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SHORT COMMUNICATION

Effects of S-adenosyl-L-methionine on Platelet Thromboxane and Vascular Prostacyclin

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ABSTRACT: We therefore designed the present study to evaluate the effect of S-adenosyl-L-methionine (SAMe) on the synthesis of platelet thromboxane and vascular prostacyclin. The experimental materials were human blood and aortic rings from untreated Wistar rats; and platelets and aortic rings from Wistar rats treated for 7 days with SAMe at 5 or 10 mg/kg/day s.c. The administration of 10 mg/Kg/day of SAMe to rats significantly increased vascular production of 6-keto-$PGF_{1\alpha}$. *In vitro* vascular production of 6-keto-$PGF_{1\alpha}$ increased in a concentration-dependent manner when SAMe was incubated in the range of 10^{-7} to 10^{-4} M. The greatest increase was 167 ± 15%, obtained in samples incubated with 5×10^{-5} M SAMe. In aortic rings, lipid peroxidase production was inhibited in a concentration-dependent manner in the SAMe range of 10^{-7} to 10^{-5} M. Maximum inhibition (75.3 ± 6.2%) was obtained with SAMe at 1.5×10^{-5} M. Vascular 6-keto-$PGF_{1\alpha}$ production showed a significant inverse linear correlation with vascular lipid peroxide production ($Y = -0.04x + 18.1$, $r = 0.7309$, $P < 0.0001$). BIOCHEM PHARMACOL 53;11:1761–1763, 1997. © 1997 Elsevier Science Inc.

KEY WORDS: prostacyclin; thromboxane; S-adenosyl-L-methionine

The S-adenosyl-L-methionine (SAMe) molecule participates in many enzymatic processes, favoring the methylation of other molecules such as proteins, nucleic acids and lipids [1]. The main clinical indications for treatment with SAMe are certain types of liver disease [2]. These diseases involve an alteration in blood hemostasis and an imbalance in prostanoid synthesis [3]. We designed the present study to evaluate the effect of SAMe on the synthesis of two prostanoids of fundamental importance for hemostasis: platelet thromboxane and vascular prostacyclin.

MATERIALS AND METHODS

The experimental materials were human blood and aortic rings from untreated Wistar rats, and platelets and aortic rings from Wistar rats treated for 7 days with SAMe (Boehringer Ingelheim España, Barcelona, Spain) at 5 or 10 mg/kg/day s.c. Blood samples were obtained from healthy male volunteers (mean age 31.3 ± 2.1 years) who had taken no medication for at least the previous 15 days. A blood sample was obtained with 3.8% sodium citrate (1:10) after an overnight fast; aortic rings were obtained from male Wistar rats (250–275 g body weight) which were killed by decapitation. A blood sample was also obtained from each animal as described above. The entire aorta was excised and divided into segments 2–3 mm thick (56.8 ± 3.2 mg), and the segments were placed in buffer containing (in mmol L): 100 NaCl, 4 KCl, 25 $NaHCO_3$, 2.1 Na_2SO_4, 20 sodium citrate, and 50 Tris (pH 8.2).

Blood samples were centrifuged at 180 *g* for 10 min at 18°C and the resulting supernatant was used as platelet-rich plasma (PRP). The platelet count was adjusted to 270000–300000 platelets/μL with autologous platelet-poor plasma, which was obtained by centrifuging blood at 1800 *g* for 10 min at 18°C. The PRP was divided into aliquots of 500 μL. Then platelets were stimulated at 37°C with 100 μmol/L arachidonic acid. After 5 min of incubation, indomethacin (100 μmol/L) was added and the sample was centrifuged at 10000 *g* for 5 min. Part of the supernatant was frozen at −80°C for thromboxane B_2 (TxB_2) determinations with radioimmunoassay (Amersham International, plc, Little Chalfont, Buckinghamshire, England), and part was incubated with thiobarbituric acid (0.5% in 20% trichloroacetic acid) at 100°C for 15 min. Absorbance was calculated spectrophotometrically at 532 nm; these values were compared against a standard curve for malondialdehyde-bis-diethylacetal, and lipid peroxide production was quantified as thiobarbituric acid reactive substances (TBARS).

Aortic rings were incubated for 5 min at 37°C in the buffer described above. Then the buffer was replaced and

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Abbreviations: MDA, malondialdehyde; PGI_2, prostacyclin; SAMe, S-adenosyl-L-methionine; TBARS, thiobarbituric acid reactive substances; TxA_2, thromboxane A_2.

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TABLE 1. Platelet thromboxane B_2 (TxB_2), aortic 6-keto-$PGF_{1\alpha}$, and thiobarbituric acid reactive substance (TBARS) production induced by 100 μmol/L arachidonic acid in SAMe-treated rats

			TBARS	
	TxB_2 (ng/mL)	6-keto-$PGF_{1\alpha}$ (ng/mg aorta)	Platelets (nmol/10^9 Plat)	Aortic rings (nmol/mg aorta)
Saline (N = 10)	224 ± 19	58.6 ± 7.8	1.09 ± 0.15	1.65 ± 0.19
Vehicle (N = 10)	245 ± 6.3	45.9 ± 9.0	1.14 ± 0.16	1.77 ± 0.20
SAMe				
5 mg/kg/day (N = 10)	260 ± 34	52.3 ± 7.5	1.18 ± 0.15	1.21 ± 0.12*
10 mg/kg/day (N = 10)	272 ± 23	71.5 ± 6.1*	0.99 ± 0.10*	0.77 ± 0.05†

*$P < 0.05$, †$P < 0.01$ vs saline.

arachidonic acid (100 μmol/L) was added. After 5 min indomethacin (100 μmol/L) was added; then the arterial tissue was dried and weighed. Part of the supernatant was frozen for 6-keto-$PGF_{1\alpha}$ determinations with radioimmunoassay (Amersham International), and part was processed as described above for platelets to determine TBARS production. In order to discard a possible interference of SAMe with the thiobarbituric acid assay, we incubated SAMe without tissue (platelets or aortic rings) in some experiments, and TBARS production was measured; modifications in absorbance were not demonstrated in any experiment (data not shown).

In all cases a control sample was prepared with SAMe (10^{-7} to 10^{-3} M) and incubated for 5 min before platelets were stimulated with arachidonic acid. All concentrations of SAMe were prepared in isotonic saline solution buffered to pH 7.4.

The data in the text and figure are given as means ± SEM for each group of experiments, which comprised 8–10, were compared with Student's t test, and P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The administration of SAMe to rats did not significantly modify TxB_2 production. Vascular production of 6-keto-$PGF_{1\alpha}$ increased significantly after the administration of 10 mg/kg/day SAMe (Table 1).

Experiments *in vitro* showed that SAMe did not significantly modify thromboxane synthesis by platelets. Maximum inhibition was 19.9 ± 3.8% with respect to samples incubated in the absence of the drug (Fig. 1). Vascular production of 6-keto-$PGF_{1\alpha}$ increased in a concentration-dependent manner when SAMe was administered in the range of 10^{-7} to 10^{-4} M. The greatest increase was 167 ± 15%, obtained in samples incubated with 5×10^{-5} M SAMe (Fig. 1).

Lipid peroxide production by platelets was maximally inhibited (28.1 ± 4.6%) by SAMe at 5×10^{-5} M. In aortic rings, lipid peroxidase production was inhibited in a concentration-dependent manner in the range of 10^{-7} to 10^{-5} M. Maximum inhibition (75.3 ± 6.2%) was obtained with SAMe at 1.5×10^{-5} M (Fig. 1). Vascular 6-keto-$PGF_{1\alpha}$ production showed a significant inverse linear correlation with vascular lipid peroxide production ($Y = -0.04x + 18.1$, $r = 0.7309$, $P < 0.0001$).

Our results show that SAMe increases prostacyclin synthesis in the arterial wall, possibly by inhibiting the synthesis of lipid peroxides, which comprise a major inhibitor of prostacyclin synthetase [4]. The doses given to rats in our experiments are equivalent to the recommended doses for the treatment of a variety of liver diseases in humans [2]. Our findings may therefore shed light on possible clinical applications. The results of our *in vitro* experiments support this conclusion: in humans, plasma concentrations of SAMe reach 10^{-6} to 10^{-5} M [5], a range within which we observed most of the effects reported here.

We have found no previous data comparable to those reported here that might explain the mechanism by which SAMe increases prostacyclin synthesis. However, liver disease usually involves a decrease in the levels of arachidonic acid and its prostaglandin derivatives on the cell membrane [6]. Moreover, SAMe increases the methylation of phosphatidylethanolamine [7], one of the natural phospholipids that releases arachidonic acid. This may represent a mechanism by which prostacyclin synthesis is enhanced by SAMe; however, if this were the case increased thromboxane synthesis would also be expected, but was not found in the present experiments.

Another possible explanation is that SAMe, rather than directly affecting prostacyclin synthesis, may prevent the inhibition of synthesis by lipid peroxides. This mechanism is involved in the action of other drugs that enhance prostanoid synthesis [8]. The inverse linear correlation between 6-keto-$PGF_{1\alpha}$ production and vascular lipid peroxide production suggests that SAMe acts through such an indirect mechanism.

The biphasic effect seen in our experiments is consistent with the behavior of SAMe in other studies of tissue lipid peroxidation [9], and with the finding that the hepatoprotective effect of the drug is weaker at high doses than at lower doses [10]. This phenomenon may reflect the unspecific nature of methylation in the millimolar range of concentrations, i.e., at doses much higher than those used in human clinical practice. Moreover, the commercial preparation of SAMe used in the present study contains traces of S-adenosylhomocysteine and methylthioad-

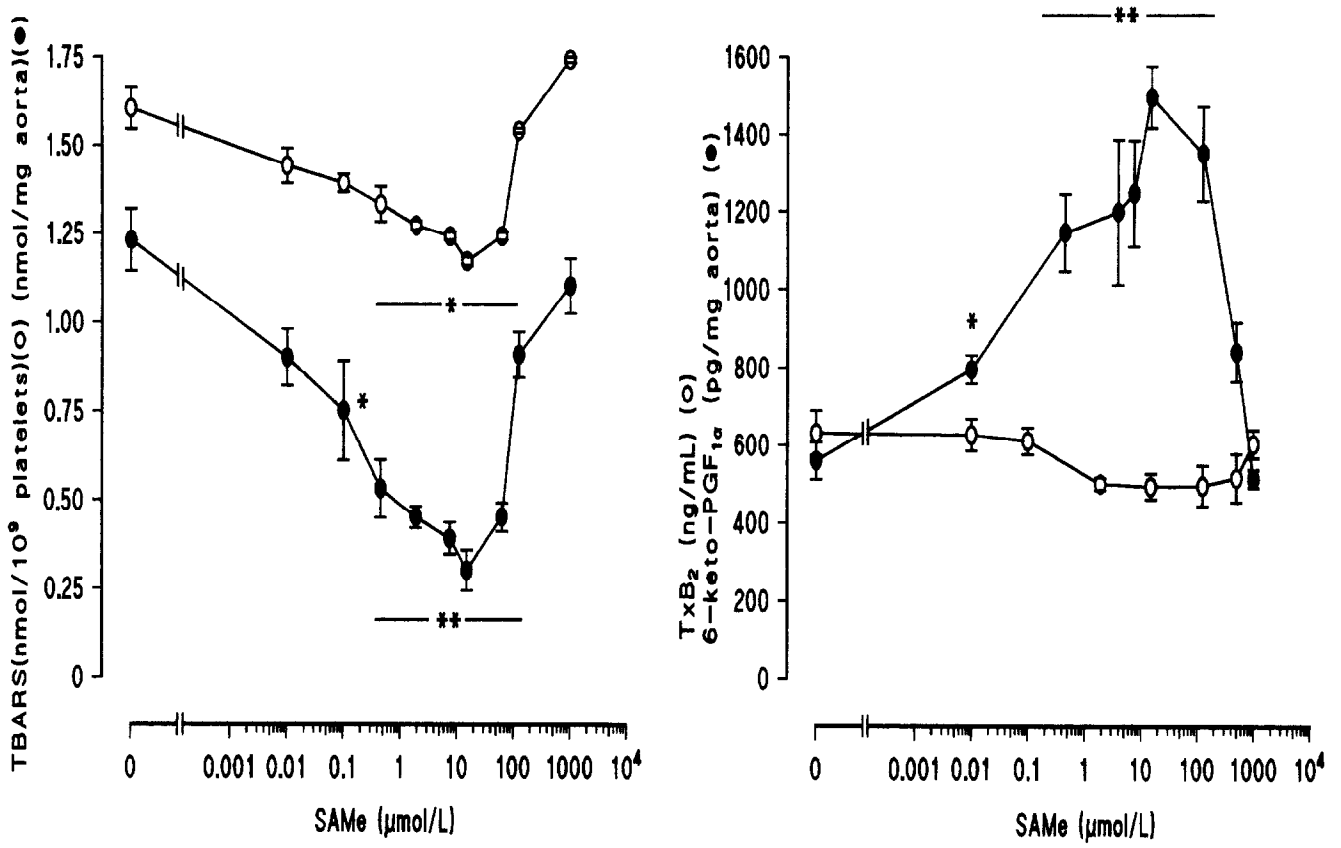

FIG. 1. Concentration-dependence of the inhibition of thiobarbituric acid reactive substances (TBARS) in human platelets and rat aortic rings (left panel), and thromboxane B_2 (TxB_2) in human platelets and 6-keto-$PGF_{1\alpha}$ in rat aortic rings (right panel), induced by 100 μmol/L arachidonic acid. *P < 0.05, **P < 0.001 vs samples without SAMe.

enosine; at 10^{-3} M of SAMe it is possible to observe some effects of these above-mentioned substances.

In conclusion, we show that S-adenosyl-L-methionine (SAMe) increases prostacyclin synthesis. The most direct consequence of this effect is likely to be the prevention of the thrombotic effect that may be caused by a deficit in prostacyclin in patients with liver disease.

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