Etiological and Preventive Implications in Alcohol Carcinogenesis

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Abstract

The current state of knowledge of the epidemiological association of alcohol and tobacco consumption with cancers of the head and neck is considered. Data are presented which indicate that in vitro metabolism of the hepatocarcinogen N-nitrosopyrrolidine is increased in microsomal fractions isolated from ethanol-consuming hamsters. Relevant studies in experimental animals are discussed in the context of possible mechanisms that could account for the increased risk of cancer in heavy drinkers who smoke.

Epidemiological Considerations

Perhaps the earliest clinical observation associating chronic alcohol consumption with cancer was that of J. C. Warren, noted Boston surgeon, who in 1836 described a case of lingual cancer in a tobacco chewer by saying, "predisposition . . . was generated by the long use of ardent spirits . . ." (103). Since then, large-scale case-control studies conducted in the United States have firmly established that chronic alcohol consumption markedly increases the risk for oral cavity cancer (6, 28, 43, 75, 100, 108, 110), esophageal cancer (39, 114), as well as cancer of the glottis and supraglottis and that this universal risk applies primarily to users of various forms of tobacco both when smoked and chewed (66, 83, 100, 109, 111) (Charts 1 and 2; Table 1). Data obtained from other countries also clearly show that alcohol consumption is strongly associated with cancers of both the UAT and URT (57, 62, 66, 83, 97, 109, 111, 116). Studies of populations known to have a low alcohol intake, such as Seventh-Day Adventists and older members of the American Jewish population, have shown that these groups have a lower incidence of these cancers compared with the general population (29, 68, 72, 110). Furthermore, the fact that cancers at these sites are found predominantly in males is consistent with their smoking and drinking habits, which historically have been greater than those of females.

Two questions which arise from consideration of the epidemiological data are (a) does consumption of alcohol in the absence of tobacco usage increase the risk for cancers of the UAT and URT and (b) does the type of alcoholic beverage consumed have any effect on the relative risk? An association of chronic alcohol consumption with cancer was that of J. C. Warren, noted Boston surgeon, who in 1836 described a case of lingual cancer in a tobacco chewer by saying, "predisposition . . . was generated by the long use of ardent spirits . . ." (103). Since then, large-scale case-control studies conducted in the United States have firmly established that chronic alcohol consumption markedly increases the risk for oral cavity cancer (6, 28, 43, 75, 100, 108, 110), esophageal cancer (39, 114), as well as cancer of the glottis and supraglottis and that this universal risk applies primarily to users of various forms of tobacco both when smoked and chewed (66, 83, 100, 109, 111) (Charts 1 and 2; Table 1). Data obtained from other countries also clearly show that alcohol consumption is strongly associated with cancers of both the UAT and URT (57, 62, 66, 83, 97, 109, 111, 116). Studies of populations known to have a low alcohol intake, such as Seventh-Day Adventists and older members of the American Jewish population, have shown that these groups have a lower incidence of these cancers compared with the general population (29, 68, 72, 110). Furthermore, the fact that cancers at these sites are found predominantly in males is consistent with their smoking and drinking habits, which historically have been greater than those of females.

Two questions which arise from consideration of the epidemiological data are (a) does consumption of alcohol in the absence of tobacco usage increase the risk for cancers of the UAT and URT and (b) does the type of alcoholic beverage consumed have any effect on the relative risk? An association of chronic alcohol consumption with esophageal cancer in the absence of tobacco consumption has been observed by Tuyns et al. (97) in France. Their data indicate that consumption of home-distilled apple brandy by people living in the Normandy region of France markedly increases the risk for esophageal cancer. The risk is, however, positively increased when tobacco usage is concurrent (96, 97). Some increase in risk for URT cancer due solely to alcohol consumption has been claimed by Rothman and Keller (75); however, they too report a marked synergy when tobacco usage is combined with chronic alcohol consumption. The data of Williams and Horn (104) indicate that if the smoking variable is controlled for, an increased risk due to alcohol consumption can be observed. Several studies have clearly established that the increased risk is associated with the total amount of alcohol consumed independent of the nature of the beverage (104, 110, 117). Because of the apparent synergy between alcohol and tobacco consumption, it is difficult to conclude from these types of studies if alcohol in and of itself can increase the risk for UAT and URT cancers. Since these cancers can occur in the absence of chronic alcohol and tobacco consumption, the observed increase in risk due to alcohol consumption alone may be due to the enhancement of some other process (such as nutrient deficiency) which is associated with carcinogenesis (114).

An association of chronic alcohol consumption with cancer of the liver has been observed (41, 42, 49, 50, 84). That this increased risk for liver cancer may be due to components other than ethanol can be inferred from the work of Gibel et al. (26). They have presented preliminary evidence that several of the higher alcohols normally found in distilled beverages may be carcinogenic. For example, studies have shown that administration either p.o. or s.c. of isoamyl, isobutyl, or n-propyl alcohol to rats resulted in the appearance of both benign and malignant tumors of the liver as well as other sites. Recent findings which indicate that certain fermented and distilled beverages contain trace quantities of nitrosamines raise the possibility that this class of carcinogens may play a role in the etiology of alcohol-related cancers (54, 95, 102).

In summary, current epidemiological evidence indicates that in the United States increased risk among smokers is associated with the amount and duration of alcohol consumption and leads us to the conclusion that alcohol acts primarily as a cocarcinogen or promoter.

How does ethanol increase the risk for cancer? It is conceivable that it results from nutritional deficiencies that are commonly associated with alcoholism (50). Since alcoholics often consume 900 or more calories a day from alcohol alone (15), it is not difficult to imagine that the rest of their caloric intake is insufficient to provide necessary nutrients. Cancers of the UAT also seem to occur most commonly in those individuals who do not eat nutritionally balanced diets (85). Alcohol consumption can lead to impaired absorption of nutrients and vitamins (101). That ethanol is capable of decreasing vitamin A levels is noteworthy in view of the participation of this vitamin in the regulation of epithelial cell differentiation (16, 98).

Our initial basis for correlating alcoholism and cancers of the UAT with diet and nutritional status resulted from consideration of the classical association between Plummer-Vinson (Patterson-Kelly) syndrome and cancer of the UAT. This disease, which was prevalent among Swedish women, was shown to be
Table 1
Relative risks for oral cavity cancer and larynx cancer in 40 to 65-year-old white males by daily consumption of alcohol and cigarettes

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Cigarettes/day</th>
<th>None</th>
<th>1-10</th>
<th>11-20</th>
<th>21+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>95% confidence interval</td>
<td>RR</td>
<td>95% confidence interval</td>
<td>RR</td>
</tr>
<tr>
<td>Oral cavity cancer</td>
<td>0</td>
<td>1</td>
<td>3.2</td>
<td>1.78-5.73</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>1-6</td>
<td>0.4</td>
<td>0.05-2.45</td>
<td>3.5</td>
<td>1.48-8.44</td>
</tr>
<tr>
<td></td>
<td>7+</td>
<td>2.5</td>
<td>0.35-18.24</td>
<td>5.1</td>
<td>1.31-19.6</td>
</tr>
<tr>
<td>Larynx cancer</td>
<td>0</td>
<td>1</td>
<td>3.5</td>
<td>1.61-7.58</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>1-6</td>
<td>1.1</td>
<td>0.06-1.19</td>
<td>8.0</td>
<td>4.27-15.03</td>
</tr>
<tr>
<td></td>
<td>7+</td>
<td>26.8</td>
<td>15.01-47.88</td>
<td>27.2</td>
<td>15.92-46.43</td>
</tr>
</tbody>
</table>

* Relative to a risk of 1.0 for nonsmokers and nondrinkers. Unless otherwise indicated, relative risks are significant at p < 0.05.

**RR**, relative risk.

* Not significant (based on less than 5 observations).

associated with chronic iron and vitamin deficiencies. High rates of UAT cancer were observed in the absence of exposure to either tobacco or any other obvious source of carcinogen (112, 114). It is of both etiological and preventive interest that since the introduction of national program of iron and vitamin supplementation in Sweden in the early 1950’s, a significant reduction in the number of cases of Plummer-Vinson syndrome with a subsequent reduction of UAT cancer has occurred (47).

The key questions which need to be addressed experimentally are: Where does ethanol exert its effects? What are the
effects? and How do the effects result in increased risk for cancer? There are 4 possible mechanisms which can be envisioned for the association between alcohol and cancer: alcohol as a solvent, alcohol-induced changes or decreases in liver metabolism, or alcohol-induced alterations in target tissue metabolism.

Etiological Considerations

Alcohol as a Solvent. The simplest of the 4 possible mechanisms is that of alcohol functioning as a solvent. This mechanism assumes that entry of tobacco-related carcinogens into target tissues is facilitated because of enhanced solubility of the carcinogen and easier passage through cellular membranes. Stenback (91) has demonstrated that administration of the carcinogen DMBA dissolved in ethanol resulted in a reduced latent period and increased skin tumor formation compared to mice treated with DMBA dissolved in acetone. Kuratsune et al. (46) have shown that although chronic treatment of mice or rats with various distilled beverages failed to cause tumor formation in either mice or rats, a tumor-promoting activity (for DMBA) similar to that caused by croton oil was found in sake and its distillation residues. This mechanism is an attractive one for oral cavity and esophageal cancer, since this area does not come into direct contact with alcohol.

Enhanced Liver Metabolism. The extensive body of literature dealing with alcohol-induced alterations in liver metabolism requires that serious attention be given to the second possibility. Chronic ethanol consumption leads to, among other things, an enhancement in the liver microsomal drug-metabolizing capabilities of both humans (40, 63) and experimental animals (63, 77, 78). In the absence of extensive destruction of liver tissue, increased production of a proximate carcinogen which is then delivered to the target tissues and further metabolized to its ultimate carcinogenic form could be envisioned. Radike et al. (74) have shown that ethanol decreases the latent period for vinyl chloride carcinogenesis in rats, demonstrating the mechanism by which ethanol could increase the risk for the development of liver cancer.

Recently, our group has demonstrated that the in vitro metabolism of the hepatocarcinogen N-nitrosopyrrolidine is increased in microsomal fractions isolated from ethanol-consuming animals (Table 2) and that postmitochondrial supernatants isolated from ethanol-consuming animals are capable of much greater conversion of nitrosopyrrolidine to a mutagen than are control preparations (Chart 3) (59). This particular mechanism must of necessity place a restriction on the nature of the carcinogen in that the carcinogens and/or their metabolites must have a high degree of site specificity, since all tissues in the body would be exposed to the results of changes in liver metabolism, and yet, only those in the head and neck area are at increased risk. The major objection to enhanced liver metabolic activation being involved in the causation of head and neck cancer is that this mechanism excludes consideration of PAH participation. Although tobacco smoke contains nitrosamines which are site specific for the UAT (4, 5, 35) and URT (34, 89) in animals, we feel that any mechanism which fails to take into account the carcinogenic potential of the PAH in tobacco smoke is a seriously compromised hypothesis. In addition, this mechanism clearly cannot apply in the case of the severely damaged alcoholic liver.

Decreased Liver Metabolism. An association between cirrhosis and the development of cancer of the liver in humans has been observed (49, 51, 71, 84). In addition, Keller (41, 42) has presented evidence that cancers of the head and neck area are often found in cirrhotic patients.

Experimental evidence in favor of this mechanism has been presented by Protzel et al. (73). In their studies, mice treated with either ethanol or carbon tetrachloride whose buccal mucosa were swabbed with benzo(a)pyrene solutions, developed more tumors which had a shorter latency period than did controls receiving only carcinogen treatment. The work of Falk and Kotin (23) has shown that the rate of bile clearance of benzo(a)pyrene is markedly decreased in rats whose livers have been damaged by exposure to carbon tetrachloride. In patients suffering from cirrhotic liver disease, the rate of metabolic clearance of a number of drugs known to be metabolized by the microsomal drug metabolizing system has been shown to be reduced (see Ref. 12 for a recent review).

The loss of metabolic capacity in the cirrhotic liver would result in a decreased ability to detoxify or render tobacco-related carcinogens inactive, causing a net increase in their systemic concentrations and resulting, in essence, in the target tissues being exposed to more carcinogen than would occur in individuals with uncompromised livers.

Alteration of Epithelial Cells. The fourth mechanism is that

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Table 2

<table>
<thead>
<tr>
<th>Nitrosopyrrolidine a-hydroxylase (nmol/min/mg protein)</th>
<th>Benzo(a)pyrene (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.01 ± 0.10</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.89 ± 0.44</td>
</tr>
<tr>
<td>Ethanol/control</td>
<td>2.9</td>
</tr>
</tbody>
</table>

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Chart 3. N'-Nitrosopyrrolidine mutagenicity of liver postmitochondrial supernatant fractions from control and ethanol consuming hamsters. Salmonella typhimurium TA 1535 was used as the indicator strain. Final protein concentration for both control and ethanol postmitochondrial supernatants varied from 1.3 to 1.5 mg/ml. Data are expressed as mean ± S.E. of 6 separate determinations. Data were corrected for spontaneous revertants which ranged from 4 to 30/plate.
chronic ethanol consumption alters intracellular metabolism of the epithelial cells at the target sites, resulting in enhanced metabolic activation of tobacco-associated carcinogens. Some of the more attractive features of this proposal are that the cancers associated with the UAT and URT are epithelial in origin. Secondly, these surface epithelial cells are most exposed to tobacco-associated carcinogens. Thirdly, site specificity of the carcinogen need not be invoked since all sites, by virtue of their anatomical location, will be exposed to tobacco smoke. This is an important point since no class of carcinogen need be initially excluded from consideration because we cannot say with any degree of certainty which of the many potential carcinogens in tobacco smoke are actually involved in the initiation of carcinogenesis in the head and neck area. The major limitation of this proposed mechanism is that so far there exists but little background information on the metabolic capacity (activation or detoxification) of the epithelial cells in this area.

Studies on Epithelial Cells. Riboflavin-deficient mice exhibit morphological alterations in the epithelium of skin and UAT similar to those observed in patients suffering from Plummer-Vinson syndrome (115). As the deficiency progresses, epithelial morphology progressively changed from atrophy to hyperkeratosis to, in several instances, hyperplasias. The experiments of Chan and Wynder (12) have shown that upon initiation with benzo(a)pyrene and promotion with croton oil that riboflavin-deficient mice develop these tumors more rapidly than do control mice receiving a nutritionally adequate diet. In parallel studies, Chan et al. (11) showed that basal levels of skin aryl carbon hydroxylase were slightly reduced in riboflavin-deficient mice. However, the skin activity of riboflavin-deficient animals was induced to a much greater extent following a single application of DMBA.

The work of Gerson and Meyer (25) has shown that feeding rats diets deficient in zinc causes similar changes in the morphology of the buccal mucosa. Lower levels of zinc have been observed in hair and tissue samples from esophageal cancer patients (55). In addition, rats on a zinc-deficient diet have been shown to be more susceptible to the esophageal carcinogen methylbenzylnitrosamine (24).

In experimental animals deficient in vitamin A, the tracheobronchial epithelium undergoes atrophic degenerative changes (31, 82, 106, 107) which are quite similar to the changes observed in animals exposed to tobacco smoke (17–19, 45, 113). Animals deficient in vitamin A have been shown to be more susceptible to the esophageal carcinogen methylenbenzyl nitrosamine (24).

Effect of Alcohol on Target Tissue Metabolism. What effects do chronic ethanol consumption and riboflavin deficiency have on target tissue metabolism? Since there are no data on the effect of either of these 2 variables on target tissue metabolism, the effects of liver structure and function will be summarized. The assumption here is that at least some of the changes that occur in liver will also occur in the epithelium of the target tissue.

Electron microscopic examination of liver from rats which have been fed ethanol for extended periods of time reveals the appearance of atypical mitochondrial forms (9, 27, 37, 44, 76). The mitochondria appear swollen and distended, and although both inner and outer membranes appear intact, there is an apparent decrease in the invagination of the inner mitochondrial membrane (cristae). Mitochondria isolated from the liver of rats which have been maintained on chronic ethanol intake show numerous biochemical changes when assayed in vitro. It has been established that mitochondria from ethanol-consuming animals have reduced respiratory control ratios, as well as lowered ADP/oxygen ratios (13, 76). These observations suggest that the coupling efficiency, i.e., the coupling of substrate oxidation and ADP phosphorylation, is decreased in mitochondria isolated from the livers of animals on a chronic alcohol diet. Mitochondria isolated from ethanol-treated rats show markedly decreased ability to oxidize exogenous fatty acids when assayed in vitro, but β-oxidation of endogenous fatty acids appears to be unimpaired (27), indicating that there are no qualitative biochemical changes in the enzymatic apparatus of mitochondria isolated from ethanol-treated rats. What appears to have been impaired is the ability of mitochondria isolated from ethanol-treated rats to translocate adenine nucleotides. Such an inhibition of adenine nucleotide transport has been reported by Gordon (27). In addition, her work has shown that there is a 2-fold increase in the fatty acyl CoA content of these mitochondria. Fatty acyl CoA esters have been shown to be potent inhibitors of adenine nucleotide translocation when added to mitochondria in vitro (2, 52, 70, 88, 105). Similar changes in the structure and function of mitochondria isolated from the livers of riboflavin-deficient animals have also been reported (7, 36, 92–94).

One would predict that if decreased flux of adenine nucleotides into the mitochondria happened in the undisrupted tissue, the intracellular distribution of ATP/ADP plus P would shift to the right and that the oxidation-reduction state of the pyridine nucleotides should be further reduced making more NADPH available for the metabolic activation process. In agreement with this view, the ratio of ATP/ADP plus P has been shown to decrease and decreases in the NAD/NADH ratio have also been reported (3, 32, 99).

Isolated microsomes from ethanol-consuming rats show increases in P-450 content (38, 56, 77) and a 2- to 4-fold increase in a specific form of cytochrome P-450 by chronic ethanol administration has been reported by Comai and Gaylor (14) and, Ohnishi and Lieber (69). Greater increases in cytochrome P-450 have been shown to occur when protein deficiency is superimposed on chronic ethanol administration (44). Marked elevation in aniline hydroxylase activity (38, 77) and smaller but significant increases in cytochrome P-450 reductase activity (13, 67) have also been reported. Microsomes isolated from riboflavin-deficient mice also show increases in aniline hydroxylase, aminopyrine demethylase, cytochrome P-450 content (8, 118), and cytochrome P-450 reductase.

In summary, both chronic ethanol consumption and riboflavin deficiency result in decreased mitochondrial function which can in turn lead to an increase in the reducing potential in the cell. Active metabolism of ethanol by the cell would increase the reducing potential still further. Parallel increases in the metabolic capabilities of the microsomal drug metabolism system would result in the potential for enhanced metabolic activation of carcinogens.

Experimental Approach

To undertake studies of the metabolic consequences of both alcohol and tobacco consumption on target tissue metabolism, a suitable animal model is a necessary prerequisite. The Syrian hamster appears to be ideally suited for these types of studies.
for several reasons. First, because of a low incidence of URT infections as well as low rates of spontaneous tumor formation in the URT, the Syrian hamster was selected as a test animal for chronic inhalation studies (33, 48, 64, 65). Second, the hamster is quite susceptible to cyclic nitrosoamine carcinogenesis and develops URT tumors when treated with N-nitrosornicotine, a tobacco-specific carcinogen (34). Third, the hamster readily consumes ethanol when it is administered as part of a Lieber-DeCarli liquid diet (60) or as an unsweetened ethanol/water mixture in place of drinking water (1).

The hamster cheek pouch has found extensive use in experimental carcinogenesis (21, 22, 81) and in investigations of the ability of a wide variety of compounds to either promote or inhibit tumor growth (20, 30, 53, 79, 80, 86, 87). Anatomically, the pouches are bilateral evaginations of the oral cavity mucosa. The epithelial portion is composed of simple, stratified, squamous epithelium essentially devoid of other cell types. These large, readily accessible structures contain sufficient quantities of epithelium to permit subcellular fractionation and biochemical characterization. The development of a rapid method for the isolation of the epithelial portion of the cheek pouch has enabled us to isolate and partially characterize the mitochondrial, microsomal, and soluble fractions of the hamster cheek pouch epithelium (Chart 4). The mitochondrial fraction has been characterized with respect to respiratory capacity, specific enzyme, and cytochrome content and found to be qualitatively similar to liver mitochondria, with the exception that the mitochondrial ATPase was insensitive to dinitrophenol (58). Isolated microsomes have been found to contain both P-450- and b5-type cytochromes. The specific content of these 2 cytochromes is approximately 25% of that found in liver microsomes. NADH and NADPH cytochrome c reductase activities, as well as aniline hydroxylase activity, have also been demonstrated in this fraction (61). The demonstration of the presence of both alcohol and aldehyde dehydrogenase activities in squamous epithelial cells is a key piece of evidence for our hypothesis for the etiology of UAT cancer since it indicates that ethanol oxidation can occur within the cells of the target tissue and can therefore exert a direct effect on intermediary metabolism.

Conclusions

The strong association of alcohol and tobacco consumption with cancers of the head and neck has been firmly established by numerous epidemiological studies. The mechanisms by which these 2 factors increase risk have so far received little attention. However, we hope the information presented here will increase our understanding of the possible mechanisms of epithelial carcinogenesis of the UAT and URT.

From the point of view of preventive medicine, strategies should be developed that would lead to a reduction of alcohol intake, particularly among those who are exposed to known or suspected UAT and URT carcinogens. It is also of interest to consider whether dietary supplements might decrease the risk for cancer of the UAT associated with chronic alcohol consumption.

We hope that this communication will bring to the attention of scientists from a variety of disciplines these strong human epidemiological and experimental leads so that additional effort to investigate the underlying mechanism(s) can be undertaken. Concentrated efforts in this direction would contribute much to our understanding of the etiology of cancer of the UAT and URT in humans and should ultimately lead to the prevention of alcohol-related cancer.

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References

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51. Lewy, I. S., and Rubin, D. Inhibition by 2,4-dinitrophenol of 1,9,10-dimethyl-1-benzanthracene carcinogenesis in the hamster cheek pouch. Oncology (Basel), 31: 334–337, 1975.


68. Pande, S. V., and Blanchard, M. C. Reversible inhibition of mitochondrial...


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