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_{1α}. In vitro vascular production of 6-keto-PGF_{1α} increased in a concentration-dependent manner when SAMe was incubated in the range of 10^{-7} to 10^{-5} M. The greatest increase was 167 ± 15%, obtained in samples incubated with 5 \times 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 \times 10^{-5} M. Vascular 6-keto-PGF_{1α} production showed a significant inverse linear correlation with vascular lipid peroxide production (Y = -0.04x + 18.1, r = 0.7309, P < 0.0001).

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:lO) 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 NaHCO₃, 2.1 Na₂SO₄, 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.

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 B₂ (TXB₂) determinations with radioimmunossay (Amersham International, plc, Little Chalfont, Buckinghamshire, England), and part was incubated with thiobarbituric acid reactive substances (TBARS). 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 NaHCO₃, 2.1 Na₂SO₄, 20 sodium citrate, and 50 Tris (pH 8.2).

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Received 10 September 1996; accepted 28 November 1996.
TABLE 1. Platelet thromboxane B_2 (TxB2), aortic 6-keto-PGF_1 alpha, and thiobarbituric acid reactive substance (TBARS) production induced by 100 μmol/L arachidonic acid in SAMe-treated rats

<table>
<thead>
<tr>
<th></th>
<th>TxB2 (ng/mL)</th>
<th>6-keto-PGF_1 alpha (ng/mg aorta)</th>
<th>Platelets (nmol/10^9 Pla)</th>
<th>Aortic rings (nmol/mg aorta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (N = 10)</td>
<td>224 ± 19</td>
<td>58.6 ± 7.8</td>
<td>1.09 ± 0.15</td>
<td>1.65 ± 0.19</td>
</tr>
<tr>
<td>Velaure (N = 10)</td>
<td>245 ± 6.3</td>
<td>45.9 ± 9.0</td>
<td>1.14 ± 0.16</td>
<td>1.77 ± 0.20</td>
</tr>
<tr>
<td>5 mg/kg/day (N = 10)</td>
<td>260 ± 34</td>
<td>52.3 ± 7.5</td>
<td>1.18 ± 0.15</td>
<td>1.21 ± 0.12*</td>
</tr>
<tr>
<td>10 mg/kg/day (N = 10)</td>
<td>272 ± 23</td>
<td>71.5 ± 6.1*</td>
<td>0.99 ± 0.10*</td>
<td>0.77 ± 0.05*</td>
</tr>
</tbody>
</table>

*P < 0.05, †P < 0.01 vs saline.

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.

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^-3 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 [3], 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 methylothioad-
enomine; at $10^{-1}$ 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.

We thank A. Pino Blanes for expert technical assistance and Karen Shashok for translating the original manuscript into English.

References