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Folate, Alcohol, and Liver Disease

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Abstract

Alcoholic liver disease (ALD) is typically associated with folate deficiency, which is the result of reduced dietary folate intake, intestinal malabsorption, reduced liver uptake and storage, and increased urinary folate excretion. Folate deficiency favors the progression of liver disease through mechanisms that include its effects on methionine metabolism with consequences for DNA synthesis and stability and the epigenetic regulation of gene expression involved in pathways of liver injury. This paper reviews the pathogenesis of alcoholic liver disease with particular focus on ethanol-induced alterations in methionine metabolism which may act in synergy with folate deficiency to decrease antioxidant defense as well as DNA stability while regulating epigenetic mechanisms of relevant gene expressions. We also review the current evidence available on potential treatments of alcoholic liver disease based on correcting abnormalities in methionine metabolism and the methylation regulation of relevant gene expressions.

Keywords

Alcohol; Folate; Liver; Methionine

1. Chronic alcoholism and liver disease

Although the prevalence of ALD has been stable over the last 10 years [1], alcohol remains one of the major etiologies of cirrhosis and liver disease-related mortality in United States and in European countries [2–4] with an overall U.S. mortality of 9.6 in 100,000 persons. ALD spans a spectrum of conditions that include hepatic steatosis, alcoholic hepatitis, and ultimately alcoholic cirrhosis which is a risk factor for hepatocellular carcinoma. Hepatic steatosis, or excessive fat in the liver, is present in at least 80% of heavy drinkers and is a reversible condition with prolonged sobriety. Alcoholic steatohepatitis is a clinical entity that can be life-threatening, and is characterized by steatosis, various degrees of inflammatory neutrophilic infiltrate, and fibrosis which has a high mortality and may be followed by rapid progression to cirrhosis. Alcoholic cirrhosis is the terminal stage of ALD characterized by excessive accumulation of collagen and extracellular matrix proteins with consequent increased sinusoidal pressure and clinical manifestations of portal hypertension including esophageal and gastric varices, splenomegaly, ascites, and hepatic encephalopathy. The major determining factor for the development of alcoholic liver disease is the average daily amount of alcohol intake and its duration. The threshold risk for ALD is a daily intake of more than 30 g of alcohol, as found in 2 shots of 80-proof liquor, 2 glasses of wine, or 2 cans of beer daily for at least 10 years [5]. Other factors that are known to be

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Conflicts of interest

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associated with ALD include female gender, ethnicity, co-infection with viral hepatitis, obesity, and cigarette smoking [6, 7]. Women are more susceptible to ALD due to their different body composition with smaller body water compartments than men, hormonal factors, and reduced activity of gastric alcohol dehydrogenase which is associated with higher blood alcohol concentrations. Hispanics tend to have higher mortality rate for alcoholic cirrhosis than Blacks and non-Hispanics Whites in the U.S. [3].

The mechanisms of alcohol-related liver injury are multifactorial and involve several pathways. The principal product of oxidative alcohol metabolism is acetaldehyde whose production is associated with changes in NADH-NAD⁺ reduction-oxidation potential in the liver. In addition to changes in redox potential, steatosis may be triggered by signaling of the endoplasmic reticulum stress pathway, by elevated levels of homocysteine [8], as well as decreased fatty oxidation and triglyceride export. In addition, the endotoxin lipopolysaccharide (LPS), a component of the outer wall of gram-negative intestinal bacteria, is translocated through the intestine to the portal tract and ultimately the liver where it triggers the production of the cytokine tumor necrosis alpha (TNF α) and a subsequent inflammatory response that forms the background for pathways of fibrosis and eventual cirrhosis [9]. Increased intestinal permeability and consequent increased LPS translocation is closely related to protein calorie malnutrition, which is one of the most frequently observed clinical features of ALD. Other nutritional deficiencies, including those of folate, thiamine, and vitamin B6, may play a significant role in the pathogenesis and progression of ALD [10, 11]. Malnutrition in ALD is due to reduced calorie intake, abnormal digestion, increased skeletal and visceral protein catabolism, and abnormal lipid metabolism [12]. A study of more than 500 male U.S. veterans showed that clinical signs of protein calorie malnutrition, as indicated by decreased body weight, skin-fold thickness, and visceral protein levels, was present in about 60% of chronic alcoholic subjects without clinical or laboratory evidence of ALD, whereas the same clinical signs of malnutrition were evident in all jaundiced subjects with advanced liver disease and their severity correlated with mortality risk [13]. Furthermore, nutritional repletion is associated with improved outcome in severe alcoholic steatohepatitis as shown by a prospective trial of use of conventional corticosteroid versus enteral nutritional supplementation. Mortality in the corticosteroid treatment group was mainly related to infections with intestinal bacteria, suggesting that the effectiveness of enteral nutrition may relate to its capacity to improve intestinal barrier function and consequently reduce bacterial translocation [14].

2. Folate deficiency in chronic alcoholism: frequency, causes, and potential effect on the liver

Incidence of folate deficiency in chronic alcoholism

An original study showed that 80% of 70 chronic alcoholics that were admitted to a large U.S. urban hospital had low serum folate levels, including 44% with serum folate levels in the severely deficient range. While most patients were under evaluation for ALD, there was no association of low serum folate levels with severity of liver dysfunction [15]. Megaloblastic bone marrows with low red blood cell folate levels, both markers of tissue folate deficiency, were found in 40% of anemic alcoholics evaluated in another urban hospital [16]. Low red blood cell folate levels were associated with elevated serum homocysteine levels in a Portuguese study of 32 chronic alcoholics [17], whereas hyperhomocysteinemia is also associated with acute alcohol withdrawal [18]. After the onset of grain fortification with folic acid in the U.S. in 1998, low red blood cell folate levels were found in 11% patients with an alcohol related illness [19], contrasting with an incidence of 5% low red cell folate levels in healthy adults [20]. Also, a 2008 post-fortification study of 77 consecutive patients seen in a U.S. emergency room for acute alcohol intoxication found

none with low serum folate levels [21]. In our recent study, the mean levels of serum folate were in normal range and similar in chronic alcoholics with or without liver disease, but were lower than mean folate levels in age and gender matched healthy control subjects [10]. Therefore, it would appear that folate deficiency as defined by low serum folate levels is rare in alcoholics in the post-folate fortification era, but mean folate levels are likely to be decreased in chronic alcoholics compared to healthy persons.

Causes of folate deficiency in chronic alcoholism and ALD

In addition to dietary deficiency [15], there are at least four established reasons for folate deficiency in chronic alcoholism, which are enumerated and discussed below. These causes are related to the different mechanisms of folate homeostasis that are illustrated in Figure 1. These include the digestion of dietary pteroylpolyglutamate folates (PteGlu_n) to their monoglutamate form (PteGlu) by intestinal glutamate carboxypeptidase II (GCP_{II}), followed by transfer of PteGlu through the portal vein to the liver where it is transported across the basolateral membranes of hepatocytes for intracellular re-polyglutamylolation and storage as PteGlu_n. After subsequent intrahepatic hydrolysis of storage PteGlu_n to methylated PteGlu, folate enters both an enterohepatic folate cycle (EHFC) with re-absorption of 99%, and the systemic folate circulation (SFC) for transport to all cells of the body. The kidney is a subsequent regulatory site, where about 90% of methylated PteGlu is re-absorbed by proximal tubular epithelial cells and the remaining 10% excreted in the urine each day.

Reduced folate absorption by the small intestine—Dietary folate exists primarily in the form of oxidized and methylated PteGlu_n, which are digested at the jejunal brush border surface by GCP_{II}. The product PteGlu exists as either as oxidized folic acid or reduced and methylated tetrahydrofolic acid (5-MTHF) is taken up and transported across the enterocytes by the reduced folate carrier (RFC) to the portal vein and eventually the liver (Figure 1). The recently discovered proton coupled folate transporter (PCFT) also plays an essential role in folate absorption [22], and both PCFT with low pH optimum and RFC with neutral pH optimum may operate cooperatively as the molecule navigates the acid microclimate of the intestinal lumen and subsequent crosses the brush border and basolateral membranes of the intestinal cell [23]. Two separate clinical studies demonstrated decreased intestinal absorption of ³H-labeled folic acid in chronic alcoholics coming to hospital emergency rooms for alcoholic withdrawal, whereas acute ingestion of ethanol does not impair folate absorption [24, 25]. In vitro mechanistic studies showed that the expression of intestinal RFC and folic acid transport by isolated jejunal brush border membranes were decreased in micropigs fed daily alcohol for one year [26, 27], while the intestinal mucosal expressions of RFC and PCFT were each decreased together with reduced transport of folate across isolated basement membranes of intestinal cells of chronic ethanol fed rats [23].

Abnormal liver uptake and low folate storage—The liver is the major storage organ for folate, which undergoes several reactions before exit to both the biliary and systemic blood circulation (Figure 1). 5-MTHF crosses the basolateral membranes of hepatocytes after its transport through the intestine to the portal vein. Whereas both folate-binding protein (FBP) and RFC were found present by immunohistochemistry on this membrane in micropigs [26, 27], the presence of a pH gradient mediated transport system for 5-MTHF across human hepatic basolateral membranes suggests a significant role for PCFT in its transport [28]. Once within the hepatocytes, 5-MTHF undergoes re-polyglutamylolation by folypolyglutamate synthetase (FPGS) for storage, then reaction with an intracellular gamma glutamyl hydrolase (GGH) prior to export of 5-MTHF into the bile or systemic circulation [29]. A portion of the liver pool of 5-MTHF is cycled through an EHFC, most enters the systemic folate circulation (SFC) for transport to the different cells of the body [30] (Figure

1). Hence chronic alcoholism may alter hepatic folate metabolism at levels of basolateral membrane transport, intrahepatic processing, and re-distribution of folate between the SFC and EHFC. The reduction of liver folate stores in ALD was demonstrated in a clinical study according to two-fold accelerated development of folate deficiency with megaloblastic anemia in such patients consuming a folate depleted diet [31] compared to that required by a healthy subject consuming a folate depleted diet [32]. Low liver folate could be caused in part by decreased liver uptake of 5-MTHF, as was shown in monkeys given alcohol for two years [33]. Another study of ethanol fed rats found decreased expression of hepatic FPGS as a contributing cause of decreased storage folate [34], whereas chronic alcohol feeding may alter the distribution of 5-MTHF between enterohepatic and systemic folate circulations [35].

Increased urinary folate excretion—Loss of folate in the urine has been documented in chronic alcoholic subjects [36], ethanol fed rats [37], and in chronic ethanol fed monkeys [38]. Urinary folate excretion is governed by renal tubular cell re-absorption by way of both FBP and RFC on proximal tubular cell brush border membranes [39]. However, long term ethanol exposure in a micropig model of ALD had no effect on folic acid transport by isolated renal tubular brush border membranes or on the expression of renal tubular RFC [26]. A more recent in vitro study of human proximal renal tubular cells found decreased transport of 5-MTHF in response to short term ethanol exposure, whereas subchronic ethanol exposure in rats increased the expressions of renal tubular FBP and RFC [40]. These studies suggest that increased urine folate excretion is caused by acute ethanol exposure, with subsequent adaptation to chronic alcoholism.

Effects of acute ethanol exposure on folate levels—In addition to these causes of folate deficiency in chronic alcoholism, the acute ingestion of alcohol appears to have immediate effects on serum folate levels and tissue folate uptake. In an experiment designed to measure the acute effect of alcohol on folate metabolism in human volunteers, the serum folate level fell within eight hours after oral or intravenous ethanol, returning rapidly to normal after cessation of alcohol exposure [41]. In another human experiment, the hematological response to folic acid supplementation was repeatedly interrupted by ingestion of ethanol in an anemic chronic and folate deficient patient [42]. The mechanisms for these phenomena remain unexplained though might be related to effects of acute ethanol on re-distribution of circulating folate to the enterohepatic cycle [35] or on increasing urine folate excretion [40]. Others showed that acetaldehyde, the initial metabolite of alcohol, causes oxidative destruction of 5-MTHF in vitro [43].

3. Vitamin dependent methionine metabolism in health, alcoholism, and ALD

As shown in Figure 2, both dietary and endogenous folate play significant roles in hepatic methionine metabolism, which in turn regulates homocysteine levels, antioxidant defenses, DNA assembly, lipid export, and all epigenetic methylation reactions that contribute to gene expression regulation. Dietary folates are metabolized in the liver and other tissues to 5-MTHF which in turn is the substrate for methionine synthase (MS) in the initial transmethylation reaction that metabolizes homocysteine to methionine and ultimately to S-adenosylmethionine (SAM). SAM is the methyl donor for all methylation reactions involving DNA, histones, and proteins. Since S-adenosylhomocysteine (SAH) is both the product and principal inhibitor of methyltransferase reaction [44], the SAM to SAH (SAM/SAH) ratio is considered a useful index of methylation capacity. SAM also plays a regulatory role in the production of 5-MTHF by down regulating methyl tetrahydrofolate reductase (MTHFR). When SAM concentration is adequate, 5,10-MTHF, the substrate for

MTHFR, reacts with thymidine synthase (TS) for the conversion of uridine (dUMP) to thymidine (dTMP), which maintains nucleotide balance and DNA stability. Conversely, decreased levels of SAM result in increased activity of MTHFR with increased production of 5-MTHF, but decreased availability of 5,10-MTHF for thymidine synthase, with resultant nucleotide imbalance and DNA instability [45]. As described below, the regulatory effect of SAM on MTHFR activity and its critical effect on the availability of 5,10-MTHF for maintenance of DNA stability has significant ramifications on cancer risk as well as development of ALD.

Whereas 5-MTHF is the initial methyl donor as substrate for MS, vitamin B12 (cobalamin) is the cofactor for this reaction by transfer of the methyl group from 5-MTHF to cobalamin, forming methylcobalamin, the coenzyme form of B12, that facilitates both the production of methionine and the regeneration of tetrahydrofolate (THF) [45]. Betaine homocysteine methyltransferase (BHMT) is an alternative pathway that ensures conversion of homocysteine to methionine when MS is compromised by alcoholism. Its methyl donor betaine is available in the diet and is also generated endogenously from choline, and betaine supplementation restores SAM levels in alcohol fed rats [46]. Vitamin B6 is also a regulator of methionine metabolism as a co-factor in the transsulfuration pathway which metabolizes homocysteine to glutathione (GSH). This pathway includes two vitamin B6 dependent enzymes, cystathionine- β -synthase (C β S) and γ -cystathionase ultimately producing the antioxidant glutathione (GSH) [45]. Alpha-aminobutyrate is produced during the conversion of cystathionine to cysteine as a byproduct of the transsulfuration pathway and may be measured as an index of cystathionase activity [10]. Of note, SAM stabilizes the C β S reaction [47], and therefore plays an important role in GSH production and antioxidant defense. Two different studies have correlated hepatic SAM and GSH levels in experimental ALD in the micropig and baboon [48, 49].

Alcohol consumption affects the described methionine metabolic pathways at different levels and folate deficiency may promote liver disease limiting its effect as substrate for hepatic methionine metabolism. For example, transcript levels of MS, BHMT, methionine adenosyl transferase (MAT1A), and C β S were each reduced in liver biopsies from cirrhotic patients, with similar findings in a separate study of biopsies from patients with alcoholic steatohepatitis [50, 51]. In ALD, SAM levels are typically reduced by decreased activities of these enzymes, thereby increasing MTHFR activity to increase endogenous 5-MTHF and consequently guarantee SAM levels [45].

Chronic alcoholics typically present with reduced vitamin B6 levels, which has been attributable to displacement of the vitamin from its protein carrier by acetaldehyde with subsequent degradation by phosphatases [52]. Since vitamin B6 is the cofactor for two transsulfuration enzymes (Figure 2), the direct consequence of vitamin B6 deficiency is impaired transsulfuration pathway with homocysteine accumulation. A study of 81 ALD patients with advanced cirrhosis showed increased homocysteine and cystathionine plasma levels compared to 55 healthy subjects, which could be ascribed to an effect of reduced vitamin B6 levels on their regulatory enzymes (Figure 2) [53]. Our subsequent study established the significance of the ratio of α -aminobutyrate/cystathionine which can be considered a marker of vitamin B6 dependent cystathionase activity. We compared 40 alcoholic with clinical evidence of liver disease, who also underwent a liver biopsy, to 26 alcoholics without liver disease, and 28 healthy subjects. The α -aminobutyrate/cystathionine ratio correlated with the severity of fibrosis and was a predictor of the presence of ALD [10], thereby demonstrating the central role of vitamin B6 deficiency in the progression of ALD.

4. Potential effects of folate deficiency in development of ALD

In view of the prevalence of low serum folate levels in chronic alcoholics and the importance of 5-MTHF as the initial methyl donor in the hepatic methionine cycle (Figure 2), it seems reasonable to assume that folate deficiency could play a role in the development of ALD potentially through the epigenetic effect of reduced supply of methyl groups for silencing gene expression and/or on DNA stability. To test this possibility, we studied the effect of ethanol containing diets with or without folate on the development of ALD in a micropig model. In contrast to a prior finding that all the liver histopathology of ALD could be induced by one year in ethanol fed micropigs [54], the onset of ALD was accelerated to 3 months in the ethanol and folate deficient diet model [48]. Compared to the micropigs fed ethanol with a control diet, those that were fed ethanol with a folate deficient diet had lower liver folate levels, lower liver SAM and SAM/SAH ratios, greater rise in serum AST levels [48], and mRNA and protein expressions of several genes associated with liver injury, including cytochrome P450 2E1 (CYP2E1) and sterol regulatory element binding protein (SREBP 1-c) [8]. Translating these observations to clinical medicine, one might therefore suppose that the incidence of ALD has decreased in the U.S. following folic acid fortification, but there is no evidence to support or refute this concept [55].

5. Effects of folate deficiency and alcoholism on DNA stability in association with progression of ALD and cancer risk

As shown in Figure 2, DNA stability is regulated by the activity of thymidine synthase (TS) which generates thymidine monophosphate (dTMP) from uridine monophosphate (dUMP) and 5,10-MTHF. However, the availability of 5,10-MTHF is dependent upon the downstream activity of MTHFR which produces 5-MTHF as the original methyl donor for the methionine cycle. Since SAM down-regulates MTHFR, its deficiency promotes MTHFR activity and the diversion of 5,10-MTHF away from thymidine synthase (TS) to the detriment of nucleotide balance, DNA synthesis, and stability. The consequences of these interactions have been described in relation to cancer risk with DNA instability as well as increased progression of ALD. For example, several large epidemiological studies demonstrated the enhanced risk of colon cancer in folate deficient alcoholics with presumed low liver SAM levels as opposed to those with normal folate levels [56, 57]. Regarding hepatocellular cancer (HCC), a known complication of ALD, a large clinical series found that its risk was lower in alcoholics with predominantly homozygous TT677 variants of MTHFR which is known to impair its activity as opposed to those with wild-type CC or heterozygous CT polymorphisms [58]. Another large study of Hispanic and Chinese individuals found significantly lower incidence of HCC in those with CT and TT variants of MTHFR [59]. These findings are consistent with the concept that decreased activity of MTHFR is protective of DNA stability by virtue of assuring adequate levels of its substrate 5,10-MTHF for the TS reaction. However, a later study of ALD patients with cirrhotic livers found increased risk of HCC in those with CC and TT MTHFR polymorphisms [60], which could have been related to lower liver SAM that is known to occur in severe liver disease [51].

A study from the ethanol fed micropig model of ALD demonstrated enhanced hepatocellular DNA strand breaks and apoptosis in association with reduced SAM levels and decreased dTMP to dUMP ratios with increased hepatocellular proliferation [61]. Our subsequent study in the folate deficient ethanol-fed micropig associated the development of ALD with reduced liver folate and SAM, increased DNA oxidation and strand breaks, as well as DNA global hypomethylation, consistent with combined effects on DNA synthesis and methylation capacity [48]. Another study related low SAM levels to DNA instability by

finding decreased MAT1A expression and SAM levels together with genome wide DNA strand breaks in the intragastric ethanol fed rat model of ALD [62].

Summarizing, the altered folate and methionine metabolism that is associated with chronic alcohol ingestion is associated with increased progression of ALD and risk of certain cancers, in particular colon cancer and HCC. The mechanisms for this observation include DNA nucleotide imbalance and stability that occur due to diversion of the TS substrate 5,10-MTHF to the MTHFR pathway, which may be upregulated due to deficiency of SAM that occurs with relative folate deficiency. These mechanisms are consistent with clinical observation of increased cancer risk in folate deficient alcoholics, and with increased DNA damage with strand breaks, oxidation, and apoptosis occurring in experimental ALD in association with decreased SAM levels. As discussed below, reduced SAM provides an additional HCC risk by way of promoting DNA hypomethylation.

6. Folate and alcohol interactions in DNA and histone methylation and risks of DNA hypomethylation

The epigenetic regulation of gene expression involves remodeling of chromatin by either the addition of methyl groups to DNA and/or the posttranslational modification of histone amino acid residues. The modification of histone amino acid residues by methylation and/or acetylation can alter the conformation of the histone to permit greater DNA expression and/or vice versa. In ALD, methylation at H3K4, H3K36, and H3K79 generally results in gene activations, but genes may be silenced by methylation at histone residues H3K9, H327, and H4K20 [63]. For example, increased H3K4 was associated with increased activation of genes involved in oxidative stress in chronic ethanol fed rats [64], whereas studies of ethanol exposed primary rat hepatocytes found decreased H3K9 methylation in association with several down regulated genes and increased H3K4 methylation in association with several up regulated genes [65]. DNA methylation is regulated by specific methyltransferases DNMT 1, 2, 3A and 3B [63], one or more of which may be influenced by the ethanol metabolite acetaldehyde [66].

DNA and histone methylation are closely linked to hepatic methionine metabolism, since the level of substrate SAM is critical as substrate for methyltransferases, while the product S-adenosylhomocysteine (SAH) is a potent inhibitor of the same reactions [44]. Experimental conditions which lead to low SAM levels, such as methionine and choline deficient diets or MAT1A deletion, are associated with DNA hypomethylation and the development of cirrhosis with increased risk for hepatocellular carcinoma (HCC) [62, 67, 68]. A folate and methyl deficient diet resulted in DNA hypomethylation in several tissues in an ethanol fed rat model together with liver-specific enhanced activity of DNMT1 [69]. A subsequent study from the same group associated a methyl deficient diet with decreased SAM and SAM/SAH ratio, together with global DNA hypomethylation and irreversible pre-malignant hepatic foci [70]. Whereas MAT1A expression and SAM levels are decreased in ALD patients [51], the MAT1A deficient mouse develops steatohepatitis in 8 months and HCC in 18 months [71]. Another study demonstrated the preventive effect of SAM on development of HCC in rats injected with precancerous liver cells [72].

Our recent work evaluated the effect of ethanol feeding on the epigenetic regulation of selected genes in a mouse model of ALD that is heterozygous for C β S (Figure 2). Since C β S regulates the transsulfuration of homocysteine, its deficiency can be expected to elevate homocysteine [73], in turn increasing the methylase inhibitor SAH through reverse SAHH pathway (Figure 2). Since ethanol exposure reduces MS expression and activity [51, 74], the combination of ethanol and C β S heterozygosity predictably would maximize homocysteine and secondarily SAH, at the same time as reducing SAM production. Summarizing our

results, intragastric ethanol feeding of heterozygous CbS mice accelerated the histopathology of ALD, while minimizing the SAM/SAH methylation ratio. Immunohistochemical staining found decreased abundance of the histone residue H3K9, whereas chromatin immunoprecipitation with antibody to H3K9 showed increased expressions of the genes relevant to steatosis and apoptosis [75].

Summarizing this section, emerging evidence links ethanol-induced alteration of hepatic homocysteine metabolism, in particular reduction of the methyltransferase substrate SAM or increase in its inhibitor SAH, to altered expressions of genes relevant to ALD. The induction of DNA hypomethylation by methyl deficient diet is linked to HCC risk, and ethanol feeding of a genetically altered mouse model of altered methionine metabolism resulted in selective gene expressions related to aberrant histone methylation.

7. Effects of methyl group supplementation in alcoholic liver disease

Studies in animal models

Exogenous folate administration has not been investigated as possible treatment for ALD but experimental evidence indicates its possible efficacy. As shown in Figure 2, folate as 5-MTHF is the original methyl donor for methionine metabolism. In one study, folate treatment in association with vitamin B12 improved liver fibrosis and was associated with reduction in homocysteine levels in a rat model of ALD [76]. As described above, folate deficiency alone does not cause liver disease but, by magnifying alterations in methionine metabolism, it can promote and accelerate ALD [48]. The rationale for use of the downstream methyl group donors SAM and betaine is to bypass the known decreased expression of MS in ALD [51, 74] (Figure 2). Experimental SAM treatment was shown to prevent ALD and its gene expressions for lipogenesis, apoptosis, and oxidative liver injury in the folate deficient ethanol fed micropig model [77, 78]. In other studies, when SAM was provided as dietary supplement to animal models of ALD or as a media supplement in cell culture, it: 1) attenuated SAM and GSH deficiency and reduced hepatic fibrogenesis in ethanol-fed baboons [49], 2) maintained adequate hepatocyte GSH levels and mitochondrial membrane fluidity [79], 3) preserved mitochondrial function [80], and 4) prevented TNF α induced depletion of GSH and necrosis in ethanol-fed rats [81].

Even though there are no clinical data on the potential efficacy of betaine as treatment for ALD, experimental evidence from animal models indicates its possible efficacy in this condition. The effect of betaine was studied in a rat model of ALD where animals developed fatty liver after 4-weeks of feeding with the Lieber-DeCarli diet containing 36% of total energy from ethanol. Betaine, at the concentration of 0.5% in the diet prevented the ethanol induced accumulation of hepatic triacylglycerol as well as liver histopathology of ALD. The mechanisms that underlie this improvement consist of lowering of homocysteine levels, increase of SAM hepatic levels, reduction of SAH levels by increased homocysteine metabolism and consequent reversal of the SAM/SAH ratio [82]. Furthermore, by improving the SAM/SAH ratio, betaine treatment restored the activity of PEMT, the enzyme that catabolizes the methylation of phosphatidylethanolamine to phosphatidylcholine which is essential for the synthesis and secretion of VLDL from the liver. Therefore, by restoring PEMT activity, betaine improved VLDL secretion and alcoholic fatty liver [82, 83]. Other possible mechanisms of action of betaine include increased metabolic rate and generation of NAD for alcohol oxidative metabolism by alcohol dehydrogenase [84], inhibition of toll-like receptor 4 in the inflammation pathway [85], protection against apoptosis [86], and preservation of oxidative phosphorylation complexes in mitochondria [87].

Clinical studies

Several clinical studies have explored the therapeutic effect of SAM in established ALD. In an Italian study, SAM or placebo was given orally at 1.2g/d for 6 months to 17 well-nourished chronic alcoholic patients with normal serum albumin levels but varied histological features of alcoholic hepatitis, and to 7 patients with non-alcoholic chronic hepatitis. Compared to levels in control liver specimens obtained at elective surgery from patients with unrelated disease, hepatic GSH levels were low in biopsies from all liver disease patients and were normalized by SAM treatment regardless of initial diagnosis [88]. A multicenter European trial tested the effect of SAM at 1.2 g/d vs placebo for 2 years on survival vs death or liver transplant in 123 ALD patients. The overall mortality or liver transplant incidence was reduced from 30% in the placebo to 16% in the SAM group, a difference that was significant after exclusion of Childs class C patients from the analysis [89]. We conducted a double-blinded, randomized, placebo-controlled trial in 37 patients with ALD who received either SAM, at the dose of 1.2 grams daily, or placebo for 6 months [90]. In contrast to prior clinical trials, this is the only study where a subgroup of patients also underwent pre- and post-treatment liver biopsy, in which advanced liver disease was documented by bridging fibrosis or cirrhosis in more than 50% of patients. Despite the fact that SAM serum levels were increased over time in the SAM group, there were no differences between the treatment groups in any clinical or biochemical parameters including liver histopathology. Therefore, we concluded that SAM was no more effective than placebo for the treatment of ALD with the limitation of relatively small numbers of patients [90]. One (unpublished) factor that may have contributed to our negative result is the relative vitamin B6 deficiency that persisted during the 6-month study and may have further impaired the transsulfuration pathway and its capability to synthesize GSH [10]. Finally, a meta-analysis of 9 different clinical trials found inconclusive evidence for the efficacy of SAM in the treatment of ALD [91].

Summarizing this section, there is abundant evidence that both methyl donors SAM and betaine are protective in the development of experimental ALD in animal models. However the efficacy of SAM as a treatment modality of established ALD has not been proven conclusively in clinical trials. It seems likely that the efficacy of SAM requires its retention and metabolism by intact hepatocytes, which would apply in prevention of disease, but not in presence of damaged hepatocytes in the presence of ALD.

Summary and conclusions

This review has described the incidence of folate deficiency in chronic alcoholism and documented the evidence for its linkage with altered hepatic methionine metabolism in the pathogenesis of ALD. In addition to dietary inadequacy, the mechanisms for folate deficiency in chronic alcoholism includes intestinal malabsorption, reduced hepatic transport and storage, and reduced renal reabsorption. The several mechanisms for altered methionine metabolism as a cause of ALD include defective DNA synthesis and stability and the reduced methylation capacity on the expressions of genes related to liver injury. In addition, both reduced DNA stability and increased DNA hypomethylation are associated in increased risk for hepatocellular cancers. Whereas both methyl donors SAM and betaine have been shown effective in the prevention of ALD and its pathogenetic mechanisms in animal models, the evidence for their efficacy in the treatment of clinically established ALD has not been demonstrated.

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Abbreviations

ALD	alcoholic liver disease
BHMT	betaine homocysteine methyltransferase
CβS	cystathionine-β-synthase
dTMP	deoxythymidine monophosphate
dUMP	deoxyuridine monophosphate
EHFC	enterohepatic folate cycle
FBP	folate-binding protein
FPGS	folylpolyglutamate synthetase
GCPII	glutamate carboxypeptidase II
GSH	glutathione
LPS	lipopolysaccharide
MAT1A	methionine adenosyl transferase 1A
MS	methionine synthase
5,10-MTHF	methylene tetrahydrofolate
5-MTHF	methyl tetrahydrofolate
PCFT	proton coupled folate transporter
PEMT	phosphatidylethanolamine transferase
PteGlu	pteroylmonoglutamate
PteGlu_n	pteroylpolyglutamates
RFC	reduced folate carrier
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SFC	systemic folate circulation
TNFα	tumor necrosis factor alpha
TS	thymidinesynthase

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Figure 1. Folate homeostasis

Dietary folate is predominantly in the form of pteroylpolyglutamate (PteGlu_n) which is then hydrolyzed on the jejunal (J) brush border by a specific gamma carboxy peptidase (1) to yield pteroylmonoglutamyl folate (PteGlu) that is methylated and then transported across enterocyte membranes by the reduced folate carrier (2) to the portal vein. Subsequent transport across membranes of hepatocytes in the liver (L) is facilitated by the reduced folate carrier (2), possibly together with folate binding protein or the proton coupled folate transporter. Within the liver, PteGlu is re-polyglutamated by an intracellular folylpolyglutamatesynthetase (3) to PteGlu_n for storage, then released back to PteGlu by a separate gamma glutamyl hydrolase (4) and transported to both the enterohepatic folate circulation (EHFC) or systemic folate circulation (SFC) for transport to all cells of the body. Urinary excretion is regulated in the kidney (K) through re-absorption of about 90% by the reduced folate carrier on renal tubular cells (2). While less than 1% of folate is excreted in stool from the EHFC, about 10% of the SFC pool is excreted daily by the kidney, requiring replacement by dietary folate.

Figure 2. Normal folate and methionine metabolism

After passage through intestine to the liver, dietary folate is metabolized to dihydrofolate (DHF) and then tetrahydrofolate (THF), which is substrate for production of 5,10-methylene tetrahydrofolate (5,10-MTHF). This compound then interacts either with thymidine synthetase (TS) for regulation of nucleotide metabolism in DNA synthesis, or with methylenetetrahydrofolate reductase (MTHFR), for production of 5-methyltetrahydrofolate (5-MTHF), which is the initial methyl donor for methionine metabolism. Homocysteine and 5-MTHF are substrates for vitamin B₁₂ dependent methionine synthase (MS) for production of methionine and THF. In the transmethylation cycle, methionine is substrate for methionine adenosyl transferase (MAT) to produce S-adenosylmethionine (SAM), which is the principal methyl donor for all histone and DNA methyltransferases (MTs). S-adenosylhomocysteine (SAH) is the both product and inhibitor of all MT reactions, and is subsequently metabolized to homocysteine by the bidirectional enzyme SAH hydrolase (SAHH). Through the transsulfuration pathway, homocysteine is metabolized by vitamin B₆ regulated pathways, cystathionine beta synthase (CβS) and cystathionase to cysteine and subsequently to the antioxidant glutathione (GSH). In addition to its role as principal methyl donor, SAM regulates both folate metabolism as an inhibitor of MTHFR and transsulfuration of homocysteine as a facilitator of CβS.