REVIEW

Cocarcinogenic effects of alcohol in hepatocarcinogenesis

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Alcohol is a major aetiological factor in hepatocarcinogenesis but our understanding of its importance as a modulating factor is just beginning to emerge. In the present review, a number of possible cofactors and mechanisms are discussed by which alcohol may enhance the development of hepatoma. These include dietary or environmental carcinogens ingested along with alcoholic beverages, alcoholic cirrhosis as a precancerous condition, and the effects of alcohol metabolism.

SUMMARY

The incidence of hepatocellular carcinoma is rising worldwide. Apart from hepatitis B and C viruses, alcohol presents a major aetiological factor in hepatocarcinogenesis, as shown in numerous epidemiological studies. While the pathogenic role of alcohol in the development of liver cirrhosis has been investigated extensively, our understanding of its importance as a modulating factor in hepatocarcinogenesis is just beginning to emerge. In the present review, a number of possible cofactors and mechanisms are discussed by which alcohol may enhance the development of hepatoma. These include dietary or environmental carcinogens ingested along with alcoholic beverages, alcoholic cirrhosis as a precancerous condition, and the effects of alcohol metabolism such as the toxicity of its metabolite acetaldehyde, increased lipid peroxidation due to reactive oxygen species, activation of procarcinogens via induction of cytochrome P450 2E1, and polymorphisms of alcohol dehydrogenase. Furthermore, alterations of DNA methylation through interactions with one carbon metabolism can lead to aberrant methylation of tumour suppressor genes and oncogenes. Alcohol metabolism also reduces hepatic retinoic acid levels and may thereby enhance cell proliferation and malignant transformation via upregulation of activator protein 1 gene expression. Synergistic effects between alcohol and hepatitis B and especially C virus have been demonstrated, although the mechanisms remain unclear. Alcohol leads to malfunction of the immune system, and suppression of natural killer cells by alcohol may favour tumour development. Thus alcohol is commonly considered a tumour promoter. However, evidence from animal studies that showed preneoplastic alterations after chronic alcohol exposure indicate that alcohol may also contribute to tumour initiation.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumour among the commonest malignant tumours today.[1] Its prevalence is increasing worldwide but differs greatly between regions, with incidences of approximately 3–4/100 000 in Western countries[2–4] and up to 120/100 000 in Asia and Southern Africa. In more than 80% of European and North American cases, HCCs develop in cirrhotic livers whereas in Asia near 50% of HCCs may occur in non-cirrhotic livers.[5 6] The increase in HCC is most likely due to the more widespread chronic infection with hepatotropic viruses, namely hepatitis B (HBV) and especially hepatitis C (HCV). Epidemiological studies have incriminated both viruses in hepatocarcinogenesis, and the contributory role of alcohol, a major aetiological factor of liver cirrhosis in Western countries, is undisputed.[1] In the following, we summarise the evidence and discuss potential mechanisms of the cocarcinogenic effect of alcohol.

EPIDEMIOLOGY

There is compelling evidence that chronic alcohol consumption increases the risk of developing HCC.[7–9] However, the exact role of alcohol in the development of HCC compared with chronic HBV and HCV infection is still incompletely defined. Numerous studies demonstrated that the incidence of HCC among alcoholics is above the expected rate.[10] Thus an epidemiological survey from the UK demonstrated an eightfold increase in the risk of developing HCC among male alcoholics.[11] The higher rate of alcohol related HCC worldwide may be partially explained by prolongation of survival time of patients with alcoholic cirrhosis due to improved disease management.

> "Chronic alcohol consumption increases the risk of developing HCC"

The effect of abstinence in the development of HCC was discussed controversially in various studies. It was shown that cessation of alcohol

Abbreviations: AA, acetaldehyde; ADH, alcohol dehydrogenase; AFB_1, aflatoxin B_1; ALD, alcoholic liver disease; ALDH, aldehyde dehydrogenase; DMN, dimethyl-nitrosamine; CYP 2E1, cytochrome P450 2E1; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MB, Mallory body; NK, natural killer; RA, retinoic acid; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; TNF-α, tumour necrosis factor α.

consumption increased the risk of developing HCC. This was explained by alterations in cell regeneration after alcohol withdrawal, which will be discussed below. However, a major plausible argument is that abstinence allows recovery from alcohol related hepatocellular damage which by prolonging survival time may by itself increase the likelihood of developing HCC in a cirrhotic liver.

ANIMAL EXPERIMENTS

Experiments in which alcohol but no carcinogen was given continuously to rodents have shown that alcohol per se is not a carcinogen as even lifelong exposure to alcohol did not lead to more cancers than in pair fed controls.[8] Most animal experiments with respect to hepatocarcinogenesis have been performed using nitrosamines as tumour inducing compounds. Unexpectedly, in almost all of these studies inhibition of hepatocarcinogenesis together with alcohol intake was shown.[8] On the other hand, the rate of extrahepatic tumours, such as tumours in the nasal cavity, trachea, and oesophagus, increased. Only with additional manipulations, such as administration of a diet low in methyl donors or carbohydrates,[12 13] or after partial hepatectomy,[14] was alcohol able to stimulate hepatocarcinogenesis. Interestingly, striking enhancement of hepatic carcinogenesis was observed when alcohol and the procarcinogen were given on an alternating basis to avoid interactions between alcohol and carcinogen metabolism. Another important determinant in animal studies of alcohol is the route of administration. If ethanol is given with drinking water, nutrient deficiencies may occur due to interactions with their absorption that may influence carcinogenesis. Administration of ethanol as a liquid diet, a technique established by Lieber and DeCarli,[15] assures constant alcohol intake and provides adequate amounts of macro and micronutrients.

PATHOMECHANISMS

For the liver, there are multiple mechanisms by which alcohol can accelerate cancer development. These include enzymes and metabolites involved in ethanol metabolism, such as cytochrome P450 2E1 (CYP 2E1) which can potentiate carcinogens, interference with methyl transfer, modulation of retinoid turnover, and the preconditioning associated with concomitant infection by HBV and HCV. Importantly, cirrhosis by itself is a precancerous condition. As no single pathomechanism can be incriminated exclusively, several must act in concert to induce HCC.

Alcohol, cirrhosis, and preneoplastic histology

Alcohol causes hepatocellular injury that can lead to enhanced fibrogenesis and finally cirrhosis, the latter being per se associated with an increased risk of developing HCC. Alcohol related HCC without pre-existing cirrhosis is rare,[10] indicating that pathogenic events leading to cirrhosis precede those causing cancer or that the structural alterations of cirrhosis favour hepatocyte dedifferentiation. Although the presence of cirrhosis can be considered the major prerequisite for the development of HCC, various other pathogenic factors may contribute significantly to the malignant transformation of hepatocytes.

> "Alcohol related HCC without pre-existing cirrhosis is rare"

Some controversy exists as to whether alcohol is a tumour inducer in hepatocarcinogenesis (fig 1). In various animal models attempts have been made to correlate the stages of initiation, promotion, and progression in hepatocarcinogenesis with specific precancerous histological features. Thus centres of focal growth have been observed which show a number of metabolic alterations—for example, enzyme altered foci and preneoplastic nodules.[16] Recently, such areas of preneoplastic tissue were also produced in rats by alternate treatment with *N*-nitrosodimethylamine as a cancer inducer and alcohol, strongly suggesting that ethanol may indeed act as a tumour promoter in hepatocarcinogenesis (fig 1).[17] Interestingly, Mallory body (MB) formation is high in HCC and the incidence of HCC is significantly higher in cirrhosis with MBs than without.[18] It was therefore hypothesised that MBs may represent an initial phenotypical alteration in the carcinogenic transformation of hepatocytes.

Another histological abnormality observed in experimental hepatocarcinogenesis is the occurrence of oval cells which originate from the portal triads after long term alcohol exposure.[19] These cells do also appear after administration of a choline deficient ethionine supplemented diet which is known to stimulate hepatocarcinogenesis.[20] Recently, the occurrence of oval cells has also been observed in patients with chronic alcoholic liver disease.[21]

Alcohol and environmental carcinogens

Alcoholics may be exposed to carcinogens or procarcinogens ingested along with alcoholic beverages which may contain nitrosamines, polycyclic hydrocarbons, asbestos fibres, and fusel oils.[22] In addition, many alcoholics are smokers and epidemiological surveys have shown a hyperadditive effect of alcohol and smoking in increasing the risk of developing HCC.[9] Similarly, dietary carcinogens and exposure to carcinogens at the working place have to be taken into account (see fig 1). With regard to the former, aflatoxin B_1 (AFB_1) is a major hepatocarcinogen which is metabolised by the alcohol inducible cytochrome P450 2E1 (CYP 2E1). AFB_1 can induce a mutation in codon 249 of the p53 tumour suppressor gene which is frequently found in human HCC.[23]

> "Many alcoholics are smokers and epidemiological surveys have shown a hyperadditive effect of alcohol and smoking in increasing the risk of developing HCC"

Although animal experiments have been controversial as to whether ethanol enhances AFB_1 induced hepatocarcinogenesis, an epidemiological study on AFB_1 exposure demonstrated that even a moderate daily consumption of 24 g ethanol increases the risk of developing HCC induced by 4 μg of dietary AFB_1 by 35-fold.[24] Vinyl chloride is also metabolised by CYP 2E1 and its exposure is associated with the development of HCC which is again increased several fold by additional alcohol consumption.[25]

Alcohol metabolism and HCC

In the liver, ethanol is predominantly metabolised by alcohol dehydrogenase (ADH) and CYP 2E1, resulting in acetaldehyde (AA) formation. AA, the extremely toxic first intermediate of ethanol metabolism, binds rapidly to cellular proteins and also possibly to DNA. These AA adducts represent neoantigens leading to the formation of specific antibodies.[26] AA has mutagenic and carcinogenic properties leading to metaplasia, inhibition of DNA repair,[27] sister chromatid exchanges,[28] stimulation of apoptosis, and enhanced cell injury associated with hyperregeneration.[29] According to the International Agency for Research on Cancer, there is sufficient evidence to identify AA as a carcinogen in animals.

Ethanol is metabolised by the successive action of ADH and aldehyde dehydrogenase (ALDH). For both ADH and ALDH, genetic polymorphisms have been described that influence the rate of conversion of ethanol to AA and of the latter to acetate.[30] It has been consistently reported that ALDH2 is the most important alcohol metabolising polymorphic enzyme affecting predisposition to alcoholism in Asian populations. With regard to ADH, the alleles ADH2*2 and ADH3*1 encode

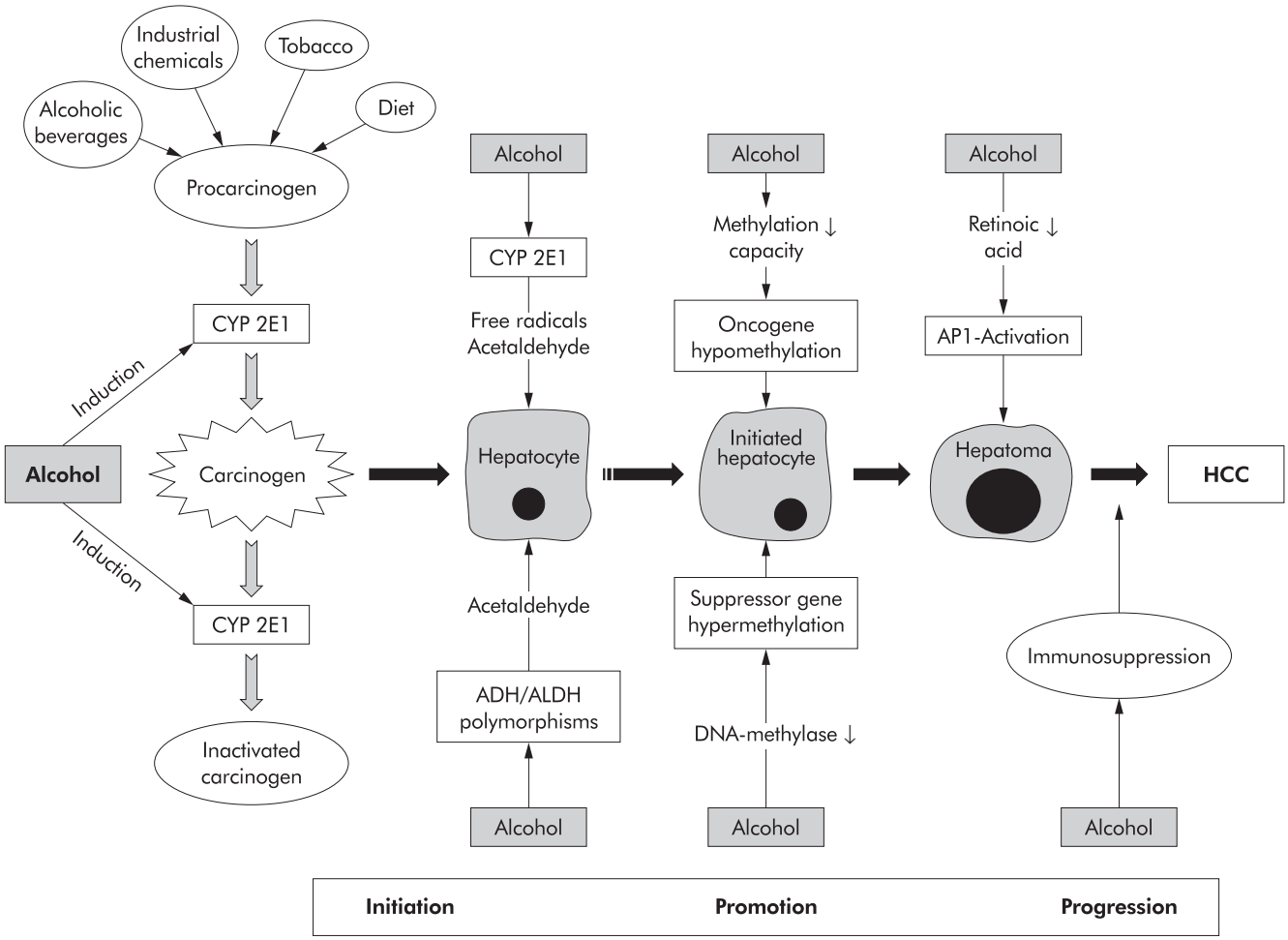

Figure 1 Alcohol as a promoter of hepatocarcinogenesis. Both activation and inactivation of procarcinogens can occur. Alcohol per se is a tumour promoter but may contribute to initiation via procarcinogen activation. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP 2E1, cytochrome P450 2E1; HCC, hepatocellular carcinoma.

for an enzyme with a high capacity to produce AA (400 kcat/min and 87 kcat/min).[31] Therefore, higher AA levels are found in patients revealing the alleles ALDH2*2, ADH2*2, and ADH3*1, either via increased AA synthesis or via decreased oxidation of AA to acetate. It has been shown that individuals with inactive ALDH2*2 or highly active ADH2*2 are at increased risk of alcoholic liver disease.[32] While no association between ADH2*2 and ALDH2 genes and HCC has been found,[33] preliminary data reveal a higher prevalence of ADH3*1 in patients with alcohol related HCC than controls (Stickel *et al*, unpublished data).

CYP 2E1 constitutes the microsomal ethanol oxidising system which is inducible by higher amounts of ethanol and other xenobiotics.[34] The degree of CYP 2E1 induction can be correlated with generation of reactive oxygen species (ROS), in particular hydroxyethyl radicals and lipid peroxides.[35] Moreover, experimental alcohol induced liver disease and CYP 2E1 can be modulated by CYP 2E1 inhibitors and inducers.[36–38] In the setting of alcoholism, additional sources of ROS formation may be NADH dependent cytochrome C reductase, aldehyde and xanthine oxidase, neutrophil NADPH oxidase, and catalase. ROS initiate predominantly lipid peroxidation but they may also react rapidly with cell constituents, including DNA, and thereby lead to DNA damage and cancer initiation.[39]

Hepatic iron plays a key role as an enhancer of ROS production. Alcohol consumption increases iron absorption from the gut with its consequent accumulation in the liver, which suggests an at least additive effect of alcohol and iron in the generation of ROS.[40] Under normal conditions, these toxic ROS are rapidly neutralised by reductive detoxification mechanisms, mainly glutathione, α-tocopherol, superoxide dismutase, catalase, and glutathione peroxidase. Eventually, the amount of ROS produced exceeds the neutralising capacity of these defence systems which may result in precancerous tissue and organ damage.[41] Therefore, the importance of oxidative stress in alcohol related liver disease and hepatic carcinogenesis has precipitated numerous experimental studies and clinical trials.[42] Although on the basis of pathophysiology the use of antioxidants in the treatment of alcoholic liver disease seems plausible, most clinical trials addressing their therapeutic effect in alcoholic liver disease have been negative.[43]

Induction of CYP 2E1 may also contribute to hepatocarcinogenesis by enhancing the conversion of various procarcinogens to eventual carcinogens such as dimethylnitrosamines (DMN), AFB_1, vinyl chloride, and dimethylhydrazine, as previously mentioned above.[44] In particular, the metabolic interaction between ethanol and nitrosamines has been investigated.[45] Alcohol induces low Km-DMN demethylase activity and CYP 2E1 which both lead to increased activation of this carcinogen in animals and humans.[46 47] However, ethanol is also an effective competitive inhibitor of DMN demethylase when administered simultaneously with DMN. The capacity of ethanol to both induce and inhibit DMN mediated hepatocarcinogenesis is strongly dependent on the presence or absence of alcohol at the time of carcinogen exposure. This phenomenon explains why some animal studies in which alcohol/nitrosamine interactions had been investigated have shown an increase in DMN induced hepatoma[17 48] and others have not.[49 50]

Recently, polymorphisms of CYP 2E1 were identified and Yu and colleagues[51] have suggested an association with hepatocarcinogenesis based on a higher prevalence (83.3% *v* 63.3%) of the CYP 2E1 c1/c1 genotype in patients with HCC compared with controls. Homozygosity of this genotype was associated with a significantly increased risk for the development of HCC in smokers. However, other investigators could not confirm the association of certain CYP 2E1 polymorphisms with the risk of HCC.[52 53]

TNF-α and intracellular signal transduction

A major feature in the pathogenesis of ALD is release of tumour necrosis factor α (TNF-α) and other cytokines, mainly from Kupffer cells that are stimulated by endotoxin absorbed from the gut.[54] In fact, elevated TNF-α levels and corresponding cytokines are a prominent feature of ALD compared with other liver diseases, finally resulting in hepatocyte proliferation or death, recruitment of inflammatory cells, and tissue remodelling.[54 55] TNF-α binds to its cellular receptors on hepatocytes and other liver cells leading to activation of various adaptor proteins and potentially to apoptosis via the caspase cascade. Thus TNF-α can trigger *jun*-N terminal kinase 1 which cooperates with other mitogens such as epidermal growth factor to promote proliferation.[56] On the other hand, activation of sphingomyelinase by TNF-α increases intracellular ceramide which inhibits the mitochondrial electron transport chain. Thereby, mitochondrial production of ROS is increased promoting lipid peroxidation and apoptosis independently of caspases. In addition, ROS, such as the superoxide anion as well as cytochrome C oxidase, are released from damaged mitochondria via activation of caspases 8 and 3 leading to apoptosis. However, increased levels of ROS also contribute to activation of the oxidative stress sensitive transcription factor nuclear factor κB. Nuclear factor κB is pivotal for initiation of a cell survival machinery involving antiapoptotic proteins such as Bcl-2, manganese superoxide dismutase, and nitric oxide synthase that protect the mitochondrial membrane potential.[57] Interestingly, experiments in mice that lack the signal transducing type I TNF receptor have demonstrated impaired liver regeneration after partial hepatectomy.[58] In summary, TNF-α may dose dependently lead to activation of cellular survival mechanisms, or to apoptosis and necrosis. This can explain why hepatocytes that are challenged by an inflammatory insult which is below the level leading to cell death may be more susceptible to proliferative stimuli and to dedifferentiation triggered by carcinogens such as AA and nitrosamines. Thus ethanol induced activation of nuclear factor κB could contribute to hepatocarcinogenesis.

Interactions with retinoids

Reduced serum and hepatic vitamin A concentrations have been shown in chronic alcoholics.[59] This is of particular importance as retinoic acid (RA) is synthesised from retinol via various enzymatic steps involving microsomal and cytosolic retinol dehydrogenases, as well as via cytosolic ADH and ALDH. RA has profound effects on cellular growth and differentiation via two families of RA nuclear receptors (RAR-α, -β, and -γ, and RXR-α, -β, and -γ) which mediate RA induced gene transcription.[60] In a series of experiments, the effects of alcohol on retinol and RA metabolism, on transcellular RA signalling, and on early events of carcinogenesis have been investigated. Chronic alcohol consumption affects several aspects of vitamin A metabolism, including retinol absorption, enhanced degradation in the liver, and increased mobilisation of retinol from the liver to other organs.[61 62] These ethanol induced changes may result in decreased hepatic concentrations of both retinol and retinyl esters which are the metabolically active precursors of RA.

"Chronic alcohol consumption affects several aspects of vitamin A metabolism, including retinol absorption, enhanced degradation in the liver, and increased mobilisation of retinol from the liver to other organs"

Furthermore, it has been demonstrated that ethanol acts as a competitive inhibitor of retinol oxidation in the liver, thereby counteracting the biosynthesis of RA.[63] Accordingly, RA levels in the liver of ethanol fed rats were decreased significantly compared with controls pair fed an isocaloric control diet containing equal amounts of vitamin A.[64] It has recently been shown that ethanol causes an additional local deficiency of RA in the liver, resulting from enhanced RA catabolism due to induction of CYP 2E1.[65] In the same study, treatment of ethanol fed rats with chlormethiazole, a specific CYP 2E1 inhibitor, restored both hepatic and plasma RA concentrations to normal levels. Enhancement of RA catabolism by ethanol in vitro was inhibited by CYP 2E1 antibodies and chlormethiazole, while catabolism of RA into polar metabolites was abolished completely by non-specific cytochrome P450 inhibitors. Lastly, chronic alcohol consumption resulted in a functional downregulation of RA receptors and an up to eightfold expression of the AP-1 (c-jun and c-fos) transcriptional complex.[63] This explains parenchymal hyperproliferation as AP-1 is a central complex downstream of various growth factors, oncogenes, and tumour promoters.[66] Most interestingly, supplementation of animals with *all-trans*-RA to normal RA levels not only leads to a decrease in AP-1 (c-jun and c-fos) gene expression but also to normalisation of hepatic proliferation, as expressed by proliferating cell nuclear antigen expression.[65] In summary, these data suggest that low hepatic RA levels due to chronic alcohol abuse may favour proliferation and malignant transformation of hepatocytes via upregulation of AP-1 (c-jun and c-fos) gene expression.

Alcohol and methylation

Hepatocarcinogenesis is a multistep process involving genetic events such as point mutations, as well as epigenetic factors, particularly aberrant DNA methylation patterns and post-transcriptional alterations. Changes in the degree of methylation of cytosine are frequently encountered in human cancers but their relevance as an epigenetic factor in carcinogenesis is only partially understood.[67] However, DNA methylation is an important determinant in controlling gene expression whereby hypermethylation has a silencing effect on genes and hypomethylation may lead to increased gene expression. In hepatocarcinogenesis, general hypomethylation may be coupled with areas of regional hypermethylation. Thus hypermethylation of tumour suppressor genes can result in decreased gene transcription of p53 and HIC-1,[68] and hypomethylation of certain oncogenes such as c-myc and c-N-ras may lead to dedifferentiation and proliferation.[69 70] Recently, it has been suggested that aberrant DNA hypermethylation may be associated with genetic instability, as determined by loss of heterozygosity and microsatellite instability in human HCC due to chronic viral hepatitis.[71 72] Iwata *et al* detected hypermethylation of the 14-3-3 sigma gene which has been implicated as a key inducer of cell cycle arrest associated with p53 in 89% of investigated human HCCs.[73] However, genetic alterations in animal models and human hepatocarcinogenesis differ substantially. Thus it was shown that activation of N-myc and c-myc oncogenes is frequent in woodchuck hepatitis virus associated HCC while no p53 mutations were found. This mutational pattern is reversed in humans where p53 are frequent and oncogene activation seems to play only a minor role.[16 74]

Importantly, modifications of the degree of hepatic DNA methylation have also been observed in experimental models of chronic alcoholism.[75 76] Hypomethylation is a plausible consequence of metabolic alterations in the setting of ethanol

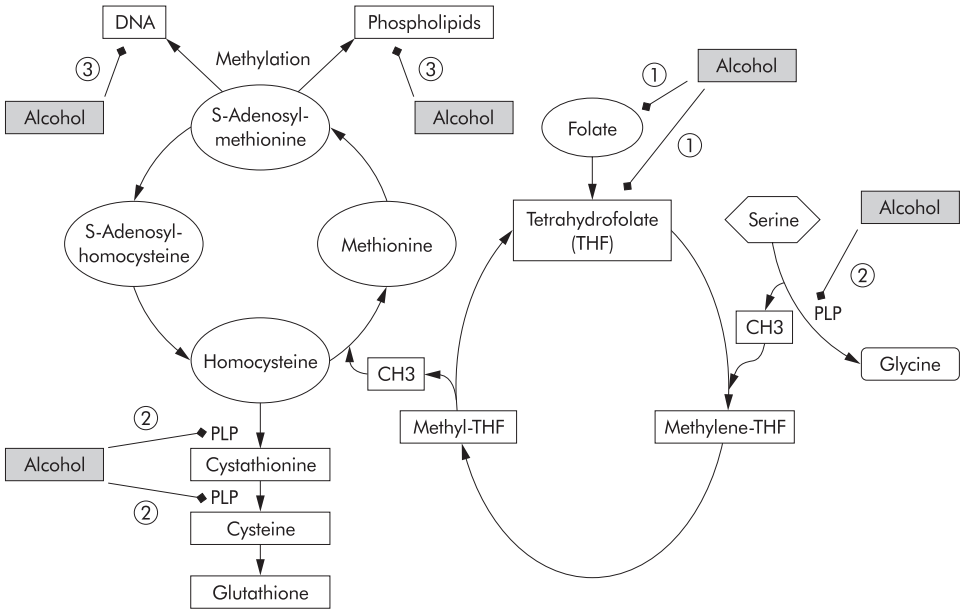

Figure 2 Interaction of alcohol with methyl transfer. Alcohol impairs one carbon metabolism via interfering with (1) folate uptake and generation of tetrahydrofolate (THF); (2) degradation of pyridoxal-5′-phosphate (PLP) at several sites; and (3) inhibition of methyl transfer to DNA via inhibition of methyltransferase, resulting in hypomethylation and consequently enhanced transcription of certain oncogenes.

consumption. In fact, alcohol has a marked impact on hepatic methylation capacity, as reflected by decreased levels of S-adenosylmethionine (SAM), an important methyl group donor, and increased levels of S-adenosylhomocystein (SAH), resulting in an up to 2.5-fold decrease in the SAM/SAH ratio.[77–79] Several mechanisms have been suggested by which ethanol could interact with one carbon metabolism and DNA methylation and thereby enhance carcinogenesis (fig 2):

(1) chronic alcohol interacts with intake, absorption, and subsequent metabolism of B vitamins involved in hepatic transmethylation reactions, namely folate and pyridoxal-5′-phosphate (vitamin B6), resulting in impaired methyl group synthesis and transfer[79–83];

(2) ethanol reduces the activity of methionine synthase which remethylates homocysteine to methionine with methyltetrahydrofolate as the methyl donor[84 85];

(3) chronic alcohol consumption decreases glutathione levels, a reductive tripeptide, which is synthesised from homocysteine via transsulphuration in the liver, and thereby enhances the susceptibility of the liver towards alcohol related peroxidative damage[85 86]; and

(4) alcohol can inhibit the activity of DNA methylase which transfers methyl groups to DNA in rats,[77] a finding which could not be confirmed in humans.[87]

To date, it is well established that dietary depletion of lipotropes, including methionine, choline, betaine, SAM, and folate, leads to DNA hypomethylation, particularly hypomethylation of oncogenes (that is, c-Ha-ras, c-Ki-ras, and c-fos) and to DNA strand breaks, all of which are associated with an increased incidence of HCC in rats.[88 89] Whether chronic alcohol consumption alone is capable of inducing a lack of methylation capacity sufficient to cause hypomethylation of DNA and genes involved in hepatocarcinogenesis is not yet known.

It has been shown that DNA and site specific hypomethylation is reversible, either spontaneously[90 91] or by therapeutic intervention.[16] In a randomised, controlled, multicentre trial, 123 alcoholic cirrhotics received SAM or placebo for two years.[92] The two groups were well matched and only six patients were lost during follow up. Mortality and the number of patients requiring liver transplantation were significantly lower in patients with Child C cirrhosis treated with SAM. However, no patient in the trial developed HCC, probably due to the short duration of surveillance. So far, a study investigating the chemopreventive effect of lipotropes—that is, SAM—in HCC has not been performed. Therefore, the role of ethanol in gene specific methylation requires further investigation.

Alcohol and hepatitis viruses

From epidemiological studies a close relationship has been noted between alcohol consumption, infection with hepatotropic viruses, and HCC. With respect to HBV, several studies have shown a high prevalence of HBV markers in patients with alcohol related HCC.[93] Brechot *et al* screened HCC liver specimens of alcoholics for HBV-DNA to find that they were all positive.[94] However, other investigators failed to confirm these data.[95 96] Thus the role of alcohol and chronic HBV infection in hepatocarcinogenesis awaits further clarification.

> "A close relationship has been noted between alcohol consumption, infection with hepatotropic viruses, and HCC"

In the case of chronic HCV, the role of alcohol abuse remains undisputed. A number of studies have demonstrated a high prevalence of antibodies to HCV among alcoholics with liver disease, ranging from 11% to 46%, even after confirmatory antibody tests or polymerase chain reaction were used, and after patients at risk (for example, recipients of blood transfusions and intravenous drug abusers) were excluded.[97] With regard to hepatocarcinogenesis, it has been shown unambiguously by various investigators that alcohol abuse coupled with HCV infection accelerates the development of HCC.[98 99] For example, Yamauchi and colleagues[98] showed that the cumulative incidence of HCC after three, five, and 10 years in cirrhotic HCV infected patients with an average daily alcohol consumption of 120 g was 13.3%, 41.3%, and 80.7% versus 0%, 8.3%, and 18.5%, respectively, compared with alcoholic cirrhotics without HCV infection. A case control study by Corrao *et al* in 115 patients with alcoholic liver disease and chronic HCV demonstrated a clear dose dependency between long term alcohol consumption and the development of cirrhosis, a necessary precondition of HCC development in chronic HCV.[100] The authors concluded that as little as 20 g/day was detrimental. While the modes of interaction between HCV and alcohol remain to be defined, there are a number of possible explanations.

(1) Chronic alcohol consumption and coinfection with HCV synergistically aggravate histological damage resulting in faster progression.[98 101 102]

(2) Alcohol appears to enhance HCV replication with subsequent direct cytopathic damage.[103 104]

(3) Alcohol may compromise the host's immune response to HCV infection, as demonstrated by Oshita and colleagues[103] who measured serum neopterin levels, a suggested indirect marker of macrophage activation. Thus in heavy drinkers coinfected with HCV, neopterin levels were significantly lower than in non-alcoholic HCV infected individuals. In addition, CD4 cells, which are important in the antiviral immune response, are particularly susceptible to alcohol related functional impairment.[105]

(4) Alcoholic patients with chronic hepatitis C show higher hepatic iron levels than patients with HCV infection alone and iron excess is an important factor in liver damage and may increase HCV replication.[106]

Alcohol and immune surveillance

Chronic alcohol consumption results in a complex alteration of the unspecific (innate) and specific (acquired) immune response.[107] Numerous studies and clinical experience have shown that chronic alcoholics are more susceptible to infections and to certain neoplasms.[108] Thus alcohol related alterations of immune surveillance could contribute to the development of cancer. Among the factors affecting the immune system in the setting of alcoholism are malnutrition, vitamin deficiencies, established cirrhosis, and alcohol itself. In this respect, the influence of alcohol on natural killer (NK) cells, which are implicated in the control of tumour development and growth, is of particular importance. Interactions between alcohol and this subset of cytotoxic cells have been investigated in cell culture, animal studies, and in human alcoholics. However, the data are conflicting which is mainly due to discrepancies in analysis of lymphoid subsets and NK cell cytotoxic activity, the presence or absence of active alcohol consumption, biased patient selection, and different nutritional status and comorbidity variables, such as coinfection with hepatitis viruses.[109]

> "Chronic alcoholics are more susceptible to infections and to certain neoplasms"

Studies in mice have demonstrated that chronic alcohol administration inhibits NK cell activity[110] and reduces NK cell number and lytic activity following a single binge equivalent of alcohol.[111] A more recent study in rats has shown that acute alcohol intoxication may lead to an up to 10-fold increase in the number of lung metastases of the NK cell controlled adenocarcinoma cell line MADB106.[112] Few studies in humans have so far been performed. In a study by Laso *et al*, alcoholic cirrhotics revealed both diminished NK cell numbers and reduced lytic activity, even when stimulated by interleukin 2, a powerful NK cell stimulating cytokine.[113] NK cell numbers were also found to be decreased in actively drinking individuals without established alcoholic liver disease.[114] Pathomechanisms are not fully understood but it has been suggested that transforming growth factor β1, which is a key profibrogenic cytokine in liver fibrogenesis and which is markedly elevated in alcoholic liver disease,[115] suppresses immune function in general and NK cell activity in particular.[116] However, there are no data on how far antigen specific lymphocyte reactivity, HLA class I and II expression, or organ specific lymphocyte subsets are altered in alcoholism.

In summary, a major impact of alcohol on the immune system is undisputed which may favour tumour development and expansion but mechanisms by which alcohol compromises antitumour immune surveillance are not yet completely understood.

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