ABSTRACT Chronic and excessive alcohol intake is associated with an increased incidence of a variety of cancers (e.g., liver, oral cavity, esophagus, colorectal and breast). Long-term alcohol intake results in impaired nutritional status of retinoic acid (RA), the most active derivative of vitamin A, which may provide a promoting environment for tumor formation. Recent studies demonstrate that chronic alcohol-induced hepatic cellular proliferation, which may convert hepatocytes from a state of resistance to a carcinogen to a state of high susceptibility, is due to alcohol-impaired RA metabolism and signaling and crosstalk with the Jun N-terminal kinases-dependent signaling pathway. Further, the restoration of hepatic RA homeostasis by treatment with either RA supplementation or inhibitors of RA catabolism can suppress alcohol-induced hepatectomy hyperproliferation and restore alcohol-deregulated apoptosis, thereby reducing the risk of alcohol-promoted hepatocellular carcinogenesis. These studies indicate the importance of RA actions in the prevention and/or treatment of alcohol-related carcinogenic process in the liver and other organs. J. Nutr. 133: 287S–290S, 2003.

KEY WORDS: • retinoids • ethanol • carcinogenesis • apoptosis • cell proliferation • rats

A number of epidemiological studies have indicated that long-term and excessive alcohol consumption is a significant risk factor for liver cancer (as well as other cancers, such as esophageal, gastric, oropharynx, colorectal, lung and breast cancer) (1). However, the mechanisms by which alcohol ingestion promotes carcinogenesis are not well defined (2). Several mechanisms have been proposed; these include increased generation of acetaldehyde that has mutagenic and carcinogenic effects, induction of microsomal cytochrome P450 (CYP) enzymes that activate various procarcinogens and generation of reactive free radicals that can directly damage DNA. Importantly, chronic alcohol intake can induce a number of biochemical and molecular alterations that contribute to increased hepatocyte proliferation and genomic instability, thereby providing a promoting environment for carcinogenesis. One of the prime candidates for such an alteration is impairment of retinoid nutritional status (3,4). In this mini-review, recent studies regarding possible protective roles of hepatic retinoid homeostasis and signal transduction pathways against alcohol-promoted hepatic carcinogenesis are discussed.

Ethanol-related carcinogenesis and protective effect of retinoids

Carcinogenesis is a multistage process consisting of initiation, promotion and progression. Initiation is rapid and occurs at a high frequency, whereas promotion is a long-term process that requires chronic exposure to a tumor promoter (e.g., long-term and excessive alcohol intake and vitamin A deficiency). Carcinogenesis for a number of tumors occurs in the context of chronic tissue injury and increased cell proliferation (5). For example, several carcinogens can induce tumors in various organs, but not in the liver, unless the exposure is associated with a proliferative stimulus (5). It has been shown that hepatocytes become hyperproliferative after chronic ethanol treatment (6,7). We have observed time-dependent changes in hepatocyte proliferation in rats fed ethanol (36% of total caloric intake) after 1- and 6-mo treatments (8,9). Alcohol acts as a promoter on the development of chemically induced hepatic cancer when alcohol is given to rats at 35% (but not at 5–10%) of total caloric intake (10). Therefore under certain conditions (e.g., in a chronic alcoholic state), the hepatocytes can escape normal proliferative controls to become proliferative, which may convert cells from a state of resistance to a carcinogen to a state of high susceptibility to a carcinogen.

It is well known that retinoids exert profound effects on development, cellular growth and differentiation. Retinoic acid (RA) plays an important role in controlling carcinogen-
Ethanol and retinoid metabolism

Several mechanisms are proposed to explain how ethanol might interfere with retinoid metabolism in the liver (Fig. 1).

First, ethanol may act as a competitive inhibitor of the oxidation of retinol to RA in the liver and other tissues [see Wang (3) for earlier publications]. This mechanism may be particularly relevant in the case of acute ethanol digestion. A recent study by Molotkov and Duester (21) demonstrated that the biosynthesis of RA in alcohol dehydrogenase (ADH) null mutant mice (ADH<sup>−/−</sup>) after a dose of retinol was reduced by 82%, which was similar to wild-type mice pretreated with ethanol (87% decrease), indicating that acute ethanol intake primarily decreases ADH-catalyzed RA synthesis.

Second, chronic ethanol intake increases the catabolism of vitamin A and RA into more polar metabolites in the liver (22). Recently, we have shown that the enhancement of catabolism of retinol and RA in ethanol-fed rats can be inhibited by chloromethiazole, an inhibitor of cytochrome P4502E1 (CYP2E1) in vitro and in vivo (23,24), indicating that CYP2E1 is the major CYP responsible for the ethanol-enhanced catabolism of RA in hepatic tissue after treatment with alcohol. A significant induction of CYP2E1 was observed in humans 1 wk after the ingestion of ethanol, and the disappearance of CYP2E1 was found to be significant 3–8 d after the withdrawal of ethanol (25). Therefore it is possible that the induction of CYP2E1 activity during chronic intermittent drinking could be a factor in the destruction of retinol and RA, even after alcohol is cleared. Because it has been reported that CYP2E1 is also present and inducible by alcohol in the esophagus, forestomach, and surface epithelium of the proximal colon (26), these studies also provide a possible explanation for why chronic and excessive alcohol intake is a risk not only for hepatic but also for extrahepatic cell proliferation and carcinogenesis. Treatment with CYP2E1 inhibitors has been shown to protect against ethanol-induced liver injury (27). Very recently, we observed that treatment of rats with a CYP2E1 inhibitor, chloromethiazole, could prevent ethanol-promoted hepatic carcinogenesis by restoring retinoid status that was impaired by ethanol (28).

Third, chronic ethanol consumption enhances the mobilization of vitamin A from the liver to other organs (29,30), although the mechanism(s) are unclear. The restoration of plasma RA to a normal concentration by chloromethiazole in ethanol-fed rats was associated with both a reduction in the otherwise elevated plasma retinyl palmitate concentrations and an increase in hepatic retinol palmitate (24), supporting the concept that RA may function as a “feedback signal” to regulate hepatic vitamin A metabolism (31). It has been demonstrated that RA treatment increases retinol esterification in the liver of vitamin A–deficient mice and rats (32). However, due to the involvement of multiple biochemical factors by ethanol, such as hepatic enzyme regulation, lipoprotein secretion, cellular retinol-binding protein (CRBP) and retinol-binding protein (RBP) functions, and so on, the mechanisms of this regulation of RA are more complicated in the case of chronic alcohol consumption and require further study.

Possible actions of retinoid against alcohol-promoted hepatic carcinogenesis

The molecular mode of the chemoprotective action of RA may involve several mechanisms (Fig. 2): i) transactivation through direct binding of RA to RA response elements in target gene promoters, thereby transcriptionally activating a series of genes with distinct antiproliferative activity; ii) trans-repression of activator protein-1 (AP-1, consisting of c-Jun and c-Fos), a transcription factor that mediates signals from growth factors, inflammatory peptides, oncogenes and tumor promoters, thereby modulating cell proliferation and carcinogenic process; and iii) induction of apoptosis, thereby eliminating cells with unreparable alterations in their genome or killing neoplastic cells.

Retinoid receptors (RARs and RXRs) regulate gene expression by binding as dimeric complexes to the RA response elements, which are located in the 5′ promoter region of susceptible genes. Mice carrying mutations in RARs or RXRs showed signs of vitamin A deficiency, which provided proof...
that these receptors control retinoid signaling and function (33). The RAR function as ligand-dependent transcription factors, and their actual down-regulation (loss or low expression of specific RARs, such as RARβ with tumor suppressor activity) or “functional” down-regulation due to a lack of RA, could interfere with retinoid signal transduction, resulting in enhanced cell proliferation and potentially malignant transformation (11). Recently, it has been reported that the expression of the RARβ gene was down-regulated by ethanol even in the presence of retinol (34), as well as in tumorigenic hepatoma cell lines (35,36). We have observed that the appearance of the placental form of glutathione-S-transferase, a biomarker of hepatic carcinogenesis, in hepatocytes was associated with the down-regulation of RXRα (28) in diethylnitrosamine-treated and ethanol-fed rats, indicating an important role of this nuclear receptor in hepatic carcinogenesis. A lack of RXRα in the epidermis of mice results in hypersensitivity to chemical carcinogen–induced skin carcinogenesis (37). Therefore RXRs not only function as heterodimers of other nuclear receptors but also as active transducers of tumor-suppressive signals (38).

The products of the two proto-oncogenes, c-Jun and c-Fos, form a complex in the nucleus, termed AP-1, that binds to a DNA sequence motif referred to as the AP-1 response element (AP-1 RE). Components of AP-1 are important in modulating carcinogenesis and the transactivation of AP-1–dependent genes is required for tumor promotion (39). Although it is unknown how alcohol initiates a signal transduction cascade, recent evidence has accumulated supporting a role of ethanol in the regulation of AP-1 gene expression. We have observed that chronic ethanol intake in rats significantly increases hepatic c-Jun and c-Fos protein concentrations compared with control rats (19). The antiproliferative and antioncogenic effects of retinoids may be mediated by inhibition of AP-1 activity (38). Retinoid receptors and AP-1 (Jun/Fos) can interfere with each others’ activities (40). For example, all three RAR subtypes (RARα, β and γ) could effectively inhibit phorbol ester–induced AP-1 activity in either an RA-dependent or -independent manner (41). Recently, we showed that treating ethanol-fed rats with RA (0.05 and 0.1 mg/kg body weight, dissolved in the ethanol-containing diet) inhibited the ethanol-induced overexpression of c-Jun, AP-1 DNA binding activities, concentrations of cyclin D1 (AP-1-dependent and a major player in cell proliferation) and the number of ethanol-induced proliferating cellular nuclear antigen (PCNA)-positive hepatocytes (9). Because the transactivation of AP-1–dependent genes is required for tumor promotion (39) and cyclin D1 plays an important role in tumorigenesis and tumor progression, including hepatocellular carcinoma (42), the identification of c-Jun and cyclin D1 as two potential targets of RA action in ethanol-fed rats indicates that retinoids play an important role in preventing certain types of ethanol-promoted cancer. Furthermore, supplementation of ethanol-fed rats with all-trans-RA greatly attenuated the ethanol-induced phosphorylation of Jun N-terminal kinases (JNK) and increased the concentrations of mitogen-activated kinase phosphatase-1 (MKP-1) in liver tissue (8). In addition, all-trans-RA blocked serum-induced JNK activation by up-regulating MKP-1 activity in normal human bronchial epithelial cells (43), and JNK was required for tumorgenesis using a multistep carcinogenesis model in mice lacking the JNK2 gene (44). These studies support our notion that JNK signaling may mediate ethanol-promoted hepatocyte proliferation and oncogenic transformation, due to alcohol-impaired RA action, and “crosstalk” with the JNK signaling pathway.

Retinoids have been implicated in the induction of cell death in many tumor-derived cultured cell systems in both retinoid receptor–dependent and –independent manners (45). Deregulated apoptosis contributes to the pathogenesis of a number of human diseases. Therefore it is possible that under certain risk conditions, such as diminished hepatic retinoid signaling due to prolonged alcohol intake, apoptosis may become deregulated, thereby promoting genomic instability and neoplasia. Recently we investigated whether hepatocellular
apoptosis can be regulated by either ethanol feeding or RA supplementation. In rats, ethanol feeding for a 1-mo period (subacute phase) significantly increased apoptosis; however, after 6 mo of ethanol feeding hepatic apoptosis was significantly decreased compared with controls (8). Interestingly, RA supplementation increased apoptosis by fourfold in ethanol-fed rats compared with ethanol treatment alone (8). Although the mechanism(s) are not well defined, these data indicate that RA plays an important role in preventing ethanol-promoted carcinogenesis by inducing apoptosis.

**Summary**

Critical events in early alcohol-induced hepatic damage are an altered homeostasis of retinoids (retinyl esters, retinol and RA) and the proliferative activation of hepatocytes, which together may play a central role in both the initiation and promotion stages of hepatic carcinogenesis. The restoration of RA homeostasis by either RA supplementation or the use of an inhibitor of RA catabolism suppresses alcohol-induced cell hyperproliferation and restores alcohol-deregulated apoptosis, thereby reducing the risk of alcohol-promoted carcinogenesis (in liver as well as peripheral organs). The mechanism of action of RA may involve “crosstalk” with alcohol-activated JNK-dependent signaling pathway and inhibition of AP-1 (c-Jun and c-Fos) activity and induction of apoptosis. A better understanding of the molecular mechanism(s) involved is needed before pursuing retinoids in the prevention and treatment of alcohol-related carcinogenesis.

**LITERATURE CITED**