Recent studies have focused on establishing a link between the pathogenesis of ethanol and the disruption of metabolic pathways in the liver. Ethanol alters hepatic methionine metabolism, leading to perturbation of S-adenosylmethionine-dependent transmethylation. Therefore, the supply of metabolically related nutrients such as folate may play a role in the hepatotoxic effects of ethanol.

Key words: methionine, liver, ethanol, transmethylation, folate

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INTRODUCTION

Ethanol is a known toxicant that has adverse effects on many tissues and organ systems and disrupts metabolic processes. One of the tissues most susceptible to the toxic effects of ethanol is the liver, where chronic exposure results in the accumulation of fat (i.e., hepatic steatosis), followed by steatohepatitis, cirrhosis, and ultimately liver failure. In addition to the direct actions of ethanol on cellular toxicity, the interaction of ethanol with various nutrients and/or their metabolism may be the basis for some of its adverse effects as well. Understanding the relationship between ethanol administration and the metabolism/function of folate and methyl groups has been the focus of research for a number of years.

FOLATE, METHIONINE, AND HOMOCYSTEINE METABOLISM

Folate, methyl group, and homocysteine metabolism (Figure 1) are interrelated metabolic pathways important in the maintenance of optimal health. Perturbation of these pathways has been associated with numerous pathologies, including cardiovascular disease, cancer development, birth defects, and neurological disorders. As a coenzyme in the one-carbon pool, folate functions to transfer one-carbon units for the synthesis of thymidylate, a key step in DNA synthesis, and to supply methyl groups for S-adenosylmethionine (SAM)-dependent transmethylation reactions. Two key enzymes linking the one-carbon pool and methyl group metabolism are 5,10-methylene-tetrahydrofolate reductase (MTHFR) and the B12-dependent enzyme methionine synthase.

MTHFR irreversibly reduces the methylene group of 5,10-methylene-tetrahydrofolate to the methanol oxidation state as 5-methyl-tetrahydrofolate, whereupon methionine synthase serves to transfer the methyl group to remethylate homocysteine and generate methionine. In addition to protein synthesis, the essential amino acid methionine provides the necessary methyl groups, originating from both folate-dependent and folate-independent sources, for biological methylation. Although the metabolism of folate and methyl groups occurs in most cells, the liver plays a central metabolic role in this regard because: a) half of the body folate pool is hepatic; b) a large percentage of all SAM-dependent transmethylation reactions occur in the liver; and c) a number of the key enzymatic and regulatory proteins related to these pathways are liver specific. Thus, perturbation of the hepatic metabolism of folate, homocysteine, and methyl groups may have profound effects on other tissues throughout the body.

Hepatic methionine adenosyltransferase activates methionine to SAM, the ubiquitous source of methyl groups for a number of transmethylation reactions, including the methylation of DNA and the synthesis of phosphatidylcholine. SAM-dependent transmethylation reactions result in the formation of S-adenosylhomocysteine (SAH), a product that is immediately hydrolyzed to homocysteine and adenosine by the action of SAH hydrolase. Because SAH is a potent inhibitor of most SAM-dependent transmethylation reactions, the intracellular ratio of SAM to SAH is an important regulatory signal for the methylation capacity of the cell. In this...
regard, a key protein in the regulation of methyl group supply and utilization is the enzyme glycine-N-methyltransferase (GNMT), an abundant cytosolic SAM-dependent methyltransferase that is less sensitive to the inhibitory action of SAH. Homocysteine can be re-methylated back to methionine by the folate-dependent pathway, or it can be irreversibly catabolized through the transsulfuration pathway by the action of cystathionine β-synthase.

The transsulfuration pathway is important in the synthesis of cysteine and glutathione, as well as in the removal of homocysteine. In addition to the folate-dependent route for homocysteine remethylation, the liver also possesses a second pathway that is independent of folate. Betaine, derived from the oxidation of choline, serves as a source of methyl groups for the remethylation of homocysteine, a reaction catalyzed by betaine homocysteine S-methyltransferase (BHMT).

As mentioned previously, two key SAM-dependent transmethylation reactions that are important in discussing the adverse effects of ethanol are the methylation of DNA and the synthesis of phosphatidylcholine from phosphatidylethanolamine, which are catalyzed by DNA methyltransferase and phosphatidylethanolamine N-methyltransferase (PEMT), respectively. DNA methylation represents an epigenetic mechanism for the regulation of gene expression, whereas adequate phosphatidylcholine production is required for the synthesis and secretion of very-low-density lipoproteins from the liver. Perturbation of these processes, such as under conditions of methyl group deficiency, have been shown to result in hypomethylation of DNA, elevated expression of specific oncogenes, lipid accumulation, and hepatocarcinogenesis.

**ETHANOL AND METHIONINE METABOLISM**

A number of animal models, including rat, baboon, and micro pig, have been utilized to examine the effects of ethanol consumption on folate and methionine metabolism. For all of these studies, animals were typically provided an ethanol-containing diet at a level of about 40% of total calories for various treatment times ranging from 16 hours to 12 months. For rat studies, a consistent finding was the depletion of the hepatic concentrations of SAM and glutathione and a decrease in the ratio of SAM to SAH. Moreover, the ability to restore methyl group supply via the folate-dependent pathway (i.e., methionine synthase) was also significantly reduced, whereas the enzymatic activity of BHMT and cystathionine β-synthase were elevated. Ethanol appears to enhance the catabolism and loss of methyl groups, thereby compromising SAM-dependent transmethylation reactions. It has been shown that ethanol consumption can result in the hypomethylation of DNA and the accumulation of lipid in the liver. A loss of methyl groups owing to ethanol consumption would be further exacerbated by an increase in the hepatic activity of methionine adenosyltransferase and the subsequent action of GNMT, as reported previously.
The administration of either SAM or betaine attenuates the effects of ethanol on SAM and glutathione depletion, as well as the development of a fatty liver.\textsuperscript{12,14,18} Findings similar to those discussed above have been reported using the baboon as a primate model.\textsuperscript{19} In addition to hepatic tissues, ethanol has also been shown to deplete SAM and the SAM/SAH ratio in rat colon, resulting in global hypomethylation of DNA.\textsuperscript{20}

In addition to rats and baboons, similar changes have been reported by Halsted et al. in a series of studies using the micropig as a model. In the first study, ethanol (40% of calories) was provided to Yucatan micropigs for up to 12 months.\textsuperscript{21} The researchers found that ethanol increased methionine catabolism, as indicated by the decrease (13%–32%) in serum methionine, serine, glycine, and cystathionine levels, whereas the circulating concentrations of homocysteine and cysteine were elevated (32% and 10%, respectively). The hepatic concentrations of SAH were elevated 41%, whereas the activity of methionine synthase and the ratio of SAM to SAH were diminished by 20% and 36%, respectively. Contrary to earlier studies, no change was observed in the hepatic concentration of SAM. These data suggest that ethanol consumption by micropigs results in diminished folate-dependent remethylation and enhanced transsulfuration. Moreover, the ratio of dUTP/dTTP was elevated, and there was a marked increase in apoptotic bodies (3.4-fold) and cell proliferation (6.2-fold).

\textbf{ETHANOL AND FOLATE STATUS}

From these findings, Halsted et al. postulated that folate status could play a significant role in modulating the sensitivity of cells/tissues to the adverse effects of ethanol.\textsuperscript{22} To examine this, the investigators used their micropig model in which control and ethanol-fed (40% of calories for 14 weeks) animals were maintained on either a folate-sufficient (14.5 \textmu g/kg body weight) or a folate-deficient (vitamin mix devoid of folate) diet. Hepatic folate concentrations were depleted approximately 50% as a result of the folate-deficient diet. As seen previously, ethanol feeding decreased the hepatic activity of methionine synthase and the concentrations of SAM and glutathione, whereas hepatic and plasma homocysteine concentrations were elevated.

Both ethanol and a folate-deficient diet increased the hepatic concentration of SAH and reduced the SAM/SAH ratio. The SAM/SAH ratio in control rats (2.74 ± 0.21) was reduced further (0.83 ± 0.11) by the combination of ethanol and folate deficiency. However, an increase in DNA hypomethylation and the activity of BHMT was only observed in the folate-deficient animals; both ethanol and folate deficiency increased DNA strand breaks. Ethanol feeding increased plasma alanine aminotransferase and aspartate aminotransferase activity, markers for liver toxicity and dysfunction. Moreover, a folate-deficient diet elevated aspartate aminotransferase activity 3.2-fold above the values exhibited by ethanol alone. In support of this interaction, only in the folate deficient-ethanol group did micropigs (5 out of 6) exhibit hepatic steatosis. Thus, it appears that a lack of dietary folate in combination with ethanol feeding enhances metabolic perturbation of methionine metabolism, with the subsequent damage to DNA and liver tissue.

In an extension of this study using samples from the same micropigs fed ethanol and a folate-deficient diet, Villanueva and Halsted\textsuperscript{23} evaluated the potential mechanistic basis for the changes in metabolite concentrations by using quantitative, real-time PCR analysis to determine the expression of key proteins and measure their enzymatic activity. For folate-dependent homocysteine remethylation, both folate deficiency and ethanol decreased the transcript abundance and activity of MTHFR. Ethanol decreased methionine synthase activity, but did not affect the transcript level, implying that the changes in its activity may be at the post-translational level. For methionine (i.e., methyl group) metabolism, the activity of methionine adenosyltransferase III was diminished by ethanol, whereas the transcript level of hepatic methionine adenosyltransferase was reduced by both ethanol and folate deficiency. For SAH hydrolase, both the transcript level and activity of the protein were reduced by folate deficiency and ethanol; there was an additive effect on activity, but not transcript abundance.

These findings would explain in part the reduced levels of methionine and SAM, as well as the elevations in SAH observed in the livers of these same animals in the previous report.\textsuperscript{22} The concomitant reduction in the ratio of SAM to SAH would significantly compromise the methylation capacity of the cell. The increased homocysteine concentrations appear to be the result of a diminished folate-dependent remethylation pathway. The changes in the regulatory protein GNMT were of interest in that both folate deficiency and ethanol reduced its transcript level; however, the activity of GNMT was increased by ethanol feeding regardless of dietary folate. Taken together, these results clearly indicate that the folate-dependent supply and utilization of methyl groups were compromised by ethanol and folate deficiency, and that the interaction between ethanol and folate was additive with respect to some of the changes observed in the regulation of key enzymes.

The decrease in expression and activity of MTHFR, in conjunction with reduced methionine synthase activity, would be expected to result in diminished levels of 5-methyl-tetrahydrofolate. Because 5-methyl-tetrahydrofolate is an allosteric inhibitor of GNMT,\textsuperscript{24} this may explain the increase in GNMT activity despite a reduc-
phosphatidylcholine. It might be expected that the ability of choline for SAM-independent synthesis of the activity of BHMT, thereby compromising the availability of very-low-density lipoprotein synthesis and secretion.

However, ethanol has been reported in rats to increase the activity of BHMT, thereby compromising the availability of choline for SAM-independent synthesis of phosphatidylcholine. It might be expected that PEMT would be elevated in a compensatory fashion, but without effect due to the depletion of methyl group supply as a result of reduced concentrations of SAM and increased GNMT activity. Thus, the lack of phosphatidylcholine synthesis would contribute to the development of hepatic steatosis. This possibility is supported by the observations that betaine supplementation attenuated the effects of ethanol on depleting SAM and inducing hepatic steatosis in the rat, although BHMT activity was not altered by ethanol feeding in the micropig model.

SUMMARY AND FUTURE STUDIES

The results from these studies clearly demonstrate that ethanol consumption has adverse effects on the expression and activity of many enzymes involved in methyl group metabolism, and folate deficiency may exacerbate these effects. These changes in the regulation of key proteins begin to provide a mechanistic explanation for the metabolic anomalies and liver toxicity reported in many previous studies. However, it must also be noted that many of these key enzymes are also regulated at a post-translational level, and thus the collective metabolic outcome from disruption of these pathways in vivo is difficult to predict based solely on enzyme activity and expression. For example, SAM is an allosteric inhibitor of MTHFR and a positive modulator of cystathionine β-synthase activity, whereas GNMT is inhibited by 5-methyl-tetrahydrofolate binding and activated by phosphorylation. Thus, it will ultimately be important in future studies to utilize a tracer kinetic approach under in vivo conditions to definitively determine the effects of ethanol alone and in combination with nutritional deficiencies (e.g., folate, B₆, B₁₂) on perturbation of these pathways, not only in the liver but in other tissues as well. Moreover, it will also be important to determine if the ability of ethanol to alter the expression and activity of key enzymes is a direct effect or if it is secondary to other changes induced by ethanol consumption.

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