0145-6008/01/2505-0137$03.00/0
ALCOHOLISM: CLINICAL AND EXPERIMENTAL RESEARCH

Vol. 25, No. 5
May Supplement 2001

Alcohol and Cancer

Helmut K. Seitz, Shohei Matsuzaki, Akira Yokoyama, Nils Homann, Satu Väkeväinen, and Xian Dong Wang

This article represents the proceedings of a symposium at the 2000 ISBRA Meeting in Yokohama, Japan. The chairs were Helmut K. Seitz and Shohei Matsuzaki. The presentations were (1) Alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 genotype and cancer risk for upper aerodigestive tract in Japanese alcoholics, by Akira Yokoyama; (2) The role of acetaldehyde in alcohol-associated carcinogenesis, by Nils Homann; (3) High salivary acetaldehyde levels after a moderate dose of alcohol in ALDH2-deficient subjects, by Satu Väkeväinen; (4) Alcohol and vitamin A interactions, by Xian Dong Wang; and (5) Alcohol and colorectal cancer, by Helmut K. Seitz.

Key Words: Alcohol Dehydrogenase, Aldehyde Dehydrogenase, Cancer, Alcohol-Associated Carcinogenesis, Acetaldehyde, Vitamin A, Colorectal Cancer.

CHRONIC ALCOHOL CONSUMPTION is associated with an increased risk for upper aerodigestive tract cancer (oral cavity, pharynx, larnynx, esophagus) as well as for cancer of the liver, colorectum, and breast. A great number of epidemiological studies have demonstrated a correlation between alcohol ingestion and the occurrence of cancer in these organs (Seitz et al., 1998). These studies clearly show that all types of alcoholic beverages are associated with an increased cancer risk, which suggests that ethanol itself is the common ingredient which causes that effect. A good overview of the different epidemiological studies was provided by an international agency on the research of cancer (Anonymous, 1988). There is increasing evidence that acetaldehyde rather than alcohol itself is responsible for the confirmed effect. Acetaldehyde is highly toxic, mutagenic, and carcinogenic. Acetaldehyde interferes at many sites with DNA synthesis and repair and consequently tumor development (Anonymous, 1985). Numerous in vitro and in vivo experiments in prokaryotic and eukaryotic cell cultures and in animal models have shown that acetaldehyde has direct mutagenic and carcinogenic effects. It causes point mutations in certain genes and induces sister chromatide exchanges and gross chromosomal aberrations (Dellarco, 1988; Helander and Lindahl-Kiessling, 1991; Obe et al., 1986). It induces inflammation and metaplasia of tracheal epithelium, delays cell cycle progression, stimulates apoptosis, and enhances cell injury associated with hyperregeneration. It also has been shown that acetaldehyde interferes with the DNA repair machinery. Acetaldehyde directly inhibits its O^6-methylguanyltransferase, an enzyme important for the repair of adducts caused by alkylating agents (Espina et al., 1988). Moreover, when inhaled, acetaldehyde causes nasopharyngeal and laryngeal carcinoma (Woutersen et al., 1986). According to the International Agency for Research and Cancer, there is sufficient evidence to identify acetaldehyde as a carcinogen in animals (Anonymous, 1985). Acetaldehyde also binds rapidly to cellular proteins and DNA, which results in morphological and functional impairment of the cell. The covalent binding to DNA and the formation of stable adducts represent one mechanism by which acetaldehyde could trigger the occurrence of replication errors and/or mutations in oncogenes or tumor suppressor genes (Fang and Vaca, 1995). The occurrence of stable DNA adducts has been shown in different organs of alcohol-fed rodents and in leukocytes of alcoholics (Fang and Vaca, 1997). Moreover, it recently has been shown that the major stable DNA adduct, N^2-ethyldeoxyguanosine, can indeed be used efficiently by eukaryotic DNA polymerases (Matsuda et al., 1999).

In addition, acetaldehyde adducts represent neoantigens that lead to the production of specific antibodies and to the stimulation of the immune system, which possibly leads to cytotoxic immune response. Acetaldehyde also has been shown to destroy folic acid in vitro (Shaw et al., 1989). Folic acid is an important cofactor in restoring S-adenosylmethionine and consequently the C1-transmethylation pool. Hypomethylation of certain functional genes has been shown to occur in early stages of cancer development. Thus, it is not surprising that a low folate diet is associated with an increased cancer risk in humans (Giovannucci et al., 1995). Accordingly, the cleavage of folate might be another

From the Laboratory of Alcohol Research (HKS), Liver Disease and Nutrition, and Department of Medicine, Salem Medical Center, Heidelberg, Germany; the Department of Internal Medicine (SM), Division of Gastroenterology, Tokai University, School of Medicine, Tokai, Japan; the National Institute on Alcoholism (AY), Kurihama National Hospital, Kurihama, Japan; the Department of Gastroenterology (NH), Medical University of Lübeck, Lübeck, Germany; the Research Unit of Alcohol Disease (SV), Helsinki University Central Hospital, Helsinki/Finland; and the USDA Human Nutrition Research Center on Aging at Tufts University (XDW), Boston, Massachusetts.

Received for publication January 11, 2001; accepted January 11, 2001.

Reprint requests: Helmut K. Seitz, MD, PhD, Alcohol Research Lab, Department of Internal Medicine, Salem Medical Center, University of Heidelberg, 11 Zeppelinstrasse, Heidelberg 6900, Germany; Fax: 49-621-383-3816; E-mail: Helmut_Karl.Seitz@urz.uni-heidelberg.de

tumor-promoting effect of acetaldehyde. Recent and striking evidence for the causal role of acetaldehyde in ethanol-associated carcinogenesis derives from genetic linkage studies in alcoholics. Individuals who accumulate acetaldehyde due to polymorphism and/or mutation in the gene coding for enzymes responsible for acetaldehyde generation and detoxification have been shown to have an increased cancer risk (Harty et al., 1997; Yokoyama et al., 1998a).

In addition to the action of acetaldehyde, other mechanisms may be involved in the alcohol-associated carcinogenic process:

1. A local toxic effect of alcohol that leads to membrane and cell injury and subsequently to cellular proliferation. Hyperproliferation itself increases susceptibility to concomitantly inhaled or ingested carcinogens. A permanent hyperproliferation is also a first step in the multistep process of malignant transformation (Seitz et al., 1998).
2. Induction of cytochrome P-4502E1 associated with an increased production of reactive oxygen species and enhanced activation of a variety of procarcinogens, which include nitrosamines (Seitz et al., 1998).
3. Alteration of the metabolism of retinol and retinoic acid (RA) that leads to a decrease in RA, an important factor for cell differentiation and regeneration (Seitz, 2000).

The aim of this symposium was to present an overview of the latest knowledge about mechanisms by which alcohol increases cancer risk. Thus we placed major emphasis on acetaldehyde, especially generation by cellular enzymes and also by bacteria. In addition, new data on the effect of alcohol on retinol and RA metabolism, especially with respect to early events in carcinogenesis, have been collected. Finally, we used new findings to present a clearer picture of the effect of alcohol on colorectal cancer.

DISCUSSION

Esophageal Cancer in Japan

Beginning in 1993, systematic screening of 2500 Japanese alcoholic males via endoscopy combined with esophageal iodine staining showed cancer in 135 patients, an extremely high rate of 5.4%. Esophageal cancer was diagnosed in 3.8% of the patients, oropharyngeal and laryngeal cancer in 0.9%, and stomach cancer in 1.3% (Yokoyama et al., 1995). Fourteen patients with esophageal cancer were multiple cancer patients who also had oropharyngeal, laryngeal, and/or stomach cancer. The high rate of esophageal cancer found in this study was influenced by the sensitive method of esophageal iodine staining. Endoscopic mucosal resection is now the treatment of choice in Japan. Patients recover quickly from this procedure and are able to have the same quality of life as before. About 80% of Dr. Yokoyama's patients with esophageal cancer were treated by endoscopic mucosal resection.

Eighty-six percent of Japanese alcoholics are also heavy smokers. Multiple regression analysis revealed that the risk for esophageal cancer slightly increased for users of stronger alcoholic beverages (odds ratio [OR] = 1.81) and more than 50 pack-years of cigarettes (OR = 1.69; Yokoyama et al., 1996c). These results stress the importance of the local effect of ethanol and its solvent action on tobacco.

*Importance of ALDH2*2 in the Role of Upper Aerodigestive Tract Cancer*

Approximately 40% of Japanese have inactive forms of aldehyde dehydrogenase-2 (ALDH2), in which the mutant allele ALDH2*2 encodes an inactive subunit. When this enzyme is inactive, the body fails to metabolize acetaldehyde, which rapidly leads to excessive accumulation of acetaldehyde. In individuals with inactive homozygous ALDH2*2/2*2 and inactive heterozygous ALDH2*1/2*2, blood acetaldehyde concentrations are approximately 19 and 6 times higher than in those with active ALDH2, respectively. After drinking alcohol, persons with inactive ALDH2 exhibit the so-called flushing responses, which include facial flushing, tachycardia, and drowsiness. By causing severe acetaldehydemia and the flushing response, the inactive ALDH2 prevents many Japanese people from drinking heavily and developing alcoholism. However, the preventive effect of heterozygous inactive ALDH2 is incomplete. Individuals with the inactive enzyme often become alcoholics. In fact, as many as 12% of Japanese alcoholics have the heterozygous form of inactive ALDH2.

Dr. Yokoyama and his colleagues were the first to report that among Japanese people, the inactive ALDH2 encoded by the ALDH2*1/2*2 gene is a strong risk factor for esophageal cancer in both every day drinkers and alcoholics (Yokoyama et al., 1996a). A comprehensive study of the *ALDH2* genotype and cancers prevalent in Japanese alcoholics showed that the frequency of inactive ALDH2 increased markedly among alcoholics with cancer of the oral cavity, oropharynx, hypopharynx, larynx, and esophagus (Yokoyama et al., 1998a). The adjusted odds of those cancers occurring in patients with inactive ALDH2 were more than 10-fold the odds for patients with active ALDH2. For stomach and colorectal cancer, 3-fold higher ORs for these cancers in alcoholics with inactive ALDH2 suggest that some of these cancers are also associated with severe increased acetaldehyde levels. The ALDH2 effect was not observed with liver cancer. Inactive ALDH2 is also a risk factor for synchronous (Yokoyama et al., 1996b) and metachronous (Yokoyama et al., 1998b) development of the multiple intraesophageal cancers of the esophagus in alcoholics. One explanation why inactive ALDH2 and increased acetaldehyde levels influence the development of cancer in the esophagus is related to ethanol metabolism by esophageal enzymes. Esophagus lacks ALDH2 activity but strongly expresses alcohol dehydrogenase (ADH)-7 (σ-ADH), which is very active at high ethanol concentrations.

After exposure to acetaldehyde derived from systemic, mucosal, or salivary production or alcoholic beverages, inefficient degradation of the acetaldehyde in the esophagus may enhance the risk. If the tissue concentrations of acetaldehyde were compared between esophagus and other tissues, the enigma might be clear.

The Role of ADH2 Polymorphism in Upper Aerodigestive Tract Cancer

Dr. Yokoyama and colleagues also investigated the role of ADH 2 polymorphism. More than 90% of the Japanese have superactive ADH2 encoded by homozygous ADH2*2/2*2 or heterozygous ADH2*1/2*2. In vitro studies showed that these superactive isozymes have 100 to 200 times higher catalytic activity than the normal ADH2 encoded by ADH2*1/2*1. The normal ADH2 is found in approximately 90% of Western people but in 7% of Japanese. Available data in Asians have consistently shown that the normal ADH2 encoded by *ADH2*1/2*1* genotype is much more prevalent in alcoholics than in nonalcoholic controls.

Dr. Yokoyama and his colleagues performed *ADH2*/*ALDH2* genotyping in Japanese male alcoholics (526 cancer free, 31 with oropharyngolaryngeal cancer, 102 with esophageal cancer, and 36 with stomach cancer). Among the alcoholic population, individuals with normal ADH2*1/2*1 were more frequently found in the alcoholics with cancer in the oral cavity, oropharynx, hypopharynx, epilarynx, and esophagus than in the cancer-free alcoholics (Yokoyama et al., 1999). Logistic regression analysis showed significantly increased risks for oropharyngolaryngeal cancer in the presence of the ADH2*1/2*1 (OR = 6.1 and 2.7, respectively) and ALDH2*1/2*2 (OR = 16.8 and 15.2, respectively), but the role of the inactive ALDH2 predominated over that of the normal ADH2. The ORs for inactive ALDH2 were 3- to 6-fold those for normal ADH2. The role of ADH2 in alcohol related carcinogenesis remains puzzling. Investigators have failed to establish any correlation between *ADH2* genotype and peak levels of blood acetaldehyde after drinking. The role of *ADH2* genotype in carcinogenesis may differ from that of acetaldehyde. Some unestablished aspects in drinking behavior or alcohol metabolism in alcoholics with normal ADH2 might influence the risk for cancer.

For patients with inactive ALDH2, the risks for multiple cancers in two or three organs were markedly higher than the risks for a single type of cancer. The positive association with inactive ALDH2 was strong for multiple stomach cancer patients who also had esophageal cancer but was not observed for those with stomach cancer alone. These findings support the hypothesis that the pathogenesis of stomach cancer concurrent with esophageal cancer is associated with acetaldehyde exposure and is distinct from other stomach cancer.

For patients with both normal *ADH2*1/2*1* and inactive *ALDH2*1/2*2* genotypes, the risks for oropharyngeal and esophageal cancers were multiply enhanced. It is interesting that Dr. Yokoyama and colleagues found development of tolerance for acetaldehyde in alcoholics with cancer with inactive ALDH2. Among the patients with inactive ALDH2, 52% of those who also had normal ADH2 had never experienced alcohol flushing compared with only 8% of those with superactive ADH2. Accordingly, individuals with a combination of normal ADH2 and inactive ALDH2 should be at high risk for acetaldehyde-related cancers, because as a result of the lack of protective alcohol flushing, they are likely to develop alcoholism and to be exposed to higher levels of acetaldehyde. Dr. Yokoyama's data can be summarized as follows: (a) The application of esophageal iodine staining for alcoholics yields a higher rate of cancer detection; (b) stronger alcoholic beverages and heavy smoking are risk factors for upper aerodigestive tract cancer; (c) inactive ALDH2 is a strong risk factor for esophageal and multiple cancers in alcoholics, which suggests a key role of acetaldehyde in carcinogenesis; (d) Japanese alcoholics can be divided into subgroups depending on their cancer susceptibility based on the *ADH2* and *ALDH2* genotypes; and (e) the ADH2 effect may be in part explained by its influence on alcohol flushing.

Microbial Production of Acetaldehyde in Saliva

The production of acetaldehyde from ethanol in bronchopulmonary washings of humans due to bacteria was shown several years ago (Miyakawa et al., 1986; Pikkarainen et al., 1981). The local acetaldehyde production in the oral cavity from ethanol was almost totally abolished after the volunteers rinsed their mouths with a local antiseptic, which indicated that these acetaldehyde levels were of microbial origin (Miyakawa et al., 1986). Recent research by Dr. Homann and colleagues revealed a substantial production of acetaldehyde in the saliva of volunteers who consumed moderate amounts of alcohol (0.5 g/kg body weight). The highest acetaldehyde levels detected ranged between 18.7 and 143.4 μM, and in all volunteers the peak value was achieved within 40 min after ethanol ingestion (Homann et al., 1997a). From this peak value, salivary acetaldehyde levels decreased to a mean value of 1.3 μM after 240 min. This acetaldehyde production was decreased significantly after treatment with the antiseptic chlorhexidine. In vitro results showed that this acetaldehyde production was associated strongly with the ethanol concentration, mainly of microbial origin, pH dependent, and capable of being inhibited by 4-methylpyrazole, an ADH inhibitor. These in vivo salivary acetaldehyde levels would be sufficient to cause severe mutagenic damages. Homann and coworkers also showed significantly increased salivary acetaldehyde levels in heavy drinkers and smokers (Homann et al., 2000a). Smoking showed a positive linear correlation, and it can be estimated that a smoker with a daily consumption of approximately 20 cigarettes has an increased salivary acetaldehyde production of about 50% to 60%.

This implies that smokers even after moderate alcohol intake produce much higher levels of carcinogenic acetaldehyde in the oral cavity than nonsmokers. Alcohol seems to interact and increase salivary acetaldehyde production only if consumed heavily (>40 g/day). When an increase is observed, it is dose-dependent and it increases salivary acetaldehyde levels on average by about 50%. Smoking and alcohol together further increase the salivary acetaldehyde production by about 100% as compared with nonsmokers and moderate alcohol consumers. Studies on human saliva showed that mainly aerobic bacteria are associated with increased acetaldehyde production. *Streptococcus salivarius*, hemolytic *viridans* group *Streptococci*, *Corrynebacterium sp.*, *Stomatococcus sp.*, and yeasts are microbes associated with an increased acetaldehyde production. Studies focusing on yeasts in saliva clearly showed that yeast colonization was significantly higher in high-acetaldehyde-producing salivas than in low-acetaldehyde-producing salivas (Tillonen et al., 1999). Among carriers, the density of the yeasts was higher in the high than in low acetaldehyde producers. Moreover, *Candida albicans* strains isolated from the high-acetaldehyde-producing salivas formed significantly higher acetaldehyde levels from ethanol than *Candida albicans* strains from low-acetaldehyde-producing salivas.

More recently, it has been shown that microbial acetaldehyde is significantly increased in patients with poor oral status (Homann et al., 2001). Thus, the microbial conversion from ethanol to acetaldehyde could be one explanation for the observed increased cancer risk in alcoholics with poor oral hygiene.

Possible Role of Microbial Acetaldehyde Production in Saliva in Ethanol-Associated Carcinogenesis

There is also experimental evidence that the microbial saliva acetaldehyde production from ethanol might be a major factor in ethanol-associated carcinogenesis. It has been shown that chronic alcohol consumption results in hyperproliferation of the esophageal mucosa and this increase is only seen when normal salivary function is guaranteed. Animals that did not produce saliva due to the removal of salivary glands revealed a normal proliferation pattern after chronic alcohol ingestion of the upper aerodigestive tract (Simanowski et al., 1993). Homann and coworkers demonstrated that the hyperproliferation seen after chronic alcohol consumption is also found after chronic acetaldehyde administration in drinking water (Homann et al., 1997b). Such an acetaldehyde administration causes hyperplastic and hyperproliferative changes in the tongue, epiglottis, and forestomach. Finally, it has been shown that acetaldehyde in concentrations observed in the colonic lumen (Jokelainen et al., 1996) may destroy folate (Shaw et al., 1989). Epidemiological studies have underlined the importance of folate in colorectal cancer, and it has been demonstrated that individuals with low folate, low methionine, and an increased alcohol consumption of more than 20 g/day had a 7-fold increased risk to develop distal colon cancer (Giovannucci et al., 1995). Homann and coworkers investigated folate levels in the colon of rats and showed that high acetaldehyde levels correlated with low folate concentrations and that this effect was abolished by the administration of antibiotics that destroyed fecal bacteria capable of producing acetaldehyde (Homann et al., 2000b). It can be concluded that besides the production of acetaldehyde from cellular ethanol metabolism, microbes also may produce acetaldehyde and this may be important in the upper aerodigestive tract. Salivary acetaldehyde levels are increased by smoking and poor oral hygiene, but acetaldehyde may also be high in the large intestine and may destroy folate.

High Salivary Acetaldehyde Levels in ALDH2-Deficient Subjects After Moderate Alcohol Ingestion

Dr. Väkeväinen and coworkers presented data about acetaldehyde in saliva of individuals with inactive ALDH2. They clearly showed a significant increase in salivary acetaldehyde levels in these individuals compared with controls with normal ALDH2. This may explain the high risk for upper aerodigestive tract cancer in Japanese alcoholics with inactive ALDH2, due to increased acetaldehyde concentration in the saliva (Väkeväinen et al., 2000). Thus, for the first time, a link between genetically determined ADH activity, acetaldehyde concentration, and cancer risk has been established.

Ethanol Effects on Retinol and RA Metabolism and Its Role in Early Carcinogenesis

Dr. Wang and colleagues have accumulated new insight into the interaction between alcohol and vitamin A metabolism. In a series of experiments, they investigated the effect of alcohol on retinol and RA metabolism as well as its effects on transcellular signaling and early events in carcinogenesis. Chronic alcohol consumption affects several aspects of vitamin A metabolism, which include vitamin A malabsorption, enhanced degradation in the liver, and an increased mobilization of vitamin A from the liver to other organs. These ethanol-induced changes can decrease liver levels of both retinol and retinol esters, which are precursors of RA (Leo and Lieber, 1982). A number of investigations also have demonstrated that ethanol acts as a competitive inhibitor of retinol oxidation in the liver, thereby reducing the biosynthesis of retinoic acid. Dr. Wang has shown that treatment of rats with high doses of ethanol significantly reduced retinol palmitate and retinol concentrations in the liver as compared with animals pair-fed an isocaloric control diet that contained the same amount of vitamin A. However, RA levels in the liver of the ethanol-fed rats were decreased to a much greater extent (Wang et al., 1998). Thus, it appears that ethanol causes a local deficiency of RA in the liver that results from an

enhancement of RA catabolism in addition to a decreased biosynthesis of RA (Wang, 1999). Dr. Wang further investigated the role of this increased catabolism of RA into 4-oxo RA and 18-hydroxy RA in rat liver. Both ethanol-exposed and non-ethanol-exposed rats were treated with or without chlormethiazole, a specific CYP2E1 inhibitor, for 1 month (Liu et al., 2001). In vitro incubation of RA in microsomal fractions of liver tissue that contained cytochrome P-450 from either ethanol-exposed or non-ethanol-exposed rats were carried out by using chemical inhibitors and antibodies against various cytochrome P-450s. The results showed that treatment with chlormethiazole in ethanol-fed rats in vivo restored both hepatic and plasma RA concentrations to normal levels (Liu et al., 2001). The enhancement of RA catabolism by ethanol in vitro was inhibited by both cytochrome P-4502E1 antibody and specific inhibitors (allylsulfide and chlormethiazole), whereas the metabolism of RA into polar metabolites was abolished completely by nonspecific cytochrome P-450 inhibitors (disulfirame and liarozole). From these experiments, it was concluded that ethanol-induced cytochrome P-4502E1 plays a major role in the degradation of RA (Liu et al., 2001). Dr. Wang further investigated the effect of chronic alcohol consumption on RA receptors in the liver and on the expression of AP1 gene (C-*jun* and C-*fos*) and found that chronic alcohol consumption resulted in a functional down-regulation of RA receptors and an increased expression up to 10-fold on AP1 gene (Wang et al., 1998). This was associated with hyperproliferation in the liver. Most interestingly, supplementation of the animals with RA which led to normal RA levels in the liver not only decreased AP1 gene expression but also normalized hepatic regeneration. These data indicate an important role of low RA levels in the liver and possibly also in other tissues due to chronic alcohol consumption in the carcinogenic process, because RA is an important factor in regulating cell regeneration and cell differentiation.

Alcohol and Colorectal Cancer

It was concluded by a recent consensus conference on nutrition and cancer that alcohol is a risk factor for colorectal cancer even at low daily intake (Scheppach et al., 1999). More than 60 epidemiological studies have been performed on alcohol and colorectal cancer, and the majority of these studies support such a correlation. Clinically it was found that alcoholics revealed a disturbed morphology in rectal biopsies with crypt destruction and inflammation, which returned completely to normal after 3 weeks of withdrawal (Brozinski et al., 1979). Seitz and colleagues showed that, indeed, chronic alcohol consumption resulted in an increased crypt cell production rate and an extension of the proliferative compartment of the crypt in rodents. This was associated with a decrease of the functional compartment (Simanowski et al., 1986, 1994). They extended their studies to humans and again found that chronic alcoholics had an increased proliferation in their crypts with extension of their proliferative compartments, a condition associated with an increased risk for cancer (Homann et al., 1996).

There is some evidence that acetaldehyde is involved in these observed morphological alterations as precursors for colorectal cancer:

1. Crypt cell production rate correlated significantly with acetaldehyde levels in the colonic mucosa (Simanowski et al., 1994).
2. Animal experiments showed an increased occurrence of colorectal tumors induced by a specific carcinogen, when cyanamide, an acetaldehyde dehydrogenase inhibitor, was applied and acetaldehyde levels were increased (Seitz et al., 1990).
3. High acetaldehyde levels occur in the colon due to bacterial production (Jokelainen et al., 1996), and this acetaldehyde is capable of destroying folate, as discussed previously (Homann et al., 2000c; Shaw et al., 1989).
4. As Dr. Yokoyama has shown, individuals with inactive form of ALDH2 exhibit an increased risk also for colorectal cancer (Yokoyama et al., 1998a).

Seitz and colleagues have investigated ADH phenotypes in the colorectal mucosa of alcoholics with and without cancer or polyps and have shown that class IV ADH (σ-ADH) is absent in the normal colorectal mucosa. However, σ-ADH is expressed in adenomatous tissue of polyps in some patients. The reason for this expression is not clear. However, because class IV ADH is able to metabolize retinol to RA, its expression in polyps may demonstrate RA deficiency and an attempt to increase RA in the precancerous conditions of polyps. Furthermore, an increased allele frequency of ADH3*1 was found in patients with colorectal cancer as compared with alcoholics of similar age without cancer.

In addition, cytochrome P-4502E1 is induced in the colorectal mucosa not only in animals (Hakkak et al., 1996) but also in humans after ethanol ingestion. This induction may lead to the production of reactive oxygen species (ROS). In a series of experiments in rodents, alcohol-induced hyperproliferation of the colorectal mucosa could be inhibited partly by the concomitant administration of vitamin E, which demonstrated that this inhibition is possibly due to a reduction of ROS by vitamin E application (Vincon et al., 2000). In conclusion, chronic alcohol consumption is associated with an increased risk for colorectal cancer. Acetaldehyde produced by mucosal ADH and by bacteria seems to be an important pathophysiological factor for alcohol-associated colorectal carcinogenesis. ROS also could be involved in the process.

Significance

The data presented in this symposium underline the importance of acetaldehyde as a carcinogenic factor in

alcohol-associated cancer development in the upper aerodigestive tract and colorectum.

It could be demonstrated that individuals with either increased production of acetaldehyde due to ADH3*1 allele or decreased degradation of acetaldehyde due to ineffective ALDH, both of which lead to acetaldehyde accumulation, have an increased risk for alcohol-associated cancer. It also was shown that ALDH2 deficiency increases salivary acetaldehyde, which supports genetic data. In addition, acetaldehyde is produced by oral and fecal bacteria, and salivary acetaldehyde is elevated in individuals with poor oral hygiene and in smokers.

Another mechanism by which alcohol affects cancer development is by its interaction with retinoids. In this context, it is of considerable importance to note that production of RA in the liver and possibly also in extrahepatic tissues during chronic ethanol consumption significantly decreased. This is primarily due to an increased metabolism of RA via induced cytochrome P-4502E1 and to an inhibition of the conversion of retinol to RA by ethanol. Low RA concentrations initiate early events in carcinogenesis such as an increased expression of AP-1 gene, which leads to hypoproliferation, a premalignant condition.

Finally, cytochrome P-4502E1 induction in the colon of rats leads to the generation of free oxygen species, which partly could be prevented by vitamin E.

REFERENCES

Anonymous (1985) Acetaldehyde. IARC Monogr Eval Carcinog Risk Chem Hum, Vol. 36.

Anonymous (1988) Alcohol drinking. IARC Monogr Eval Carcinog Risk Chem Hum, Vol. 44.

Brozinski S, Fami K, Grosberg JJ (1979) Alcohol ingestion induced changes in the human rectal mucosa: Light and electromicroscopic studies. Dis Colon Rectum 21:329–335.

Dellarco VL (1988) A mutagenicity assessment of acetaldehyde. Mutat Res 195:1–20.

Espina N, Lima V, Lieber CS, Garro AJ (1988) In vitro and in vivo inhibitory effect of ethanol and acetaldehyde on O6-methylguanine transferase. Carcinogenesis 9:761–766.

Fang JL, Vaca CE (1995) Development of a 32P-postlabeling method for the analysis of adducts arising through the reaction of acetaldehyde with 2'-deoxyguanosine-3'-monophosphate and DNA. Carcinogenesis 16:2177–2185.

Fang JL, Vaca CE (1997) Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. Carcinogenesis 18:627–632.

Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC (1995) Alcohol, low methionine-low folate diets, and risk of colon cancer in men. J Natl Cancer Inst 87:265–273.

Hakkak R, Korourian S, Ronis MJ, et al (1996) The effects of diet and ethanol on the expression and localisation of cytochromes P450 2E1 and P450 2C7 in the colon of male rats. Biochem Pharmacol 51:61–69.

Harty IC, Caporaso NE, Hayes RB, Winn DM, Bravo-Otero E, Blot WJ, Kleinman DV, Brown LM, Armenian HK, Fraumeni JF Jr, Shields PG (1997) Alcohol dehydrogenase 3 genotype and risk of oral cavity and pharyngeal cancers. J Natl Cancer Inst 89:1698–1705.

Helander A, Lindahl-Kiessling K (1991) Increased frequency of acetaldehyde-induced sister-chromatide exchanges in human lymphocytes treated with an aldehyde dehydrogenase inhibitor. Mutat Res 264:103–107.

Homann N, Bosch FX, Simanowski UA, Seitz HK (1996) Chronic heavy ethanol ingestion has striking effects on cell proliferation and differentiation in the human rectum (abstract). Gastroenterology 110:530.

Homann N, Jousimies-Somer H, Jokelainen K, Heine R, Salaspuro M (1997a) High acetaldehyde levels in saliva after ethanol consumption: Methodological aspects and pathogenetic implications. Carcinogenesis 18:1739–1743.

Homann N, Kärkkäinen P, Koivisto T, Nosova T, Jokelainen K, Salaspuro M (1997b) Effects of acetaldehyde on cell regeneration and differentiation of the upper gastrointestinal tract mucosa. J Natl Cancer Inst 89:1692–1697.

Homann N, Tillonen J, Meurman J, Rintamäki H, Lindqvist C, Jousimies-Somer H, Salaspuro M (2000a) Increased salivary acetaldehyde levels in heavy drinkers and smokers: A microbiological approach to oral cavity cancer. Carcinogenesis 21:663–668.

Homann N, Tillonen J, Rintamäki H, Salaspuro M, Lindqvist C, Meurman J (2001) Poor dental status increases the acetaldehyde production from ethanol in saliva. A possible link to the higher risk of oral cancer among alcohol consumers. Oral Oncol, 37:153–158.

Homann N, Tillonen J, Salaspuro M (2000b) Heavy alcohol intake leads to local colonic folate deficiency in rats: Evidence of microbial acetaldehyde production from ethanol as the pathogenic substance. Int J Cancer 86:169–173.

Jokelainen K, Matysiak-Budnik T, Mäkisalo H, Höckerstedt K, Salaspuro M (1996) High intracolonic acetaldehyde levels produced by a bacteriocolonic pathway for ethanol oxidation in piglets. Gut 39:100–104.

Leo MA, Lieber CS (1982) Hepatic vitamin A depletion in alcoholic liver injury. N Engl J Med 304:597–600.

Liu C, Russell RM, Seitz HK, Wang XD (2001) Ethanol enhances retinoic acid metabolism into polar metabolites in rat liver via induction of cytochrome P4502E1. Gastroenterology, 120:179–189.

Matsuda T, Terashima I, Matsumoto Y, Yabushita H, Matsui S, Shibutani S (1999) Effective utilization of N2-ethyl-2'-deoxyguanosine triphosphate during DNA synthesis catalyzed by mammalian replicative DNA polymerases. Biochemistry 38:929–935.

Miyakawa H, Baraona E, Chang JC, Lesser MD, Lieber CS (1986) Oxidation of ethanol to acetaldehyde by bronchopulmonary washings: Role of bacteria. Alcohol Clin Exp Res 10:517–520.

Obe G, Jonas R, Schmidt S (1986) Metabolism of ethanol in vitro produces a compound which induces sister-chromatid exchanges in human peripheral lymphocytes in vitro: Acetaldehyde not ethanol is mutagenetic. Mutat Res 174:47–51.

Pikkarainen PH, Baraona E, Jauhonen P, Seitz HK, Lieber CS (1981) Contribution of oropharynx flora and of lung microsomes to acetaldehyde in expired air after alcohol ingestion. J Lab Clin Med 97:631–636.

Scheppach W, Bingham S, Boutron-Ruault MC, Gerhardsson-de-Verdier M, Moreno V, Nagengast FM, Reifen R, Riboli E, Seitz HK, Wahrendorf J (1999) WHO consensus statement on the role of nutrition in colorectal cancer. Eur J Cancer Prev 8:57–62.

Seitz HK (2000) Ethanol and retinoid metabolism. Gut, 47:748–750.

Seitz HK, Pöschl G, Simanowski UA (1998) Alcohol and cancer. Recent Dev Alcohol 14:67–95.

Seitz HK, Simanowski UA, Garzon FT, Rideout JM, Peters TJ, Koch A, Berger MR, Einecke H, Mainwald M (1990) Possible role of acetaldehyde in ethanol-related rectal cocarcinogenesis in the rat. Gastroenterology 98:406–413.

Shaw S, Jayatilleke E, Herbert V, Colman N (1989) Cleavage of folates during ethanol metabolism. Biochem J 257:277–280.

Simanowski UA, Seitz HK, Baier B, Kommerell B, Schmidt-Gayk H, Wright NA (1986) Chronic ethanol consumption selectively stimulates rectal cell proliferation in the rat. Gut 27:278–282.

Simanowski UA, Suter P, Russell RM, Heller M, Waldherr R, Ward R, Peters TJ, Smith D, Seitz HK (1994) Enhancement of ethanol induced rectal mucosal hyperregeneration with age in F344 rats. Gut 35:1102–1106.

Simanowski UA, Suter P, Stickel F, Maier H, Waldherr R, Russell RK, Seitz HK (1993) Esophageal epithelial hyperregeneration following

chronic ethanol ingestion: Effect of age and salivary gland function. J Natl Cancer Inst 85:2030–2033.

Tillonen J, Homann N, Rautio M, Jousimies-Somer H, Salaspuro M (1999) Role of yeasts in the salivary acetaldehyde production from ethanol among risk groups for ethanol-associated oral cavity cancer. Alcohol Clin Exp Res 23:1409–1415.

Väkeväinen S, Tillonen J, Agarwal D, Srivastavana N, Salaspuro M (2000) High salivary acetaldehyde after a moderate dose of alcohol in ALDH 2-deficient subjects: Strong evidence for the local carcinogenic action of acetaldehyde. Alcohol Clin Exp Res 24:873–877.

Vincon P, Wunderer J, Simanowski UA, Preedy VR, Peters TJ, Waldherr R, Seitz HK (2000) Inhibition of alcohol-associated hyperregeneration in the rat colon by alpha-tocopherol. Alcohol Clin Exp Res 24:213A.

Wang X-D (1999) Chronic alcohol intake interferes with retinoid metabolism and signaling. Nutr Rev 57:51–59.

Wang X-D, Liu C, Chung J, Stickel F, Seitz HK, Russell RM (1998) Chronic alcohol intake reduces retinoic acid concentration and enhances AP1 (c-Jun and c-Fos) expression in rat liver. Hepatology 28: 744–750.

Woutersen RA, Appelmann LM, Van Garderen-Hoetmer A, Feron VJ (1986) Inhalation toxicity of acetaldehyde in rats: III. Carcinogenicity study. Toxicology 41:213–231.

Yokoyama A, Muramatsu T, Ohmori T, Higuchi S, Hayashida M, Ishii H (1996a) Esophageal cancer and aldehyde dehydrogenase-2 genotypes in Japanese males. Cancer Epidemiol Biomarkers Prev 5:99–102.

Yokoyama A, Muramatsu T, Ohmori T, Makuuchi H, Higuchi S, Matsushita S, Yoshino K, Maruyama K, Nakano M, Ishii H (1996b) Multiple primary esophageal and concurrent upper aerodigestive tract cancer and the aldehyde dehydrogenase-2 genotype of Japanese alcoholics. Cancer 77:1986–1990.

Yokoyama A, Muramatsu T, Ohmori T, Matushita S, Yoshimizu H, Higuchi S, Yokoyama T, Muruyama K, Ishii H (1999) Alcohol and aldehyde gene polymorphisms influence susceptibility to esophageal cancer in Japanese alcoholics. Alcohol Clin Exp Res 23:1705–1710.

Yokoyama A, Muramatsu T, Ohmori T, Yokoyama T, Okuyama K, Takahashi H, Hasegawa Y, Higuchi S, Maruyama K, Shirakura K, Ishii H (1998a) Alcohol-related cancers and aldehydrogenase-2 in Japanese alcoholics. Carcinogenesis 19:1383–1387.

Yokoyama A, Ohmori T, Makuuchi H, Maruyama K, Okuyama K, Takahashi H, Yokoyama T, Yoshino K, Hayashidi M, Ishii H (1995) Successful screening for early esophageal cancer in alcoholics using endoscopy and mucosa iodine staining. Cancer 76:928–934.

Yokoyama A, Ohmori T, Muramatsu T, Higuchi S, Yokoyama T, Matsushita S, Matsumoto M, Maruyama K, Hayashida M, Ishii H (1996c) Cancer screening of upper aerodigestive tract in Japanese alcoholics with reference to drinking and smoking habits and aldehyde dehydrogenase-2 genotype. Int J Cancer 68:313–316.

Yokoyama A, Ohmori T, Muramatsu T, Yokoyama T, Okuyama K, Makuuchi H, Takahashi H, Higuchi S, Hayashida M, Maruyama K, Ishii H (1998b) Short-term follow-up after endoscopic mucosectomy of early esophageal cancer and aldehyde dehydrogense-2 in Japanese alcoholics. Cancer Epidemiol Biomarkers Prev 7:473–476.