S-Adenosyl-L-Methionine Protects the Liver Against the Cholestatic, Cytotoxic, and Vasoactive Effects of Leukotriene D₄: A Study With Isolated and Perfused Rat Liver

RAFAEL N. CINCU, CARLOS M. RODRIGUEZ-ORTIGOSA, IDOIA VESPERINAS, JORGE QUIROGA, AND JESUS PRIETO

Cysteinyl-leukotrienes can cause cholestasis and liver damage when administered at nanomolar concentrations. Using the isolated and perfused rat liver we analyzed whether S-adenosyl-L-methionine (SAME) may protect this organ against the noxious effects of leukotriene-D₄ (LTD₄). We observed that a 2 nmol bolus of this compound decreased bile flow (−12.6% ± 1.6%, P < .02), and bile salt excretion (−23.5% ± 2.2%, P < .02; both compared with baseline values), caused the release of glutamic-oxaloacetic transaminase (GOT) and lactic dehydrogenase (LDH) to the hepatic effluent, and increased significantly the perfusion pressure as compared with a control group not receiving LTD₄ (6.0 ± 1.1 vs. 0.2 ± 0.02 mm hg, respectively; P < .001). The cholestatic effect of LTD₄ was attenuated by infusion of SAME which, at rates of 67 and 100 μg/min, totally prevented the decrease in bile salt excretion. Likewise, in SAME infused livers, the release to the effluent of GOT and LDH was lower than in the group receiving LTD₄ only, and was even lower than in the control group. We also found that the increase in perfusion pressure induced by LTD₄ was prevented by SAME in a dose-dependent manner. Of interest, SAME increased the biliary excretion of the eicosanoid in a dose-related fashion. We conclude that SAME reverts the cholestatic, cytotoxic, and hemodynamic effects of LTD₄ on the liver, and that these protective effects might be partly because of a stimulation of the biliary excretion of the leukotriene. (HEPATOLOGY 1997;26:330-335.)

Cysteinyl-leukotrienes (cLTs), specifically leukotriene C₄ and leukotriene D₄ (LTD₄), have been associated with the induction of liver injury.¹ A significant increase in the production of cLTs occurs in some experimental models of fulminant hepatitis, as those induced by frog virus 3² or D-galactosamine plus endotoxin.³ Moreover, a fulminant hepatitis similar to that induced by administration of D-galactosamine plus endotoxin can be induced by administration of LTD₄,³ suggesting that LTD₄ is directly involved in the mediation of liver damage.

Previous studies from our laboratory and from other groups, using isolated and perfused rat liver, have shown that the administration of leukotriene C₄ or LTD₄, at nanomolar concentrations, increases the perfusion pressure and induces cholestasis.⁴⁻⁶ Very little is known on the mechanism underlying the cholestatic effect of cLTs, and, although leukotriienes are thought to be implicated in the cholestasis accompanying sepsis and inflammatory processes,⁷⁻⁸ there are few data on substances that can protect the liver against the cholestatic and toxic effects of cLTs.

S-adenosyl-L-methionine (SAME) is a naturally occurring metabolite that originates from methionine and adenosine triphosphate through the SAME synthetase pathway.⁹ It has been shown that SAME has a protective role in experimental cholestasis induced by alcohol,¹⁰ acetaminophen,¹¹ D-galactosamine,¹² carbon tetrachloride,¹³ or ethinyl estradiol.¹⁴ In this study we analyze whether SAME may alleviate the cholestatic, cytotoxic, and hemodynamic effects induced by LTD₄ on the isolated and perfused rat liver.

MATERIALS AND METHODS

SAME was a gift from Europharma (Madrid, Spain). Taurocholic acid (sodium salt), methionine, and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). Synthetic standards of cLTs were from Cayman Chemical (Ann Arbor, MI), and [³H]LTD₄ was from Amersham (Little Chalfont, UK). Scintillation liquid (Normascint F-1) and methanol (high-performance liquid chromatography [HPLC] grade) were obtained from Schurralt (Barcelona, Spain). All other chemicals were purchased from Merck (Darmstadt, Germany).

Livers were isolated from male Wistar rats (range, 240-255 g body weight), and they were used in a recirculating antegrade perfusion system. Liver isolation was as previously described.¹ Briefly, the bile duct was first cannulated with a polyethylene tubing PE-50, followed by cannulation of the portal vein with a catheter Abbocath (Abbott, Sligo, Ireland) 16-G. Then, the liver was perfused in situ with oxygenated and heparinized Krebs-Ringer bicarbonate (KRB) buffer at 37°C, the vena cava was cut, and the thoracic vena cava was cannulated with a polyethylene cannula (1.5 mm inner diameter), defining the final path of perfusion from vena porta to vena cava. At that moment, the liver was transferred to an acrylic platform, and placed into a thermostated and humidified chamber. The liver was perfused at 37°C with 250 mL of KRB buffer (116 mmol/L ClNa, 4.7 mmol/L CIK, 1.2 mmol/L PO₄H₂K, 1 mmol/L SO₄Mg, 1.25 mmol/L Cl₄Ca, 25 mmol/L CO₃HNa, 5 mmol/L glucose, 0.2 mmol/L pyruvate, 2 mmol/L lactate, and 1% bovine serum albumin), at a rate of 30 mL/min. Oxygenation was guaranteed by gassing the perfusion liquid with 93% O₂, 5% CO₂. The pH of the medium was adjusted at 7.35-7.40 by an automatic autoburette (Radiometer,
Copenhagen, Denmark), and the perfusion pressure was continuously registered with a pressure transducer (Letica, Barcelona, Spain). Animals were treated avoiding unnecessary suffering, and in accordance with the guidelines of our institution.

The design of the experiments was the following. After a 50-minute stabilization period, and a further 30-minute basal period, a bolus of 2 nmol of LTD₄ (or 500 µL of KRB buffer in the control group) was injected, and the experiment was continued for another 30 minutes (experimental period).

Five groups of perfused livers were studied: control group (KRB buffer injection in minute 80; n = 6); LTD₄ group (injection of 2 nmol of LTD₄ in minute 80; n = 7); and three SAMe-treated LTD₄ groups (n = 6-7 experiments in each group), in which SAMe was continuously infused from minute 0 onwards at rates of 33, 67, or 100 µg/min (equivalent to 63, 127, and 190 nmol/min) and LTD₄ was administered as indicated above. An additional group was included, in which 190 nmol/min of methionine (equimolar with the high dose of SAMe) were infused since minute 0 and LTD₄ was injected in minute 80, to check the specificity of SAMe.

Also, we studied the hepatic metabolism of the exogenously added LTD₄, and the effect of the continuous infusion of SAMe, at the three same rates as before, on such metabolism. Thus, four additional groups of livers were analyzed (n = 3 to 5 experiments). In one of them, which served as a control group, a bolus of [³H]LTD₄ (5.8 × 10⁻¹¹ mol/g liver) was injected at minute 50. In the other three groups, the same amount of [³H]LTD₄ was injected at minute 50, and a continuous infusion of SAMe at 33, 67, or 100 µg/min was maintained from minute 0 onwards. Bile and perfusate fractions were collected every 10 minutes starting at minute 50, and the radioactivity present in these fractions was counted in a scintillation counter (LKB, Turku, Finland). Furthermore, in the groups in which [³H]LTD₄ alone or plus 100 µg/min of SAMe was injected, we performed RP-HPLC separation of the bile fractions collected, to check the metabolic fate of LTD₄ and the effect of SAMe on this metabolism.

RP-HPLC of the biliary metabolites of [³H]LTD₄ was performed on a Nova Pak column (10 × 0.4 cm, 5-μm particles, Waters, Milford, MA). The mobile phase consisted of methanol, water, and acetic acid (65/35/0.1 by volume) at pH 5.6. In these conditions, the retention times of main leukotrienes were: LTC₄ ≈ 5 minutes, LTD₄ ≈ 9 minutes, LTE₄ ≈ 11 minutes, and N-acetyl-LTE₄ ≈ 7 minutes. Twenty-second fractions of the HPLC eluent were collected and then counted in a LKB scintillation counter.

In all experimental groups, a continuous infusion of sodium taurocholate (40 µmol/h) was also maintained throughout the experiments, to counteract the loss of bile salts.

To assess the viability of the livers, samples of the hepatic effluent (2.5 mL) were obtained every 10 minutes following the stabilization period. In these samples, the levels of glutamic-oxaloacetic transaminase (GOT), lactate dehydrogenase, and glucose were measured by enzymatic conventional methods, and K⁺ concentration was determined in an automatic analyzer (Beckman, Palo Alto, CA). Also, the bile produced in these 10-minute periods was collected in pre-weighted polypropylene tubes. Bile flow was calculated by gravimetry (assuming a bile density = 1), and total bile salts were quantified by the 3α-hydroxysteroid dehydrogenase method.

Mann-Whitney, Wilcoxon, and Kruskal-Wallis nonparametric statistical tests were used for comparisons between experimental groups.³ Values are expressed as the mean ± SEM.

RESULTS

Bile flow throughout the experiments is represented in Fig. 1A. As it can be seen, in control group vehicle administration did not modify the levels of bile flow. In contrast, administration of 2 nmol of LTD₄ induced a significant drop in bile flow with respect to the basal values in the three experimental periods (mean decrease: −12.6% ± 1.6%, P < .02). Continuous infusion of SAMe attenuated the cholestatic effect of LTD₄ (mean reduction in the three groups infused with SAMe: −4.8% ± 0.4%, P < .001 with respect to the LTD₄ group) (Table 1).

Biliary excretion of bile salts in all groups of livers is shown in Fig. 1B. There were no significant differences between groups in samples corresponding to basal period, although the highest values were found in the SAMe-treated livers. In control group, vehicle administration did not modify the biliary excretion of bile salts. In contrast, LTD₄ (2 nmol) caused a significant decrease in bile salt excretion (mean reduction in the three experimental periods: −23.5 ± 2.2%, P < .02 with respect to the basal value). Infusion of SAMe was effective in preventing the LTD₄-induced reduction in bile salt excretion. Thus, in the group infused with 100 µg/min of SAMe, the levels of biliary bile salts after LTD₄ injection were significantly higher than those in livers in rats treated with only LTD₄. Infusion with 33 or 67 µg/min of SAMe also diminished the cholestatic effect of LTD₄, but the differences in bile salt excretion with the group receiving only LTD₄ were not significant (Table 2). Methionine infusion at a concentration equivalent to the highest one of SAMe (190 nmol/min), also prevented the cholestatic effects of LTD₄. Thus, the decreases in bile flow (−5.87% ± 2.2%), and in biliary bile salt excre-
tion (−3.42% ± 3.9%) were not significantly different from those found in control group.

As shown in Fig. 2, the release of hepatocyte enzymes to the effluent increased after LTD₄ administration, as compared with control group (Fig. 2). When LTD₄ injection was given to SAMe-infused livers, the hepatic release of GOT and lactate dehydrogenase (LDH) was lower than in livers receiving LTD₄ alone. Moreover, in livers treated with SAMe and LTD₄, the release of GOT was even lower than in the control group, thus indicating that SAMe not only protects the liver against the cytotoxic effects of cLTs but improves the viability of the organ in our system. On the other hand, when 190 nmol/min of methionine were infused instead of SAMe, the release of GOT to the hepatic effluent was similar to that measured in the control group (values at the end of the experiment: 93.3 ± 11.3 vs. 81.5 ± 11.4 mIU/min/g liver, respectively; nonsignificant), whereas the levels of LDH at the end of the experiment in the methionine group were between those found in control group and in LTD₄ group (1.56 ± 0.29, 1.02 ± 0.31, and 2.26 ± 0.64 IU/min/g liver, respectively; n.s.).

As shown in Fig. 3, in the control group the injection of the vehicle was followed by a very small (1.1 ± 0.1 mm h²g) and short-lasting (≈ 1 minute) increase in the perfusion pressure. In contrast, after 2 nmol bolus injection of LTD₄, the perfusion pressure in the group that was not receiving SAMe changed following a different pattern: during the first minute after LTD₄ injection, there was a quick rise of 4.8 ± 0.8 mm h₉g (P < .01 as compared with the control group); 3 to 4 minutes later, the pressure returned to levels close to basal values, and then increased again to values significantly greater than those observed in the control group (6.0 ± 1.1 vs. 0.2 ± 0.02 mm h₉g, respectively; P < .001). This second wave of increased pressure lasted for more than 15 minutes.

Continuous infusion of SAMe did not prevent the initial rise of the perfusion pressure, but reduced both the intensity and the length of the second wave of increased pressure in a dose-related manner (Fig. 3). Thus, during this second period (between 4 and 20 minutes after LTD₄ injection) the maximum increase of the perfusion pressure in the groups infused with 33, 67, or 100 µg/min of SAMe was respectively: 4.2 ± 1.1, 3.2 ± 0.3, and 1.4 ± 0.7 mm h₉g; the last value being significantly lower (P < .01) than that found in the group that received only LTD₄. In the livers receiving a continuous infusion of 190 nmol/min of methionine, values of 4.3 ± 0.3 mm h₉g were observed during the second wave of increased pressure; these values were not significantly different from those obtained in the group that received LTD₄ alone (Fig. 3).

Because, as indicated above, SAMe appeared to block a diversity of effects of LTD₄ in the liver, we performed additional experiments to establish whether SAMe might block the uptake of LTD₄ by liver cells or whether it could affect the transport and biliary elimination of this compound. To this aim we analyzed the metabolism and biliary excretion of exogenously added [³H]LTD₄, and the effect on these processes of SAMe infusion at three different rates. As it can be seen in Fig. 4A, SAMe caused an increase in the biliary excretion of radioactivity, in a dose-dependent manner. This finding was associated with a dose-dependent reduction in the radioactivity remaining in the perfusate (Fig. 4B), indicating that SAMe might facilitate the clearance of leukotrienes by increasing the biliary excretion of these compounds.

To examine the metabolic fate of injected [³H]LTD₄, and the effect of SAMe on this metabolism, RP-HPLC fractionation of biliary metabolites of [³H]LTD₄ was performed. As represented in Fig. 5, the HPLC profile of radio-

### Table 1. Bile Flow Along the Experiments in All Groups (μL/min/g Liver)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th></th>
<th></th>
<th>Experimental Period</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minute 60</td>
<td>Minute 70</td>
<td>Minute 80</td>
<td>Minute 90</td>
<td>Minute 100</td>
<td>Minute 110</td>
</tr>
<tr>
<td>Control</td>
<td>1.79 ± 0.13</td>
<td>1.76 ± 0.13</td>
<td>1.79 ± 0.14</td>
<td>1.80 ± 0.14</td>
<td>1.82 ± 0.15</td>
<td>1.81 ± 0.15</td>
</tr>
<tr>
<td>LTD₄</td>
<td>1.96 ± 0.17</td>
<td>1.86 ± 0.14</td>
<td>1.87 ± 0.13</td>
<td>1.65 ± 0.11*</td>
<td>1.64 ± 0.13*</td>
<td>1.62 ± 0.14*</td>
</tr>
<tr>
<td>LTD₄ + SAMe (33 µg/min)</td>
<td>2.18 ± 0.13</td>
<td>2.04 ± 0.10</td>
<td>1.95 ± 0.10</td>
<td>1.81 ± 0.10*</td>
<td>1.84 ± 0.09*</td>
<td>1.86 ± 0.09*</td>
</tr>
<tr>
<td>LTD₄ + SAMe (67 µg/min)</td>
<td>1.99 ± 0.08</td>
<td>1.87 ± 0.08</td>
<td>1.81 ± 0.08</td>
<td>1.73 ± 0.09*</td>
<td>1.77 ± 0.08*</td>
<td>1.81 ± 0.08</td>
</tr>
<tr>
<td>LTD₄ + SAMe (100 µg/min)</td>
<td>2.19 ± 0.14</td>
<td>2.04 ± 0.11</td>
<td>2.00 ± 0.11</td>
<td>1.88 ± 0.10*</td>
<td>1.86 ± 0.10*</td>
<td>1.85 ± 0.10*</td>
</tr>
</tbody>
</table>

NOTE. Experimental groups correspond to those described in Table 1.

* P < .05 as compared with the value of the same group at minute 60.

### Table 2. Biliary Excretion of Bile Salts Along the Experiments in All Groups (nmol/min/g Liver)

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal Period</th>
<th></th>
<th></th>
<th>Experimental Period</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minute 60</td>
<td>Minute 70</td>
<td>Minute 80</td>
<td>Minute 90</td>
<td>Minute 100</td>
<td>Minute 110</td>
</tr>
<tr>
<td>Control</td>
<td>25.2 ± 3.9</td>
<td>28.8 ± 1.4</td>
<td>28.6 ± 1.8</td>
<td>28.1 ± 2.5</td>
<td>26.5 ± 1.3</td>
<td>27.9 ± 3.3</td>
</tr>
<tr>
<td>LTD₄</td>
<td>28.6 ± 3.0</td>
<td>27.3 ± 2.8</td>
<td>28.9 ± 2.6</td>
<td>22.0 ± 2.3†</td>
<td>22.3 ± 2.6†</td>
<td>22.4 ± 2.4†</td>
</tr>
<tr>
<td>LTD₄ + SAMe (33 µg/min)</td>
<td>29.3 ± 2.0</td>
<td>29.7 ± 2.2</td>
<td>30.1 ± 2.0</td>
<td>26.7 ± 2.2†</td>
<td>26.9 ± 2.0*</td>
<td>29.7 ± 2.6</td>
</tr>
<tr>
<td>LTD₄ + SAMe (67 µg/min)</td>
<td>33.2 ± 2.1</td>
<td>32.7 ± 2.3</td>
<td>31.3 ± 1.6</td>
<td>29.6 ± 1.8†</td>
<td>32.8 ± 2.2</td>
<td>32.5 ± 1.9</td>
</tr>
<tr>
<td>LTD₄ + SAMe (100 µg/min)</td>
<td>30.2 ± 1.2</td>
<td>30.6 ± 1.2</td>
<td>31.3 ± 1.5</td>
<td>30.4 ± 1.1†</td>
<td>31.3 ± 1.2†</td>
<td>32.7 ± 1.2†</td>
</tr>
</tbody>
</table>

NOTE. Experimental groups correspond to those described in Table 1.

* P < .05 as compared with the value of the same group at minute 60.

† P < .05 in respect to the same time of group LTD₄.
pared with SAMe, induces only a partial protection against leukotrienes.

The observation that cLTs, at nanomolar concentration, induce a reduction in bile flow has been reported by others.6 The effect of LTD4 on bile flow and on bile salt excretion found in the present study is similar to that previously reported by our group using LTC4 in the same experimental model.4 The mechanisms through which cLTs induce cholestasis are not clear. Although it has been proposed16 that cLTs can cause fluid extravasation and edema around bile ducts, thus impairing bile flow, our finding that cLTs reduce bile salt excretion suggests that these substances affect bile salt kinetics.4 Because cLTs do not impair sinusoidal uptake nor canalicular excretion of bile salts,17-20 it seems possible that they might influence intrahepatocytic transport of bile salts, as we have previously suggested.4

SAMe has been shown to improve bile flow and bile salt excretion in experimental models of cholestasis produced by ethynylestradiol or total parenteral nutrition in the rat,14,21,22 and similar effects have been observed in the present work in LTD4-induced cholestasis. The increase in bile flow observed in SAMe-treated livers could be mediated, at least in part, by a "solvent drag" effect, but SAMe has also been reported to improve both Na+/K+-ATPase activity and membrane fluidity of hepatocytes in experimental cholestasis,14,22 being these factors involved in solute transport across the hepatocellular membrane. In addition to these effects, the present report shows that SAMe increases the biliary excretion of LTD4 and its metabolites in a dose-dependent manner.

**Fig. 2.** Hepatic release to the effluent of (A) GOT and (B) LDH throughout the experiments, as described in legend of Fig. 1. The data represent the mean of six to seven separate experiments in each group. Arrows indicate the time at which LTD4 (2 nmol) or KRB vehicle (in control group) was injected: a, P < .01 with respect to the SAMe-treated groups; b, P < .01 with respect to the rest of the groups; c and d, P < .05 and P < .01, respectively, in comparison with the control group; e, P < .05 with respect to the SAMe-treated groups; and f, P < .05 with respect to the groups infused with 67 or 100 μg/min of SAMe.

**Fig. 3.** Changes in perfusion pressure over baseline value in the experimental period in control livers, in livers treated with LTD4 alone, in livers treated with LTD4 plus continuous infusion of SAMe at three different doses (33, 67, or 100 μg/min), and in livers treated with LTD4 plus continuous infusion of 190 nmol/min of methionine (equimolar to the highest dose of SAMe). Arrow indicates the time at which LTD4 (2 nmol) or vehicle (in control group) was injected. Previously, perfusion pressure was similar in all groups. The data represent the mean of six to seven separate experiments.

**DISCUSSION**

In the isolated and perfused rat liver, we show that a bolus injection of 2 nmol of LTD4 causes reduction in bile flow and bile salt excretion, increased release of GOT and LDH to the effluent and a rise in the perfusion pressure. All these changes are significantly attenuated, or totally reversed, by SAMe. The effects of SAMe are not totally paralleled by its precursor methionine that was capable of reducing the cholestatic effects of LTD4, but only partially protected against the cytotoxic and hemodynamic effects of this compound. It should be considered that methionine is a precursor of SAMe, but the synthesis of this substance consumes adenosine triphosphate.9 These two effects (on antioxidant cell defenses on one hand, and on cell energy stores on the other) could provide an explanation for the fact that methionine, as compared with SAMe, induces only a partial protection against leukotrienes.
in the isolated and perfused liver: a sharp and short peak of increased pressure occurring immediately after the bolus injection of LTD₄, and a lasting wave of increased pressure starting approximately 3 minutes after the end of the first peak (Fig. 3). The sequential changes of pressure here reported have not been previously recognized, possibly because other studies have used continuous infusion of the eicosanoid ⁵,⁶,¹⁷ instead of bolus injection. In our study, the first peak is probably caused by the vasoconstrictor action of LTD₄ on hepatic vasculature, ²⁷,²⁸ whereas the second wave of increased pressure could be caused by other as yet uncharacterized actions of cLTs on the hepatic parenchyma and/or vasculature.

SAMe infusion did not prevent the first increase in perfusion pressure induced by LTD₄, but reduced the second peak in a dose-dependent manner (Fig. 3). Because vascular endothelium and hepatocytes possess specific receptors for cLTs,²⁹,³⁰ the different effect of SAMe on the first and second peaks of pressure suggests that this substance does not block the binding of cLTs to the cell receptor, but inhibits later metabolic events leading to the second wave of increased pressure. Our experiments show that SAMe does not inhibit the uptake of LTD₄ by liver cells. On the contrary, it reduces the radioactivity remaining in the perfusate after injection of [³H]LTD₄, and increases the transport of the compound to bile. Thus, further studies are needed to clarify the effects of cLTs on the intrahepatic vascular resistance. These studies could be of relevance in the understanding of the pathophysiology of portal hypertension.

Acknowledgment: We thank P. Alzuguren and R. Bilbao for their technical assistance.

**Fig. 4.** Effect of SAMe infusion on the excretion of radioactivity in (A) bile and in (B) perfusate. At minute 50, 5.8 × 10⁻¹² mol/g liver of [³H]LTD₄ were injected in the isolated rat liver, and the radioactivity of both bile and perfusate 10-minute fractions was counted. SAMe was infused from minute 0 onwards, at doses of 33, 67, and 100 μg/min. Arrow marks the time at which [³H]LTD₄ was administered. * P < .05 and ** P < .01 in respect to the values in group in which only [³H]LTD₄ was given.

**Fig. 5.** RP-HPLC extraction of radioactivity present in bile of livers that received a bolus injection of 5.8 × 10⁻¹² mol/g liver of [³H]LTD₄ at minute 50. Left column: livers perfused with KRB alone. Right column: livers perfused with KRB and 100 μg/min of SAMe. Only the radioactive profiles of bile samples corresponding to minute 70 (highest level of biliary radioactivity, see Fig. 4), minute 90, and minute 110 have been represented. The scale in ordinates is different in each group to better compare the radioactive profile.
REFERENCES


23. Bilzer M, Lauterburg BH. Peptidoleukotrienes increase the efflux of glutathione from perfused rat liver. Prostagland Leuk Essent Fatty Acids 1993;49:715-721.


