Pathogenesis and treatment of alcoholic liver disease: progress over the last 50 years

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Abstract

Fifty years ago the dogma prevailed that alcohol was not toxic to the liver and that alcoholic liver disease was exclusively a consequence of nutritional deficiencies. We showed, however, that liver pathology developed even in the absence of malnutrition. This toxicity of alcohol was linked to its metabolism via alcohol dehydrogenase which converts nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide-reduced form (NADH) which contributes to hyperuricemia, hypoglycemia and hepatic steatosis by inhibiting lipid oxidation and promoting lipogenesis. We also discovered a new pathway of ethanol metabolism, the microsomal ethanol oxidizing system (MEOS). The activity of its main enzyme, cytochrome P4502E1 (CYP2E1), and its gene are increased by chronic consumption, resulting in metabolic tolerance to ethanol. CYP2E1 also detoxifies many drugs but occasionally toxic and even carcinogenic metabolites are produced. This activity is also associated with the generation of free radicals with resulting lipid peroxidation and membrane damage as well as depletion of mitochondrial reduced glutathione (GSH) and its ultimate precursor, namely methionine activated to S-adenosylmethionine (SAMe). Its repletion restores liver functions. Administration of polyenylphosphatidylcholine (PPC), a mixture of unsaturated phosphatidylcholines (PC) extracted from soybeans, restores the structure of the membranes and the function of the corresponding enzymes. Ethanol impairs the conversion of β-carotene to vitamin A and depletes hepatic vitamin A and, when it is given together with vitamin A or β-carotene, hepatotoxicity is potentiated. Our present therapeutic approach is to reduce excess alcohol consumption by the Brief Intervention technique found to be very successful. We correct hepatic SAMe depletion and supplementation with PPC has some favorable effects on parameters of liver damage which continue to be evaluated. Similarly dilinoleoylphosphatidylcholine (DLPC), PPC's main component, also partially opposes the increase in CYP2E1 by ethanol. Hence, therapy with SAMe +DLPC is now being considered.

Key words: alcoholic hepatitis, nonalcoholic steatohepatitis (NASH), cytochrome P450 (CYP2E1), S-adenosylmethionine (SAMe), polyenylphosphatidylcholine (PPC).

Introduction

In a prospective survey of 280 subjects with alcoholic liver injury [1] it was found that, within 48 months of follow-up, more than half of those with cirrhosis, and two thirds of those with cirrhosis plus alcoholic hepatitis, had died. This dismal outcome is more severe than that of many cancers, yet it is attracting much less concern, both among the public and the medical profession. This may be due, at least in part, to the general perception that not much can be done about this major public health issue. One purpose of this review is to analyze how concepts about alcoholic liver disease have evolved and how the present state of knowledge allows for a more optimistic outlook in terms of treatment and outcome.

Malnutrition versus toxicity

Because experimentally, nutritional deficiencies cause liver damage, it was postulated that this is also the mechanism

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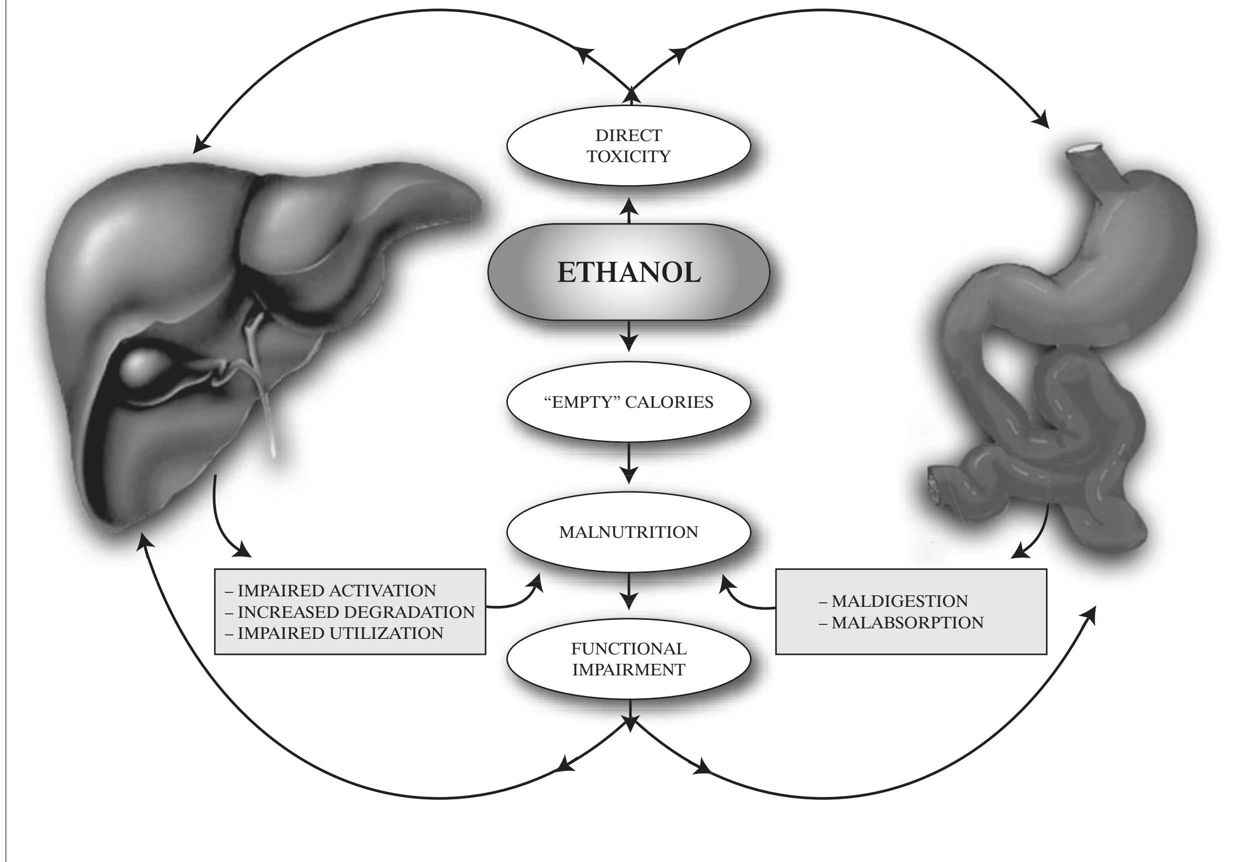
Figure 1. **Interaction of direct toxicity of ethanol on liver and gut with malnutrition secondary to dietary deficiencies, maldigestion, and malabsorption, as well as impaired hepatic activation, inhibition and increased degradation of nutrients (modified from** [176]**)**



whereby alcoholic liver disease develops. Accordingly, fifty years ago the dogma prevailed that alcohol was not toxic to the liver and that alcoholic liver disease was exclusively a consequence of nutritional deficiencies. As a result, the prevailing therapy was nutritional with enriched diets. This view was clearly expressed by Best et al. [2], the co-discoverer of insulin, who stated that "There is no more evidence of a specific toxic effect of pure ethyl alcohol upon liver cells than there is for one due to sugar". This opinion was based on his experiments in rats given alcohol in drinking water. They did not develop any liver damage unless the diet was deficient. However, rats do not develop significant blood levels of alcohol when given in the drinking water. When we overcame the aversion of the rats for alcohol by incorporating 35% of calories as alcohol into nutritionally adequate liquid diets, obvious liver pathology, including fatty liver, developed compared to the control animals pair-fed with an isocaloric diet in which alcohol was replaced by carbohydrates [3]. This was also demonstrated in volunteers given alcohol with enriched diets which did not prevent the development of the first stage of alcoholic liver disease, namely alcoholic fatty liver [3,4] indicating that alcohol exerts some direct toxic effect on the liver independent of malnutrition (*Fig. 1*). This demonstration of the hepatotoxicity of alcohol did, of course, not preclude that one of the mechanisms whereby alcohol affects the liver may also be its interference with nutritional factors.

Pathogenesis and treatment of alcoholic and non-alcoholic fatty liver

1. Alcohol and nutrition

Unlike other drugs, ethanol is a substantial source of energy, with 7.1 kcal (29.7 kJ) per gram, a value that exceeds the energy content of carbohydrates or proteins. On average, ethanol accounts for half an alcoholic's caloric intake. It therefore displaces normal nutrients, causing malnutrition (*Fig. 1*), including deficiencies of folate, thiamine, and other vitamins. Secondary malnutrition also occurs through malabsorption due to gastrointestinal complications, such as pancreatic insufficiency and impaired hepatic metabolism of nutrients (*Fig. 1*). In addition, alcohol promotes the degradation of nutrients, as exemplified by its effects on vitamin A [5].

Indeed, in addition to the study of the hepatotoxicity of ethanol, we investigated its associated effect on nutrients with implications for the liver. This included the discovery that liver microsomes harbor previously unrecognized pathways for retinol metabolism [6] which were shown to play a role in the homeostatic control of hepatic vitamin A levels [7]. Using purified cytochrome P-450 isozymes, including the human CYPIIC8 [8], retinol [6,8] and retinoic acid [8,9] metabolizing systems were reconstituted; administration of ethanol or various drugs was shown to result in the induction of the activity of these

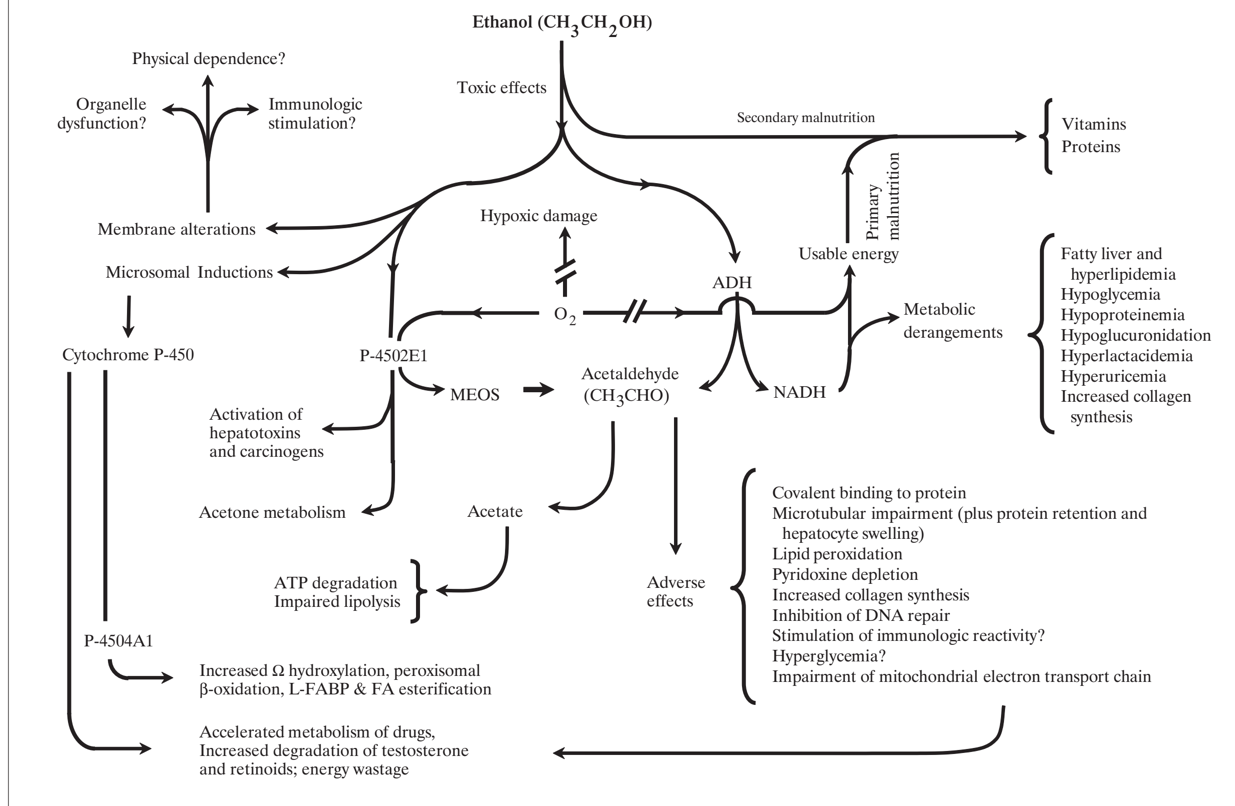


Figure 2. **Toxic and metabolic disorders caused by ethanol abuse. Most of the toxic and metabolic disorders that result from long-term ethanol abuse can be explained on the basis of the metabolism of ethanol by alcohol dehydrogenase (ADH) and the associated generation of reduced NADH or by the microsomal ethanol oxidizing system (MEOS), with the induction of cytochrome P-4502E1 and other microsomal enzymes, as well as by the toxic effects of acetaldehyde produced by both pathways. FA denotes fatty acids, and L-FABP denotes liver fatty-acid-binding protein (from** [177])

systems [10,11], a possible mechanism for the striking hepatic vitamin A depletion which was discovered to result from chronic ethanol consumption in rats and non-human primates [12], as well as man [13]. This hepatic vitamin A depletion was found to be associated with significant lysosomal lesions [14].

Depletion of vitamin A called for its replenishment but it was found that vitamin A supplementation in alcohol users is complicated because of the marked exacerbation of vitamin A hepatotoxicity by chronic ethanol consumption. Indeed, amounts of ethanol and vitamin A which, by themselves, do not produce fibrosis, when combined, resulted in necrosis and fibrosis in the liver [15], with development of severe mitochondrial injury [16]. Furthermore, it was discovered in non-human primates that alcohol interferes with the clearance of the retinol precursor β-carotene, possibly by impairing its conversion to vitamin A, resulting in enhanced hepatic and blood levels in baboons [17] and also in man [18], with associated potentiation of their hepatotoxicity [17]. In the aggregate, the above listed studies have led to the recognition of a narrowed therapeutic window for vitamin A and β-carotene in moderate and in heavy drinkers; in turn, this has prompted a redefinition of the optimal conditions for their therapeutic use, in order to avoid adverse interactions with ethanol in terms of hepatotoxicity and carcinogenicity, as reviewed by Leo and Lieber [19].

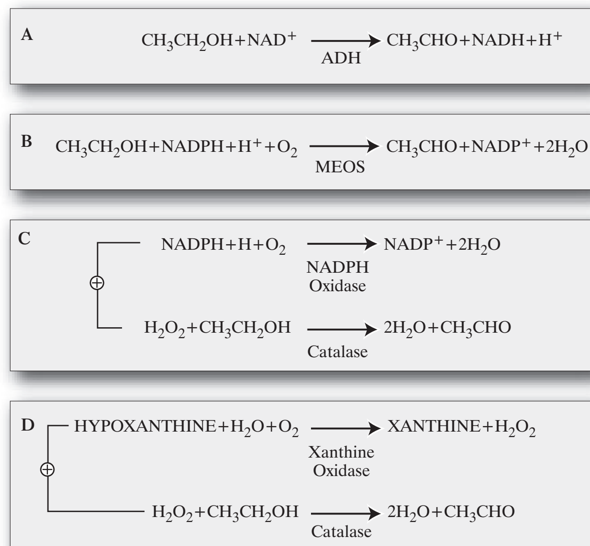
These studies also established the fact that, even in the presence of an adequate diet, alcohol can produce a fatty liver at blood levels which do not necessarily produce intoxication. Increases in dietary long-chain triglycerides enhanced the alcohol-induced steatosis and this effect was attenuated by medium-chain triglycerides [20] as confirmed more recently [21]. Decreasing dietary fat reduced steatosis but, below a level of 10% of calories as dietary fat, there was a reversal of this effect, probably due to the high carbohydrate content of the corresponding diet [22]. These, as well as other effects of alcohol were shown to be due to ethanol's prevailing metabolism in the liver (*Fig. 2*).

2. Toxic effects of hepatic alcohol oxidation

a. Role of Alcohol Dehydrogenase (ADH)

Oxidation of ethanol through the ADH pathway produces acetaldehyde, which is converted to acetate (*Fig. 3*); both reactions reduce nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide-reduced form (NADH). Excess NADH causes a number of metabolic disorders [5], including inhibition of the Krebs cycle and of its fatty acid oxidation. The inhibition of fatty-acid oxidation favors steatosis and hyperlipidemia. The effects of ethanol were reproduced *in vitro* by an alternative NADH-generating system (sorbitol-fructose) and

Figure 3. **Ethanol oxidation by (A) alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD^+); (B) the hepatic microsomal ethanol oxidizing system (MEOS), which involves cytochrome P4502E1 and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH); (C) a combination of NADPH oxidase and catalase; and (D) xanthine oxidase and catalase (from** [5]**)**



were blocked by a H^+ acceptor (methylene blue) [23,24]. The preventive effect of methylene blue against ethanol-induced fat accumulation was more recently confirmed [25]. Our liquid diet rat model of alcoholic liver disease consistently reproduced a fatty liver but it did not progress to inflammation, fibrosis and cirrhosis. This is probably due to the fact that, even with the liquid diet, the alcohol intake is limited to 35% of total calories whereas alcoholics who develop cirrhosis usually consume at least 50% of their calories as alcohol. Such a high intake of alcohol was then achieved in non-human primates, namely baboons [26]. Under these conditions, not only fatty liver, but also fibrosis and cirrhosis developed despite adequate diets.

It was also known that catalase, located in the peroxisomes, is capable of oxidizing ethanol *in vitro* in the presence of an H_2O_2-generating system [27] (*Fig. 3*), but under physiological conditions, catalase appears to play no major role. In both the rat and baboon models, it was also shown that in addition to the metabolic abnormalities caused by the ADH activity, a new pathway of ethanol metabolism, namely the microsomal ethanol oxidizing system (MEOS), plays a key role in the progression of the disease (vide infra).

b. Role of Acetaldehyde Dehydrogenase

As mentioned, the oxidation of ethanol results in the production of the highly toxic metabolite, namely acetaldehyde which is rapidly further metabolized to acetate, mainly by a mitochondrial low Km aldehyde dehydrogenase (ALDH2), the activity of which is lacking in about 25-50% of Asians. In these individuals, even small amounts of alcohol which have almost no effect on Caucasians can produce a rapid facial flush, frequently associated with tachycardia, headache and nausea [28]. This propensity for flushing is genetically determined and caused by decreased acetaldehyde disposition secondary to the lack of ALDH2 activity [29-32]. In fact, the flushing reaction seen in susceptible Asians mimics to a lesser degree the disulfiram reaction caused by the elevation of acetaldehyde following aldehyde dehydrogenase inhibition. The latter reaction has gained therapeutic use as a reinforcement for abstinence in alcoholism rehabilitation programs. In addition, the aversive cardiovascular effects of acetaldehyde may contribute to the relatively lower incidence of cirrhosis in "flushers" [33]. The flushing phenotype may also confer some resistance to the development of alcoholism [34]. Conversely, however, some Japanese alcoholics with an ALDH2 deficiency and, presumably, higher hepatic acetaldehyde levels during drinking, develop alcoholic liver disease at a lower cumulative intake of ethanol than controls [35]. The ALDH2 activity is also significantly reduced by chronic ethanol consumption [36]. The decreased capacity of mitochondria after chronic alcohol consumption to oxidize acetaldehyde, associated with unaltered or even enhanced rates of ethanol oxidation and hence acetaldehyde generation (e.g. because of MEOS induction, vide infra) results in an imbalance between production and disposition of acetaldehyde. The latter causes the elevated acetaldehyde levels observed after chronic ethanol consumption in man [37] and in baboons [38], with a tremendous increase of acetaldehyde in hepatic venous bloods, reflecting the high tissue level.

Acetaldehyde's toxicity is due, in part, to its capacity to bind to proteins, such as microtobules [39], collagen [40] and microsomal protein [41], including CYP2E1 [42]. By binding to the tubulin of microtubules, acetaldehyde blocks the secretion of proteins. The increases in protein, lipid, water [43] and electrolytes cause hepatocytes to enlarge or "balloon", a hallmark of alcoholic liver disease. Acetaldehyde-protein adducts promote collagen production and may also act as neoantigens, which stimulate an immune response [44,45]. Formation of adducts results in hepatic protein retention, contributing to the hepatomegaly [46], and a score of other toxic manifestations, including decreased GSH and impairment of other antioxidant mechanisms, as reviewed elsewhere [5,47]. The effects include inhibiting the repair of alkylated nucleoproteins [48], decreasing the activity of key enzymes, and markedly reducing oxygen utilization in mitochondria damaged by long-term ethanol consumption [38,49]. Impaired oxygen utilization was also confirmed in baboons *in vivo* [38]. The impaired oxidation capacity of the mitochondria also interferes with the oxidation of acetaldehyde [50], thereby contributing to the vicious circle of progressive acetaldehyde accumulation in turn causing greater mitochondrial injury.

c. Microsomal Ethanol Oxidizing System (MEOS)

1) Discovery of this new pathway

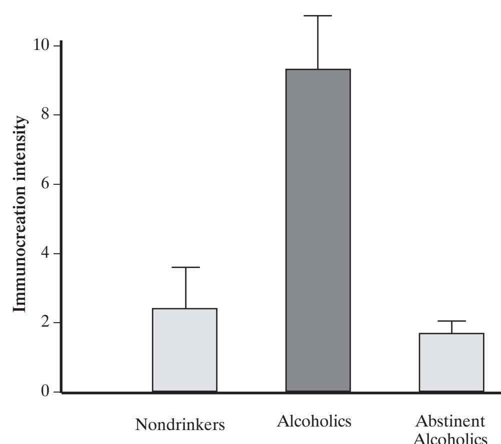
As reviewed elsewhere [51,52], the discovery of this pathway was prompted by the observation that, after chronic ethanol consumption, there is an adaptive increase of ethanol metabolism which could not be explained on the basis of ADH, therefore raising the possibility of the existence of an additional pathway. The first indication of a possible interaction of ethanol with the endoplasmic reticulum of the hepatocyte (also called

microsomal fraction when obtained by ultracentrifugation) was provided by the observation that in rats and humans, ethanol feeding results in a proliferation of the smooth endoplasmic reticulum (SER) [53-55] which was confirmed by an increase in the amount of enzyme activities of the hepatic microsomal membranes [56], including a rise in microsomal glucose-6-phosphatase activity after chronic ethanol administration [57]. This increase in SER resembled that seen after the administration of a wide variety of hepatotoxins [58], barbiturates, and other therapeutic agents [59] and food additives [60]. Because many of the substances that induce a proliferation of the SER are metabolized, at least in part, by the cytochrome P450 enzyme system that is located in the SER, the possibility that ethanol may also be metabolized by a similar process was raised. Indeed, such a system was demonstrated in liver microsomes *in vitro* and found to be inducible by chronic ethanol feeding *in vivo* [22,61]. The proposal that this new pathway (named MEOS) plays a significant role in ethanol metabolism initiated a decade of lively debate: some invoked ADH contaminating the liver microsomes [62], whereas others asserted that this microsomal ethanol oxidation was due to a hydrogen peroxide-dependent reaction promoted by contaminating catalase [63]. Indeed, it had been postulated that the combination of H_2O_2 generation from NADPH oxidase and of catalase could account for microsomal ethanol oxidation [64,65] (*Fig. 3*), especially because a H_2O_2-generating system (glucose-glucose oxidase) can be substituted for NADPH. Actually, the latter is not unexpected, because not only do microsomes contain catalase, but commercial glucose oxidase (used to generate NADPH) is contaminated with catalase. It also had been reported that microsomes from acatalasemic mice fail to oxidize ethanol [66], but this claim was subsequently retracted [67]. In fact, hepatic microsomes of acatalasemic mice subjected to heat inactivation displayed decreased catalase activity, but NADPH-dependent MEOS remained active and unaffected [26,68].

2) Differentiation of the MEOS from ADH and catalase

Eventually, MEOS was solubilized and separated from ADH and catalase activities by diethylaminoethyl cellulose column chromatography [69,70]. Furthermore, whereas catalase reacts peroxidatically primarily with methanol and ethanol, but not with alcohols of longer aliphatic chains [71], the NADPH-dependent MEOS was found capable of metabolizing n-propanol as well as n-butanol. This was shown in hepatic microsomal preparations and in reconstituted systems that contained the microsomal components cytochrome P450, NADPH-cytochrome P450 reductase and phospholipids, and exhibited no ADH or catalase activity [68-70,72]. Such a system was also reconstituted in acatalasemic mice [68]. Furthermore, MEOS activity was inhibited by carbon monoxide and the effect was reversed by lights of wavelengths 430 to 460 nm, which showed the involvement of cytochrome P450 [73]. Finally, successful reconstitution was accomplished with either partially purified or highly-purified microsomal P450 from alcohol [74] or phenobarbital-treated [75] rats. Based on these and various other studies, and regardless of the original claim to the contrary [76], it was finally agreed by the principal contenders involved that catalase cannot account for the microsomal ethanol oxidation

Figure 4. **Hepatic cytochrome P4502E1 levels in alcoholics and in nondrinkers. Cytochrome P4502E1 was quantitated by scanning of Western blots of microsomes obtained from percutaneous liver biopsies, using anti-2E1 antibodies. (data from** [79]**)**



[77,78], and that the MEOS is distinct from ADH and catalase and dependent on cytochromes P450, as reviewed elsewhere [51,52].

Thus, it was established that the key enzyme of the MEOS is an ethanol-inducible cytochrome P450 2E1 (CYP2E1) which was found (*Fig. 4*) to be increased 4- to 10-fold in liver biopsies of recently drinking subjects [79], with a corresponding rise in mRNA [80]. This induction contributes to the metabolic tolerance to ethanol that develops in the alcoholic (in addition to the central nervous tolerance).

3) "Ethanol-specific" cytochrome P450

That chronic ethanol consumption results in the induction of a unique P450 enzyme was shown by Ohnishi and Lieber [74] using a liver microsomal P450 fraction isolated from ethanol-treated rats. An ethanol-inducible form of P450 (LM-3a) was purified from rabbit liver microsomes which catalyzed ethanol oxidation at rates much higher than other P450 isozymes [81,82]. Similar results have been obtained with cytochrome P450j, a major hepatic P450 isozyme purified from ethanol- or isoniazid-treated rats [83,84]. A P450j-like isozyme was also described in humans [85,86]. In a new nomenclature system, it was proposed that the ethanol-inducible form be designated as CYP2El [87].

Despite the discovery of CYP2E1 and its prevailing role in microsomal ethanol oxidation, the term MEOS was maintained because cytochromes P450 other than CYP2E1, as well as hydroxy radicals, can contribute to ethanol metabolism in the microsomes (vide infra). Thus, the term MEOS characterizes total microsomal ethanol oxidation, not only that catalyzed by CYP2E1.

The rat CYP2E1 gene was isolated, characterized, and localized to chromosome 7 [88] and the human gene to chromosome 10 [89]. In rabbits, two genes may be involved [90].

The rat hepatic CYP2E1 gene is transcriptionally activated within 1 day after birth [91]. This activation is accompanied by a demethylation of cytosine residues located within the 5' flanking region of the gene, suggesting that methylation of specific

residues in the 2E1 gene is responsible for the lack of transcription of the 2E1 gene in fetal liver. Cytochrome P4502E1 remains relatively stable during the remainder of the life span, and a tissue-specific relation between the hypomethylation of the human CYP2E1 gene and its hypoexpression was observed [92]. CYP2E1 can be strikingly induced by a variety of substrates.

4) Physiologic role of CYP2E1

As revealed in the studies cited above, the most common cause for CYP2E1 induction is early alcoholic liver injury, such as alcoholic steatosis and alcoholic steatohepatitis (ASH). CYP2E1, however, is also induced in nonalcoholic steatohepatitis (NASH), which can be understood on the basis of the physiologic role of CYP2E1. Indeed, CYP2E1 has a dual role (*Fig. 5*), namely one of detoxification and one of nutritional support. That CYP2E1 contributes to the defense mechanisms of the body against the penetration of toxic xenobiotics is suggested by its location and inducibility at port of entries into the body, and by its broad substrate specificity.

Regarding xenobiotics such as alcohol, CYP2E1 may play a dual detoxification role: through its location in the liver, it may oppose the ethanol resulting from gastrointestinal fermentation to enter the systemic circulation. Moreover, through its marked inducibility at high ethanol concentration, it may prevent alcohol that has entered the circulation from reaching excessive levels. This inducibility was shown not only in experimental animals [93] but also in man [94]. In volunteers, chronic administration of substantial amounts of ethanol resulted in a progressively increased rate of clearance of alcohol from the blood. This accelerated blood alcohol disappearance occurred exclusively at relatively high concentrations of ethanol, the kinetics of which clearly indicate that a system is involved with a Km of approximately 10 mM, which is consistent with that of MEOS (and its CYP2E1) and at least 1-2 orders of magnitude higher than that of ADH. Thus, despite the fact that, at low ethanol concentrations, liver ADH has a much higher capacity for ethanol oxidation than the CYP2E1 system, it is the MEOS, and particularly its CYP2E1, which accounts for the metabolic adaptation to high concentrations of ethanol that develops upon chronic substantial ethanol consumption.

In terms of the nutritional role, CYP2E1 is inducible by fasting in the rat [95]. The increase may be due, at least in part, to ketones. Indeed, in rats [96], rabbits [97], and humans [98], acetone is actively utilized, being metabolized by a microsomal acetone mono-oxygenase identified as CYP2E1 [99, 100]. Acetone is both an inducer and a substrate of CYP2E1 [101,102]. The existence of a gluconeogenic pathway for acetone was shown by the incorporation of [^{14}C] acetone into glucose and aminoacids during fasting and diabetic ketoacidosis [98,103,104]. The conversion of acetone to acetol and then to methylglyoxal, both intermediates in the gluconeogenic pathway, was demonstrated *in vitro* [96,97,105]. The contribution of CYP2E1 to the biotransformation of acetone was shown *in vivo* by the increase in blood acetone after the CYP2E1 inhibitors diallyl sulfide, diallyl sulfoxide, and diallyl sulfone [106]. It is noteworthy, as already pointed out by Reichard et al., [103] that acetone may be a significant gluconeogenic precursor in fasting humans, accounting for 10% of the gluconeogenic demands.

The role of CYP2E1 in fatty acid metabolism also supports the concept of a nutritional role for CYP2E1. Indeed, CYP2E1, in addition to its ethanol oxidizing activity, catalyzes fatty acid ω-1 and ω-2 hydroxylations [107-109]. Ethanol feeding also results in an increased activity of CYP4A1 [110]. The CYP4A subfamily catalyzes ω-hydroxylation at the terminal carbon of fatty acids. Further oxidation of the ω and ω-1 hydroxyacids by alcohol and aldehyde dehydrogenases results in the production of dicarboxylic and oxo-carboxylic acids.

5) Gender difference

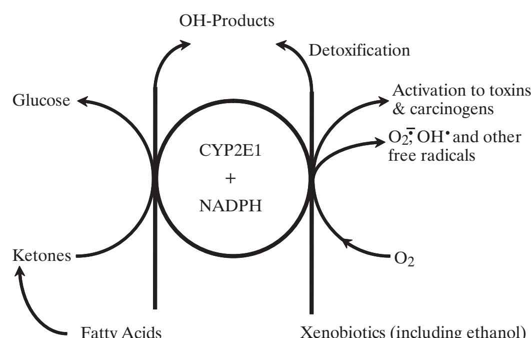
It is of interest that dicarboxylic acids (products of the CYP4A-mediated pathway) play a regulatory role in the hepatic disposition of non-esterified fatty acids by activating a peroxisomal proliferator nuclear receptor alpha (PPNRα) which increases the transcription of key fatty acid disposal pathways, such as the microsomal CYP4A1, the peroxisomal acyl-CoA oxidase and the liver fatty acid-binding protein (L-FABPc) in the cytosol [111]. This, in turn, results in enhanced microsomal ω-oxidation and peroxisomal β-oxidation of fatty acids and in a greater stimulatory effect of L-FABPc on microsomal acylglycerol synthesis [112].

The resulting changes exacerbate the gender difference in the alcohol-induced lipid abnormalities and in the vulnerability to alcoholic liver disease. It was known that gender differences in ethanol distribution [113], bioavailability [114] and hepatic metabolism [115], with increased production of acetaldehyde [116], may contribute to women's vulnerability to alcohol consumption. In addition, gender differences in alcohol-induced derangements of hepatic lipid metabolism, affecting the disposition of potentially toxic fatty acids, have also been reported in animal experiments [117]. Indeed, chronic alcohol administration increased ω-oxidation more effectively in male than in female rats [110], explaining the potentially deleterious accumulation of non-esterified fatty acids in the liver of the alcohol-fed females [117]. This was also associated with a much smaller ethanol-induced increase in cytosolic L-FABPc and microsomal esterification in females than in males, whereas the inhibition of mitochondrial β-oxidation was similar in both genders. Related findings have been observed in humans [118] and contribute to the greater vulnerability of women to the development of alcoholic liver injury.

6) CYP2E1 and oxidative stress

The increase of CYP2E1 has also been shown to play a key role in the pathogenesis of alcoholic liver injury, including ASH, because of the oxidative stress it generates [51]. Actually, CYP2E1 is also invariably elevated in the liver of patients with NASH [119] because fatty acids (which increase in obesity) and ketones (which increase in diabetes) are also substrates for CYP2E1 (vide supra and *Fig. 5*); their excess up-regulates CYP2E1. Although the pathogenesis of NAFLD and NASH has not yet been fully elucidated, a popular mechanism is the "Two Hit" theory [120], the first hit being the accumulation, by several causes (such as obesity), of fatty acids in the liver. The second hit is the peroxidation of these fatty acids because of the oxidative stress produced by different factors, such as CYP2E1 induction [121].

Figure 5. **Physiologic and toxic roles of cytochrome P4502E1 (CYP2E1), the main enzymes of the microsomal ethanol oxidizing system (MEOS). Many endogenous and xenobiotic compounds, including ethanol, ketones, and fatty acids, are substrates for CYP2E1 and induce its activity through various mechanisms, resulting in an array of beneficial as well as harmful effects. NADPH = Reduced form of nicotinamide adenine dinucleotide phosphate (from** [52]**)**



7) Mitochondrial injury

The damage caused by oxidative stress in both ASH and NASH includes mitochondrial injury, which in turn exacerbates the oxidative stress [51]. That mitochondrial damage is a key component of alcoholic liver injury is well established [5], but NASH is also associated with mitochondrial structural defects whereas NAFLD is not. This mitochondrial dysfunction contributes to the oxidative stress in NASH [122].

Like many other useful adaptive systems, when the adaptation becomes excessive, adverse consequences prevail. CYP2E1 leaks oxygen radicals as part of its operation (*Fig. 5*) and when they exceed the cellular defense systems, they result in oxidative stress with its pathologic consequences. This is true when excess alcohol has to be metabolized, as in ASH, or when CYP2E1 is confronted by an excess of ketones and fatty acids associated with diabetes and/or obesity, resulting in NASH.

8) NASH

No therapy for NASH has been proven clearly effective [123-126]. Preliminary data with antioxidants such as vitamin E and C in the treatment of NASH are promising [127], but need to be confirmed. In view of the pathogenic role of CYP2E1 in both ASH and NASH, inhibitors are being considered to oppose the up-regulation of CYP2E1 activity by either ethanol, fatty acids or ketones, which results in increased generation of reactive radicals and toxic metabolites. Indeed, it has been suggested that CYP2E1 inhibitors may eventually provide useful tools for the prevention and treatment of the hepatotoxicity associated with heavy drinking as well as overeating. Indeed, experimentally, a decrease in the inducibility of CYP2E1 was found to be associated with a reduction in associated liver injury [128,129] but when the liver pathology was semiquantitated, it was only partially ameliorated by the CYP2E1 inhibitors used [130,131]. Indeed, the CYP2E1 inhibition was incomplete. Whereas CYP2E1 inhibitors completely blocked lipid peroxidation, they only partially prevented other lipid abnormalities [128]. Thus far, no corresponding human data are available, mainly because of a lack of very effective agents suitable for chronic human use. Several inhibitors [132] have been used or are being developed [133] but there is still a need to obtain CYP2E1 inhibitors which are both effective and also innocuous enough to be used chronically in humans.

It is noteworthy that preliminary results in a rat model of NASH [134] revealed that the combination of SAMe and DLPC, two physiological antioxidants, reduces the oxidative stress resulting from the CYP2E1 induction generated by an excess of lipids, thereby significantly attenuating key changes associated with NASH. Accordingly, since SAMe and DLPC are innocuous, clinical trials with NASH patients can now be considered.

Using the same NASH experimental models, acarbose, a glucosidase inhibitor, was also found to attenuate the NASH [135].

Progression of fatty liver to inflammation and fibrosis

1. Role of products of lipid peroxidation

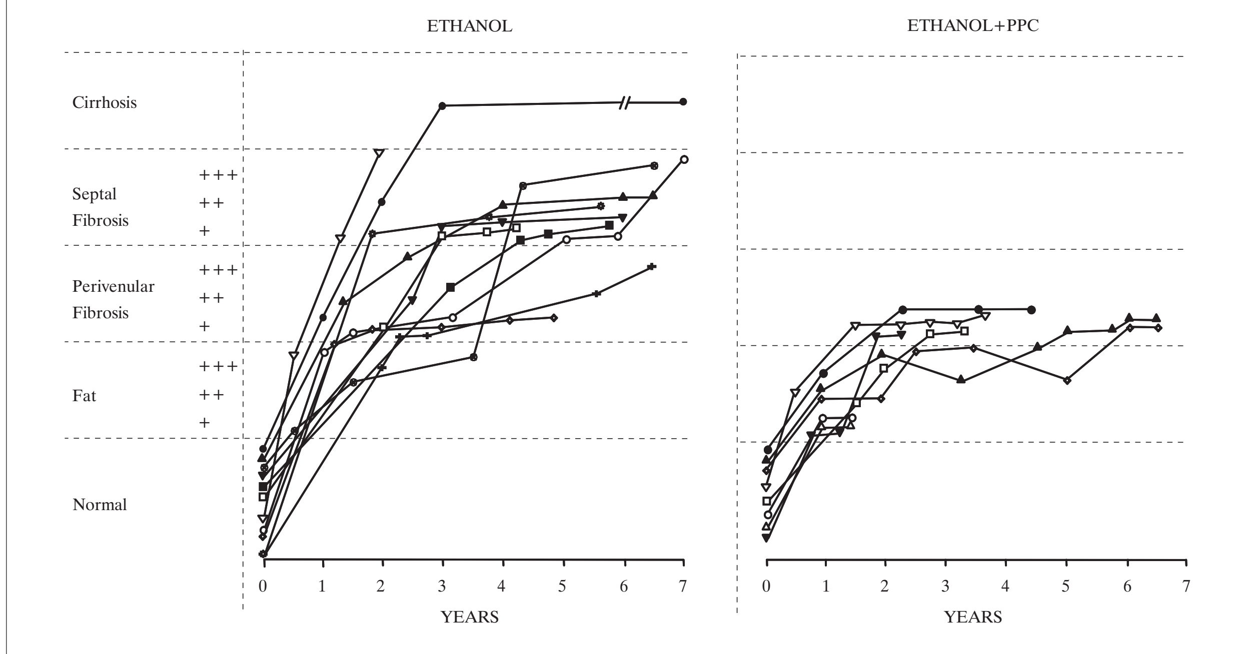
The oxidative stress caused by CYP2E1 induction, acetaldehyde and mitochondrial injury (vide supra) results in lipid peroxidation and membrane damage. In addition, lipid peroxidation products such as 4-hydroxynonenal stimulate fibrogenesis which is also increased through feedback inhibition of collagen synthesis because acetaldehyde forms adducts with the carboxyl-terminal propeptide of procollagen [40].

2. Role of inflammation, cytokines and anticytokine therapy

Oxidative stress promotes inflammation which is aggravated by an increase of the proinflammatory cytokine TNF-α in the Kupffer cells. TNF-α causes metabolic disturbances that are similar to the complications of alcoholic hepatitis, and it is also an important mediator of endotoxic shock and sepsis. Kupffer cells are a major source of cytokines. They also harbor CYP2E1 and its increase after chronic alcohol consumption [136] may act as a major stimulator. Indeed, in both acute and chronic liver diseases, Kupffer cells become activated to produce cytokines and reactive oxygen radicals [137]. Of potential therapeutic interest is the observation that DLPC (the active PC species of PPC) decreases stellate cell activation [138] and that it selectively modulates the LPS-induced activation of Kupffer cells by decreasing the production of the cytotoxic TNF-α, while potentiating the release of the protective IL-1β [139]. This dual action of DLPC on cytokines may provide a potent mechanism against liver injury. In addition to the reduction in TNF-α, the enhanced release of IL-1β could oppose the hepatotoxicity of TNF-α, either directly or indirectly through an IL-1β related increase in the tolerance to TNF-α mediated toxicity.

Recently, it has also been shown that ethanol induces transforming growth factor α (TGF-α) production in hepatocytes, leading to stimulation of collagen synthesis by hepatic stellate cells [140]. These results suggest that TGF-α derived from ethanol-exposed hepatocytes may contribute to the development of hepatic fibrosis in alcoholic liver disease. This effect was spe-

Figure 6. **Sequential development of alcoholic liver injury in baboons fed ethanol with adequate diet (left panel) and prevention of septal fibrosis and cirrhosis by supplementation with polyenylphosphatidylcholine (PPC) (right panel). Liver morphology in animals pair-fed control diets (with or without PPC) remained normal (not shown) (from** [146])



cifically inhibited by anti-TGF-α antibodies. It is noteworthy that experimentally DLPC also decreased TGF-β1-induced collagen mRNA by inhibiting p38 MAPK in hepatic stellate cells [141]. These are activated through induction of CYP2E1, acetaldehyde and by endotoxin. TNF-α is decreased by DLPC, but also in stellate cells not only in Kupffer cells (vide supra) [141]. In addition, collagen accumulation reflects not only enhanced synthesis, but results from an imbalance between collagen degradation and collagen production. Thus, cirrhosis might, in part, represent a relative failure of collagen degradation to keep pace with synthesis. Interestingly, PPC may affect this balance. Indeed, addition of PPC to transformed lipocytes was found to prevent the acetaldehyde-mediated increase in collagen accumulation, possibly by stimulation of collagenase activity [142]. The active ingredient was identified as DLPC [143]. The role of collagenase was also shown indirectly in man by the correlation of the development of alcoholic fibrosis with increased activity of the circulating tissue inhibitor of metalloproteinase (TIMP). Indeed, serum TIMP was significantly increased in alcoholic cirrhosis and may play a role in its pathogenesis through inhibition of collagenase activity. In addition, it can serve as a marker of precirrhotic states, since this test was more sensitive in detecting either perivenular fibrosis or septal fibrosis and offered better discrimination from fatty liver than serum procollagen peptide (PIIIP) [144]. The stimulation of collagenase activity [142] may explain, at least in part, why PPC attenuates the development of fibrosis (including cirrhosis) after chronic alcohol administration [145], an effect confirmed using more purified lecithin extracts, which again pointed to DLPC as the active ingredient of PPC [146] (*Fig. 6*). In the latter studies, the control livers remained normal whereas 10 of 12 baboons fed alcohol without PPC developed septal fibrosis or cirrhosis, with transformation of 81±3% of the hepatic lipocytes to collagen producing transitional cells. By contrast, none of the 8 animals fed alcohol with PPC developed septal fibrosis and cirrhosis, and only 48±9% of their lipocytes were transformed.

Antifibrotic therapy through correction of altered nutrient activation or of the deficiency in selective nutrients

The nutritional approach to liver disease has been improved by recognizing the importance not only of providing a sufficient intake of nutrients but also of correcting the impairment in the activation of key nutrients by alcohol.

1. Methionine and S-adenosylmethionine (SAMe)

a. Pathogenesis of the deficiency and its consequences

Correction of methionine deficiency has been proposed for the treatment of liver diseases, especially the alcoholic variety, but excess methionine was shown to have some adverse effects, as reviewed elsewhere [147]. Whereas in some patients with alcoholic liver disease, circulating methionine levels are normal, in others elevated levels were observed and it was reported that the blood clearance of methionine after an oral load was slowed, suggesting impaired metabolism. Indeed, for most of its functions, methionine must be activated to SAMe and, in cirrhotic livers, Duce et al. [148] reported a decrease in the activity of the enzyme involved, namely SAMe synthetase, also called methionine adenosyltransferase [149] (*Fig. 7*). Furthermore, long-term ethanol consumption in non-human primates was associated

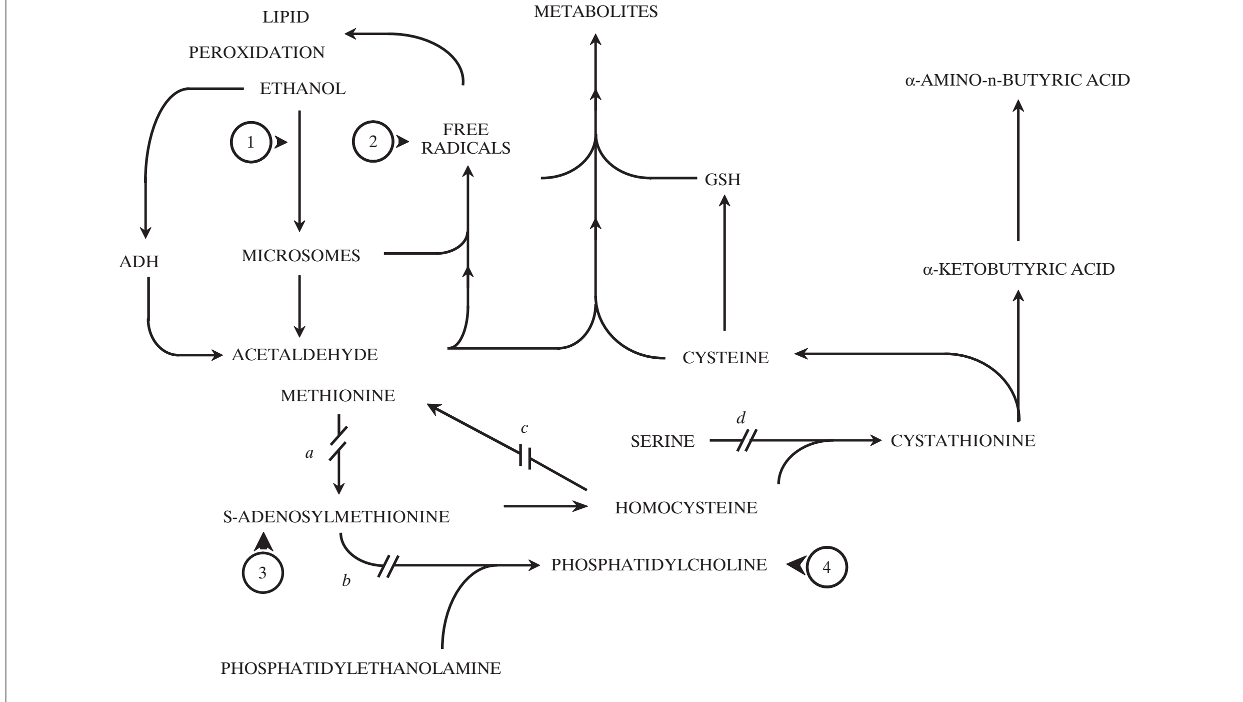


Figure 7. **Link between enhanced lipid peroxidation and accelerated acetaldehyde production as well as increased free radical generation by the induced microsomes, with sites of possible therapeutic interventions. Metabolic blocks caused by liver disease (a, b) or folate (c), B_{12} (c), or B_6 (d) deficiencies are illustrated, with corresponding depletions in S-adenosylmethionine, phosphatidylcholine, and reduced glutathione (GSH). New therapeutic approaches include: ① down-regulation of microsomal enzyme induction, especially of cytochrome P450 2E1 (CYP2E1); ② decrease of free radicals with antioxidants, and replenishment of ③ S-adenosylmethionine as well as "phosphatidylcholine. ADH = Alcohol dehydrogenase. (from** [147]**)**

with a significant depletion of hepatic SAMe [145] which may have a number of adverse effects since SAMe is the principal methylating agent in various transmethylation reactions which are important for nucleic acid and protein synthesis, membrane fluidity and the transport of metabolites and transmission of signals across membranes. Because of impaired methyltransferase activity, SAMe depletion exacerbates the membrane injury induced by alcohol. Furthermore, SAMe plays a key role in the synthesis of polyamines and provides a source of cysteine for glutathione production (*Fig. 7*).

b. Therapeutic applications of SAMe, including clinical trials

In baboons, correction of the ethanol-induced hepatic SAMe depletion with oral SAMe administration resulted in a corresponding attenuation of ethanol-induced liver injury, as shown by a less striking glutathione (GSH) depletion, lesser increases in plasma AST and glutamic dehydrogenase (GDH) activities and fewer megamitochondria [145]. SAMe treatment is also beneficial in man, as shown in a prospective, multicenter, double-blind, placebo-controlled trial [128] performed in 220 inpatients with chronic liver diseases (chronic active hepatitis and cirrhosis, including primary biliary cirrhosis) in whom serum markers of cholestasis and subjective symptoms significantly improved after SAMe. Oral administration of 1200 mg/day of SAMe for 6 months also resulted in a significant increase of hepatic GSH in patients with alcoholic as well as nonalcoholic liver disease [150]. Noteworthy is a two-year randomized, placebo-controlled, double-blind, multicenter clinical trial of SAMe in patients with alcoholic liver cirrhosis (Child class A and B) in whom SAMe improved survival (29% vs 12%, P=0.025) or delayed liver transplantation [151].

2. Antioxidant therapy

a. Polyenylphosphatidylcholine therapy

This promising therapy involves using polyunsaturated PPC or specifically its main PC species namely DLPC. An early event in the development of ALD is a decrease in hepatic levels of phospholipids, especially PC. This phospholipid deficiency is associated with altered mitochondrial membranes and correlates with morphologic changes and impaired function. Administration of PPC to primates corrected the ethanol-induced decrease in phospholipid levels and prevented progression to alcohol-induced septal fibrosis and cirrhosis [144], with an associated reduction in the number of activated stellate cells. These had been found to be strikingly increased in alcohol-fed baboons, contributing to the progression of fibrosis [152]. A similar transformation of stellate cells was observed in patients with alcoholic liver disease [153], and an attenuation of the activation of stellate cells to myofibroblast-like cells was also observed *in vitro* [138].

A possible explanation for the beneficial effect of PPC, especially DLPC, is the ability of liver cells to directly incorporate the phospholipids into cell membranes. This influ-

ences membrane structure and function. Indeed, the activity of membrane-bound enzymes was normalized: cytochrome oxidase, a key enzyme of the mitochondrial electron-transport chain, which requires a normal phospholipid milieu for optimal activity, and which is severely depressed by chronic ethanol consumption, was restored to normal by addition of PC *in vitro* [154] or by PPC supplementation *in vivo* [155], with improvement of hepatic mitochondrial respiration. It is noteworthy that misoprostol can also attenuate several functional alterations in liver mitochondria during alcohol consumption [156].

PPC promotes collagen breakdown [146] and it also acts as an antioxidant [157] which results in a protection against oxidative stress, as determined by normalization of 4-hydroxynonenal, F2-isoprostanes, and GSH levels [157]. Furthermore, the activity of phosphatidylethanolamine methyltransferase (PEMT), a key enzyme for the regeneration of hepatic PC, and which is depressed in alcoholic liver disease (*Fig. 7*), is also restored with PPC treatment [146].

All these favorable effects of PPC provided a basis for some of the reported therapeutic effects of unsaturated phospholipids in the treatment of liver diseases, including patients with alcoholic hepatitis [158]. Furthermore, PC was shown [146] to interfere with collagen formation from stellate cells which have a predominant role in alcohol-induced fibrogenesis. Subsequently, PPC was found to improve liver tests in patients with hepatitis C [159], and to ameliorate alcoholic liver disease in a pilot study of patients with underlying fibrosis [160]. This was followed by a randomized, prospective, double-blind, placebo-controlled clinical trial conducted in twenty VA medical centers with 789 patients (97% male; mean age 48.8 years) averaging 16 drinks/day (1 drink =14 g alcohol) for nineteen years [161]. A baseline liver biopsy confirmed the presence of perivenular or septal fibrosis or incomplete cirrhosis. Alcohol intake was reduced to approximately two-and-one-half drinks/day as a result of the Brief Intervention approach [162,163]. Accordingly, there was no more progression of the fibrosis and therefore no way to test whether PPC could oppose such a progression except for a subgroup who were still consuming 6 or more drinks a day and who displayed beneficial effects against fibrosis. Ascites, an important secondary clinical measure of liver disease, was also less frequently observed during follow-up of PPC treated patients. Furthermore, improvement in transaminases and bilirubin favoring PPC was seen in the subgroup of HCV^+ drinkers. We found that 25% of all patients with alcoholic liver disease are HCV^+, with an even higher incidence in some U.S. urban areas [164]. In addition to antiviral medications, agents that oppose oxidative stress and fibrosis such as PPC are also being tested for hepatitis C treatment since these two processes contribute much to the pathology and mortality associated with the virus.

b. Silymarin and other antioxidants

Silymarin was found to be effective against various liver injuries in rodents. In patients with alcoholic liver disease, some randomized controlled trials with silymarin showed beneficial effects such as improved survival [165]. However, other studies did not verify such an effect [166]. To clarify this issue, silymarin was studied under controlled conditions in non-human primates and it was found to oppose the alcohol-induced oxidative stress and to retard the development of alcohol-induced hepatic fibrosis [161]. The negative outcome observed in some clinical trials possibly reflects poor compliance. Thus, in view of the innocuity of silymarin, it might be justified to verify its effect in additional clinical studies. Theoretically, other antioxidants, such as alpha-tocopherol, might be useful. Indeed, in patients with cirrhosis, diminished hepatic vitamin E levels have been observed [167]. However, therapeutic results were disappointing [168].

3. Anti-inflammatory therapy

a. Corticosteroids

Because of the potential role of inflammatory factors in the pathogenesis of fibrosis and cirrhosis, anti-inflammatory therapy with corticosteroids has been used. It improved survival rates in encephalopathic patients but not in those with milder illnesses [169]. In patients who had either spontaneous hepatic encephalopathy or a high hepatic discriminant function (based on elevated prothrombin time and bilirubin concentration), prednisolone improved survival by 2 months [170]. However, Christensen and Gluud [171] performed a meta-analysis of 15 treatment trials and concluded that steroids are of no benefit in alcoholic hepatitis. Obviously, multiple factors affect the outcome and present a great challenge to the clinician.

b. Colchicine

Colchicine has also been evaluated as a treatment for alcoholic cirrhosis because of its anti-inflammatory and antifibrotic effect [172]. At the end of this study, patients in the colchicine group had a significantly better 5-year survival. However, these beneficial effects were not confirmed in a recent multicenter trial [173].

Conclusions

The better understanding of the pathogenesis of alcoholic liver disease resulting from extensive research carried out over the last half century has raised prospects for better treatment as well. Our present therapeutic approach is to first reduce excess alcohol consumption by the Brief Intervention technique which, in a recent controlled study, resulted in a significant and striking decrease of alcohol consumption from an average 18 to only 2½ drinks per day sustained for up to 5 years [162]. New tools have also been developed for early recognition and detection of heavy drinkers, based on circulating biologic markers, mainly carbohydrate-deficient transferrin, and preferably in association with γ-glutamyltransferase (GGT) [174]. These biochemical markers, combined with screening for signs of medical complications, help overcome patient denial and facilitate early detection. Craving can be opposed by such pharmacologic agents as naltrexone [175].

Thus, effective prevention and therapy against steatosis and its progression to more severe injury can be achieved by a multifactorial approach: control of alcohol consumption, avoidance of obesity and excess dietary long-chain fatty acids, and replenishment of SAMe and the phosphatidylcholines (using PPC).

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