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REVIEW

ALCOHOL AND CANCER

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Abstract — Epidemiological data have identified chronic alcohol consumption as a significant risk factor for upper alimentary tract cancer, including cancer of the oropharynx, larynx and the oesophagus and of the liver. The increased risk attributable to alcohol consumption of cancer in the large intestine and in the breast is much smaller. However, although the risk is lower, carcinogenesis can be enhanced with relatively low daily doses of ethanol. Considering the high prevalence of these tumours, even a small increase in cancer risk is of great importance, especially in those individuals who exhibit a higher risk for other reasons. The epidemiological data on alcohol and other organ cancers is controversial and there is at present not enough evidence for a significant association. Although the exact mechanisms by which chronic alcohol ingestion stimulates carcinogenesis are not known, experimental studies in animals support the concept that ethanol is not a carcinogen but under certain experimental conditions is a cocarcinogen and/or tumour promoter. The metabolism of ethanol leads to the generation of acetaldehyde (AA) and free radicals. Evidence has accumulated that acetaldehyde is predominantly responsible for alcohol associated carcinogenesis. Acetaldehyde is carcinogenic and mutagenic, binds to DNA and proteins, destructs folate and results in secondary hyperproliferation. Acetaldehyde is produced by tissue alcohol hydrogenases, cytochrome P 4502E1 and through bacterial oxidative metabolism in the upper and lower gastrointestinal tract. Its generation or its degradation is modulated due to functional polymorphisms of the genes coding for the enzymes. Acetaldehyde can also be produced by oral and faecal bacteria. Smoking, which changes the oral bacterial flora, and poor oral hygiene also increase acetaldehyde. In addition, cigarette smoking and some alcoholic beverages such as calvados contain acetaldehyde. Other mechanisms by which alcohol stimulates carcinogenesis include the induction of cytochrome P-4502E1, which is associated with an enhanced production of free radicals and enhanced activation of various procarcinogens present in alcoholic beverages: in association with tobacco smoke and in diets, a change in the metabolism and distribution of carcinogens: alterations in cell cycle behaviour such as cell cycle duration leading to hyperproliferation: nutritional deficiencies, such as methyl-, vitamin E-, folate-, pyridoxal phosphate-, zinc- and selenium deficiencies and alterations of the immune system eventually resulting in an increased susceptibility to certain virus infections such as hepatitis B virus and hepatitis C virus. In addition, local mechanisms may be of particular importance. Such mechanisms lead to tissue injury such as cirrhosis of the liver, a major prerequisite for hepatocellular carcinoma. Also, an alcohol-mediated increase in oestradiols may be at least in part responsible for breast cancer risk. Thus, all these mechanisms functioning in concert actively modulate carcinogenesis leading to its stimulation.

INTRODUCTION

Chronic alcohol consumption is a strong risk factor for cancer in the upper aerodigestive tract (UADT) (oral cavity, pharynx, hypopharynx, larynx, oesophagus) (Seitz *et al*., 1998, 2004) and also a major aetiological factor in hepatocarcinogenesis (Stickel *et al*., 2002). In addition, alcohol increases the risk for cancer of the colorectum and the breast (Seitz *et al*., 1998, 2003).

A great number of epidemiological studies have demonstrated a correlation between alcohol ingestion and the occurrence of cancer in these organs (Tuyns, 1978, 1983; Bruguere *et al*., 1986; Maier *et al*., 1990; Kune and Vitetta, 1992; Seitz *et al*., 1998, 2003; Doll *et al*., 1999; Scheppach *et al*., 1999; Stickel *et al*., 2002). These studies clearly show that the ingestion of all types of alcoholic beverages is associated with an increased cancer risk which suggests that ethanol itself is the crucial compound which causes that effect. The exact mechanism(s) of ethanol-associated carcinogenesis, however, have remained obscure, as ethanol itself is not a carcinogen (Ketcham *et al*., 1963).

Multiple mechanisms are involved in alcohol-associated cancer development, including the effect of acetaldehyde (AA), the first metabolite of ethanol oxidation, the induction of cytochrome P-4502E1 (CYP2E1) leading to the generation of reactive oxygen species (ROS), and enhanced procarcinogen activation, as well as the modulation of cellular regeneration and nutritional deficiencies. Since it is far beyond the scope of this review to discuss all factors of alcohol-associated carcinogenesis in detail, major emphasis is laid on genetic and nutritional aspects.

In addition, it is primarily focused on major general pathogenetic mechanisms and to a lesser degree on tissue specific ethanol actions. For more details see recent review articles (Seitz *et al*., 2003, 2004; Stickel *et al*., 2002; Salaspuro, 2003).

EPIDEMIOLOGY

Nearly 100 years ago, Lamu noticed an increased incidence of oesophageal cancer in absinth drinkers (Lamu, 1910). Since then, extensive epidemiological data has accumulated which identified alcohol as a major risk factor for UADT cancer (Tuyns, 1978, 1983; Bruguere *et al*., 1986; Maier *et al*., 1990; Seitz *et al*., 1998, 2003; Doll *et al*., 1999). In a carefully designed French study, Tuyns was able to demonstrate that alcohol consumption of more than 80 g/day (approximately one bottle of wine) increases the relative risk (RR) of oesophageal cancer by a factor of 18, while smoking alone of more than 20 cigarettes daily leads to an increased RR by a factor of 5. Taken together, both factors act synergistically resulting in an increased RR of 44 (Tuyns, 1978). More recently, an epidemiological study by Maier *et al*. (1990) showed that 90% of all patients with head and neck cancer

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consumed alcohol regularly in quantities twice the amount of a control group with a significant dose–response relationship. If the RR for an individual with a daily alcohol consumption of 25 g was assumed to be 1, this figure rose to 32 if alcohol consumption exceeded 100 g/day. Bruguere *et al.* (1986) found RR values of 13.5 for oral cancer, 15.2 for oropharyngeal carcinoma, and of 28.6 for hypopharyngeal carcinoma when 100–159 g of alcohol were consumed daily. It is noteworthy that even with these high daily alcohol dosages, the alcohol-associated cancer risk is not saturable. Alcohol consumption exceeding 1.5 bottles of wine daily results in a 100-fold increased risk for oesophageal cancer (Tuyns, 1983). In an epidemiological study of the American Cancer Society (ACS) on more than 750 000 individuals, Bofetta and Garfinkel (1990) found an increased risk for oesophageal cancer already at a dose of 12 g alcohol daily (RR = 1.37) rising to an RR of 5.8 following 72 g alcohol daily. A follow-up study of the ACS came to the same conclusions (Thun *et al.*, 1997). Similar dose-dependent data have also been demonstrated in case–control studies involving non-smokers (Tuyns, 1983). It has been estimated that 25–68% of UADT cancers are attributed to alcohol and that up to 80% of these tumours can be prevented by abstaining from alcohol and smoking (La Vecchia *et al.*, 1997).

Hepatocellular carcinoma (HCC) is the most frequent primary liver tumour today (El-Serag and Mason, 2000). Its prevalence is increasing worldwide but differs greatly between regions. In more than 80% of cases from Europe and North America, HCC develop in cirrhotic livers, and alcohol is here one of the greatest risk factors for developing liver cirrhosis, whereas in Asia nearly 50% of HCC occur in non-cirrhotic livers (Oka *et al.*, 1990; Simonetti *et al.*, 1997). The increase in HCC is most probably due to the expansion of chronic infection with hepatitis B and C. Epidemiological studies have incriminated both viruses in hepatocarcinogenesis, at which the contributory role of alcohol is undisputed (El-Serag and Mason, 2000). There is compelling evidence that chronic alcohol consumption increases the risk of developing HCC (Caselmann and Alt, 1996; Seitz *et al.*, 1998; Kuper *et al.*, 2000; Inoue and Seitz, 2001). 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 (Ohnishi, 1992). Thus an epidemiological survey from the UK demonstrated an eight-fold increase in the risk of developing HCC among male alcoholics (Prior, 1988). The higher rate of alcohol-related HCC worldwide may partially also be explained by the prolongation of survival time of patients with alcoholic cirrhosis due to an improved disease management.

Furthermore, chronic alcohol consumption accelerates hepatocarcinogenesis in hepatitis B (Onishi *et al.*, 1982) and formation of hepatic cirrhosis in hepatitis C (Inoue and Seitz, 2001).

In 1974, Breslow and Enstrom were the first to raise the possibility of an association between beer consumption and the occurrence of rectal cancer. Several correlations, over 40 case–controlled and almost 20 prospective cohort studies, followed (Kune and Vitetta, 1992; Seitz *et al.*, 1998, 2003). Taking all these data together, chronic alcohol consumption seems to be associated with an increase in the occurrence of adenomatous polyps in the large bowel (Kune and Vitetta, 1992; Seitz *et al.*, 1998, 2003), influences the adenoma — carcinoma sequence (Boutron *et al.*, 1995) and finally results in a 1.5- to 3.5-fold risk of rectal cancer and to a lesser extent of colonic cancer (Scheppach *et al.*, 1999).

A great number of epidemiological studies have clearly identified chronic alcohol consumption even in moderate amounts as a risk factor for breast cancer. (Rohan *et al.*, 2000; Bowlin *et al.*, 1997; Colditz, 1993; Feigelson *et al.*, 2001; Friedenreich *et al.*, 1993; Longnecker, 1994). 84% of the 69 case–control and 76% of the 21 cohort studies published so far show a positive association between ethanol intake and breast cancer. So have all six meta-analyses to date. (Ellison *et al.*, 2001; Hiatt, 1990; Longnecker, 1994; Longnecker *et al.*, 1988; Smith-Warner *et al.*, 1998; Steinberg and Goodwin, 1991). It was calculated that 4% of all newly diagnosed breast cancer cases in the US are primarily due to alcohol (Longnecker, 1994).

ANIMAL EXPERIMENTS

The results of animal experiments on alcohol and cancer depend on the experimental design, the type of carcinogen used, its time, duration of exposure and dosage, as well as the route of alcohol administration. In summary, when alcohol is applied locally to the oral or oesophageal mucosa, it increases the occurrence of tumours probably due to an irritant effect of alcohol (Seitz *et al.*, 1998). When ethanol is given systemically in most of the studies, with some exceptions, the stimulating effect on chemically induced carcinogenesis is noted (Seitz *et al.*, 1998). Both an enhancement of tumour initiation and tumour promotion has been reported. Experiments in which alcohol is given chronically to rodents have shown that alcohol is not a carcinogen, as animals with a chronic life-long exposure to alcohol do not develop more cancers than do controls (Ketcham *et al.*, 1963). Since ethanol modulates chemically induced carcinogenesis, it has to be defined as a tumour promoter and/or cocarcinogen.

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 has been shown (Seitz *et al.*, 1998). However, the rate of extrahepatic tumours, especially of the UADT, increased. Only with additional manipulations, such as administration of a diet low in methyl donors or carbohydrates (Wainfan and Poirier, 1988; Porta *et al.*, 1995, 1982), or after partial hepatectomy (Takada *et al.*, 1986), alcohol was able to stimulate hepatocarcinogenesis. Striking enhancement of hepatocarcinogenesis was observed when alcohol and procarcinogen were given on an alternating basis to avoid interactions between alcohol and carcinogen metabolism.

Experimental data on alcohol and colorectal cancer are controversial and complex, depending on the experimental design of the study. It can be concluded that alcohol stimulates colorectal carcinogenesis primarily during the preinduction and induction phases and that an interaction between ethanol and procarcinogenic metabolism occurs which influenced tumour incidences. For more details see recent review article (Seitz *et al.*, 2003).

POSSIBLE MECHANISMS OF ALCOHOL

Local effects of alcohol

Alcohol acts as a solvent that enhances the penetration of carcinogenic compounds into the mucosa. Ethanol may facilitate the uptake of environmental carcinogens, especially from tobacco smoke, through cell membranes that are damaged and changed in their molecular composition by the direct effect of alcohol. Furthermore, chronic alcoholism leads to atrophy and lipomatous metamorphosis of the parenchyma of the parotid and submaxillary gland and this alteration results in a functional impairment of saliva flow and its increased viscosity. Thus, the mucosal surface will be insufficiently rinsed and is, therefore, exposed to higher concentrations of locally acting carcinogens in addition to a prolongation of the contact time of the substances with the mucosa (Maier *et al.*, 1986). Other local mechanisms include the direct toxic effect of highly concentrated alcoholic beverages on the epithelium, the altered motility of the oesophagus due to alcohol and the enhanced gastro-oesophageal reflux, which may lead to oesophagitis and metaplasia (Seitz *et al.*, 1998). In the past, various alcoholic beverages contained carcinogenic compounds such as polycyclic hydrocarbons, asbestos fibres and nitrosamines, which have now almost been completely eliminated (Seitz *et al.*, 1998). However, calvados, closely associated with oesophageal cancer risk contains considerable amounts of AA (Salaspuro, 2003).

Acetaldehyde

There is increasing evidence that AA, rather than alcohol itself, is responsible for the cocarcinogenic effect of alcohol (Seitz *et al.*, 2001). In the gastrointestinal tract, AA can be generated from ethanol through mucosal and/or bacterial alcohol dehydrogenase (ADH) (Seitz and Oneta, 1998). AA is highly toxic, mutagenic and carcinogenic. AA interferes at many sites with DNA synthesis and repair and can, consequently, result in tumour development (Anonymous, 1985). Numerous *in-vitro* and *in-vivo* experiments in prokaryotic and eukaryotic cell cultures, as well as in animal models, have shown that AA has direct mutagenic and carcinogenic effects. It causes point mutations in the hypoxanthine-guanine-phosphoribosyl transferase locus in human lymphocytes, induces sister chromatid exchanges and gross chromosomal aberrations (Obe *et al.*, 1986; Dellarco, 1988; Helander and Lindahl-Kiessling, 1991). It induces inflammation and metaplasia of tracheal epithelium, delays cell cycle progression and enhances cell injury associated with hyperregeneration (Simanowski *et al.*, 1994; Seitz *et al.*, 2001). Thus, when AA was administered in drinking water to rodents (Homann *et al.*, 1997), the mucosa lesions of the UADT observed resembled those following chronic alcohol ingestion (Simanowksi *et al.*, 1993). It has also been shown that AA interferes with the DNA repair machinery. AA directly inhibits O6 methyl-guanyltransferase, an enzyme important for the repair of adducts caused by alkylating agents (Espina *et al.*, 1988). Moreover, when inhaled, AA causes nasopharyngeal and laryngeal carcinoma (Woutersen *et al.*, 1986). AA also binds rapidly to cellular proteins and DNA which results in morphological and functional impairment of the cell and to an immunological cascade reaction. The binding to DNA and the formation of stable adducts represent one mechanism by which AA could trigger the occurrence of replication errors and/or mutations in oncogenes or tumour 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). In addition, it has been shown recently that the major stable DNA adduct, N^2-ethyldeoxyguanosine can indeed be used efficiently by eukaryotic DNA polymerase (Matsuda *et al.*, 1999). These AA-associated effects occurred at AA concentrations from 40 to 1000 μmol/l, which are similar to concentrations observed in human saliva following alcohol ingestion (Homann *et al.*, 1997). According to the International Agency for Research on Cancer (IARC) there is sufficient evidence to identify AA as a carcinogen in experimental animals (Anonymous, 1985).

Recent and striking evidence of the causal role of AA in ethanol-associated UADT carcinogenesis derives from genetic linkage studies in alcoholics. Individuals who accumulate AA due to polymorphism and/or mutation in the gene coding for enzymes responsible for AA generation and detoxification have been shown to have an increased cancer risk. In Japan, as well as in other Asian countries, a high percentage of individuals carry a mutation of the acetaldehyde dehydrogenase 2 (ALDH2) gene. In humans, there are at least four classes of ALDH isoenzymes. Mitochondrial class 2 ALDH (ALDH2) is primarily responsible for AA oxidation. Human ALDH2 enzyme is polymorphic, with two distinct alleles: ALDH2*1 and ALDH2*2. ALDH2*2 results from a single point mutation in chromosome 6 coding the normal ALDH2*1 allele. Individuals homozygous for the mutated ALDH2*2 allele are completely devoid of ALDH2 activity, whereas heterozygous individuals showing the ALDH2*1,2 genotype reveal only 30–50% of the normal ALDH activity. Blood AA levels of ALDH2*2 homozygous individuals are six to 20 times higher than in ALDH2*1 individuals, in which AA is hardly detectable after alcohol consumption. The elevated AA concentrations cause unpleasant side-effects (flush syndrome) which protects those individuals from alcoholism. However, heterozygous individuals may become heavy drinkers or even alcoholics (Seitz *et al.*, 2001).

Yokoyama *et al.* (1996, 1999) were the first to report that the heterozygous mutation of the ALDH2 gene (ALDH2*1,2) is a strong risk factor for oesophageal cancer both in every day drinkers and alcoholics. A comprehensive study of the ALDH2 genotype and cancer prevalence in Japanese alcoholics showed that the frequency of inactive ALDH2 increased remarkably among alcoholics with cancer of the oral cavity, oropharynx, hypopharynx, larynx, oesophagus and colorectum (Yokoyama *et al.*, 1998). It is important to note that these individuals also have high AA levels in their saliva and thus deliver AA directly to the surface mucosa of the UADT (Väkeväinen *et al.* 2000).

In addition to the mutation of the ALDH2 gene, polymorphisms of alcohol dehydrogenase 1B (ADH1B) and alcohol dehydrogenase 1C (ADH1C) may also modulate AA levels. While the ADH1B*2 allele encodes for an enzyme which is approximately 40 times more active than the enzyme encoded by the ADH1B*1 allele, ADH1C*1 transcription leads to an ADH isoenzyme 2.5 times more active than that from ADH1C*2. However, the ADH1B*2 allele frequency is

high in Asians but low in Caucasians. It protects from alcoholism, because of the high amount of AA produced and its toxic side-effects (Borras *et al.*, 2000; Seitz *et al.*, 2001). Because of the low ADH1B*2 allele frequency and the lack of ALDH2 mutations in Caucasians, ADH1C polymorphism and its role in alcohol-associated carcinogenesis can ideally be investigated in Caucasian populations.

Studies on ADH1C polymorphism in Caucasians and UADT cancer have shown contradictory results. Whereas an increased risk of oropharyngeal and laryngeal cancer in individuals with the ADH1C*1 allele has been reported (Harty *et al.*, 1997; Coutelle *et al.*, 1997), others could not confirm such an association in case–control studies (Olshan *et al.*, 2001; Sturgis *et al.*, 2001). We studied 107 alcoholic patients with oropharyngeal, laryngeal, hypopharyngeal and oesophageal cancer to compare their ADH1C genotype with 103 age-matched alcoholics without cancer, and found a significantly increased cancer risk in individuals with the ADH1C*1 allele (Visapää *et al.*, 2004). This was found to be associated with significantly elevated AA levels in the saliva of individuals homozygous for ADH1C*1 (Visapää *et al.*, 2004). Increased salivary AA levels in these individuals similar to that in individuals with ineffective ALDH activity may explain their increased cancer risk, as AA comes into direct contact with the mucosa. In this context, it is interesting to note that AA-fed rats showed a severe hyper-regeneration of the upper gastrointestinal mucosa (Homann *et al.*, 1997b) which is very similar to the morphological changes observed after chronic alcohol consumption (Simanowski *et al.*, 1993). These changes were only observed when the animals had functionally intact salivary glands. After sialoadenectomy, this proliferation disappeared, which supports the hypothesis that salivary AA is involved in carcinogenesis. In this context it has to be pointed out that chronic alcohol consumption alters salivary morphology and function (Maier *et al.*, 1986).

Morphometric analyses in rats who were fed alcohol over 6 months have shown an enlargement of the size of the nuclei of the basal cells of the oral mucosa associated with an increased percentage of cells in the S-phase and a reduction of the epithelial thickness indicating mucosal atrophy and hyperproliferation (Maier *et al.*, 1994). A similar finding of hyperproliferation was reported for the oesophageal mucosa in rats chronically fed ethanol (Simanowski *et al.*, 1993).

Acetaldehyde can also be produced by oral bacteria. Significant amounts of AA can be detected in the saliva of healthy volunteers after ingestion of a moderate dose of alcohol, which is 10–20 times higher compared to systemic blood AA levels even at a higher alcohol intake (Homann *et al.*, 1997a). Salivary AA concentrations following ethanol ingestion can be significantly reduced by using the antiseptic chlorhexidine prior to alcohol intake emphasizing the important role of oral bacteria in AA production (Homann *et al.*, 1997b). It has been shown that alcoholics with oropharyngeal cancer had very high salivary AA concentrations (Jokelainen *et al.*, 1996). This may be due to the fact that smoking (Homann *et al.*, 2000) and poor oral hygiene (Homann *et al.*, 2001), both frequently observed in alcoholics, result in high salivary AA concentrations due to bacterial AA production. Very recently it has been shown that smoking changes the oral bacterial flora rapidly from Gram-negative to Gram-positive bacteria, which leads to AA concentrations 50–60% higher compared to those observed without smoking (Salaspuro, 2003). Indeed, Gram-positive bacteria are capable of producing higher amounts of AA than are Gram-negative bacteria. In addition, *Candida albicans* also frequently belongs to the microbial environment of smokers and converts alcohol to AA. The data imply that smokers exposed to moderate amounts of alcohol produce higher AA concentrations compared to non-smokers. Apart from that, poor oral hygiene is associated with bacterial overgrowth, parodontitis and caries and also increases salivary AA concentrations. In this context it seems worth mentioning that non-pathogenic *Neisseria* species isolated from oral cavity produce significant amounts of AA (Salaspuro, 2003).

Acetaldehyde can also be produced by faecal bacteria. It has been shown that the amount of AA per gram of tissue is highest for the colonic mucosa than for all other tissues in the body. This is primarily due to the production of AA from faecal bacteria, as animal studies with germ-free rats have shown (Seitz *et al.*, 1990). AA has toxic effects on the colon mucosa resulting in a decreased number of cells in the functional compartment of the colonic crypt. This AA-mediated toxicity is answered by a secondary compensatory hyper-regeneration with increased crypt cell production rates and an extension of the proliferative compartment towards the lumen of the crypt (Simanowski *et al.*, 1986, 1994). Such a change in crypt cell dynamics represent a condition associated with increased risk for colorectal cancer (Deschner *et al.*, 1983, 1984; Lipkin *et al.*, 1984). The alcohol-associated hyper-regeneration of the colonic mucosa is especially pronounced with increasing age (Simanowski *et al.*, 1994). This may have practical implication as age alone is a risk factor for colorectal cancer. The hyperproliferative colorectal mucosa observed in animals was recently confirmed in alcoholics (Simanowski *et al.*, 2001). Here again, the significant extension of the proliferative compartment of the rectal crypt has been documented. Although AA production form faecal bacteria obviously dominates in the colon, it has been recently observed that individuals with ADH1C*1 allele homozygocity also exert an increased risk for colorectal cancer as they may produce more AA, as already discussed above. (Seitz, personal communication).

Induction of CYP2E1

Chronic alcohol consumption leads to an induction of cytochrome P-4502E1 (CYP2E1), which metabolizes ethanol to AA. This cytochrome enzyme is also involved in the metabolism of various xenobiotics, including procarcinogens (Seitz *et al.*, 1998). It has been shown in the liver that the concentration of CYP2E1 can be correlated with the generation of hydroxyethyl radicals and, thus, with lipid peroxidation. Induction of CYP2E1 resulted in enhanced hepatic injury, and inhibition of CYP2E1 was associated with an improvement of these lesions (Seitz *et al.*, 1998). It has been concluded that this is mainly due to a stimulation and inhibition, respectively, of free radical formation. The role of CYP2E1 induction and cell injury has been studied in detail in the liver. However, for the upper gastrointestinal tract data on the role of CYP2E1 induction and alcohol-related cancer are limited.

Chronic alcohol consumption resulted in a marked induction of CYP2E1 in the gastrointestinal mucosa of rodents (Shimizu *et al.*, 1990) and in men (Baumgarten *et al.*, 1996).

In humans, the extent of CYP2E1 induction is individually determined, but may already be significant following the ingestion of 40 g alcohol per day (corresponding to 400 ml 12.5 vol% wine) over 1 week (Oneta *et al.*, 2002). The role of free radicals in UADT cancer has been demonstrated in an animal study. Eskelson *et al.* (1993) reported that chronic alcohol consumption increases the development of tumours induced by N-nitrosemethylbenzylamine in the oesophagus which was associated with an increased free radical production and which was offset by administration of alpha tocopherol (Eskelson *et al.*, 1993). Interestingly, colorectal hyper-regeneration observed after chronic alcohol administration to rats, most probably due to AA, was also attenuated by the concomitant administration of alpha tocopherol (Vincon *et al.*, 2003).

The induction of CYP2E1 also increases the conversion of various xenobiotics, including procarcinogens (nitrosamines, aflatoxin, vinylchloride, polycyclic hydrocarbons, hydrazines) to their ultimate carcinogens (Seitz *et al.*, 1998). Induction of CYP2E1 in the UADT may be particularly relevant with respect to procarcinogens present in tobacco smoke and the well-known synergistic effect of drinking and smoking on UADT carcinogenesis. Thus, the microsomal activation of nitrosopyrrolidine, present in tobacco smoke, to its ultimate carcinogen is significantly enhanced in the oesophagus after alcohol ingestion in rats (Farinatti *et al.*, 1985). The interaction between ethanol and procarcinogen metabolism is complex and may depend, among others, on the degree of CYP2E1 induction, on the chemical structure of the procarcinogen and on the presence or absence of ethanol in the body during procarcinogen metabolism. The events in this setting are reviewed elsewhere (Seitz *et al.*, 1998). In most of the studies published, the co-administration of ethanol with nitrosamines has resulted in a strong increase in tumours in extrahepatic target organs (Anderson, 1992). The results are very similar to those observed when the CYP2E1 inhibitor disulfiram was administered. The tumours that occurred were cancers of the nasal cavity and the trachea in hamsters; lung, kidney and forestomach tumours in mice; and oesophageal and nasal cavity tumours in rats. It has been a consistent, reproducible and general finding. The mechanism behind this observation may be an inhibition of the first pass metabolism of nitrosamines in the liver by alcohol, leading to an increased exposure of extrahepatic tissues to nitrosamines.

Measurements of dimethylnitrosamine (DMN) metabolism in liver slices and oesophageal epithelium suggest that the changes in alkylation of oesophageal DNA can be the result of a selective inhibition of DMN metabolism in the liver (Swann *et al.*, 1983). This is in agreement with the observation that no increased methylation of hepatic DNA was detected when radioactively labelled DMN was given in ethanol-fed and control rats. However, labelling of the oesophagus DNA was enhanced after alcohol (Kouros *et al.*, 1983). Furthermore, ethanol administration also increased DMN derived O^6-methylguanidine in gastrointestinal mucosal DNA of monkeys (Anderson *et al.*, 1996). Following the administration of the oesophageal carcinogen N-nitrosemethylbenzylamine, the formation of O6-methyldeoxyguanosine in the oesophagus was increased threefold by water with 20% alcohol. Various alcoholic beverages such as brandy, scotch, whiskey, white wine or beer had the same effect. However, red burgundy and calvados exhibited the most striking increase in DNA alkylation (Yamada *et al.*, 1992).

Nutritional deficiencies

In heavy drinkers, the entire nutritional status is impaired due to primary and secondary malnutrition. Various deficiencies of vitamins and trace elements that occur in chronic alcoholics may contribute to alcohol-associated carcinogenesis (Seitz und Suter, 2002). The increased oxidative stress observed during ethanol metabolism leads to an increased requirement for glutathion and alpha-tocopherol. In addition, chronic alcoholism increases the requirements for methyl groups and dietary methyl deficiency may enhance hepatic carcinogenesis (Stickel *et al.*, 2002; Stickel and Seitz, 2004). Folate deficiency, primarily the consequence of a low intake and of destruction by AA, is common in alcoholics and contributes to an inhibition of transmethylation which is an important factor in the regulation of genes involved in carcinogenesis (Stickel and Seitz, 2004). The role of nutritional deficiencies in alcohol-associated UADT cancer is unclear. Various studies have pointed out the role of iron-deficiency (Plummer–Vinson syndrome) in cancer of the hypopharynx. Also, some indirect evidence exists that the risk from exposure to many carcinogenic agents may be reduced by regular consumption of fruit and green vegetables, which is a rare dietary component in alcoholics. The deficiency of zinc and selenium may also contribute to cancer development (Seitz *et al.*, 1998). Besides the impact of zinc on nitrosamine activation by CYP2E1 (Barch *et al.*, 1984), zinc deficiency may also lead to disturbances in vitamin A metabolism, as zinc is an important factor in the conversion of retinol (ROL) to retinal, as well as in the synthesis and secretion of retinol-binding protein in the liver. Zinc deficiency also reduces glutathion transferase, an enzyme important in the detoxification of carcinogens *in vivo*. Furthermore, zinc depletion is associated with increased cell proliferation in the oesophageal mucosa (Cho, 1961).

Interactions with retinoids

Reduced serum and hepatic vitamin A concentrations have been shown in chronic alcoholics (Leo and Lieber, 1982). This is of particular importance as retinoic acid (RA) is synthesized from retinol via various enzymatic steps involving microsomal and cytosolic ADH and ALDH. RA has profound effects on cellular growth and differentiation via two families of RA nuclear receptors (RAR-α, -β and -χ and RXR-α, -β and -χ), which mediate RA induced gene transcription (Chambon, 1996). In a series of experiments, the effects of alcohol on ROL 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 mobilization of retinol from the liver to other organs (Leo and Lieber, 1999; Seitz, 2000). These ethanol-induced changes may result in decreased hepatic concentrations of both retinol and retinyl esters which are the metabolically active precursors of RA. Furthermore it has been demonstrated that ethanol acts as a competitive inhibitor of ROL oxidation in the liver, thereby counteracting the biosynthesis of RA (Wang *et al.*, 1998). Accordingly, RA levels in the liver of ethanol-fed rats were decreased significantly compared with control pairs fed an isocaloric

control diet containing equal amounts of vitamin A (Liu *et al.*, 2001). 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 CYP2E1 (Chung *et al.*, 2001). In the same study, treatment of ethanol-fed rats with chlormethiazole, a specific CYP2E1 inhibitor, restored both hepatic and plasma RA concentrations to normal levels. Enhancement of RA catabolism by ethanol *in vitro* was inhibited by CYP2E1 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 down-regulation of RA receptors and an up to eightfold expression of the AP-1 (c-jun and c-fos) transcriptional complex (Wang *et al.*, 1998). This explains parenchymal hyperproliferation as AP-1 is a central complex downstream of various growth factors, oncogenes and tumour promoters (Chiu *et al.*, 1988). 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 normalization of hepatic proliferation, as expressed by proliferating cell nuclear antigen expression (Chung *et al.*, 2001). In summary, these data suggest that low hepatic RA levels due to chronic alcohol misuse may favour proliferation and malignant transformation of hepatocytes via upregulation of AP-1 (c-jun and c-fos) gene expression.

It is noteworthy that the supplementation of β-carotene to smokers for the prevention of lung cancer resulted in the opposite observation. Smokers who received β-carotene developed more lung cancer than placebo controls (The alpha tocoperol, β-carotene study group, 1994). When these data were re-evaluated it was found that only alcohol consumers of more than 11 g/day developed lung cancer under β-carotene supplementation (Albanes *et al.*, 1996). This may be due to the fact that alcohol-induced CYP2E1 generates toxic and carcinogenic metabolites from ROL (Dan *et al.*, 2004).

Alcohol and methylation

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 (Counts and Goodman, 1995). 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 (Kanai *et al.*, 1999), and hypomethylation of certain oncogenes such as c-myc and c-N-ras may lead to dedifferentiation and proliferation (Shen *et al.*, 1998; Wainfan *et al.*, 1989).

Recently, it has been suggested that aberrant DNA hypermethylation may be associated with genetic instability, as determined by the loss of heterozygosity and microsatellite instability in human HCC due to chronic viral hepatitis (Kanai *et al.*, 2000; Kondo *et al.*, 2000). Iwata *et al.* (2000) 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 HCC. 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 (Hui and Makuuchi, 1999).

Importantly, modifications of the degree of hepatic DNA methylation have also been observed in experimental models of chronic alcoholism (Choi *et al.*, 1999; Garro *et al.*, 1991). Hypomethylation is a plausible consequence of metabolic alterations in the setting of ethanol 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-adenosylhomocysteine (SAH), resulting in an up to 2.5 fold decrease in the SAM/SAH ratio (Lieber *et al.*, 1990; Trimble *et al.*, 1993; Stickel *et al.*, 2000). Several mechanisms have been suggested by which ethanol could interact with one carbon metabolism and DNA methylation and thereby enhance carcinogenesis. (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 (Lumeng and Li, 1974; Labadarios *et al.*, 1977; Savage and Lindenbaum, 1986; Gloria *et al.*, 1997; Stickel *et al.*, 2000). (2) Ethanol reduces the activity of methionine synthetase which remethylates homocysteine to methionine with methyltetrahydrofolate as the methyl donor (Barak *et al.*, 1993; Lieber, 1994). (3) Chronic alcohol consumption decreases glutathione levels, a reductive tripeptide, which is synthesized from homocysteine via trans-sulfuration in the liver, and thereby enhances the susceptibility of the liver towards alcohol related peroxidative damage (Speisky *et al.*, 1985; Lieber, 1994). (4) Alcohol can inhibit the activity of DNA methylase, which transfers methyl groups to DNA in rats (Lieber *et al.*, 1990), a finding which could not be confirmed in humans (Miyakawa *et al.*, 1996).

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 (Zapisek *et al.*, 1992; Pogribny *et al.*, 1995). Further, low methionin, low-folate diets and alcohol increases the risk for colorectal cancer in men (Giovanucci *et al.*, 1995).

Alcohol and immune surveillance

Chronic alcohol consumption results in a complex alteration of the unspecific (innate) and specific (acquired) immune response (Cook, 1998). Numerous studies and clinical experience have shown that chronic alcoholics are more susceptible to infections and to certain neoplasms (Roselle *et al.*, 1993). 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—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 co-infection with hepatitis viruses (Szabo, 1999).

Studies in mice have shown that chronic alcohol administration inhibits NK cell activity (Gallucci *et al.*, 1994) and reduces NK cell number and lytic activity following a single binge equivalent of alcohol (Wu *et al.*, 1994). 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 (Ben-Eliyahu *et al.*, 1996). Few studies in humans have so far been performed. In a study by Laso *et al.* (1997) alcoholic cirrhotics revealed both diminished NK cell numbers and reduced lytic activity, even when stimulated by interleukin 2, a powerful NK cell-stimulating cytokine (Laso *et al.*, 1997). NK cell numbers were also found to be decreased in actively drinking individuals without established alcoholic liver disease (Cook *et al.*, 1991). 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 (Stickel *et al.*, 2001), suppresses immune function in general and NK cell activity in particular (Rook *et al.*, 1986). However, there are no data on how far antigen 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.

SUMMARY AND CONCLUSION

Chronic alcohol consumption and heavy smoking are the major risk factors for upper aerodigestive tract cancer including oropharynx, hypopharynx, larynx and oesophagus. Alcoholic liver cirrhosis is also a precancerous condition. Furthermore, chronic alcohol ingestion even at moderate dosage enhances carcinogenesis in the colorectum and breast, especially in individuals with increased susceptibility to developing cancer. Evidence has accumulated that acetaldehyde is predominantly responsible for the alcohol associated carcinogenesis since acetaldehyde is carcinogenic, mutagenic, binds to DNA and protein, destructs folate and results in secondary hyperregeneration. Acetaldehyde is produced by various alcohol dehydrogenases in the liver and in the gastrointestinal tract and by gastrointestinal bacteria. Acetaldehyde is degraded by acetaldehyde dehydrogenases to acetate. Both generation and the degradation of acetaldehyde is modulated due to polymorphisms or mutations of the genes responsible for the enzymes involved. In addition, cigarette smoke and some alcoholic beverages also contain acetaldehyde. Chronic alcohol consumption also induces cytochrome P-4502E1 in gastrointestinal mucosa cells and in the liver, resulting in an increased generation of reactive oxygen species and in an increased activation of various dietary and environmental carcinogens such as those present in tobacco smoke. Nutritional deficiencies commonly observed in the alcoholic may further enhance alcohol-associated carcinogenesis. A disturbed methyl transfer due to alcohol results among others in an inadequate DNA synthesis. The significant decrease in retinol and retinoic acid observed in the liver in patients with alcoholic liver disease leads to an activation of the AP1-gene resulting in cellular hyperregeneration and possibly in an associated increase of polar metabolites of retinoic acid with increased toxicity.

In summary, the following risk factors for alcohol associated carcinogenesis exist:

(1) For the upper aerodigestive tract: smoking, poor oral hygiene and poor dental status, highly concentrated alcoholic beverages, additional supplementation of vitamin A and β-carotene, ADH1C*1,1 homozygocity, ALDH 2*2,2-mutation, precancerous conditions such as Barret's oesophagus and gastro-oesophageal reflux.

(2) For the liver: cirrhosis, hepatitis B- and C infection, haemochromatosis, exposure to aflatoxins and vinylchloride.

(3) For the colorectum: chronic inflammatory bowel disease, polyps, deficiency of folate, ADH1C*1 homozygocity, ALDH2*2 mutation.

(4) For the breast: high oestradiol concentrations (especially in midcycle), ADH1C*1 genotype? Family history?

From these data one can conclude that individuals who already have the above-mentioned types of cancer or who have an increased risk of developing those cancers due to other risk factors should avoid chronic alcohol ingestion and should limit their alcohol intake to not more than twice weekly and in moderate dosage (20–30 g for men and 10–20 g for women). In this context, alcohol should be consumed with meals and highly concentrated alcoholic beverages should be avoided.

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