Effects of S-Adenosyl-l-Methionine on Hepatic and Renal Oxidative Stress in an Experimental Model of Acute Biliary Obstruction in Rats

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We used an animal model of extrahepatic biliary obstruction of 7 days’ duration to study the production of thiobarbituric acid reactive substances (TBARS), total glutathione (TG), reduced glutathione (GSH), and oxidized glutathione (GSSG), and the enzymatic activities of GSH-peroxidase, GSSG-reductase, and GSH-transferase. Four groups of six rats each were treated with saline, drug solvent, S-adenosyl-l-methionine (SAM) 5 mg/kg/d, subcutaneously, or SAM 10 mg/kg/d, subcutaneously. Extrahepatic biliary obstruction increased TBARS. SAM had the dose-dependent effects of inhibiting TBARS production and increasing TG content, mainly as a result of the increase in GSH. The activity of GSH-peroxidase and GSH-transferase was also significantly increased. In renal tissue these effects were statistically significant only in animals given the higher dose of SAM. In liver we found a reduction in biochemical values indicative of liver damage. We conclude that effect of SAM on hepatorenal function is strongly influenced by the drug’s ability to reestablish equilibrium after oxidative tissue stress. (HEPATOLOGY 1997; 26:121-127.)

S-adenosyl-l-methionine (SAM) is a physiological compound synthesized from methionine and adenosine triphosphate by the enzyme SAM synthetase.1 The compound participates in three important metabolic processes: transmethylation, transsulfuration, and aminopropylation.2 Through transmethylation reactions, SAM restores plasma membrane fluidity in hepatocytes;3 transsulfuration reactions enhance the detoxicant capacity of hepatocytes by increasing the amount of endogenous cysteine, glutathione, and taurine.2,4 Recent clinical and experimental studies have shown that treatment with SAM has important benefits in intrahepatic cholestasis caused by liver disease associated with acute or chronic cholestasis, pregnancy, and the use of certain drugs and hepatotoxins.5-7 The drug was also found to decrease fibrogenesis in a model of experimental cirrhosis produced by CCl4 administration.8 From an experimental standpoint, extrahepatic biliary obstruction caused by bile duct ligation reliably reproduces the cholestasis syndrome,9-11 and thus provides an optimal model for the study of hepatoprotective drugs. We used this model to investigate the hepatoprotective effects of SAM12 with biochemical and histopathological analyses. The present study was designed to evaluate the protective effect of two doses of SAM in a model of acute cholestasis. We measured biochemical parameters and oxidative stress, which was estimated as the equilibrium between oxidating factors (thiobarbituric acid reactive substances and oxidized glutathione [GSSG]) and antioxidant defenses (levels of reduced glutathione and activity of enzymes that control glutathione).

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 300 to 350 g at the start of the study were used. All rats were housed under habitual conditions in a temperature-controlled room (22°C) with a 12-hour light/dark cycle and free access to food and standard laboratory chow. At all times the animals received humane care in compliance with the criteria of the National Research Council.

Study Design. Two experimental groups were used: 1) animals that were sham-operated and 2) animals subjected to extrahepatic biliary obstruction (EBO) by double ligature of the common bile duct. For surgery the animals were anesthetized with ethyl ether and an abdominal midline incision was made. Muscle and peritoneal structures were dissected to expose the bile duct, which was carefully dissected and ligated with braided silk at two levels.13 Surgery in the sham-operated group was performed with the same procedure except that the bile duct was not ligated.

Each of these two groups was divided into four treatment groups (n = 6 rats per group): 1) isotonic saline solution (pH 7.4), 2) SAM solvent (L-lysine, pH 7.4), 3) SAM 5 mg/kg/d (Boehringer Ingelheim España SA, Barcelona, Spain), and 4) SAM 10 mg/kg/d. In groups 1 and 2 we administered a volume equivalent to that used for the higher dose of SAM. In all, 48 animals were used.

In all groups the route of administration was subcutaneous via a single daily injection at 9.00 am for 7 days. This route was chosen because it appears to lead to more stable levels of the drug in equilibrium.14 Twenty-four hours after the last injection each animal was weighed, anesthetized with ethyl ether, and killed by exsanguination (abdominal aorta puncture). Blood and tissues were collected and processed as described below.

Hepatic and Renal Function Parameters. Blood samples were collected with 10 IU/mL sodium heparin as an anticoagulant, then centrifuged at 1,800g for 15 minutes at 4°C to obtain plasma. The

Abbreviations: SAM, S-adenosyl-l-methionine; TBARS, thiobarbituric acid reactive substances; GSSG, oxidized glutathione; GSH, reduced glutathione; EBO, extrahepatic biliary obstruction; TG, total bilirubin; TB, total glutathione; GSHpx, GSH-peroxidase; NADPH, nicotinamide-adenine dinucleotide phosphate; GSHf, GSH-transferase.

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Received September 30, 1996; accepted February 28, 1997.

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plasma was used to measure total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (AP), and total protein (TP) as parameters indicative of hepatic function. Renal function was assessed on the basis of creatine and blood urea nitrogen (BUN) concentrations. All biochemical analyses were performed with optimized spectrophotometric techniques, using a Hitachi 737 autoanalyzer (Boehringer Mannheim, Mannheim, Germany) with selective multichannel capacity.13

Oxidative Stress in Liver and Kidney Tissues. Lipid peroxidation was determined by quantifying the reaction products with thiobarbituric acid (TBARS) under basal conditions and after induction with ferrous salts.16 The fraction enriched in cell membranes was obtained from both tissues with the method of Bossman and Hemsworth.17 Briefly, the tissue was diluted (1:10 wt:vol) in buffer containing NaCl 0.1 mol/L, KCl 5 × 10−4 mol/L, CaCl2 3.1 × 10−3 mol/L, MgSO4 1 × 10−2 mol/L, glucose 4.9 × 10−3 mol/L, NaHCO3 2.4 × 10−2 mol/L, KH2PO4 5.5 × 10−4 mol/L, and saccharose 0.32 mol/L. The sample was homogenized and centrifuged at 4°C for 15 minutes at 1,000g, and the supernatant was removed. This fraction was centrifuged at 4°C for 20 minutes at 12,000g, and the resulting pellet was resuspended in the buffer described above without saccharose at a proportion suitable for measurements of TBARS production.

To determine TBARS the tissue was divided into aliquots of 830 μL, and 100 μL of dilution buffer was added to one tube to measure basal lipid peroxidation. To other tubes we added 100 μL of one of a series of increasing concentrations of ferrous sulfate or ascorbic acid (FeAs) to measure induced lipid peroxidation. All samples were shaken and incubated at 37°C for 45 minutes, and 500 μL of 0.5% thiobarbituric acid in 20% trichloroacetic acid was then added. The samples were shaken again and incubated at 100°C for 15 minutes, then centrifuged at 4°C for 2,000g for 15 minutes. Spectrophotometric absorbance was determined in the supernatant at 532 nm (Perkin Elmer C-33201 spectrophotometer, Brook Instrument Division, IL). Blank samples were processed in the same way except that they were incubated at 4°C. The final results were expressed as μmoles of TBARS per milligram of protein, determined with the Lowry method.18 The results for FeAs were expressed as the concentration of ascorbic acid that produced 50% of the maximum concentration of ascorbic acid. The results for FeAs were expressed as the concentration of ascorbic acid that produced 50% of the maximum concentration of ascorbic acid. The results for GSHpx were expressed as units/min, and for GSSGrd were expressed as units/min divided by milligrams of protein.

Glutathione peroxidase activity (GSHpx) was measured according to the method described by Hissin and Hill.19 Enzyme activity was determined from the decrease in absorbance every 30 seconds at 340 nm, using a Hitachi 737 autoanalyzer (Boehringer Mannheim). The increase in absorbance was recorded every 30 seconds for 5 minutes.

Glutathione reductase activity (GSSGrd) was determined according to the technique described by Flohe and Gunzler.20 The amounts of sample and buffer were the same as in the GSHpx analyses. One hundred microliters of NADPH was added, and the microcuvettes were shaken by inversion and incubated as described above for GSHpx. Then 100 μL GSSG was added, the microcuvettes were again shaken by inversion, the preparation was read at 340 nm, and the decrease in absorbance was recorded every 30 seconds for 5 minutes.

Glutathione transferase activity (GSTf) was determined with the method described by Warholm et al.21 One hundred microliters of GSTf was added to the volumes of sample and buffer indicated for the other enzyme determinations. The mixture was shaken by inversion and incubated at 37°C for 3 minutes. Then 50 μL 1-chloro 2,4 dinitrobenzene was added, the mixture was shaken by inversion, and the preparation was read at 340 nm as for the other enzyme activities.

The results for GSHpx and GSSGrd were expressed as units/min, taking into account a molar extinction coefficient for NADPH of 6.22 cm2/μmol. The results for GSTf were calculated with a correction coefficient of 0.1042.

Statistical Analyses. All data in the text, tables, and figures are means ±SEM of the results in each group. Statistical analyses were performed with Statgraphics software for personal computers (Statgraphics Computer Program, STSC Inc., Rockville, MD). Distributions of the results was normal, and the Tukey test was used for multivariate analysis when significant differences between groups were found. A P value of < .05 was considered significant.

RESULTS

Effect of SAM in Sham-Operated Rats. Rats given SAM showed no significant variations in body weight or behavior during the 7-day experimental period. None of the animals died during the experimental period.

No significant alterations were found in any of the biochemical parameters, tests of hepatic function (Table 1 and Fig. 1), or tests of renal function (Table 2) in any of the four treatment groups.

In liver tissue TG increased in a dose-dependent way (Fig. 2). The effect was most notable in the GSH fraction, although at the higher dose of SAM (10 mg/kg/d) the percentage of GSSG also increased significantly (Fig. 2). Glutathione peroxidase and GSSGrd activities were also affected by SAM. Glutathione peroxidase activity increased in a dose-dependent way with both doses of the drug. GSHf activity showed significant increases only with the higher dose of SAM (Fig. 3).

Basal TBARS content was not modified; however, we noted a dose-dependent inhibition in TBARS production induced with ferrous salts (Fig. 4). The concentrations of ferrous salts that led to 50% of the maximum formation of TBARS (EC50) are shown in Table 3.

In kidney tissue, TG content increased only with the higher dose of SAM. The effect was caused entirely by an increase in GSH (Fig. 5). Moreover, of the enzyme activities tested, only GSTf increased significantly, and only in rats given the higher dose of SAM (Fig. 6). Ferrous-induced TBARS was inhibited only with the higher dose of the drug. Although,
ferrous EC$_{50}$ value are not statistically different (Fig. 7, Table 3).

**Effects of SAM in Rats With Extrahepatic Biliary Obstruction.** Rats with EBO lost weight (mean 27 ± 8.38 g) in comparison with sham-operated animals. No significant changes in body weight were seen after the administration of saline, solvent, or either dose of SAM. None of the animals died during the experimental period.

In rats with EBO the biochemical parameters indicative of cholestasis increased (total bilirubin, GGT, AP); no differences in comparison with the control group were found in any other biochemical value (Table 1 and Fig. 1). Tests of renal function showed no alterations.

The administration of SAM significantly decreased total bilirubin, GGT, and AP in EBO rats, with no significant differences between the groups given 5 or 10 mg/kg/d. There were no changes in parameters of renal function (Table 2).

In liver tissue from EBO rats there was a significant increase in GSGrd, and a significant inhibition of GSHtf. In addition we observed a significant increase in basal TBARS production and TBARS production induced with ferrous salts (Figs. 2 to 4, Table 3).

In kidney tissue, EBO significantly decreased the reduced and oxidized forms of glutathione, and significantly inhibited GSHpx activity. The production of TBARS induced with ferrous salts was also significantly increased in rats treated with saline or solvent (Figs. 4 to 6, Table 3).

In liver tissue, SAM increased glutathione levels in a dose-dependent way, leading to concentrations higher than those in sham-operated control rats (Fig. 2). If we consider the ratio % GSGrd/TG as a good indicator of oxidant stress, solvent of SAM does not significantly modify it (3.39% ± 0.8% in control group vs. 4.83% ± 0.7% in solvent group, in sham-operated rats; 9.21% ± 1.0% in control group vs. 9.45% ± 0.9% in solvent group, in EBO rats). SAM reduces this ratio both in sham-operated rats and EBO rats (sham-operated rats: 2.05% ± 0.2% with SAM-5 mg/kg/d and 2.15% ± 0.2% with SAM-10 mg/kg/d; EBO: 6.53% ± 0.5% with SAM-5, 6.40% ± 0.7% with SAM-10). Glutathione peroxidase and GSHtf activities also increased significantly in a dose-dependent way (Fig. 3). Another dose-dependent effect of treatment with SAM was the inhibition of TBARS production induced with ferrous salts (Fig. 4, Table 3).

Kidney tissue from SAM-treated rats showed increased levels of glutathione only with the higher dose of the drug. The increase reflected mainly the higher percentage of GSH (Fig. 5). The only enzyme activity that was increased by the higher dose of SAM was GSHtf (Fig. 6). This dose also significantly inhibited TBARS production induced by ferrous salts (Fig. 7, Table 3).

**DISCUSSION**

Our results show that in the rat, EBO produces oxidative stress in the liver characterized by increased TBARS and fraction GSGrd not accompanied by an antioxidant defense reaction. Oxidative stress has been related with different models of liver damage, including cholestasis. This oxidative stress alteration may be responsible for the liver tissue damage caused by cholestasis: an increase in TBARS has been shown to alter membrane functioning, to deregulate glycoprotein content in the cell membrane, and to modify the activity of certain cellular enzymes. Other mechanisms that

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**Table 1. Mean Values of Biochemical Determinations of Hepatic Function in Sham-Operated Rats and Rats With Extrahepatic Biliary Obstruction**

<table>
<thead>
<tr>
<th></th>
<th>SOR</th>
<th>Saline</th>
<th>EBO</th>
<th>SOR</th>
<th>Solvent</th>
<th>EBO</th>
<th>SAM 5 mg/kg</th>
<th>EBO</th>
<th>SAM 10 mg/kg</th>
<th>EBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP (IU/L)</td>
<td>306</td>
<td>737 ± 206*</td>
<td>112</td>
<td>35.92</td>
<td>796</td>
<td>30.21*</td>
<td>140</td>
<td>7.75</td>
<td>590</td>
<td>47.90b</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>0.8</td>
<td>23.7 ± 4.89*</td>
<td>1.09</td>
<td>0.23</td>
<td>23.56</td>
<td>2.46*</td>
<td>1.35</td>
<td>0.44</td>
<td>12.83</td>
<td>14.10</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>133</td>
<td>248 ± 46.4*</td>
<td>53.3</td>
<td>3.27</td>
<td>159</td>
<td>18.42*</td>
<td>49.8</td>
<td>4.65</td>
<td>359</td>
<td>8.57</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>34.0</td>
<td>83.7 ± 18.86*</td>
<td>17.3</td>
<td>1.95</td>
<td>46.0</td>
<td>1.75*</td>
<td>23.6</td>
<td>1.28</td>
<td>53.33</td>
<td>21.23</td>
</tr>
<tr>
<td>TB (mg/dL)</td>
<td>0.1</td>
<td>13.2 ± 0.08*</td>
<td>0.07</td>
<td>0.008</td>
<td>9.5</td>
<td>2.57*</td>
<td>0.3</td>
<td>0.07</td>
<td>5.38</td>
<td>1.91</td>
</tr>
<tr>
<td>TP (mg/dL)</td>
<td>6.0</td>
<td>6.8 ± 0.16</td>
<td>5.66</td>
<td>0.20</td>
<td>6.5</td>
<td>0.20</td>
<td>6.6</td>
<td>0.20</td>
<td>6.0</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* P < .0001 versus SOR; ap < .05, bp < .005, cp < .0001, versus saline and solvent EBO rats.

**NOTE.** To convert mg/dL to μmol/L, multiply TB by 17.1. To convert IU/L to μkat/L, multiply AP, ALT, and GGT by 0.0166.

Abbreviations: AP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TB, total bilirubin; TP, total proteins, solvent L-lysine; SAM, S-adenosyl-L-methionine.

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**Fig. 1.** Plasma total bilirubin, gamma-glutamyl-transpeptidase, and alkaline phosphatase, from 7 days sham-operated rats (□) and rats with EBO (□), without drug treatment. * P < .0001 versus SOR.
TABLE 2. Mean Values of Biochemical Determinations of Renal Function in Sham-Operated Rats and Rats With Extrahepatic Biliary Obstruction

<table>
<thead>
<tr>
<th></th>
<th>SOR</th>
<th>EBO</th>
<th>SOR</th>
<th>EBO</th>
<th>SOR</th>
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<th>SOR</th>
<th>EBO</th>
<th>SOR</th>
<th>EBO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr (mg/mL)</td>
<td>0.5 ± 0.04</td>
<td>0.26 ± 0.07</td>
<td>0.53 ± 0.01</td>
<td>0.17 ± 0.04</td>
<td>0.5 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>0.69 ± 0.01</td>
<td>0.56 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/mL)</td>
<td>13.5 ± 4.08</td>
<td>14.2 ± 1.63</td>
<td>15.82 ± 0.84</td>
<td>18.71 ± 1.27</td>
<td>11.5 ± 0.73</td>
<td>20.4 ± 0.92</td>
<td>8.06 ± 0.53</td>
<td>8.56 ± 1.46</td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: Cr, creatinine; BUN, blood urea nitrogen; solvent, t-lysine; SAM, S-adenosyl-L-methionine.

may also be involved in liver damage are alterations in plasma membrane fluidity, reduced Na⁺-K⁺-ATPase activity, and alterations in cytoskeletal function. Moreover, all these alterations are closely related with increased oxidative stress.

Treatment with SAM led to recovery of the equilibrium between oxidative damage and antioxidant defenses in rats: the drug reduced TBARS production and increased GSH contents and reduced %GSSG/TG ratio. These changes were accompanied by improvements in biochemical indicators of liver function. Our results are partially in agreement with those of Pastor et al., who showed that SAM decreased lipid peroxidation but not increased glutathione content in liver from obstruction biliary extrahepatic rats. Our study is different to these, first because they maintained the obstruction biliary extrahepatic by 28 days (secondary biliary cirrhosis), and second because they measured TG and we determined which fraction is oxidized and reduced (TG may be no affected while is possible an increase of oxidation).

Three hypotheses can be proposed to explain how SAM improves liver function in EBO. Firstly, in cholestasis, oxidative stress in the cell membrane is stimulated, as shown in this and in earlier studies. Oxidative stress impairs most membrane functions, affecting transport activities as well as the behavior of the membrane as a phospholipid substrate. Treatment with SAM reduced oxidative stress in the experimental model tested in our study (Figs. 4 and 7) suggesting that inhibition of this process improves membrane functioning and leads to recovery of normal values in biochemical parameters of liver function.

Secondly, liver damage modifies the activity of Na⁺-K⁺-
Ca²⁺-ATPase-dependent enzyme, which is needed to maintain membrane permeability and transport functions in hepatic cells and probably in other cell types, e.g., erythrocytes. The methylation of SAM prevents the inhibition of Na⁺-K⁺-Ca²⁺-ATPase-dependent activity, and may thus improve the altered biochemical profile in liver diseases.

Finally, in liver damage, cellular levels of glutathione are depleted, as was indicated by several investigators. Depletion has been related with reduced SAM-synthetase activity. This phenomenon has been explained as a result of the oxidation of the sulfhydryl groups in SAM-synthetase. Because we found excess oxidation in liver tissue in our experimental model, the increase in oxidative stress may have paralleled the excess oxidation of SAM-synthetase. Inhibition of enzyme activity may have increased glutathione depletion (Fig. 3), leading to a vicious cycle: lower glutathione > increased SAM-synthetase inhibition > lower glutathione. Exogenous SAM may act as a biochemical “bridge” for SAM-synthetase, increasing glutathione production (Figs. 3 and 6) and the capacity of this molecule for recovery (Figs. 3 and 6). This in fact is substantiated by our findings and those of others.

These three possible explanations have one factor in common: glutathione. High levels of this peptide inhibit oxidative stress and prevent SAM-synthetase oxidation. Thus the increase in glutathione levels and activity may be the main element in the hepatoprotective action of exogenous SAM in our experimental model.

A factor that should also be considered is the possibility that SAM directly affects tissue oxidative status. The results

Fig. 2. TG (GSH + GSSG), reduced form (GSH), and oxidized form (GSSG) of glutathione, in liver tissue from 7 days SORs and rats with EBO. (1) Saline, (2) solvent, (3) SAM 5 mg/kg/d s.c. (4) SAM 10 mg/kg/d s.c. *P < .05, †P < .01, ‡P < .005, §P < .001, ¶P < .0005, #P < .0001, versus solvent, except saline-OBE (versus saline-SOR).

Fig. 3. Glutathione-peroxidase (GSH-peroxidase), -reductase (GSSG-reductase) and -transferase (GSH-transferase activities, in liver tissue from 7 days SORs and rats with EBO. (1) Saline, (2) solvent, (3) SAM 5 mg/kg/d s.c., (4) SAM 10 mg/kg/d s.c. *P < .05, †P < .01, ‡P < .005, §P < .001, ¶P < .0005, #P < .0001, versus solvent, except saline-OBE (versus saline-SOR).
in our sham-operated rats suggest that SAM affects these parameters, and lend support to our findings in EBO animals.

In kidney tissue we also found that EBO animals caused oxidative stress (increased TBARS and fraction GSSG). Some investigators have related hyperbilirubinemia caused by liver cholestasis with the renal lesions that appear in this model. Others have emphasized the increased blood levels of bile acids in EBO rats, as earlier investigators have pointed out. In addition, Coratelli and Pasavant have related the decrease in bile salts in the intestinal lumen with increased reabsorption of endotoxins, a phenomenon that may account for at least some of the kidney lesions found in liver disease.

Regardless of the cause, increased oxidative stress in renal and hepatic oxidative status may have indirect repercussions on vascular function. Free radicals and TBARS inhibit the synthesis of vasodilating prostaglandins, and markedly decrease the half-life of nitric oxide, a potent endogenous vasodilator. This effect may impair blood flow, a change closely linked with renal lesions and cholestatic liver disease. Evidence suggests that ischemia and subsequent reperfusion in other tissues is related with increased TBARS levels or decreased glutathione (or both), in the model of experimental cholestasis used in the present study, we found a decrease in vascular prostacyclin (vasodilating prostaglandin) synthesis and an increase in that of platelet thromboxane (vasoconstricting prostaglandin).

We could find no mention in similar studies of renal oxidative status in models of liver damage. Our findings appear to show that increased oxidative stress in the liver of EBO rats is associated with a systemic tissue imbalance in oxidation and reduction. Several hypotheses can be proposed to account for the consequences of this increased oxidative stress in the kidney: 1) SAM may curtail the action of hepatic factors related with the kidney-liver axis; 2) the direct effect on glutathione or TBARS levels (or both) may make the kidney more resistant to increased oxidation in cholestasis, as noted above; and 3) vascular mediators may limit the effects of the imbalance through the relationship described above between oxidative status, prostanooids, and nitric oxide. SAM increases the vascular synthesis of prostacyclin in vivo; moreover, the effects of the drug are enhanced in the presence of nitric oxide precursors, and SAM may thus have some effect on renal blood perfusion.

The finding that only the higher dose (10 mg/kg/d) of SAM affected renal oxidative status suggests that kidney

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**TABLE 3. Concentrations of Ferrous Salts That Produce 50% of the Maximum TBARS (EC50) in Sham-Operated Rats and in Rats With Extrahepatic Biliary Obstruction**

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
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<tbody>
<tr>
<td></td>
<td>SOR</td>
<td>EBO</td>
<td>SOR</td>
<td>EBO</td>
</tr>
<tr>
<td>Saline</td>
<td>23.7±0.29</td>
<td>9.8±0.4</td>
<td>40.7±2.1</td>
<td>7.1±0.6</td>
</tr>
<tr>
<td>Solvent</td>
<td>25.4±2.1</td>
<td>9.01±1.5</td>
<td>47.0±6.2</td>
<td>6.6±0.4</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>20.8±2.5</td>
<td>19.1±2.1</td>
<td>48.1±6.1</td>
<td>10.5±0.9</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>91.5±8.8</td>
<td>87.7±8.6</td>
<td>51.7±7.1</td>
<td>11.9±1.5</td>
</tr>
</tbody>
</table>

**Fig. 5.** TG (GSH+GSSG), reduced form (GSH), and oxidized form (GSSG) of glutathione, in renal tissue from 7 days SORs and rats with EBO. (□) Saline, (☐) solvent, (☒) SAM 5 mg/kg/d s.c., (☐) SAM 10 mg/kg/d s.c. *P < .05, †P < .01, ‡P < .005, ††P < .001, †‡P < .0005, †††P < .0001, versus solvent, except saline-OBE (versus saline-SOR).

**Fig. 6.** Glutathione-peroxidase (GSH-peroxidase), -reductase (GSSG-reductase), and -transferase (GSH-transferase activities, in renal tissue from 7 days SORs and rats with EBO. (□) Saline, (☐) solvent, (☒) SAM mg/kg/d s.c. *P < .05, †P < .01, ‡P < .005, ††P < .001, †‡P < .0005, †††P < .0001, versus solvent, except saline-OBE (versus saline-SOR).

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Abbreviation: TBARS, thiobarbituric acid reactive substances.

* P < .01 vs. solvent.
†† P < .005 vs. solvent.
†‡ P < .0001 vs. solvent.
tissue is more resistant to the effects of this drug than is the liver. Further studies with higher doses of SAM are therefore necessary. One earlier study tested a dose of 20 mg/kg/d, but did not analyze oxidative status or renal function.

In conclusion, the administration of SAM in rats with acute extrahepatic biliary obstruction was an effective antioxidant (reduced fraction of GSSG and decreased TBARS). The drug also limited the oxidative tissue damage in the kidney seen in this experimental model.

Acknowledgment: We thank M. M. Luque Fernandez and A. Pino Blanes for their excellent technical assistance, and Karen Shashok for translating the original manuscript into English.

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22. Muriel P, Favari L, Soto C. Erythrocyte alterations correlate with CCl4 also limited the oxidative tissue damage in the kidney seen in this experimental model.


