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REVIEW ARTICLE

Oral micro-organisms in the etiology of cancer

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Abstract
We present a novel concept on carcinogenesis mediated by oral microbiota. Oral micro-organisms are capable of metabolizing alcohol to acetaldehyde. This finding casts light on the observed association between poor oral hygiene and oral cancer. Ethanol, as such, is not carcinogenic, but its first metabolite acetaldehyde is indisputably carcinogenic. Several gastro-intestinal microbial species possess the enzyme alcohol dehydrogenase (ADH), which is also the enzyme responsible for alcohol metabolism in the liver. In oral microbiota, we observed that species such as the ubiquitous viridans streptococci and *Candida* also possess ADH. Ethanol can be detected in the mouth hours after the consumption of alcoholic beverages. Patients with poor oral health status have shown higher salivary acetaldehyde concentrations than those with better oral health. It is thus understandable that ADH-containing micro-organisms in the mouth present a risk for carcinogenic acetaldehyde production, with subsequent potential for the development of oral cancer, particularly among heavy drinkers. In this article, we briefly review this area of investigation and conclude by highlighting some future possibilities for the control of carcinogenesis.

Key Words: *Acetaldehyde, alcohol, carcinogenesis, oral cancer, oral hygiene, oral microbiota*

Introduction

Oral cancer ranks eighth in the global incidence of all malignant diseases. Although most patients with oral cancer live in the developing world [1], in industrialized countries, too, as in Finland, the incidence of oral cavity cancer has increased recently, while that of lip cancer has decreased [2]. Of all forms of cancer, the 5-year survival of oral cancer remains poor, i.e. approximately at the 50% level, even though treatment modes have developed. Similar to most malignant diseases, the etiology and specific mechanisms involved in oral cancer development remain unclear.

The principal risk factors of oral cancer are use of tobacco, heavy alcohol consumption and, as observed more recently, poor oral hygiene [3]. The probable link between these risk factors is acetaldehyde, which is the first metabolite of ethanol [4]. The enzyme alcohol dehydrogenase (ADH) metabolizes ethanol into acetaldehyde, principally in the liver after the consumption of alcohol. Acetaldehyde is further oxidized into less harmful acetate mainly by the enzyme aldehyde dehydrogenase-2 (ALDH2) (Figure 1). However, the first reaction has also been observed to take place in the gastro-intestinal tract and in the oral cavity [5,6].

Some gut micro-organisms have ADH and are thus capable of metabolizing ethanol to acetaldehyde. However, microbial ADH enables the micro-organisms to produce energy under anaerobic conditions by fermenting glucose. This results in endogenic alcohol as the end product. The reaction is two-way. Acetaldehyde has been detected in endoscopic samples of the gastro-intestinal tract and in saliva after ethanol has been given to the test subjects [5–7]. In this review article, we briefly summarize the novel findings about acetaldehyde production by oral microbiota and its suggested role in alcohol-related carcinogenesis. Furthermore, we point out some practical implications of the findings and outline recommendations for future studies.

Oral microbiota

The mouth is a unique micro-cosmos. Numerous microbial species form high density plaques (biofilm

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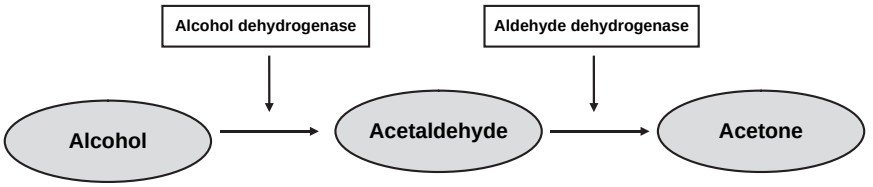

Figure 1. Metabolic pathway of alcohol. The intermediate metabolite acetaldehyde is carcinogenic.

structures), with characteristically large numbers of anaerobic, opportunistic and pathogenic micro-organisms. Diet greatly modifies the characteristics of oral microbiota. For example, intake of sugar facilitates extracellular polysaccharide synthesis by oral streptococci, the result being the development of a glue-like biofilm to which other micro-organisms attach themselves. In all, 1 mg of dental plaque may contain 10^{11} bacteria, and close to 1000 bacterial species have been identified in the oral microbiota [8].

There are different kinds of surface in the mouth where biofilms may form, including keratinized and non-keratinized epithelium, teeth with their hard tissue (dental enamel, dentin, and root cement), and often the artificial materials used for dental restorations or prostheses. Furthermore, saliva flushes all these surfaces, thus modifying the composition of microbiota. Saliva contains both specific and unspecific defense mechanisms and is thus important for health. Hence patients with reduced saliva flow are at increased risk not only for dental diseases but also for systemic illnesses. Patients with oral cancer subjected to surgical and other forms of treatment may have a greatly mutilated oral cavity. Irradiated patients may have practically no saliva left to flush the mucosa from debris and clearing microbiota. Consequently, cancer patients are rendered highly liable for additional disorders of the mouth, including cancer relapse. This may be partly due to uncontrolled microbial growth, as we discuss later in this article.

Alcohol and oral microbiota

Ethanol can be detected in the mouth for hours after the consumption of alcoholic beverages. Ethanol concentrations in saliva and blood decrease at the same rate due to the even distribution of absorbed alcohol in body fluids. Hence, salivary ethanol concentration remains the same as in blood [9]. However, it is not known how the intake of alcohol modifies oral microbiota. It may be anticipated that frequent consumption of alcoholic beverages modifies the composition of oral biofilms and causes selection pressure towards alcohol-tolerating species. This in turn may partly explain why heavy alcohol use often links with poor oral hygiene, in addition to the clinical observation that alcoholic patients frequently seem to have neglected cleaning their teeth [3]. Consequently, heavy alcohol consumption may lead to oral microbiota, where species capable of metabolizing alcohol prevail.

Alcohol-related carcinogenesis

Ethanol is not carcinogenic, while acetaldehyde is a known carcinogen; its carcinogenicity indisputably shown in experiments on animals [10,11]. Acetaldehyde causes mutations by forming adducts with DNA [12,13]. As discussed earlier, the link between heavy alcohol consumption and increased risk of cancer is explained by the fact that ethanol is being metabolized to acetaldehyde. The scientific evidence for this is convincing, and the effect is synergistic between use of alcohol and smoking. Tobacco smoke also contains acetaldehyde. For example, in a study on smokers, Salaspuro & Salaspuro [14] observed a 7-fold higher acetaldehyde concentration in saliva after ethanol challenge than in non-smokers. It is well known that alcohol consumption and smoking often go hand-in-hand. Consumption of strong alcoholic drinks further increases the risk for cancer, particularly esophageal cancer. Compared to wine, beer, and cider, strong spirits have been found to contain significantly higher concentrations of acetaldehyde (and obviously ethanol) – a fact that further emphasizes the causality between heavy drinking and cancer [15,16].

ADH and ALDH2 enzymes are controlled by multiple genes [17], genes that exist in several variants, and the enzymes encoded by certain variants may result in increased acetaldehyde concentrations. This partly explains the individual differences in carcinogenesis. ALDH2 isoenzyme is deficient in 30%–50% of the Asian populations, and leads to flushing due to the toxic effect of non-metabolized acetaldehyde if the person in question drinks alcoholic beverages. The risk for digestive tract cancer is markedly increased among ALDH2-deficient individuals. In a study from Japan, inactive ALDH2 allele was found to be the strongest contributing factor in the development of multiple oncogenic alterations in the esophagus, with an odds ratio of 17.6 (95% CI 4.7–65.3) [18]. There is therefore strong evidence for a gene-environmental interaction between the ALDH2 allele and alcohol consumption, and for the risk of cancer developing.

Ultimately, increased local acetaldehyde exposure appears to be critical [18]. Furthermore, in ALDH2-deficient individuals, acetaldehyde in saliva has been shown to originate partly from the parotid glands, where oxidation of ethanol can take place, in addition to the acetaldehyde production by oral microbiota [19].

ADH converts ethanol to acetaldehyde and its allele ADH3*1 is associated with increased conversion of ethanol to acetaldehyde. In a population-based case-control study from the State of Washington investigating the distribution of ADH genotypes and their relationship with oral squamous cell cancer, ADH3*2 allele emerged as the highest risk profile. Among ADH3*2 homozygotes, the risk of oral cancer increased 5.3% with each additional alcoholic drink per week, compared with 2.5% and 1.2% among persons carrying the ADH3*1/*2 and ADH3*1/*1 genotypes, respectively. The authors concluded that the ADH3*2 allele confers increased susceptibility on the effect of alcohol on cancer risk in their study population [20]. Consequently, there is apparent genetic susceptibility for sensitivity to the effect of acetaldehyde in carcinogenesis.

Oral health and alcohol-related carcinogenesis

Poor oral hygiene associates with the increase in the number of alcohol-metabolizing micro-organisms with a subsequent increase in acetaldehyde production after the consumption of alcoholic drinks. Homann et al. [31] investigated the composition and quantities of oral microbiota with regard to microbial acetaldehyde production in over 300 subjects whose alcohol consumption and use of tobacco had been recorded. Acetaldehyde production in saliva was found to correlate significantly with smoking and drinking (Figure 2). Gram-positive aerobic bacteria (streptococci, *Corynebacterium*, *Stomatococcus*) and yeasts were found to associate with higher acetaldehyde production in the subjects.

Homann et al. [3] then further investigated the association between dental status and acetaldehyde production from ethanol in saliva in 132 subjects,

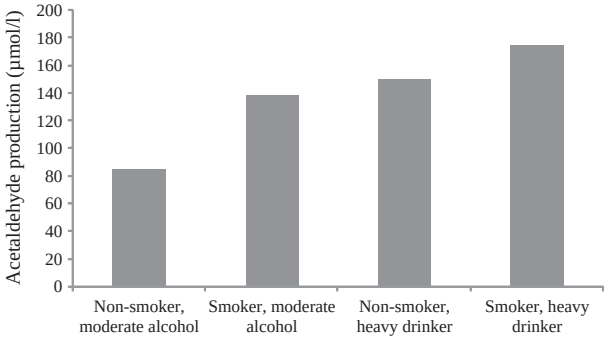

Figure 2. Production of salivary acetaldehyde is directly linked with the amount of alcohol use and smoking. Modified from Homann et al. [31].

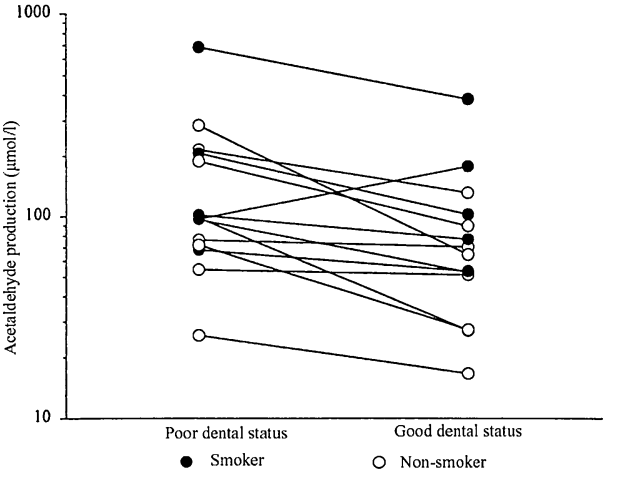

Figure 3. Salivary acetaldehyde production increases when the level of oral hygiene decreases to an extent that poor oral hygiene means an increased cancer risk due to acetaldehyde. Modified from Homann et al. [3].

adjusting the results for smoking, alcohol consumption, and age and gender. Dental status was scored "poor" if the recorded DS index was higher than 4 and DMF index at least 30. As shown in Figure 3, a significant difference was observed in acetaldehyde production between subjects with the "poor" and "good" dental status scoring.

More abundant microbial colonies have been observed on the epithelium of malignant tissue than at healthy sites of mucosa [21]. This may explain some of the disease relapses often seen in patients treated for oral cancer. As stated, ADH has been detected in several species of oral microbiota, including the bacteria *Streptococcus viridans* and *Neisseria*. In our laboratory, these bacteria were shown to readily produce acetaldehyde after incubation with ethanol (Figure 4) [22].

Oral candidiasis is also prevalent among patients with oral cancer. Following radiotherapy and/or cytostatic drug treatment, strains of *non-albicans Candida* increase in number and may be more pathogenic than the normally prevalent *C. albicans* [23]. Several *Candida* strains have also been shown to possess ADH and they thus metabolize alcohol

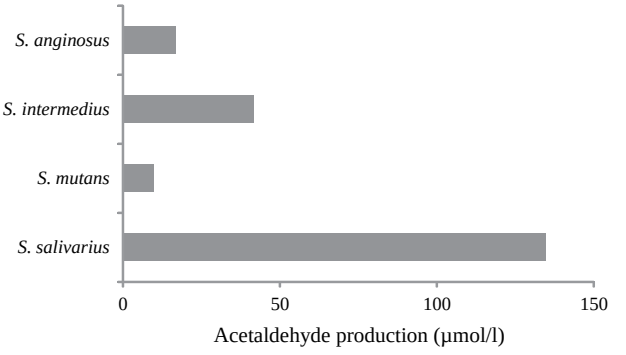

Figure 4. Examples of the *in vitro* acetaldehyde production from ethanol by some species of oral streptococci. Modified from Kurkivuori et al. [22].

into acetaldehyde [24]. It is anticipated, however, that of the hundreds of microbial species in the mouth, many more individual species than so far investigated may possess ADH. Oral microbiota reflecting the health status of the mouth could thus add *in toto* to the net acetaldehyde production when alcohol-containing drinks are consumed [25].

In this context, we stress that oral microbiota might also contain microbial strains that protect the individual from acetaldehyde-mediated carcinogenesis. Nosova et al. [26] have shown *in vitro* that aerobic bacteria of the human colon may also remove acetaldehyde and thus regulate the intracolonic acetaldehyde levels. These researchers observed that microbial ADHs were inhibited by 4-methylpyrazole by the same competitive inhibition as hepatic ADH, but with nearly 1000 times lower susceptibility. Hence, individual variations in human colonic flora may contribute to the risk of alcohol-related gastrointestinal morbidity [27]. The same is assumed in the oral microbiota and with respect to oral diseases, but there are no studies in this area.

Lactobacilli and bifidobacteria strains have revealed a limited capacity to oxidize ethanol to acetaldehyde *in vitro*. In particular, the probiotic *Lactobacillus* GG ATCC 53103 has shown the highest acetaldehyde-metabolizing capacity, which increases significantly with increasing bacterial concentrations [28]. This finding indicates possible clinical implications for controlling microbial acetaldehyde production even in the oral cavity, since probiotic lactobacilli have also been found effective in the mouth, albeit these bacteria have not been investigated in the present perspective [29]. However, taken together, the associations between oral microbiota, ethanol metabolism, and carcinogenesis call for further investigation – particularly more clinical studies – before any final conclusion can be drawn. Nevertheless, the evidence is conclusive that local acetaldehyde production takes place in the mouth. This may add indeed to carcinogenesis.

Inhibiting or eliminating microbial acetaldehyde production

Similar to what has been observed with colonic bacteria, 4-methylpyrazole (which is an ADH inhibitor) could reduce the local production of acetaldehyde from ethanol in saliva [30]. Antiseptic and anti-plaque agents, such as chlorhexidine, which affect oral microbiota, also reduce the number of acetaldehyde-producing bacteria. Such chemicals thus lower acetaldehyde concentration in saliva (Figure 5) [31]. However, there are no clinical studies on this topic and obviously long-term use of chlorhexidine-like substances is not warranted owing to potential side effects [32].

The mechanisms behind the interspecies inhibition discussed above need to be investigated in the context of probiotics, and there are no *in vivo* or clinical studies published on this topic. An enhancing mode of treatment would be to selectively adopt the interspecies microbial inhibition process; this would be an interesting area of research. There may also be future potential in the development of new means for controlling oral microbiota with respect to acetaldehyde production once the mechanisms of probiotic and other interspecies inhibitory actions become clear.

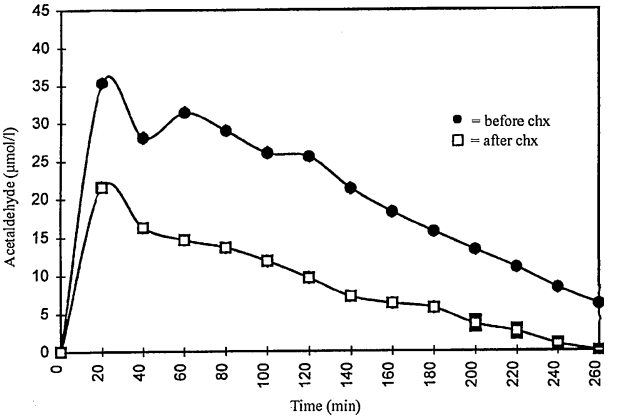

Figure 5. Chlorhexidine rinses can be used to reduce salivary acetaldehyde production in test subjects. The effect is mediated by chlorhexidine-caused inhibition of oral microbiota. Modified from Homann et al. [31].

However, it is also possible to eliminate acetaldehyde locally. The amino acid l-cysteine binds covalently to acetaldehyde, resulting in an inactive sulphur compound [33]. Salaspuro et al. [34] gave l-cysteine or placebo-containing slow-release tablets to nine volunteers in a study during which the subjects were given ethanol to drink. Salivary acetaldehyde concentrations were reduced by 59% in the cysteine series in comparison to placebo. A similar result was observed in a study on salivary acetaldehyde and smoking [35]. This approach may thus offer new perspectives in the prevention of oral cancer, too.

Conclusions

Research data indicate that the observed association between poor oral hygiene and oral cancer may be a more severe threat to the patient than has been understood hitherto. The capability of several oral microbial species to produce carcinogenic acetaldehyde from ethanol is alarming. It is of particular concern that the highly prevalent viridans streptococci are efficient acetaldehyde producers. The direct practical implication is in emphasizing the need for proper oral hygiene maintenance daily in patients who use alcohol. Smoking adds to the risk.

Future investigation will show whether giving patients preparations containing cysteine, which eliminates local acetaldehyde in the mouth, or probiotics, which inhibit acetaldehyde producing

micro-organisms, affects the incidence of oral cancer. It would also be interesting to study whether these approaches might help control cancer relapses. However, the data are still sparse and it is thus too early to give any straightforward recommendations, but it seems prudent to conclude that poor oral hygiene increases the risk for carcinogenesis among heavy alcohol users via the pathogenic mechanism discussed here.

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