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PROTECTIVE ROLE OF S-ADENOSYL-L-METHIONINE ON LIVER INJURY INDUCED BY D-GALACTOSAMINE IN RATS

GIORGIO STRAMENTINOLI, MARIA GUALANO and GAETANO IDEO*
BioResearch Co., Dept. of Biochemistry, 20060 Liscate Milano, Italy and
* Clinica Medica III, University of Milano, Italy

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Abstract—A decrease of S-Adenosyl-L-methionine (SAMe) levels and of the activity of its synthesizing enzyme is demonstrated in liver of rats treated with D-galactosamine. These effects appear to be reversed by the i.m. administration of SAMe. A protective role of SAMe on liver is also obtained by SAMe treatment of intoxicated animals, as demonstrated both by the histological analysis of the liver and GOT and GPT levels in serum. The activity of SAMe, in this respect, is comparable to that one of prednisolone when the drugs are given at the daily dose of 60 mg/kg and 100 mg/kg, respectively.

The acute administration of D-galactosamine has been shown to induce severe liver cell injury [1–4]. This effect has been reported by some investigators to be similar to the damage observed in viral hepatitis [1, 2], both from the morphological and functional point of view. However, on the basis of ultrastructural and biochemical analyses it was concluded by other scientists [3, 4] that the liver damage observed after D-galactosamine treatment differs from the one seen in human hepatitis in that the former leads to accumulation of liver triglycerides, hyperplasia of the smooth endoplasmic reticulum and cell necrosis.

The mechanism underlying the liver cell injuries caused by D-galactosamine has been investigated in detail by various authors who have focused their attention to several metabolic effects such as depletion of either hepatic glycogen [2] or ATP [5], decrease of uridine phosphates with accumulation of UDP-hexosamines [6] and decrease of protein synthesis [7].

In the present communication we are reporting the lowering of SAMe concentration in the liver after D-galactosamine administration. Since the uptake of SAMe molecule by red blood and liver cells has been recently demonstrated [8–10], the effects of SAMe administration on liver injuries induced by D-galactosamine was evaluated. Evidence is here presented that SAMe administration to D-galactosamine treated animals restores its liver levels to control values. Moreover, both the histological analysis and GOT and GPT levels in serum indicate a liver protection by SAMe similar to the one observed with prednisolone [11, 12].

MATERIALS AND METHODS

Materials. L-[Methyl-^{3}H]methionine (11 Ci/mmole), and [Methyl-^{14}C]S-Adenosyl-L-methionine (55 mCi/mmole) were obtained from the Radiochemical Centre, Amersham. [Acetyl ^{3}H]*N*-acetylserotonin was prepared from serotonin-creatinine sulfate (Regis Chemicals Co., Chicago) and [^{3}H]acetic anhydride (Radiochemical Centre, Amersham), as previously described [13]. Hydroxyindole-*O*-methyltransferase (HIOMT) was partially purified from frozen ox pineal glands as previously described [14]. Crystalline reduced glutathione (GSH) and D-galactosamine hydrochloride were from Merck, Darmstadt. Adenosine-5-triphosphate-disodium-3 H_2O (ATP) was from Boehringer Mannheim GmbH, and *N*-acetylserotonin (NAS) from Aldrich Chemical Co., Milwaukee. Unlabelled SAMe in a stable form was obtained from BioResearch Co., Liscate, Milano. Prednisolone (Neo Delta) was obtained from Amelix, Firenze, Italy.

Animals. Sprague–Dawley male rats (Charles River, 150–170 g body wt) fed an unrestricted commercial diet (C. River) were used. All the animals were sacrificed and blood was collected from the trunks. Liver tissue was rapidly excised and portions were prepared for light microscopy by fixation in 10% buffered formalin; other portions were used for SAMe determination and for the determination of methionine adenosyl-transferase (EC 2.5.1.6., MAE) activity.

Assays. Both liver and blood to be used for SAMe determination were immediately deproteinized by homogenization in 10% TCA dissolved in 0.05 N HCl; the tissue–TCA ratio was 1:1 (v/v) for the blood and 1:10 (w/v) for the liver. After centrifugation at 3000 *g* at 4° for 10 min, 1-ml aliquots of the clear supernatant were mixed with 20 μl of [Methyl-^{14}C]SAMe (10 nCi). The solution was then washed three times with 2 vol. of peroxide-free ether previously saturated with 0.05 N HCl. SAMe concentration was then assayed by the method of Baldessarini and Kopin [15].

To evaluate MAE activity, portions of liver were homogenized with 4 vol. of ice-cold 0.01 N acetic acid and then centrifuged at 15,000 *g* at 4° for 30 min [16]. The supernatant was used to measure the enzymatic activity according to the method described by Matthysse *et al.* [17]; methionine concentration in the incubation mixture was 268 μM instead of 67 μM. Serum enzymes GOT and GPT

were assayed using GOT and GPT test Biochemical-Test-Combination (Boehringer Mannheim GmbH). Enzyme activity is reported as mU/ml. Protein concentration was determined by the method of Lowry *et al.* [18].

Animal treatment. Two doses of D-galactosamine hydrochloride (400 mg/kg) in isotonic saline were administered by i.p. injection at 12-hr intervals, and the animals were sacrificed 48 hr after the first administration of D-galactosamine. SAMe was dissolved in phosphate buffer 0.04 M pH 8.0 and then injected i.m. three times a day for 5 days at the doses of 10 and 20 mg/kg in a volume of 0.1 ml/100 g body wt. Prednisolone dissolved in gum Arabic was given orally at the dose of 100 mg/kg once a day for 5 days in a volume of 1.0 ml/100 g body wt.

Statistical analysis. Significance was determined by the Student's '*t*' test.

RESULTS AND DISCUSSION

The decrease of SAMe levels in rat liver and brain after treatment with a number of substances has been reported by various authors. Pyrogallol and tropolone were shown to induce that effect in rat liver [19], and depletion of SAMe in some tissues was observed after repeated injections of L-dopa [20]. The methylation of these molecules by SAMe in the liver has been suggested to be responsible for the described effect.

Depletion of SAMe in the liver has been also reported after the administration of compounds such as CCl_4, selenite and diethylnitrosamine, which are known to induce morphological and biochemical modifications in liver tissue [21–23].

The data shown in Table 1 indicate that D-galactosamine, known for its toxic effects on liver, also produces a 55 per cent depletion of SAMe in the liver, as compared to control rats, 48 hr after its administration, although no modifications are observed in the blood SAMe levels.

The activity of the methionine-activating enzyme (MAE) synthesizing SAMe was measured: a lower activity was found in liver of D-galactosamine treated rats in comparison to control animals (Table 2), indicating that the decrease of SAMe levels may be correlated with the impairment of its hepatic synthesis. The mechanism of the enzyme inactivation is still to be investigated. The administration of SAMe before and after D-galactosamine induced intoxication resulted in the maintainance of endogenous SAMe at control values (Table 3).

In order to correlate cell injury induced by D-galactosamine with SAMe levels in the liver, histology of the tissue was performed, and GOT and GPT serum levels were evaluated both in the animals treated with D-galactosamine and in those receiving D-galactosamine and SAMe. The same parameters were also evaluated in D-galactosamine treated animals receiving prednisolone, which has been described to protect the liver from damages induced by D-galactosamine [11, 12]. As shown in Table 4, GOT and GPT values were strikingly enhanced 48 hr after D-galactosamine injection as

Table 1. Effect of D-galactosamine treatment on liver and blood levels of SAMe

Treatment	Liver SAMe (μg/g)	Blood SAMe (μg/ml)
Saline	35.6 ± 2.3	2.33 ± 0.09
D-galactosamine	16.0 ± 2.5*	2.43 ± 0.08

D-galactosamine was given by i.p. injection in two doses of 400 mg/Kg at 12-hr intervals. The rats were sacrificed 48 hr after the first D-galactosamine injection.

Values are expressed as means ± S.E. for seven rats in each group.

* $P < 0.001$.

Table 2. Effect of D-galactosamine treatment on liver MAE activity

Treatment	MAE activity (nmoles/30'/mg protein)
Saline	31.7 ± 1.3
D-galactosamine	16.7 ± 1.4*

For treatment see Table 1.

Values are expressed as means ± S.E. for seven rats in each group.

* $P < 0.001$.

Table 3. Effect of SAMe administration on liver content of SAMe in D-galactosamine treated rats

Treatment	Liver SAMe (μg/g)
D-galactosamine	16.0 ± 2.5
D-galactosamine + SAMe 10 mg/Kg	32.9 ± 6.2*
D-galactosamine + SAMe 20 mg/Kg	40.1 ± 3.8†

SAMe was injected intramuscularly three times a day for 5 days. D-galactosamine was given on the third day as reported in Table 1. The rats were sacrificed 48 hr after the first D-galactosamine injection. Values are expressed as means ± S.E. for seven rats in each group.

* $P < 0.005$ vs D-galactosamine treated rats.

† $P < 0.001$ vs D-galactosamine treated rats.

Table 4. Effect of SAMe administration and prednisolone on serum enzymes of D-galactosamine treated rats

Treatment	Serum enzymes (mU/ml) GOT	GPT
Saline	113 ± 5	15 ± 1
D-galactosamine	1683 ± 243	579 ± 75
D-galactosamine + prednisolone 100 mg/Kg	146 ± 10*	25 ± 2*
D-galactosamine + SAMe 10 mg/Kg	400 ± 125*	93 ± 29*
D-galactosamine + SAMe 20 mg/Kg	200 ± 20*	34 ± 5*

SAMe was injected intramuscularly three times a day for 5 days. Prednisolone was given orally once a day for 5 days. D-galactosamine was given on the third day as reported in Table 1. The rats were sacrificed 48 hr after the first D-galactosamine injection. Values are expressed as means ± S.E. for seven rats in each group.

* $P < 0.001$ vs D-galactosamine treated rats.

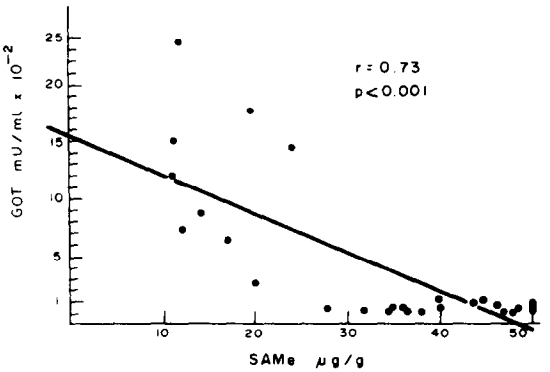

Fig. 1. Correlation between GOT values in serum and liver SAMe levels. Regression line calculated by the method of the least squares.

compared to the controls, in good agreement with the results reported by other investigators [24]. The rats pretreated with prednisolone at 100 mg/kg were completely protected from the damage induced by D-galactosamine. A similar protection was observed in the animals treated with SAMe. There was a considerable decrease of GOT and GPT levels in comparison with those of the animals treated with D-galactosamine alone. The relationship between GOT levels in serum and SAMe liver concentration was determined, and a linear correlation ($P < 0.001$) was found between the two parameters.

The protective action of SAMe was also supported by the histological data. The liver histology 48 hr after D-galactosamine administration showed some changes, in good agreement with those described by Keppler *et al.* [1], whereas neither areas of necrosis nor inflammatory infiltration were observed in the liver of rats treated with D-galactosamine and prednisolone or D-galactosamine and SAMe.

The mechanisms involved in liver protection by SAMe require to be further investigated owing to the variety of metabolic pathways implying SAMe as the substrate [25–27]. The possibility of keeping the cell levels of this compound within standard values might influence in a favourable way any of the metabolic stages where SAMe is involved.

REFERENCES

1. D. O. R. Keppler, R. Lesch, W. Reutter and K. F. A. Decker, *Exp. Molec. Path.* **9,** 279 (1968).
2. D. O. R. Keppler and K. F. A. Decker, *Eur. J. Biochem.* **10,** 219 (1969).
3. A. Meadline, F. Schaffner and H. Popper, *Exp. Molec. Path.* **12,** 201 (1970).
4. R. S. Koff, G. Gordon and S. M. Sabesin, *Proc. Soc. exp. Biol. Med.* **137,** 696 (1971).
5. C. O. Record, K. G. M. M. Alberti and D. H. Williamson, *Biochem. J.* **130,** 37 (1972).
6. D. O. R. Keppler, J. F. M. Rudigier, E. Bischoff and K. F. A. Decker, *Eur. J. Biochem.* **17,** 246 (1970).
7. R. S. Koff, J. J. Fitts and S. M. Sabesin, *Proc. Soc. exp. Biol. Med.* **138,** 89 (1971).
8. G. Stramentinoli, C. Pezzoli and M. Galli-Kienle, *Biochem. Pharmac.* **27,** 1427 (1978).
9. C. Pezzoli, G. Stramentinoli and M. Galli-Kienle, communication to the 3° Congresso Nazionale della Società Italiana di Biochimica, Siena 3–5 Ottobre 1977.
10. V. Zappia, G. Ruggiero, A. Andreana, M. Porcelli, C. Esposito and P. Galletti, communication to the 3° Congresso Nazionale della Società Italiana di Biochimica, Siena 3–5 Ottobre 1977.
11. P. J. Grases, W. Reutter and R. Lesch, *G.E.N.* **27,** 197 (1972).
12. R. W. Pickering, G. W. Lynn James and F. L. Parker, *Arzneim.-Forsch. Drug Res.* **25,** 1591 (1975).
13. I. J. Kopin, C. M. B. Pare, J. Axelrod and H. Weissbach, *J. biol. Chem.* **236,** 3072 (1961).
14. J. Axelrod and H. Weissbach, *J. biol. Chem.* **236,** 211 (1961).
15. R. J. Baldessarini and I. J. Kopin, *J. Neurochem.* **13,** 769 (1966).
16. Y. Natori, *J. biol. Chem.* **238,** 2075 (1963).
17. S. Matthysse, R. J. Baldessarini and M. Vogt, *Analyt. Biochem.* **48,** 410 (1972).
18. O. H. Lowry, N. J. Resebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
19. R. J. Baldessarini, *Biochem. Pharmac.* **15,** 741 (1966).
20. L. A. Ordonez and R. J. Wurtman, *Biochem. Pharmac.* **22,** 134 (1973).
21. G. Stramentinoli, C. Pezzoli and E. Catto, *Min. Med.* **66,** 1541 (1975).
22. J. L. Hoffman, *Archs Biochem. Biophys.* **179,** 136 (1977).
23. Y. S. S. Buehring, L. A. Poirier and E. L. R. Stokstad, *Cancer Res.* **36,** 2775 (1976).
24. R. W. Pickering G. W. Lynn James and F. L. Parker, *Arzneim.-Forsch. Drug Res.* **25,** 898 (1975).
25. J. B. Lombardini and P. Talalay, *Adv. Enz. Reg.* **9,** 349 (1971).
26. *Transmethylation and Methionine Biosynthesis* (Eds S. K. Shapiro and F. Schlenk), University of Chicago Press, Chicago (1965).
27. *The Biochemistry of Adenosyl Methionine* (Eds F. Salvatore, E. Bocek, V. Zappia, H. G. Williams-Ashman and F. Schlenk), Columbia University Press, New York (1977).