Abnormal Hepatic Methionine and Glutathione Metabolism in Patients With Alcoholic Hepatitis

Taunia D. Lee, Mamatha R. Sadda, Michel H. Mendler, Teodoro Bottiglieri, Gary Kanel, José M. Mato, and Shelly C. Lu

Background: Abnormal methionine metabolism occurs in animals fed ethanol and in end-stage cirrhotic patients. Expected consequences of these abnormalities include reduced hepatic S-adenosylmethionine and glutathione (GSH) levels, impaired transmethylation, and reduced homocysteine catabolism, resulting in the often-observed hyperhomocysteinemia in cirrhotic patients. These parameters have not been examined simultaneously in patients with less advanced alcoholic liver disease.

Methods: Six patients hospitalized for alcoholic hepatitis were studied. Plasma was analyzed for homocysteine, methionine, and GSH levels. Liver biopsies diagnosed acute alcoholic hepatitis and underlying fibrosis. Liver specimens were processed for messenger RNA (mRNA) levels and various metabolites and were compared with those of six normal controls.

Results: Three patients had cirrhosis, and three had only portal fibrosis. Plasma levels of homocysteine and methionine were increased in two of the three patients with cirrhosis but not in the patients with fibrosis. All patients had markedly lower plasma GSH levels (mean ± SD: 0.27 ± 0.19 μM, which is at least 10-fold lower than the normal range). Hepatic S-adenosylmethionine levels were reduced by 50%, whereas methionine, GSH, and cysteine levels were reduced by 70–80%. The mRNA levels of most enzymes involved in methionine metabolism and GSH synthesis were decreased, whereas albumin expression was unchanged. Despite the well known induction of cytochrome P450 2E1 in chronic alcoholics, its mRNA levels were nearly 70% lower in these patients.

Conclusions: In alcoholic hepatitis, abnormal hepatic gene expression in methionine and GSH metabolism occurs and often contributes to decreased hepatic methionine, S-adenosylmethionine, cysteine, and GSH levels. It may be important to replenish these thiols in patients hospitalized with alcoholic hepatitis.

Key Words: Methionine Metabolism, Alcoholic Hepatitis, S-Adenosylmethionine, GSH.
determining various cellular processes, changes in methionine metabolism can have far-reaching effects.

It has long been realized that patients with alcoholic cirrhosis often have hypermethioninemia and delayed plasma clearance of methionine (Horowitz et al., 1981; Kinsell et al., 1947). Subsequent studies showed that the hypermethioninemia could be attributed to a 50–60% decrease in the activity of hepatic MAT (Cabrero et al., 1988; Duce et al., 1988). This likely contributes to decreased hepatic GSH levels in cirrhotic patients, because SAMe administration prevented the decrease in GSH (Ven demiale et al., 1989). Two genes encode for MAT: MAT1A is largely expressed in normal differentiated liver, whereas MAT2A is expressed in all extrahepatic tissues and is induced during rapid liver growth and dedifferentiation (Mato et al., 2002). Two mechanisms account for the decrease in hepatic MAT activity in cirrhosis. One is due to posttranslational covalent modification of critical cysteine residues present only in the isoenzymes encoded by MAT1A under conditions of nitrosative or oxidative stress (Mato et al., 2002), and the other is diminished MAT1A expression (Avila et al., 2000). In addition to decreased MAT1A expression, cirrhotic patients also exhibited lower hepatic methionine synthase (MS), betaine homocysteine methyltransferase (BHMT), cystathionine β-synthase (CBS), and glycine-N-methyltransferase (GNMT) expression (Avila et al., 2000). These changes were observed in the livers of patients with cirrhosis due to various etiologies who underwent liver transplantation, and whether they occur in patients with less advanced alcoholic liver disease is unclear. Furthermore, the expected outcome of decreased hepatic SAMe biosynthesis, namely, decreased hepatic SAMe levels, has yet to be demonstrated in patients with alcoholic liver disease. In this study we examined the hepatic expression of various genes involved in methionine metabolism in a group of patients hospitalized for mild to moderate alcoholic hepatitis and found abnormal expression of these enzymes to be very common regardless of the presence of cirrhosis. We also found hepatic levels of SAMe, methionine, cysteine, and GSH to be significantly reduced. Our findings suggest that thiol supplementation may be an important consideration in the treatment of these patients.

MATERIALS AND METHODS

Patients

Six patients (four men and two women, aged 46 ± 6 years) hospitalized at Rancho Los Amigos from July 2001 to April 2002 for stable alcoholic hepatitis were studied. All were chronic alcohol users (ethanol consumption >40 g/day for women and 60 g/day for men for at least 1 year). For alcoholic hepatitis, the criteria were recent binge drinking; compatible physical findings (one or more of the following: jaundice, enlarged liver, hepatic bruit, abdominal pain, loss of appetite, or nausea); and a compatible biochemical profile. The diagnosis of alcoholic hepatitis was confirmed on liver biopsy showing typical features of acute sclerosing hyaline necrosis (Edmondson et al., 1963). The degree of cirrhosis was determined on liver biopsy. Only stable alcoholic hepatitis was considered, as defined...
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by a Maddrey score (Maddrey et al., 1978) of less than 32. Patients who have a Maddrey score greater than 32 have a mortality rate of 50% during their hospitalization if they are untreated by either prednisone or pentoxifylline (Akriviadis et al., 2000). The following were excluded: patients with Child's class C cirrhosis with or without a recent history (within 1 month) of decompensated liver disease (history of ascites, encephalopathy, or variceal bleeding); patients receiving hepatotropic or known hepatotoxic medications; patients with hepatitis B or C virus, hemochromatosis, autoimmune hepatitis, or cholangiopathy. During the same period, 10 patients who satisfied the criteria were admitted to the hospital, and 6 consented to be in the study. Percutaneous liver biopsies were performed 2 to 23 days after hospital admission by using a 16-gauge 10-cm-long Klatkin needle. Fasting blood samples were drawn the same day as the liver biopsy and were analyzed for routine liver biochemistry, as well as various metabolites (described below). Each liver specimen was divided in two; one was processed for routine histology, and the other was immediately frozen in liquid nitrogen and stored at −80°C for subsequent RNA isolation and measurement of metabolites as described below.

The liver biopsy samples were evaluated by one pathologist, who was unaware of the clinical characteristics, by using the fibrosis Knodell score (Knodell et al., 1981) as modified by Ishak et al. (1995) (0–6) and a modified Chedid score (Chedid et al., 1991) for alcoholic hepatitis. This included the following: sinusoidal collagen (0–3; distribution in zone 1 or 3, perisepal, or diffuse), necroinflammatory change (0–3; mononuclear, polynuclear, or mixed type), steatosis (0–4; macrovesicular, microvesicular, or mixed), Mallory bodies (0–3), megamitochondria (0–1), cholestasis (0–1), iron (hepatocytes; 0–4), and Kupffer cells (0–2).

Healthy liver tissue was obtained from healthy liver included in the resected liver specimens of six patients with metastatic colon or breast carcinoma. Written informed consent was obtained from each patient. These tissues were immediately frozen in liquid nitrogen and stored at −80°C.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Rancho Los Amigos Medicine Center and the University of Southern California School of Medicine's human research review committees.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Studies

Levels of messenger RNA (mRNA) for genes involved in methionine metabolism and stress markers were assessed with RT-PCR. Total RNA was extracted from frozen liver biopsy samples by using TRIzol (Invitrogen, Frederick, MD). Subsequently, 2 μg of total RNA was reverse transcribed by using RETROscript (Ambion, Austin, TX) and amplified with Platinum PFX polymerase (Invitrogen) by using the PTC-200 DNA Engine (MJ Research, Watertown, MA). Primers for MS (Avila et al., 2000), BHMT (Avila et al., 2000), CBS (Avila et al., 2000), GNMT (Avila et al., 2000), MATLA (Cai et al., 1996), MAT2A (Cai et al., 1996), cytochrome P450 2E1 (CYP2E1; Rodriguez-Antonio et al., 2001), and albumin (Wang et al., 2001) have been described previously. Primers flanking intronic sequences for the glutamate cysteine ligase catalytic subunit [GCLC; GenBank (NIH, Bethesda, MD) accession No. GI 4557624], glucose-regulated protein 94 (GRP94; GenBank accession No. GI X15187), and GSH synthetase (GS; GenBank accession No. GI BC007927) were designed to circumvent amplification of possible genomic DNA contaminants. Primers for the glutamate cysteine ligase (GCL) modifier subunit were designed from GenBank No. GI 4504010. As an internal standard, 18S rRNA was amplified simultaneously by using QuantumRNA Classic II 18S (Ambion). The PCR reaction was composed of PFX amplification buffer, 0.3 mM deoxynucleoside triphosphate (New England BioLabs, Beverly, MA), 1 mM MgSO4, 18S internal standard primer and competitor mix, and 1 unit of polymerase per 50-μl reaction. Table 1 describes the primers and PCR conditions used for these genes. Amplified products were resolved on a 2% agarose gel containing ethidium bromide. Images were scanned and densitometry (Gel Documentation System, Scientific Technologies, Carlsbad, CA, and NIH Image 1.60) quantitated relative gene products. The ratio of gene product over an 18S internal control was calculated from two to three separate PCR reactions for each sample, and values for alcoholic liver disease were compared with those of the healthy livers.

### Table 1. Oligonucleotide Primers and PCR Cycling Parameters for Human Methionine Metabolism Enzymes, Transsulfuration Enzymes, and Stress Markers

<table>
<thead>
<tr>
<th>Primer sequence</th>
<th>Product size</th>
<th>18s (P:C)</th>
<th>Cycle#</th>
<th>PCR Parameters</th>
<th>Final Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT1A</td>
<td>5'-GTGAGACCTCCTCTAAAGT-3’</td>
<td>167</td>
<td>3:7</td>
<td>27 (94°C–1 min, 54°C–1 min, 72°C–1 min)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>MAT2A</td>
<td>5'-TCCCGGTCTGCACTGTC-3’</td>
<td>205</td>
<td>2:8</td>
<td>28 (94°C–1 min, 54°C–1 min, 72°C–1 min)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>MS</td>
<td>5'-AGTCGCCGCTCCGTCGCTACG-3’</td>
<td>550</td>
<td>2:8</td>
<td>39 (95°C–20 sec, 62°C–15 sec, 72°C–35 sec)</td>
<td>72°C–30 sec</td>
</tr>
<tr>
<td>BHMT</td>
<td>5'-GTGATGCAGACCTCCTCTA-3’</td>
<td>462</td>
<td>2:8</td>
<td>28 (95°C–20 sec, 55°C–1 min, 72°C–35 sec)</td>
<td>72°C–10 min</td>
</tr>
<tr>
<td>CBS</td>
<td>5'-ACATGCAAGTCTCCAGTCA-3’</td>
<td>489</td>
<td>2:8</td>
<td>34 (95°C–20 sec, 60°C–30 sec, 72°C–35 sec)</td>
<td>72°C–7 min</td>
</tr>
<tr>
<td>GNMT</td>
<td>5'-AGGAGGTCTCCTAGGTCGAGG-3’</td>
<td>605</td>
<td>2:8</td>
<td>39 (95°C–20 sec, 64°C–15 sec, 72°C–50 sec)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>GCLC</td>
<td>5'-CTCTTCAACCGGATCATATTAC-3’</td>
<td>502</td>
<td>1:9</td>
<td>37 (94°C–1 min, 58°C–1 min, 72°C–30 sec)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>GCLM</td>
<td>5'-ATGTCAGACTTACCTAGTAC-3’</td>
<td>237</td>
<td>1:9</td>
<td>38 (94°C–1 min, 58°C–1 min, 72°C–30 sec)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>GS</td>
<td>5'-GCCATCGGGCCGTGAGG-3’</td>
<td>488</td>
<td>1:9</td>
<td>38 (94°C–1 min, 60°C–1 min, 72°C–1 min)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>5'-ACAGACAGACACACAGCA-3’</td>
<td>509</td>
<td>1:9</td>
<td>39 (94°C–1 min, 60°C–30 sec, 72°C–1 min)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>GRP94</td>
<td>5'-TTCTGTCGAGGCTCACT-3’</td>
<td>559</td>
<td>2:8</td>
<td>27 (94°C–1 min, 62°C–30 sec, 72°C–1 min)</td>
<td>72°C–3 min</td>
</tr>
<tr>
<td>Albumin</td>
<td>5'-TGCTGATGTCTGAGGTCAGG-3’</td>
<td>157</td>
<td>6:4</td>
<td>17 (94°C–1 min, 61°C–1 min, 72°C–1 min)</td>
<td>72°C–3 min</td>
</tr>
</tbody>
</table>

18S (P:C), 18S ribosomal RNA primer/competer ratio; MAT1A, methionine adenosyltransferase 1A; MAT2A, methionine adenosyltransferase 2A; MS, methionine synthase; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; GNMT, glycine-N-methyltransferase; GCLC, glutamate cysteine ligase catalytic subunit; GCLM, glutamate cysteine ligase modifier subunit; GRP94, glucose-related protein 94; GS, glutathione synthetase; CYP2E1, cytochrome P450 2E1.
umes of perchloric acid (0.4 M) and centrifuged at 11,000 rpm at 4 °C for 10 min. An equal volume of borate buffer (pH 9) was added to the acid extract, and the sample was derivatized with SBDF before injection into the HPLC system.

Methionine levels were determined by HPLC via lithium ion exchange chromatography (HPLC) coupled to fluorescence detection with modification of a previously described method (Araki and Sako, 1987). Plasma samples were treated with tri- nihydrochloride to liberate homocysteine bound to proteins and were then reacted with 7-fluoro-2,1,3-benzoxaazole-4-sulfonamide (SBDF) before injection into the HPLC. Separation of the SBDF-homocysteine derivative was achieved with the HPLC system.

RESULT

Plasma and Liver Thiols, Methionine, and SAmE Levels

Total homocysteine, cysteine, and GSH were determined by high-performance liquid chromatography (HPLC) coupled to fluorescence detection with modification of a previously described method (Araki and Sako, 1987). Plasma samples were treated with tri-n-butylphosphine to liberate homocysteine bound to proteins and were then reacted with 7-fluoro-2,1,3-benzoxaazole-4-sulfonamide (SBDF) before injection into the HPLC. Separation of the SBDF-homocysteine derivative was achieved by using a C18 Aqua column, 150 × 3 mm (Phenomenex, Torrence, CA), at a flow rate of 0.25 μl/min. The mobile phase consisted of 0.1 M sodium acetate adjusted to pH 3.8. Liver samples were homogenized in 10 volumes of perchloric acid (0.4 M) and centrifuged at 11,000 rpm at 4°C for 10 min. An equal volume of borate buffer (pH 9) was added to the acid extract, and the sample was derivatized with SBDF before injection into the HPLC system.

Methionine levels were determined by HPLC via lithium ion exchange chromatography with postcolumn derivatization. Methionine eluted from the ion exchange column by pH gradient and lithium citrate buffers was reacted with ninhydrin and detected by absorbance photometry at 570 and 440 nm. The data produced by the DX 500 Amino Acid Analyzer ( Dionex, Sunnyvale, CA) were quantitated with Dionex (Sunnyvale, CA) PeakNet software. Hepatic SAmE levels were determined in the clear acid extract by HPLC as previously described (Bottiglieri, 1990).

Statistical Analysis

For comparison between healthy livers and those with alcoholic liver disease, the two-tailed nonpaired Student’s t test was used. For changes in mRNA levels, ratios of the gene of interest to internal 18S rRNA densitometric values were compared. Significance was defined by p < 0.05.

RESULTS

Patient Characteristics and Liver Histopathology

Table 2 describes the characteristics and clinical data of these patients. The clinical diagnosis for all of these patients at the time of hospital admission was alcoholic hepatitis. As can be seen from Table 3, half of the patients had cirrhosis (fibrosis scale of 6); patients 1 and 4 had Child’s grade B, and patient 5 had Child’s grade A. The other three patients had only portal fibrosis. Vitamin B6 deficiency was common; it was evident in half of these patients.

Plasma Methionine, Homocysteine, Cysteine, and GH S Levels

Table 4 summarizes the levels of plasma and hepatic methionine, homocysteine, cysteine, and GSH in these patients. For plasma levels, the values were compared with the normal range established from 12 healthy men (aged 41 ± 11 years) and 14 healthy women (aged 42 ± 10 years). For hepatic levels, values were compared with those of healthy livers. Note that plasma GSH levels were more than 90% below the normal range. Plasma methionine, cysteine, and homocysteine levels were not significantly different from the normal range. When examined individually, the homocysteine level was increased in patients 1 (13.7 μM) and 4 (23.2 μM) and was slightly increased in patient 6 (7.8 μM).
patients and five controls were compared because of an insufficient sample amount. Levels of methionine, SAMe, cysteine, homocysteine, and GSH were determined for female. Hepatic levels from six patients with alcoholic hepatitis were compared with six normal liver controls except in the case of methionine, for which only four as described in METHIONINE METABOLISM IN ALCOHOLIC HEPATITIS.

Hepatic levels of key metabolites by using percutaneously obtained liver biopsy specimens, which, to our knowledge, has not been done previously.

Table 3. Liver Histopathology

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal fibrosis (0–6)</td>
<td></td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Sinusoidal collagen (0–3)</td>
<td></td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sinusoidal collagen zoning</td>
<td></td>
<td>Diffuse</td>
<td>1, 3</td>
<td>Diffuse</td>
<td>1, 3</td>
<td>Diffuse</td>
<td>Periseptal</td>
</tr>
<tr>
<td>Necroinflammatory change (0–3)</td>
<td></td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cellularity</td>
<td></td>
<td>PMN</td>
<td>PMN</td>
<td>Mono</td>
<td>Both</td>
<td>Mono</td>
<td>Both</td>
</tr>
<tr>
<td>Fatty change (0–4)</td>
<td></td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fatty change type</td>
<td></td>
<td>Macro</td>
<td>Macro</td>
<td>Macro</td>
<td>Macro</td>
<td>Macro</td>
<td>Macro</td>
</tr>
<tr>
<td>Mallory bodies (0–3)</td>
<td></td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

PMN, polymorphonuclear cells; mono, mononuclear cells; macro, macrovesicular fat.

Table 4. Plasma and Hepatic Methionine, SAMe, Cysteine, Homocysteine, and GSH Levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma levels</th>
<th>Methionine (µM)</th>
<th>SAMe (µM)</th>
<th>Cysteine (µM)</th>
<th>Homocysteine (µM)</th>
<th>GSH (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td></td>
<td>10–42</td>
<td>Not done</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td>220 ± 78</td>
<td>6.8 ± 3.1</td>
<td>4.0 ± 2.0</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td>182 ± 56</td>
<td>5.3 ± 2.0</td>
<td>3.5 ± 1.9</td>
</tr>
<tr>
<td>Alcoholic patients</td>
<td></td>
<td>41 ± 8</td>
<td>Not done</td>
<td>212 ± 42</td>
<td>10.8 ± 6.7</td>
<td>0.27 ± 0.19*</td>
</tr>
<tr>
<td>Hepatic levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal controls</td>
<td></td>
<td>39 ± 21</td>
<td>23.6 ± 7</td>
<td>558 ± 350</td>
<td>ND</td>
<td>1472 ± 675</td>
</tr>
<tr>
<td>Alcoholic patients</td>
<td></td>
<td>12 ± 5*</td>
<td>12.5 ± 11.1*</td>
<td>119 ± 117*</td>
<td>ND</td>
<td>465 ± 199*</td>
</tr>
</tbody>
</table>

Results represent mean ± SD. Plasma levels from six patients with alcoholic hepatitis were compared with those of normal controls (n = 12 for male and n = 14 for female). Hepatic levels from six patients with alcoholic hepatitis were compared with six normal liver controls except in the case of methionine, for which only four patients and five controls were compared because of an insufficient sample amount. Levels of methionine, SAMe, cysteine, homocysteine, and GSH were determined as described in "Materials and Methods." Hepatic homocysteine levels were too low for detection in the alcoholics and two of the six controls.

ND, not detected.

*p < 0.05 versus control by unpaired Student’s t test.

μM). Both patients 1 and 4 had low B₆ levels. Hepatic levels of these metabolites were all lower. SAMe levels were approximately 50% lower than those of controls, and methionine, cysteine, and GSH levels were all 70–80% lower than those of controls (Table 4).

Hepatic Expression of Enzymes Involved in Methionine Metabolic Pathways

Figure 2 summarizes the RT-PCR results in these patients as compared with healthy liver controls. Hepatic expression of MAT1A, MS, BHMT, CBS, and GNMT was significantly lower (MAT1A by 50%; MS by 35%; and BHMT, CBS, and GNMT by 62–70%) in patients with alcoholic hepatitis as compared with normal controls. Patients with only fibrosis tended to have more normal expression (patient 3 in particular), but patient 2 had a much lower expression of all these genes despite having only fibrosis. Not all genes were reduced. MAT2A and albumin levels were not significantly different from controls.

Figure 3 summarizes the RT-PCR results of the GSH synthetic enzymes, CYP2E1 and GRP94. It is interesting to note that both GCLC and GS were 50% lower than in controls, but the GCL modifier subunit was unchanged from controls. Surprisingly, we found CYP2E1 expression to be 66% lower than controls and found GRP94, a marker for endoplasmic reticulum stress (Chedid et al., 1991; Ishak et al., 1995; Knodell et al., 1981), to be 56% lower than controls.

DISCUSSION

Alcoholic liver disease is one of the most common liver diseases encountered. A tremendous effort has been undertaken to better understand the pathogenesis of the disease (Tsukamoto and Lu, 2001). One area that has received increasing notice is altered methionine metabolism. It has been known for some time that cirrhotic patients have decreased hepatic MAT activity (Mato et al., 1997) because of inactivation of the isoenzymes (MAT I/III) encoded by MAT1A and a decrease in the expression of MAT1A (Avila et al., 2000; Mato et al., 2002). An expected consequence is a decrease in the biosynthesis of SAMe, which is likely to affect the availability of cysteine, the rate-limiting precursor for GSH synthesis (Lu, 1999) and transmethylation reactions. However, direct demonstration of this has been lacking. In addition to MAT1A, the expression of many other enzymes important in methionine metabolism was lower in cirrhotic patients (Avila et al., 2000). However, these patients were end-stage cirrhotics who underwent liver transplantation, and whether these changes occur in earlier stages of liver disease has not been examined. Our study focused on a homogeneous group of patients admitted to the hospital for mild to moderate alcoholic hepatitis and examined whether altered methionine metabolism occurs in these patients and, if so, what the likely consequences are. We examined gene expression and levels of key metabolites by using percutaneously obtained liver biopsy specimens, which, to our knowledge, has not been done previously.
We examined plasma methionine, SAMe, cysteine, homocysteine, and GSH levels. Hyperhomocystinemia occurs commonly in patients and animals with alcoholic liver injury (Cravo et al., 1996; Halsted et al., 1996; Hultberg et al., 1993; Stickel et al., 2000). Plasma homocysteine is believed to derive largely from the sinusoidal release of homocysteine by a mechanism that is poorly understood (Tsukamoto and Lu, 2001). Hyperhomocystinemia, a well recognized independent risk factor for atherosclerosis (Refsum et al., 1998), can be a result of genetic factors, such as CBS deficiency; nutritional deficiencies in folate, vitamin B6, or vitamin B12; and impaired liver function (Mudd et al., 1995; Selhub et al., 1993). Vitamin B6 deficiency was common in our patients and may have partially accounted for the increase in plasma homocysteine in those patients. In contrast to previous studies that showed increased plasma methionine levels in cirrhotics (Horowitz et al., 1981; Kinsell et al., 1947), our patients did not have significantly increased methionine levels, which may be related to the severity of their liver disease. Consistent with previous reports (Burgunder and Lauterburg, 1987; Chawla et al., 1984), our patients also had markedly lower plasma GSH levels. However, plasma cysteine levels were not lower.

Much more striking abnormalities were found in the hepatic levels of these metabolites. SAMe levels were 50% lower, whereas methionine, cysteine, and GSH levels were 70–80% lower. Results of the RT-PCR provided potential mechanisms for these changes. MAT1A expression was reduced by 50%; this can partly explain the decrease in SAMe level. Both BHMT and MS were also lower and could have contributed to a decrease in the resynthesis of methionine and a further limiting substrate for SAMe synthesis. The fact that plasma methionine levels were normal or high normal whereas the hepatic methionine levels were low is consistent with an impairment in systemic methionine handling due to reduced hepatic MAT activity and reduced

![Fig. 2. RT-PCR of liver MAT1A, MAT2A, MS, BHMT, CBS, GNMT, and albumin in patients with alcoholic hepatitis as compared with normal liver controls.](image-url)
resynthesis of methionine from homocysteine. There is another possibility—namely, impaired methionine uptake—that has not been examined and warrants consideration. CBS expression was also lower by 62%, which could have contributed to a decrease in cysteine levels. Lower SAMe levels can further exacerbate this because CBS is activated by SAMe (Mato et al., 1997). Although lower hepatic GSH levels are well known to occur in patients with alcoholic liver disease (Tsukamoto and Lu, 2001; Vendemiale et al., 1989), expression of GSH synthetic enzymes has not been examined. It is interesting to note that we found the catalytic subunit of GCL and the second enzyme in GSH synthesis, GS, both decreased by 50%, whereas the modifier subunit of GCL was unchanged. Thus, the decrease in hepatic GSH levels is likely to be multifactorial, including nutritional deficiency, decreased precursor availability, and decreased capacity to synthesize GSH. We previously showed that GCLC was induced in rats fed ethanol (Lu et al., 1999). This illustrates that frequently what is observed in animal models may not apply to humans. Nutritional deficiency may have contributed to the decrease in both methionine and cysteine levels, but this cannot explain the decrease in GCLC expression, because protein/calorie malnutrition was shown to induce GCL activity, at least in rat liver (Kim et al., 2003). The molecular mechanisms for the changes in the expression of these genes are unknown and deserve further study. Although a decrease in the expression of these enzymes may not necessarily translate to a decrease in enzyme activity, no study has shown that these enzymes are activated posttranslationally, so it is most likely that a decrease in mRNA expression would translate to a decrease in enzyme activity. Because of the limitation in the amount of liver tissue, we were unable to determine enzymatic activities.

The decrease in MAT1A and hepatic SAMe levels may play important pathogenetic roles. MAT1A knock-out mice have reduced hepatic SAMe (by 76%) and GSH (by 40%) levels (Lu et al., 2001). At 3 months, these animals are
more prone to choline-deficient diet–induced fatty liver and carbon tetrachloride–induced hepatotoxicity (Lu et al., 2001; Martínez-Chantar et al., 2002). At 8 months, the MAT1A knock-outs develop spontaneous steatohepatitis that resembles the histology of alcoholic hepatitis (Lu et al., 2001). By 18 months, most knock-outs have developed spontaneous hepatocellular carcinoma (Martínez-Chantar et al., 2002). Thus, this model illustrates the importance of maintaining normal MAT1A expression and SAMe levels in the liver, because chronic hepatic SAMe deficiency can result in a liver that is prone to further injury and malignant transformation.

We also examined CYP2E1 and GRP94 expression in our patients. CYP2E1 is constitutively expressed in liver. It is highly inducible by ethanol, solvents, and various dietary conditions (including high dietary unsaturated fats), and it is also under complex hormonal regulation (Chen et al., 1999; Woodcroft et al., 2002). The well known effect of ethanol on CYP2E1 induction is believed to be largely posttranslational, because of decreased enzyme degradation stemming from substrate stabilization (Chien et al., 1997). Others have also shown increased gene transcription in response to chronic ethanol treatment, especially when the blood ethanol level is high (Chen et al., 1999). In our patients, the CYP2E1 mRNA levels were reduced by nearly 70% as compared with controls. One possible mechanism for the decrease is malnutrition, which has been shown to result in reduced CYP2E1 mRNA levels (Kim et al., 2003).

We examined GRP94 as a marker for endoplasmic reticulum stress (Little et al., 1994) because of a recent report that it may be induced by abnormal homocysteine catabolism (Werstuck et al., 2001). However, we found a 56% reduction in the GRP94 mRNA levels in our patients. In mice, dietary energy restriction led to reduced hepatic GRP94 mRNA levels (Spindler et al., 1990), and this may also have played a role in our patients.

In summary, the mRNA levels of many but not all of the enzymes important in methionine and GSH metabolic pathways are lower in patients with mild to moderate alcoholic hepatitis, with or without cirrhosis. These changes can contribute to the decrease in hepatic methionine, SAMe, cysteine, and GSH levels. CYP2E1 and GRP94 expression were also significantly lower, and they illustrate the difficulty in extrapolating results from animal models to patients with alcoholic liver injury. Finally, the striking reduction in hepatic thiol and plasma GSH levels suggests that there may be a role for thiol supplementation in the treatment of these patients to prevent further worsening of the liver injury.

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REFERENCES


