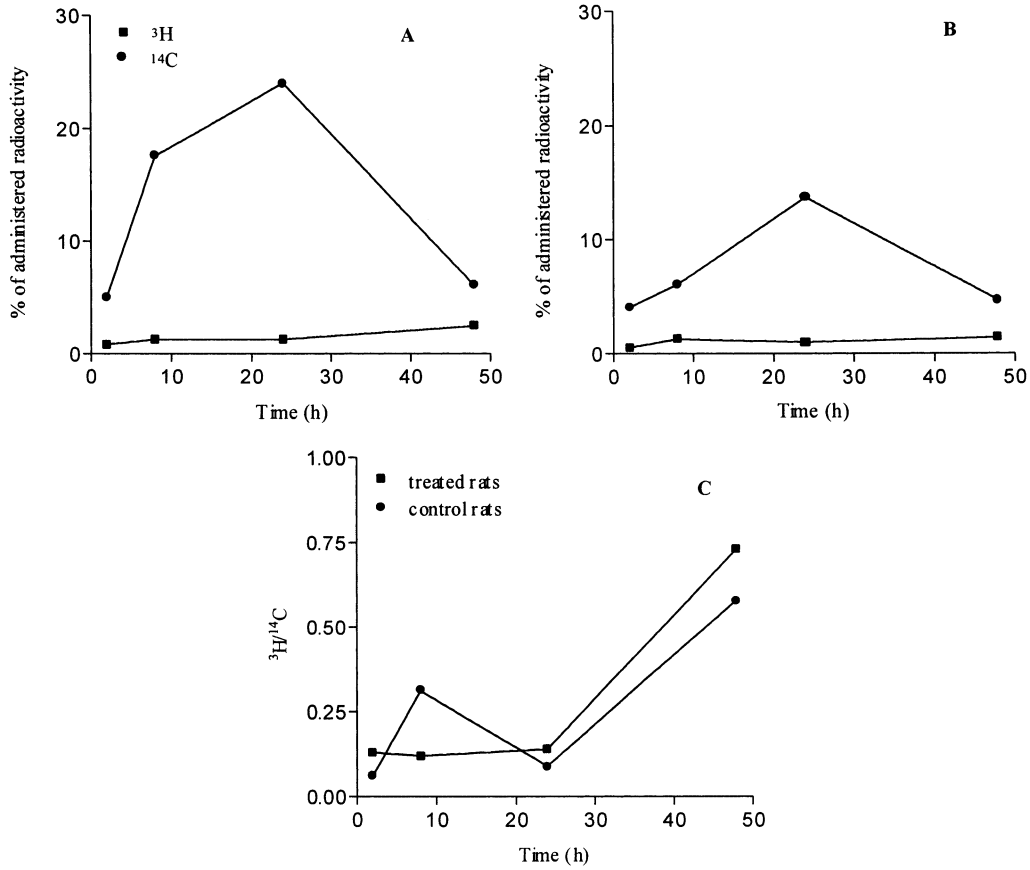

Fig. 4. Radioactivity levels in liver of rats orally treated with [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (A) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (B). $^{3}H/^{14}C$ liver ratio at different times following oral administration of [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (■) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (●) (C). At different times the animals were sacrified and the radioactivity levels were evaluated as described in the Section 3. The results are expressed as percentage of the total administered radioactivity. All the values represent the average of the results obtained from five animals.

ml of distilled water (final pH 1.0 with 2 M H_2SO_4) (Morana et al., 2000). This solution was slowly added to 15 mM [methyl-^{14}C, 8-^{3}H]SAM sulfate (0.23 mmol, ^{3}H 0.90 mCi, ^{14}C 0.45 mCi). A white precipitate of [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (1:5 molar ratio) was obtained.

3.3. Preparation of [methyl-^{14}C, 8-^{3}H]SAM sulfate p-toluensulfonate

The preparation of the double salt [methyl-^{14}C, 8-^{3}H]SAM sulfate *p*-toluensulfonate was realized using [methyl-^{14}C, 8-^{3}H]SAM sulfate (0.23 mmol, ^{3}H 0.90 mCi, ^{14}C 0.45 mCi) according to the procedure of Fiecchi (Fiecchi, 1976).

3.4. Purity assessment

The purity of the sulfonium compound was checked by HPLC analysis of the double-labelled molecule. A Beckman System Gold liquid chromatograph equipped with an Ultrasil column (10 μm particle size, 250 × 4.6 mm ID) and an ultraviolet detector, operating at the fixed wavelenght of 254 nm, was used. Elution was carried out with 0.4 M ammonium formate buffer, pH 4.0 at a flow rate of 1 ml/min. The

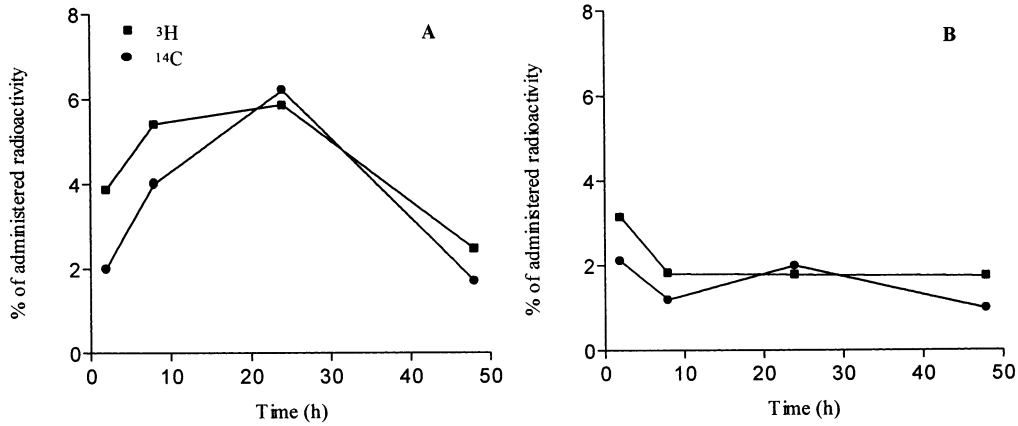

Fig. 5. Radioactivity levels in kidney of rats treated with [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (A) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (B). At different times the animals were sacrified and the radioactivity levels were evaluated as described in the Methods section. The results are expressed as percentage of the total administered radioactivity. All the values represent the average of the results obtained from five animals.

following retention times were observed for adenine and SAM: adenine 5.2 min, SAM 6.5 min.

3.5. In vivo experiments

Male Wistar rats, 200 ± 10 g body wt., 12 weeks old were used. The dose of the sulfonium compound requested for the administration to 20 'treated rats' was prepared by sonificating 100 μmol of [methyl-^{14}C, 8-^{3}H] SAM *N*-ole-1-oyltaurate (spec. act.: ^{3}H 4 μCi/μmol, ^{14}C 2 μCi/μmol) in 40 ml of physiological solution (Sonicator Heat System).

The dose of the sulfonium compound requested for the administration to 20 'control rats' was prepared by dissolving 100 μmol of [methyl-^{14}C, 8-^{3}H] SAM sulfate *p*-toluensulfonate (spec. act.: ^{3}H 4 μCi/μmol, ^{14}C 2 μCi/μmol) in 40 ml of physiological solution.

Each animal received by gavage 2 ml of sample containing 2 mg (10 mg/kg body wt.) of [methyl-^{14}C, 8-^{3}H]SAM either as *N*-ole-1-oyltaurate or as sulfate *p*-toluensulfonate (^{3}H 20 μCi, ^{14}C 10 μCi). At fixed time intervals the animals were sacrificed and the organs were promptly frozen in liquid nitrogen. Blood, faeces, urine and contents of stomach and intestine were collected.

3.6. Preparation of biological samples for radioactive measurement

Aliquots of solid biological samples were previously homogenized in water (1:4 w/v) and then dissolved by digestion at 50 °C for 12 h in a shaking

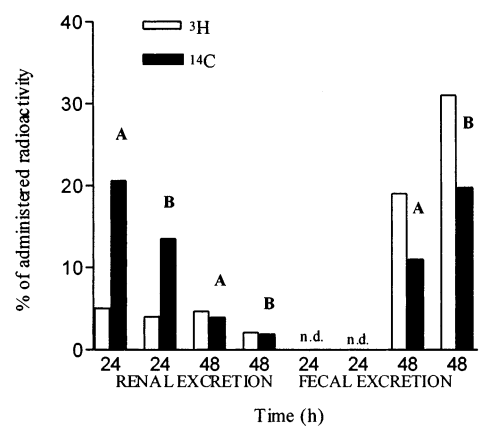

Fig. 6. Renal and fecal excretion of labelled catabolites in rats treated with [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (A) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (B). The rats were kept in metabolic cages. Urine and faeces samples were collected up to 48 h, and analysed for radioactivity. The results are expressed as percentage of the total administered radioactivity. All the values represent the average of the results obtained from five animals. (n.d., not detected)

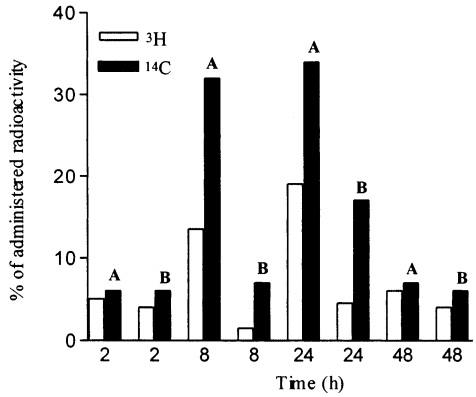

Fig. 7. Total radioactivity present in liver, kidney and plasma of rats treated with [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (A) or [methyl-^{14}C, 8-^{3}H]SAM sulfate, *p*-toluensulfonate (B). All the values represent the average of the results obtained from five animals.

water bath with BTS-450. The samples were then blanched by addition of isopropyl alcohol and H_2O_2. Liquid samples were directly measured. Ready Organic for solid samples and Ready Gel for liquid samples were used as scintillation liquids. Absolute radioactivity was measured in a Beckman LS 7800 scintillation counter, equipped with an automatic quench correction system (ACQ).

4. Results and discussion

In order to follow the gastro-intestinal absorption of the compounds, the radioactivity levels in stomach, gastric content, intestine and intestinal content, expressed as percentage of the total administered radioactivity, were evaluated at different times after administration. As shown in Fig. 1 less than 15% of the radioactivity administered could be detected in the gastro-intestinal tract over the whole experimental time, with the highest level for the ^{14}C isotope, thus indicating a preferential absorption of the ^{14}C-methylic moiety. ^{3}H and ^{14}C radioactivity was higher in treated rats suggesting that the liposoluble form was more readily absorbed. The radioactivity measured in the gastric content of both groups of animals was negligible over the total experimental time while the analysis of the small intestinal content, either for treated or control rats, revealed that almost the total radioactivity disappeared 8 h after administration (Fig. 2). This observation was supported by the low isotopic levels detected in faeces at the same time. The ^{3}H/^{14}C ratio in the small intestinal content, measured 2 h after oral administration of [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate was 1.97, very similar to that of the precursor molecule. This evidence, that we did not observe in control rats, suggests that the liposoluble form of SAM could be more resistant to the action of the enzymes present in this compartment.

The ^{3}H and ^{14}C radioactivity in plasma peaked between 8 and 16 h after treatment (Fig. 3). Over the period of the experiment, ^{3}H and ^{14}C levels were up to three times higher in treated rats demonstrating that the liposoluble compound is provided with better bioavailability. In treated rats, the values of the ^{3}H/^{14}C ratio over the first 4 h were similar to that of the precursor molecule (2.10 at 2 h, and 2.12 at 4 h) suggesting that the intact SAM could be one of the most abundant labelled molecular species present in the blood stream at those times (Fig. 3C). The subsequent increase of the isotopic ratio can be explained with the preferential uptake of the ^{14}C-metabolites by the various organs, particularly by liver.

In order to gain further informations on the distribution of the administered [methyl-^{14}C, 8-^{3}H]SAM, the radioactivity in liver and kidney was evaluated. Fig. 4 shows the radioactivity levels in the liver. The ^{14}C-peak for treated rats (25% of the ^{14}C administered radioactivity) was reached at 24 h and it was two times higher than the radioactivity detected in the controls at the same time. The ^{3}H uptake was constantly lower over the whole experimental time. The organ shows a preferential uptake of the ^{14}C-methyl fragments as indicated by the very low values of the isotopic ratio (Fig. 4C).

The radioactivity detected in the kidney of treated rats was up to three times higher than the control rats and the ^{3}H and ^{14}C-peaks were reached after 24 h from the oral administration of the [methyl-^{14}C, 8-^{3}H]SAM *N*-ole-1-oyltaurate (Fig. 5). The data from the kidney confirm the higher bioavailability of the liposoluble form of the SAM.

Fig. 6 shows the renal and fecal excretion of the radioactivity up to 48 h after administration. The

excretion in the urine of the ^{14}C-catabolites was more consistent over the first 24 h while a lower level of ^{3}H radioactivity was measured. The radioactivity excreted in the faeces from 24 to 48 h after administration represented 19 and 11% of the total ^{3}H and ^{14}C radioactivity in treated rats, and 31 and 20% in control rats. In particular, the slow faecal excretion (it was not possible to detect radioactivity in the faeces up to 24 h after administration) confirms the effective gastro-intestinal absorption of SAM and/or its metabolites.

The total ^{3}H and ^{14}C radioactivity present in plasma, liver and kidney is shown in Fig. 7. At 2 h, when the absorption and distribution processes are at the beginning, the differences between treated and control rats were not significant. At 8 and 24 h, the levels of radioactivity of the treated animals were up to five times higher than the control animals while at 48 h these differences were attenuated.

5. Conclusions

The results have shown that the liposoluble SAM *N*-ole-1-oyltaurate compound has an improved bioavailability over the SAM sulfate *p*-toluensulfonate used as control compound. It is interesting to underline that the $^{3}H/^{14}C$ ratio in the content of the small intestine of treated rats was, over the first 8 h, very close to that of the parent compound, suggesting the presence of a high percentage of the intact molecule. This behaviour, that we did not observe in control rats, can be explained with the protective effect of the *N*-ole-1-oyltaurate anion which forms micelles that surround the SAM, thus avoiding or limiting its degradation. Previous studies of liposolubility and stability of the SAM *N*-ole-1-oyltaurate confirm this structural organization consisting in hydrophobic chains directed towards the external environment and polar heads, surrounding the SAM, inside (Morana et al., 2000).

At 24 h, the total ^{3}H and ^{14}C radioactivity present in plasma, liver and kidney of the treated rats was 19 and 34%, respectively. Considering that this organs represent only the 8% of the total body weight, it is reasonable to suppose that SAM is preferably concentrated. The liver is undoubtedly the main target organ of the sulfonium compound, since the greatest amount of radioactivity is localized in this organ, either for the treated or the control rats. The high ^{14}C radioactivity indicates that methylation processes are one of the fundamental biochemical events that involve SAM at this level.

The renal and fecal excretion at 48 h in treated rats, is less than 40% indicating that the sulfonium compound is efficaciously absorbed and actively metabolized producing molecular species with low turn-over. The differences between the treated and the control rats indicate, also at this level, that in the animals which received the liposoluble SAM, the absorption and the metabolism of the sulfonium compound are more efficient.

In conclusion, all the data collected suggest that SAM *N*-ole-1-oyltaurate could represent an interesting alternative to the SAM sulfate *p*-toluensulfonate in oral therapy.

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