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PROTECTIVE ROLE OF *S*-ADENOSYL-L-METHIONINE AGAINST ACETAMINOPHEN INDUCED MORTALITY AND HEPATOTOXICITY IN MICE

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Abstract—Acetaminophen toxicity is demonstrated to be related to the protein binding of a reactive metabolite formed by the action of a P_{450} mixed function oxidase. Results of the present study indicate that *S*-adenosyl-L-methionine (SAMe) protects against mortality induced by high doses (710 mg/kg) of acetaminophen. The liver toxicity induced by acetaminophen also appears to be reduced by SAMe, as shown both by GOT and GPT plasma activities and by histologic observation of the liver. The results of experiments on *in vivo* and *in vitro* binding of the radioactivity from acetaminophen to liver microsomal proteins suggest that SAMe protection is related to its metabolism to thiol derivatives in the transmethylation–trans-sulfuration pathway.

Acetaminophen, which is known to be a useful analgesic and antipyretic drug, produces liver necrosis in man [1] and other animals [2, 3] when taken in overdose. The biochemical mechanism connected with acute hepatic necrosis has been elucidated by Mitchell *et al.* [3–6]. These authors observed that the hepatic lesion is caused by microsomal enzyme dependent metabolism of acetaminophen to a highly reactive intermediate which binds irreversibly to the cell macromolecules when liver glutathione is decreased. Very large doses of glutathione [7] or L-cysteine [6] and cysteamine [8–10], are capable of preventing hepatic necrosis following toxic doses of acetaminophen both in animals and in man.

S-Adenosyl-L-methionine (SAMe), now available as a stable disulfate-di-*p*-toluenesulfonate salt [11], has recently been shown to protect liver from injuries produced in animals by hepatotoxic agents and to be active in some hepatic disorders [12, 13]. In particular, it was demonstrated [14] that both liver histological changes and enhancement of plasma GOT and GPT activities induced by D-galactosamine treatment are not observed in animals pretreated with SAMe. Protection by SAMe was also observed against impairment of bile flow induced by ethynylestradiol administration [15, 16].

The results of the present study show that SAMe is capable of preventing mortality induced by large doses of acetaminophen and of decreasing liver damage induced by this toxic agent.

MATERIALS AND METHODS

^{3}H-Labelled acetaminophen was obtained from New England Nuclear Co. (sp. act. 400 mCi/mmol). Acetaminophen was purchased from Sigma Chemicals Co. (St. Louis, MO). SAMe disulfate-di-*p*-toluenesulfonate, *S*-adenosylhomocysteine (SAH) and methylthioadenosine (MTA) were obtained from BioResearch Co. (Liscate, Milano, Italy). Methionine, L-cysteine and cysteamine were purchased from Merck (Darmstadt), and homocysteine thiolactone from Fluka AG. All the experiments were carried out using male Charles River mice weighing 22–24 g. Acetaminophen was dissolved in a 0.9% NaCl solution, alkalinized to pH 11.3 with NaOH. All other drugs were injected in isotonic saline solution. Total glutathione levels in the liver were evaluated by the spectrophotometric method of Tietze [17]. Covalent binding *in vivo* of the reactive intermediate of acetaminophen to hepatocyte macromolecules was determined as described by Jollow *et al.* [4] after protein precipitation. *In vitro* covalent binding of an acetaminophen metabolite to liver microsomal proteins in mice was determined with the method described by Potter *et al.* [5]. Cytochrome P_{450} and *b*5 concentrations in mice were evaluated by the spectrophotometric method of Omura and Sato [18]. Protein concentration was determined using the method of Lowry [19]; serum enzymes GOT and GPT were determined using the GOT and GTP Biochemical-Test Combination (Boehringer Mannheim GmbH). For the histological assessment, liver samples were fixed in 10% formalin, enclosed in paraffin, cut to 7 μm and stained with hematoxylin–eosin.

RESULTS

Effects on mortality

Table 1 shows the effect of SAMe administration against the mortality induced by i.p. injection of 710 mg/kg acetaminophen in mice. SAMe effect was tested at two doses, 10 and 20 mg/kg.

The drug was administered 5 min before and 20 min after acetaminophen injection, according to the scheme reported by Mitchell *et al.* [6] for cysteine. Under these conditions the mortality induced by acetaminophen was reduced from 43 per cent to 8.3 per cent and 7.9 per cent with i.m. doses of 10 and 20 mg/kg of SAMe, respectively.

The protective effects of both methionine and

Table 1. Effect of SAMe administration on acetaminophen induced mortality in mice

Treatment	No. of treated animals	No. of dead animals	% of dead animals
Acetaminophen	128	55	43.0
Acetaminophen + SAMe (10 mg/kg, i.m.)	24	2	8.3
Acetaminophen + SAMe (20 mg/kg, i.m.)	38	3	7.9
Acetaminophen + methionine (7.5 mg/kg, i.m.)	24	4	16.7
Acetaminophen + cysteine (150 mg/kg, i.p.)	38	2	5.3

SAMe, methionine and cysteine were administered 5 min before and 20 min after injection of acetaminophen (710 mg/kg, i.p.)

cysteine were also evaluated administering the drug 5 min before and 20 min after acetaminophen. Mortality was lowered to 16.7 per cent with methionine at the dose of 7.5 mg/kg, i.m., and to 5.30 per cent with cysteine at the dose of 150 mg/kg, i.p. No protective effects against acetaminophen-induced mortality were observed treating animals only 20 min after the intoxication with 1, 5, 10, 20 or 40 mg/kg body wt of SAMe (data not shown).

Effects on hepatotoxicity

Protection by SAMe against acetaminophen hepatotoxicity was estimated evaluating GOT and GPT serum activities. As reported by other authors [7, 9], both these enzymatic activities resulted to be increased after acetaminophen administration. As shown in Table 2, the treatment with cysteamine at the dose of 150 mg/kg, i.p., was capable to reduce serum GOT and GPT values; the same effect was observed after i.m. injection of 20 mg/kg of SAMe 5 min before and 20 min after 500 mg/kg acetaminophen.

Twenty-four hours after acetaminophen intoxication, the mouse liver appeared to be severely damaged (Fig. 1A). A massive necrosis was evident where confluent areas of centrolobular eosinophilic hepatocytes, either anucleated or with pyknotic nucleus, bridged adjacent hepatic lobules.

Livers of mice treated with acetaminophen and with 20 mg/kg of i.m. SAMe 5 min before and 20 min after intoxication were remarkably protected (Fig. 1B). Only a limited number of centrolobular hepatocytes having eosinophilic cytoplasm with pyknotic nucleus was observed.

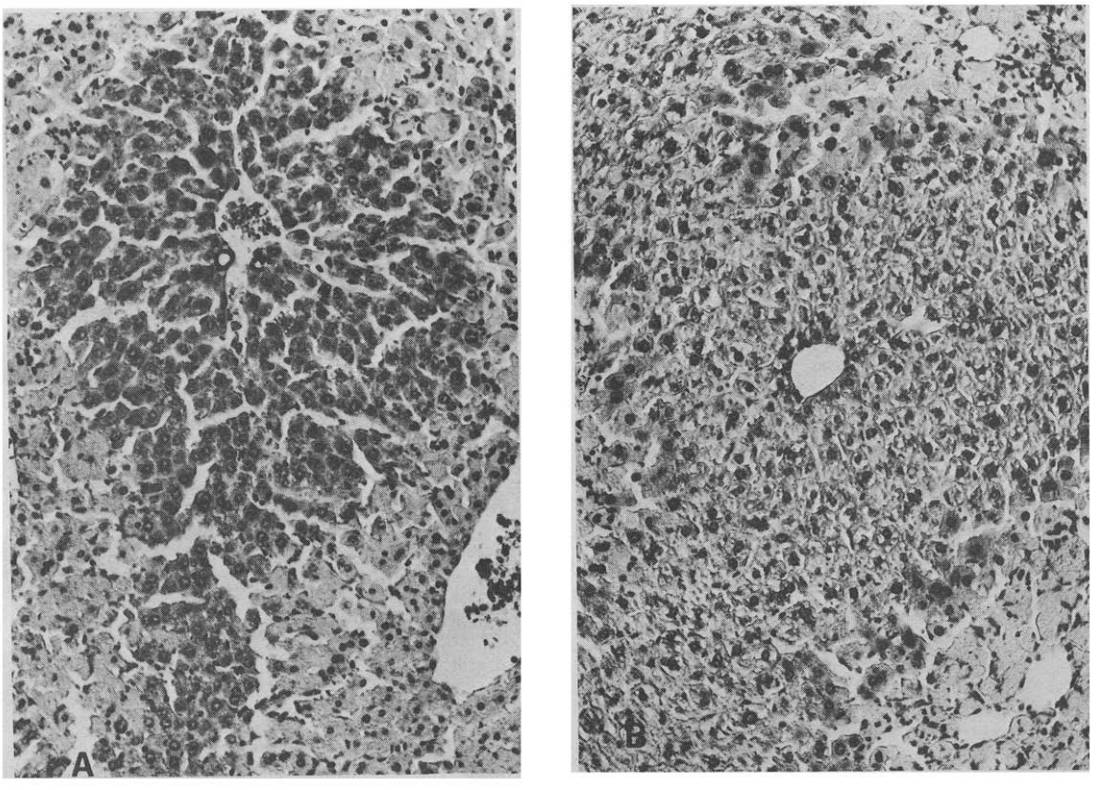

Fig. 1. Effects of SAMe on acetaminophen-induced hepatotoxicity in mice. Doses of acetaminophen and SAMe are reported in the text. The animals were killled 24 hr after acetaminophen injection and liver sections were stained with hematoxylin-eosin. × 170. A = acetaminophen; B = acetaminophen + SAMe.

Table 2. Effect of SAMe administration on serum enzymes of acetaminophen treated mice

Treatment	Serum enzymes (U/l) GOT	GPT
Saline	109.9 ± 11.96	18.2 ± 0.73
Acetaminophen	1748.0 ± 428.2*†	1394.7 ± 137.9***†
Acetaminophen + SAMe (20 mg/kg, i.m.)	404.9 ± 71.4*‡	433.6 ± 73.6***‡
Acetaminophen + cysteamine (150 mg/kg, i.p.)	257.9 ± 68.4*‡	383.3 ± 179.6**‡

SAMe was administered 5 min before and 20 min after i.p. injection of acetaminophen (500 mg/kg). Cysteamine was given only 5 min before acetaminophen administration.
The animals were killed 24 hr after acetaminophen injection.
The data represent the mean values ± S.E. for 7 animals in each group.
*P < 0.01; **P < 0.005; ***P < 0.001; † = vs saline; ‡ = vs acetaminophen.

Effects on cytochrome P-450, cytochrome b5 *and GSH levels*

The treatment with acetaminophen at the dose of 710 mg/kg, i.p., decreased the cytochrome P_{450} content by 36 per cent and cytochrome *b*5 content by 41 per cent (Table 3) 48 hr after administration, as reported by other authors [10, 20] for the rat. The treatment with SAMe, 5 min before and 20 min after acetaminophen, did not affect the cytochrome P_{450} and *b*5 hepatic levels.

In contrast, cysteine administered 5 min before acetaminophen at the dose of 150 mg/kg, i.p., restored the cytochrome P_{450} content to control values.

Glutathione levels were evaluated in livers of rats treated with 400 mg/kg, i.p., of acetaminophen. In agreement with the data reported by Mitchell *et al.* [6], levels of glutathione fell to 20 per cent of the control value within 60 min from the injection. No effects of SAMe were observed on GSH hepatic levels when it was adminstered 5 min before and 20 min after acetaminophen at the same dose protecting against mortality.

Effects on protein binding

The effect of SAMe administration on the previously reported protein binding of acetaminophen was considered. Table 4 shows the covalently bound ^{3}H-acetaminophen in liver microsomes of animals treated with either acetaminophen alone or acetaminophen and SAMe. This binding was evaluated 2 hr after the i.p. injection of 500 mg/kg of ^{3}H-acetaminophen (5 μCi) since it had been reported [4] that, at this time interval, covalent binding reaches the maximum value. The effect of SAMe was compared with that obtained with cysteamine (Table 4) previously shown to decrease the amount of acetaminophen metabolite bound to hepatic proteins [21]. A relevant decrease of ^{3}H-acetaminophen binding to microsomal proteins (52 per cent) was observed in animals treated with 20 mg/kg of i.m. SAMe in comparison with the decrease (74 per cent) induced by cysteamine treatment at the dose of 150 mg/kg, i.p.

No decrease of ^{3}H-acetaminophen binding to microsomes by SAMe was instead observed in the *in vitro* experiments. Therefore, in respect to this activity three metabolites of SAMe were checked: *S*-adenosylhomocysteine, *S*-methylthioadenosine and homocysteine (Table 5). Only addition to the incubation mixture of homocysteine could induce a remarkable inhibition (80 per cent) of the binding, comparable to that caused by cysteamine at the same concentration.

DISCUSSION

It has been well established that hepatotoxicity of acetaminophen is related to its P_{450} mediated metabolism to a reactive intermediate [22]. Glutathione, through an attack of its nucleophilic sulfur atom, binds to this compound giving a readily eliminable conjugate [22]. After doses of acetaminophen that exceed glutathione availability, binding of the reactive intermediate to liver proteins occurs

Table 3. Effect of SAMe administration on cytochrome P_{450} and *b*5 concentrations in livers of acetaminophen treated mice

Treatment	Cytochrome P_{450} (nmole/mg protein)	Cytochrome *b*5 (nmole/mg protein)
Saline	1.25 ± 0.06	0.42 ± 0.02
Acetaminophen	0.80 ± 0.03*	0.25 ± 0.004*
Acetaminophen + SAMe (10 mg/kg, i.m.)	0.74 ± 0.08	0.26 ± 0.01
Acetaminophen + SAMe (20 mg/kg, i.m.)	0.67 ± 0.05	0.25 ± 0.01
Acetaminophen + cysteine (150 mg/kg, i.p.)	1.11 ± 0.08†	0.30 ± 0.008†

The animals were killed 48 hr after i.p. injection of acetaminophen (710 mg/kg).
SAMe was administered 5 min before and 20 min after acetaminophen injection.
Cysteine was given 5 min before acetaminophen administration.
The data represent the mean values ± S.E. for 4 animals in each group.
*P < 0.005 vs saline; †P < 0.025 vs acetaminophen.

Table 4. Effect of SAMe administration on ^{3}H-acetaminophen covalently bound to mouse liver microsomes

Treatment	Microsomal bound ^{3}H-acetaminophen (nmole/mg protein)
Acetaminophen	2.04 ± 0.39
Acetaminophen + SAMe (20 mg/kg, i.m.)	0.99 ± 0.12*
Acetaminophen + cysteamine (150 mg/kg, i.p.)	0.53 ± 0.27†

The animals were killed 2 hr after i.p. injection of 500 mg/kg (5 μCi) of ^{3}H-acetaminophen. SAMe was administered 5 min before and 20 min after acetaminophen injection. Cysteamine was given 5 min before acetaminophen treatment. The data represent the mean values ± S.E. for 6 animals in each group.
*$P < 0.05$; †$P < 0.025$.

accompanied by liver necrosis. Cysteine [6], methionine [23] and cysteamine [9, 21] have been shown to protect animals against liver necrosis and mortality after overdoses of acetaminophen. However, side effects and toxicity [8, 24] discourage the clinical use of these compounds.

S-Adenosyl-L-methionine has been recently shown to counteract the effects of two hepatotoxic substances: D-galactosamine [14] and ethynylestradiol [15, 16]. Moreover, the compound shows a very low toxicity and it is available as a pharmaceutical preparation [11]. On the basis of previous observations suggesting that exogenously administered SAMe enters the hepatocytes where it is metabolized [25], and that its metabolism implies formation of sulfides and thiols such as *S*-adenosylhomocysteine, cystathionine, cysteine and taurine [26], the protective action of this molecule against acetaminophen induced mortality in mice was tested. Protection of the order of that obtained with cysteine at the dose of 150 mg/kg and higher than that with 7.5 mg/kg of methionine was observed in animals treated with 10 or 20 mg/kg SAMe, the latter dose being equimolecular with that of methionine. The only administration of SAMe after 20 min from acetaminophen intoxication did not result in protection against mortality: this was probably because the liver concentration of acetaminophen reaches its maximum value after 20 min from i.p. injection of the toxic agent [3].

Both the evaluation of GOT and GPT serum activities and liver histology of animals given a subacute dose of acetaminophen (500 mg/kg) indicated that its hepatotoxic effects are decreased by SAMe administration to a degree which is similar to that obtained with cysteamine treatment.

In order to clarify the mechanism of SAMe protective action, modifications of the various steps of acetaminophen metabolism were taken into consideration. Since it had been previously reported by Potter *et al.* [5] that the metabolism of acetaminophen into the toxic metabolite is mediated by a P_{450} mixed function oxidase, the levels of P_{450} were measured in acetaminophen treated animals: a significant decrease was observed in comparison to the control animals. A surprising elevation of P_{450} levels to normal values was noted in the animals receiving cysteine in addition to acetaminophen. Actually, the reduction in acetaminophen hepatotoxicity induced by cysteine has not been attributed to an inhibitory effect on the P_{450} mediated acetaminophen metabolism, but to its action on glutathione availability [6]. No difference was instead observed between cytochrome P_{450} levels in animals treated with acetaminophen alone and those receiving either 10 or 20 mg/kg SAMe in addition to the toxic agent. SAMe was also observed to have no effects in preventing fall of liver glutathione concentrations caused by the treatment with acetaminophen. Since the protein binding of the reactive metabolite of acetaminophen, which has been considered the factor of hepatocellular damage, is confined to a small portion of the totally formed metabolite, the major amount being eliminated via the glutathione conjugate, it may be assumed that liver protection by SAMe is not ascribable to a decreased synthesis of this compound. The

Table 5. Effect of SAMe and its metabolites on *in vitro* covalent binding of acetaminophen to liver microsomal proteins in mice

Reaction mixture		Acetaminophen bound (nmole/mg protein/15 min)
Complete		1.70 ± 0.11
+ *S*-Adenosylmethionine	100 μM	1.62 ± 0.02
+ *S*-Adenosylhomocysteine	100 μM	1.71 ± 0.14
+ *S*-Methylthioadenosine	100 μM	1.68 ± 0.24
+ Homocysteine	100 μM	0.36 ± 0.11*
+ Cysteamine	100 μM	0.15 ± 0.10†

Mouse liver microsomes were prepared and incubated with ^{3}H-acetaminophen (1 mM, 3000 d.p.m./nmole) and with the compounds tested for binding inhibition according to Potter *et al.* [5]. Values of the binding of an acetaminophen metabolite are the mean ± S.E. of four determinations.
*$P < 0.005$; †$P < 0.001$.

possibility was, therefore, considered that SAMe may affect the protein binding of the metabolite. Actually, a decreased binding was observed in animals given SAMe in addition to ^{3}H-acetaminophen (Table 4). On the other hand, SAMe failed to act on the binding in the *in vitro* experiments (Table 5) at a concentration at which cysteamine almost completely inhibited protein arylation. Since it had been previously demonstrated that exogenous SAMe is catabolized by the liver cells [25, 27], the possibility was examined that the decreased protein binding observed *in vivo* might depend on the formation of a nucleophilic metabolite of SAMe, capable of binding to the reactive acetaminophen derivative thus favouring its elimination. As shown in Table 5, both *S*-adenosylhomocysteine originating from SAMe in transmethylation reactions, and *S*-methylthioadenosine produced from decarboxylated SAMe in the polyamine synthesis [28] did not prevent the protein binding of acetaminophen. Homocysteine, instead, was shown to be as effective as cysteamine in this regard.

It has been reported by Eloranta *et al.* [29] that most of the CO_2 formed from *S*-adenosylmethionine by liver homogenates is associated with the demethylation–trans-sulfuration route which appears to be activated by SAMe [30].

Even if our data do not exclude the possibility that SAMe protection against acetaminophen toxicity depends on a direct action of the drug on microsomal macromolecules, by which the binding of the toxic derivative would be prevented, the reported results suggest that the protection is related to the transmethylation–trans-sulfuration pathway through which thiolic derivatives, capable of favouring the elimination of the toxic agent, are formed.

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