Beneficial Role of Dietary Folic Acid on Cholesterol and Bile Acid Metabolism in Ethanol-Fed Rats*

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ABSTRACT. Objective: Cholesterol metabolism is altered by chronic ethanol consumption. In previous articles, we demonstrated the antioxidant capacity of folic acid, which may be useful in the prevention of damage provoked by ethanol. We want to determine the effects of ethanol on cholesterol and bile metabolism and whether a folic acid-supplemented diet could change alterations provoked by a chronic ethanol intake in rats. Method: We used four experimental groups: (1) control, (2) alcohol, (3) alcohol supplemented with folic acid, and (4) control supplemented with folic acid. In all the experimental groups, we measured hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and cholesterol and bile acids in serum, liver, bile, and feces. Results: We have found that the alcohol-fed groups showed high hepatic HMG-CoA reductase activity, total hepatic and serum cholesterol concentration, bile cholesterol secretion concentration, and cholesterol enterohepatic circulation. Total serum and hepatic cholesterol levels decreased when alcohol-fed rats were supplemented with folic acid. The hepatic bile acid concentration increased in both chronic ethanol groups. Folic acid supplementation significantly increased bile cholesterol secretion, the bile acids in bile, and fecal bile acid excretion in ethanol-exposed rats. The independent bile acid fraction showed no significant differences between both ethanol groups with respect to Na⁺, K⁺, and Cl⁻ concentrations. Conclusions: Folic acid increases bile flow, bile acid synthesis from cholesterol, and bile acid excretion via feces, thus provoking a decrease in serum and hepatic cholesterol. However none of these actions were observed in supplemented control rats. This, therefore, could be yet another beneficial effect of folic acid on alcoholic patients. (J. Stud. Alcohol Drugs 70: 615-622, 2009)

CHRONIC INGESTION OF ETHANOL has adverse effects on lipid metabolism, both in hepatic and extrahepatic tissues leading to the development of fatty liver, as well as hypertriglyceridemia and hypercholesterolemia (Carrasco et al., 2002; Lieber, 1992; Mini and Rajamohan, 2004). Numerous studies have correlated chronic ethanol consumption and the development of liver disease (Halsted, 1995; Polo et al., 2003; Savolainen et al., 1990). The changes induced on the liver and serum lipids by alcohol are a reflection of the interaction of ethanol metabolism with liver lipids. These changes also depend on the direct effect of ethanol and its oxidation products. After prolonged alcohol abuse, the associated malnutrition and the development of liver injury also contribute to these effects (Lieber, 1992). One of the alterations that alcohol usually provokes is serum hypercholesterolemia, which has been identified as an independent risk factor for cardiovascular disease (Walldius and Jungner, 2006).

Microsomal 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the major rate-controlling enzyme in cholesterol biosynthesis. In the liver, its activity could be altered by changes in the rates of enzyme synthesis or degradation, or by changes in the state of activity in preformed enzymes, such as what might occur through a phosphorylation–dephosphorylation reaction (Law et al., 1996). It has been reported that faster changes in cholesterol synthesis were linked to the state of enzyme phosphorylation (van der Gaag et al., 1999). Long-term alcohol intake leads to severe alterations of the rate-limiting step enzyme in cholesterol biosynthesis. Seitz et al. (1994) found that long-term ethanol consumption led to a significant increase of the mRNA for low-density lipoprotein receptors and HMG-CoA reductase.

Bile acids (BAs) are steroidal amphipathic molecules derived from the catabolism of cholesterol and are part of the cholesterol elimination route. They modulate bile flow and lipid secretion, and, therefore, BAs act in the improvement of overall liver function (Vanisree and Sudha, 2006). BAs recirculate through the liver, bile ducts, small intestine, and portal vein in an enterohepatic circuit, some being excreted in feces. There are some interactions between alcohol and BAs (Axelson et al., 1991), but there are few reports on the subject (Neuman et al., 1995; Plevris et al., 1991).

Folate deficiency is common in chronic alcoholic patients. Several studies have reported the beneficial effects of folates...
on endothelial functions, thus decreasing cardiovascular risk. To date, most studies have focused on the homocysteine-lowering effect of folate (van der Gaag et al., 2000). Similarly, observational studies have demonstrated an association between folate levels and cardiovascular morbidity and mortality (Verhaar et al., 2002). In liver, folate acts directly to produce antioxidant effects because it quenches and reacts with the reactive oxygen species generated by ethanol metabolism (Cano et al., 2001). It also prevents EF-2, RhoGDI-1, ER-60 protease, and gelsolin depletion caused by ethanol (Garcia-Rodriguez et al., 2003). However, we were unable to find any studies on the effects of folic acid on cholesterol or BA metabolism.

This study was therefore designed to investigate the effects of chronic alcohol consumption on cholesterol and BA metabolism. In addition, because folic acid has been credited with a beneficial role in preventing a range of disorders caused by ethanol (Baron et al., 1998; Kim, 1999), we studied whether supplementation of alcohol-fed rats with this vitamin partially or totally prevents the effects of ethanol on cholesterol and BA metabolism in adult rats. These results could show that folic acid can be used as a concomitant treatment in cholesterol problems, as well as in cardiovascular disease.

Method

Experimental design

Animals. This experimental work conformed to The Guide for the Care and Use of Laboratory Animals published by the National Research Council (Institute of Laboratory Animal Resources, 1996). Male Wistar rats (250-300 g) were housed in stainless-steel cages (four animals/cage) in a well-ventilated room and maintained under controlled temperature conditions (22º-23º C), with 12-hour/12-hour light/dark cycles. Diets were prepared according to the Institute of Laboratory Animal Resources (1978), which included the following (in g/kg): casein = 200; sucrose granulated = 510; cornstarch = 140; fiber, cellulose = 50; corn oil = 50; AIN-76 mineral mix = 35; AIN-76 vitamin mix = 10; choline bitartrate = 2; and DL-methionine = 3. The diet ingredients were mixed and homogenized in a double-cone blender (Rest, Haan, Germany) and given to the animals as pellets. Rats were randomly distributed into four groups of 10 rats each and received different treatments for 8 weeks: control group (C), water and rat basal diet; alcohol group (A); ethanol and rat basal diet; alcohol + folic acid group (AF), ethanol and supplemented diet that contained 8 ppm of folic acid versus the control diet, which contained 2 ppm of this vitamin; and control + folic acid group (CF), water and supplemented diet.

Ethanol treatment. Ethanol treatment was previously described by our research group (Fernández-Borrachero et al., 1998). Alcohol-fed animals were started on tap water that contained 5% v/v alcohol in the first week (initial time). Alcohol concentration was increased to 10% in the second week, 15% in the third week, and 20% in the fourth week; finally, a 30% solution was maintained for 1 month. The diet and ethanol solution were provided ad libitum. Control groups drank water ad libitum during the 8 weeks. The average intake of lipid and folate and body and liver weights were measured at the end of the experimental period. Each measurement was recorded at 9:00 AM. Ethanol intake was calculated as previously described by Veale and Myers (1968).

Bile flow rate. At the end of the experimental period, the rats were fasted for 24 hours and then anesthetized with intraperitoneal urethane 28% w/v (0.5 ml/100 g of body weight). To determine the bile flow rate, the abdomen was opened by a midline incision and the bile duct was cannulated using a polyethylene catheter. After 30-60 minutes, bile was collected for 1 hour into preweighed tubes for cholesterol and BA determination. Bile volume was determined gravimetrically with an assumed density of 1 g/ml. BA output was calculated by multiplying the bile flow rate by BA concentration. BA and total cholesterol in bile was determined by using an enzymatical procedure with commercial kits (Sigma-Aldrich, St. Louis, MO; Roda et al., 1982; Svensson, 1982). The dependent and independent BA fractions were also determined in bile. In the independent BA fraction, Na+, K+, and Cl– secretions were measured by a flame photometer (Digiflame 2000; GDV, Italy).

Liver samples. At the end of the experiments, the liver was perfused through the portal vein with ice-cold physiological serum until most of the blood content of the livers was washed out. The liver was quickly removed, debrided of adipose and connective tissues in ice-cold saline, and weighed. The samples were immediately stored at -80º C. The organosomatic index was calculated as the ratio between liver weight and 100 g of body weight.

Blood samples. Total blood was collected by heart puncture, and portal vein blood was obtained by cannulation with a polyethylene catheter. Serum from both blood sources was separated by centrifugation (4º C for 10 minutes at 800 g), and BAs and total cholesterol were determined in both sera.

Feces samples. To collect feces, the rats were housed individually in metabolic cages with free access to food and liquid diet during the 48th hour. BA excretion was determined using the same methods for bile, serum, and liver. However, for BA excretion determination, we used an extraction method (Crowell and Macdonald, 1980) that had been slightly modified by our group.

Preparation of hepatic microsomes

For microsome preparation (microsomal fraction) (Molina et al., 1989), the livers were excised, weighed, and washed in
an ice-cold homogenization medium. All subsequent operations were carried out at 4°C. Livers were homogenized in a Potter-Elvehjem homogenizer (Genscript Corp., Piscataway, NJ) with an ice-cold homogenization medium containing 0.25 M NaCl, 50 mM imidazol (pH 7.4), 20 mM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol, and 50 mM NaF. In the presence of NaN (an inhibitor of the dephosphorylation), the measured activity of the enzyme reflected the activity that was presented initially in the tissue (active form). Each homogenate was centrifuged in a Beckman centrifuge J2-21 class, IA-17 rotor (Beckman Coulter, Fullerton, CA) for 20 minutes at 15,000 g to remove particulate matter. The supernatant was collected, and centrifugation (15,000 g) was repeated. The 15,000 g supernatant was centrifuged at 105,000 g for 60 minutes in a Sorvall OTD 50 B ultracentrifuge (Thermo Fisher Scientific, Inc., Waltham, MA). The resulting microsomal pellets were immediately frozen in liquid nitrogen and stored at -80°C until assays. The storage time did not result in a significant loss of enzyme activity (results not shown).

**HMG-CoA reductase activity**

Reductase activity was measured as described by Shapiro et al. (1974), with slight modifications by Molina et al. (1997). This method measures the formation of radioactive mevalonate from labeled HMG-CoA. One hundred microliters of pre-incubated medium containing 0.2-0.4 mg microsomal protein, as well as the components present in the homogenization medium, were incubated at 37°C for 15 minutes. Then, 50 μl of solution—containing 0.25 M NaCl, 50 mM imidazol (pH 7.4), 20 mM EDTA, 5 mM dithiothreitol, and 50 mM NaF—were added to the pre-incubated mixture and then incubated for 30 minutes at 37°C. The reaction was stopped by adding 25 μl of 5 N HCl. 14C-mevalonate that was formed was converted into labeled lactone, isolated by thin-layered chromatography and counted by liquid scintillation using an internal standard of 3H-mevalonate to correct for incomplete recovery.

**Protein assay**

Microsomal protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as standard.

**Lipids analysis**

The microsomal fraction was divided into aliquots for assays of total and esterified cholesterol. Total cholesterol in microsomes, serum, and bile was determined by an enzymatic procedure based on the oxidase/peroxidase cholesterol method with commercial kits from Atom (Atom Diagnostico Clinico y Laboratorio, Barcelona, Spain; Allain, 1974; Svensson, 1982). To determine esterified cholesterol, we previously used enzymatic reagents that degrade free cholesterol (Yamaguchi et al., 1981) so that the esterified cholesterol could be measured by using 1 ml of reagent solution for the total cholesterol test. Hepatic BA concentration was determined using the same methods that were used to measure bile and serum.

**Statistical analyses**

The results are expressed as mean ± SEM. Data were analyzed using a statistical program (GraphPad InStat 3; GraphPad Software, La Jolla, CA) by the unpaired Student’s t test between C and CF groups, and A and AF groups. A p value less than .05 was considered to be statistically significant.

**Results**

Table 1 illustrates that ethanol-fed rats (A and AF) had similar liver weight, hepatic organosomatic index (%), and body weight. They also showed similar lipid and ethanol intake. The same occurred in control groups (C and CF). Confirming the treatment, supplemented rats (AF and CF) showed a significant increase in folic intake above A and C groups, respectively.

Hepatic HMG-CoA reductase activity (Figure 1) was similar in non-alcohol-treated rats (C and CF). Folic acid administration did not alter HMG-CoA reductase activity in ethanol-receiving animals.

Figure 2 illustrates the results of bile flow per 15 minutes (μl/15 minutes) in basal conditions without stimulation. The alcohol folic group presented a significant increase in bile flow with respect to the alcohol group. There were no differences between the C and CF groups.

The serum cholesterol level decreased significantly in alcohol-fed rats when they received folic acid; the same also occurred with total and free hepatic cholesterol (Table 2). Alcohol-fed rats (A and AF) showed a high concentration of bile cholesterol secretion and cholesterol enterohepatic circulation. Bile cholesterol secretion in AF rats was significantly higher than in the A groups (Table 2). However, in control rats, folic acid administration did not alter any of these parameters.

Table 3 illustrates that all the groups had similar BA serum concentrations. However, both chronic ethanol groups had high hepatic BA concentrations that were significantly higher in folic acid-supplemented rats. With respect to biliary BA concentration, the supplemented ethanol-treated rats presented a significantly higher concentration than the alcohol-fed rats. There were no significant differences in enterohepatic BA values (Table 3) between groups. BAs in fecal excretion presented significantly higher levels in
TABLE 1. Body weight, liver weight, and organosomatic index liver at the end of experimental period; lipid, ethanol, and folate intakes during the whole experimental procedure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 10) Mean (SEM)</th>
<th>Alcohol group (n = 10) Mean (SEM)</th>
<th>Alcohol + folic acid group (n = 10) Mean (SEM)</th>
<th>Control + folic acid group (n = 10) Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>426.6 (9.3)</td>
<td>328.7 (9.6)</td>
<td>328.5 (9.8)</td>
<td>435.4 (9.7)</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>9.8 (0.3)</td>
<td>9.6 (0.4)</td>
<td>9.1 (0.3)</td>
<td>9.9 (0.3)</td>
</tr>
<tr>
<td>Organosomatic index liver, %</td>
<td>2.29 (0.05)</td>
<td>2.92 (0.07)</td>
<td>2.77 (0.06)</td>
<td>2.27 (0.05)</td>
</tr>
<tr>
<td>Lipid intake, g/rat/day</td>
<td>1.0 (0.04)</td>
<td>0.6 (0.02)</td>
<td>0.6 (0.03)</td>
<td>1.1 (0.03)</td>
</tr>
<tr>
<td>Ethanol intake, g/kg body weight/day</td>
<td>–</td>
<td>15.9 (1.4)</td>
<td>16.7 (1.7)</td>
<td>–</td>
</tr>
<tr>
<td>Folate intake, μg/rat/day</td>
<td>38.67 (1.53)a</td>
<td>22.48 (0.95)b</td>
<td>103.34 (4.23)</td>
<td>178.6 (4.35)</td>
</tr>
</tbody>
</table>

Notes: Lipid, ethanol, and folate intakes were the average of all the experimental procedures. Results are mean (SEM) of 10 animals in each group. Alcohol vs alcohol + folic acid group: ap < .001; control vs control + folic acid group: bp < .001.

FIGURE 1. HMG-CoA reductase activity (nmol/mg/minute). Results are mean ± SEM of 10 animals in each group. C = control group; A = alcohol group; AF = alcohol + folic acid group; CF = control + folic acid group. ***A vs C: p < .001; aaaa A vs CF: p < .001; bA vs AF: p < .05.
ethanol-supplemented rats than in nonsupplemented ones. In control rats, folic acid administration did not alter any of these parameters (Table 3).

The independent BA fraction showed no significant differences between the groups, with respect to mean (SEM) Na⁺, K⁺, and Cl⁻ concentrations (Na⁺: C = 161.98 [2.4], A = 161.48 [1.6], AF = 164.66 [4.12], CF = 162.83 [3.4]; K⁺: C = 5.4 [0.14], A = 4.95 [0.01], AF = 4.85 [0.3], CF = 5.15 [0.24]; and Cl⁻: C = 160.23 [6.88], A = 167.46 [6.9], AF = 175.01 [8.5], CF = 163.16 [7.67]).

![Figure 2](image_url)  
**FIGURE 2.** Bile flow per 15 minutes (μl/15 minutes) in basal conditions without stimulation. Results are mean ± SEM of 10 animals in each group. C = control group; A = alcohol group; AF = alcohol + folic acid group; CF = control + folic acid group; microl = microliters. A vs AF: *p < .05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n = 10) Mean (SEM)</th>
<th>Alcohol group (n = 10) Mean (SEM)</th>
<th>Alcohol + folic acid group (n = 10) Mean (SEM)</th>
<th>Control + folic acid group (n = 10) Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum total cholesterol, mg/dl</td>
<td>96.7 (8.7)</td>
<td>112.17 (9.5)</td>
<td>82 (7.0)</td>
<td>94.78 (8.3)</td>
</tr>
<tr>
<td>Hepatic, mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>17.24 (1.8)</td>
<td>31.8 (3.4)</td>
<td>14.9 (0.9)</td>
<td>15.8 (1.2)</td>
</tr>
<tr>
<td>Ester cholesterol</td>
<td>9.7 (0.9)</td>
<td>8.12 (0.56)</td>
<td>7.03 (0.8)</td>
<td>8.02 (0.65)</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>7.54 (0.8)</td>
<td>23.68 (2.4)</td>
<td>7.87 (0.73)</td>
<td>7.78 (0.67)</td>
</tr>
<tr>
<td>Biliary total cholesterol secretion, mg/60 minutes</td>
<td>0.078 (0.004)</td>
<td>0.15 (0.013)</td>
<td>0.26 (0.02)</td>
<td>0.076 (0.005)</td>
</tr>
<tr>
<td>Enterohepatic circulation total cholesterol, mg/dl</td>
<td>46.0 (3.1)</td>
<td>68.34 (5.6)</td>
<td>67.1 (3.6)</td>
<td>44.7 (3.4)</td>
</tr>
</tbody>
</table>

**Notes:** Results are mean (SEM) of 10 animals in each group. Alcohol vs alcohol + folic acid group: *p < .05; **p < .001.
Discussion

Confirming the treatment, supplemented rats showed a significant increase in folic intake. This fact did not, however, alter body or hepatic weight.

Chronic ethanol consumption increases serum and hepatic cholesterol levels, despite the fact that there is a decrease in lipid intake. This trend is the same as that found by other authors (Asha and Indira, 2004; Daher et al., 2003; Lamisse et al., 1994). We have observed that supplementation with folic acid diminished these values and that it played a beneficial role in lipid metabolism, as well as in reducing cardiovascular disease risk.

In our study, HMG-CoA reductase activity increases in ethanol-fed rats. A similar increase had previously been reported by other authors in patients with liver disease (Shoda et al., 2001) and in ethanol liver microsomes in chicks (Sanchez-Amate et al., 1991). In AF rats, we found high levels of this enzyme; therefore, folic acid supplementation in alcohol-treated rats did not affect HMG-CoA reductase activity (the major rate-controlling enzyme in cholesterol biosynthesis). However, we found a rise in serum and hepatic cholesterol levels in ethanol-fed groups that decreases when the animals were treated with folic acid. This slight increase in serum cholesterol levels may be attributable to an increase in HMG-CoA activity, biliary cholesterol secretion, and enterohepatic cholesterol circulation. Supplemented alcohol-treated rats secreted significantly more cholesterol in bile than their nonsupplemented counterparts. They also presented a higher enterohepatic cholesterol circulation than did control groups, which was similar to the A groups. Thus, proportional to the bile cholesterol secretion, in AF groups, there is a decrease in enterohepatic cholesterol circulation with respect to the A groups.

In alcohol-treated rats, we observed an increase in hepatic cholesterol, consistent with a high level of HMG-CoA activity. This increase was observed as hepatic-free cholesterol because all the groups presented the same hepatic cholesterol esters values. Supplementation with folic acid decreased these values, playing a beneficial role in cholesterol homeostasis. This fact suggests that, in ethanol-fed rats (A), hepatic cholesterol esterification is insufficient, perhaps because the level of enzyme acyl-CoA: cholesterol acyltransferase—responsible for hepatic cholesterol esterification—does not increase. The accumulation of free cholesterol in hepatocytes is very important, because the only way to diminish it is by esterification of free cholesterol that is ultimately available for secretion in lipoprotein or bile. Therefore, cholesterol esterification plays an important role in protecting the cell from free cholesterol accumulation (Stone et al., 1989).

BAs are derived from the catabolism of cholesterol. Because they modulate bile flow, they are an important element in cholesterol metabolism. However, the role of BAs in modulating chronic ethanol cytotoxicity has not yet been explored (Montet et al., 2002). In our experiment, after ethanol treatment, bile flow was similar to that in control rats. When Alvaro et al. (1995) measured the bile flow and biliary bile salt secretion before and after exposure to low or high doses of ethanol in vitro, they found an inhibitory effect of ethanol on bile secretion in the isolated perfused rat liver. However, the dose they used was different from ours and it was, as previously described, an in vitro study. Our data are in consonance with the in vivo study of Montet et al. (2002). Surprisingly, in our research, ethanol (concomitantly with folic acid) induced a significant increase in bile secretion.

The increase in bile flow and the significant enhancement of total hepatic BA concentration in AF groups clearly indicated true choleretic properties of the folic acid in the biliary salt dependent fraction. Kajiyama et al. (1998) determined the bile volume and total BA production in rats following intraperitoneal administration of folic acid. These authors reported that folic acid has a newly described pharmacologic effect, stimulating BA-dependent choleresis. However, in a recent prospective clinical study with patients who had periampullary tumors (Karakayali et al., 2003), no choleretic effect of intravenous folic acid administration was found. In

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<th>Control + folic acid group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum BA, µmol/L</td>
<td>37.7 (5.6)</td>
<td>32.9 (3.7)</td>
<td>26.2 (2.6)</td>
<td>33.7 (4.6)</td>
</tr>
<tr>
<td>Hepatic BA, µmol/g</td>
<td>1.96 (0.4)</td>
<td>5.4 (0.55)</td>
<td>8 (0.9)</td>
<td>2.13 (0.22)</td>
</tr>
<tr>
<td>Biliary BA secretion, µmol/15 minutes</td>
<td>1.3 (0.16)</td>
<td>1.6 (0.18)</td>
<td>2.4 (0.34)</td>
<td>1.4 (0.15)</td>
</tr>
<tr>
<td>Enterohepatic circulation BA, µmol/L</td>
<td>167.53 (19.84)</td>
<td>168.07 (18.78)</td>
<td>147.54 (15.94)</td>
<td>168.36 (19.84)</td>
</tr>
<tr>
<td>Fecal BA excretion, µmol/24 hours</td>
<td>42.78 (7.44)</td>
<td>37.35 (8.06)</td>
<td>94.55 (9.3)</td>
<td>44.25 (7.93)</td>
</tr>
</tbody>
</table>

Notes: Results are mean (SEM) of 10 animals in each group. BA = bile acid. Alcohol vs alcohol + folic acid group: *p < .05; **p < .001.
any case, we have observed that folic acid had a remarkably high choleretic action when we supplemented it to ethanol-treated rats. Control-supplemented rats did not change their choleretic properties. Therefore, it would appear that the choleretic effect of folic acid depends on the subject’s basal conditions. In this case, the ethanol-fed rats are folic acid deficient.

In our study, chronic ethanol consumption and folic acid treatment did not modify the serum BA concentration. Jonsson et al. (1992) determined the BA concentrations in serum and fecal excretion of BAs in patients with liver cirrhosis as a consequence of alcohol abuse, finding serum BA elevations. However, in our research, no changes in the synthesis of BAs or in synthesis pathways were observed. Ackehed et al. (1996) found that fecal BA excretion in patients increased after alcohol abuse, but we did not find differences between the ethanol-fed groups and the control groups. However, a significant increase in the fecal BA excretion in the alcohol folic acid group was observed. This change was not observed in supplemented control rats.

Based on the data obtained, we suggest that the alterations provoked by ethanol consumption in the biliary metabolism depend on biliary acid hepatic synthesis. Ethanol produces an accumulation of BA in the liver and does not modify BA secretion and fecal excretion. Consequently, serum BA is not modified in the ethanol-fed groups. Supplemented alcohol-treated rats showed a significant increase in hepatic BA concentration, bile flow, and BA concentration with respect to the nonsupplemented rats. They presented the lowest enterohepatic BA circulation value; therefore, proportionally to BA secretion, in AF groups there is a significant decrease in enterohepatic BA circulation with respect to all of the other groups. The serum BA levels were also slightly lower than in the other groups. This may be the result of a slight decrease in enterohepatic BA circulation and a large increase in fecal BA excretion. These results indicated that folic acid supplementation could exert a regulatory effect on maintaining the biliary acids’ homeostasis during ethanol ingestion.

St.-Pierre et al. (2001) reported that BAs have been involved in the regulation of all the key enzymes involved in cholesterol homeostasis. Our ethanol-fed rats (groups A and AF) showed high HMG-CoA reductase activity. It is probable that 7α-hydroxylase was also induced because hepatic biliary acid increased in these groups.

Jimenez et al. (1986) concluded that the cholestasis observed following diethyl maleate administration coincided with falls in the biliary secretion of Na⁺, Cl⁻, and bicarbonate. In our study, no significant changes were observed in the Na⁺, K⁺, and Cl⁻ concentrations in bile. We suggest that the hypercholeresis observed in the ethanol-folic acid group was in part the result of a higher biliary salt-dependent fraction contribution.

Our findings suggest that supplemental folate benefits only individuals with a low plasma folate concentration (chronic ethanol treatment) that occurs with other actions of folate (Martinez et al., 2006). In all of the experiments, C values were similar to CF values.

In conclusion, ethanol produced an increase in cholesterol synthesis, bile cholesterol secretion, enterohepatic cholesterol circulation, and BA hepatic synthesis. Folic acid supplementation decreased serum and hepatic cholesterol values in alcohol-treated rats, despite the lack of change in HMG-CoA activity. This could be explained by the fact that folic acid increases BA hepatic synthesis, fecal BA excretion, and bile cholesterol secretion and decreases enterohepatic BA and cholesterol circulation. The liver is the major source of serum cholesterol, and its concentration depends on the balance of the hepatic synthesis and catabolism, wherein BA plays a very important role. Therefore, folic acid increases bile flow, BA synthesis from cholesterol, and BA excretion by feces, thus provoking a decrease in serum and hepatic cholesterol. However, these actions are observed only in ethanol-consuming rats, not in their supplemented control counterparts. This might be another beneficial effect of folic acid on alcoholic patients.

References


