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Local Acetaldehyde: Its Key Role in Alcohol-Related Oropharyngeal Cancer

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Keywords

Acetaldehyde · Alcohol · ALDH2 · Cancer · Oropharynx

Abstract

Background: Alcohol consumption and ethanol in alcoholic beverages are group 1 carcinogens, that is, carcinogenic to humans. However, ethanol itself is neither genotoxic nor mutagenic. Based on unique gene-epidemiologic and gene-biochemical evidence, the first metabolite of ethanol oxidation – acetaldehyde (ACH) – acts as a local carcinogen in the oropharynx. This review is focused on those facts, which highlight the importance of the oropharynx and local ACH in the pathogenesis of alcohol-related oropharyngeal cancer.

Summary: The strongest evidence for the local carcinogenicity of ACH in man provides a point mutation in the aldehyde dehydrogenase 2 (*ALDH2*) gene, which has randomized millions of alcohol consumers to markedly increased ACH exposure via saliva. This novel human cancer model is associated with manifold risk for oropharyngeal cancer and most importantly it is free from confounding factors markedly hampering epidemiological studies on alcohol-related cancer. The oropharynx is an ideal target organ for the cancer risk assessment of ACH. There is substantial epidemiological data on alcohol-related oropharyngeal cancer risk and also on salivary ACH concentrations among major risk groups for oropharyngeal cancer. Normal human saliva does

not contain measurable levels of ACH. However, alcohol ingestion results within seconds in a concentration-dependent accumulation of ACH in saliva, which continues for up to 10–15 min after each sip of alcoholic beverage. This instant ACH exposure phase is followed by a long-term phase derived from ethanol diffused back to saliva from blood circulation. Microbes representing normal oral flora play a major role in local ACH formation from ethanol. In *ALDH2*-deficient subjects excess ACH during the long-term ACH exposure phase is most probably derived from salivary glands.

Key Message: *ALDH2* gene mutation proves the causal relationship between local ACH exposure via saliva and oropharyngeal cancer and provides new means for the quantitative assessment of local ACH exposure in relation to oropharyngeal cancer risk. Instant ACH formation from ethanol represents approximately 70–100% of total local ACH exposure. Ethanol present in “non-alcoholic” beverages and food forms an epidemiological bias in studies on alcohol-related upper digestive tract cancer. **Responses:** One should quit smoking, adopt sensible drinking habits, and maintain good oral hygiene. Genetic risk groups could be screened and educated. Consumption of beverages and foodstuffs containing low ethanol levels as well as alcoholic beverages containing high ACH levels should be minimized. To that aim, labeling of alcohol and ACH concentrations of all beverages and foodstuffs should be mandatory.

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Introduction

Strong genetic-epidemiologic and genetic-biochemical evidence supports the crucial role of locally formed acetaldehyde (ACH) from ethanol as a major pathogenic factor behind alcohol-related upper digestive tract cancer. Although the International Agency for Research on Cancer (IARC) classifies alcohol consumption and ethanol in alcoholic beverages as group 1 carcinogens to humans, an ethanol molecule in itself is neither genotoxic, mutagenic, nor carcinogenic. However, its first metabolite ACH is a group 1 carcinogen to humans when associated with the consumption of alcoholic beverages [1]. Group 1 classification especially concerns the upper digestive tract, where the causal relationship between alcoholic beverage consumption and cancer of the oral cavity, pharynx, and esophagus has been established.

A major part of ingested alcohol is metabolized in the liver via ACH to acetate, which is further oxidized in the peripheral tissues to carbon dioxide and water. It is less well recognized that hepatic cytoplasmic and mitochondrial aldehyde dehydrogenase (ALDH) enzymes break down ACH so efficiently that after a dose of alcohol neither peripheral nor hepatic venous blood contain measurable levels of ACH [2]. On the other hand, after a dose of alcohol, mutagenic ACH concentrations are found in the saliva and gastric juice of humans and in colonic contents of experimental animals. This locally formed ACH is by and large produced by microbes representing normal gastrointestinal flora [3, 4].

The local carcinogenic potential of ACH is supported by epidemiological findings indicating that alcohol-related cancer risk is increased mainly in those organs in which local ACH levels are elevated after alcohol administration. These organs include the oral cavity, pharynx, larynx, esophagus, stomach, large intestine, and cirrhotic liver. On the contrary, alcohol-related cancer risk is not increased in organs, which do not express elevated ACH levels in the presence of ethanol. Such organs are for instance the prostate, pancreas, lung, brain, kidney, bone, endocrine organs, and blood cells. The only exception is breast cancer, the incidence of which is dose-dependently associated with increased alcohol consumption. However, there is no evidence of locally elevated ACH levels in mammary glands in the presence of ethanol. It is generally agreed that the pathogenesis of alcohol-related breast cancer is still hypothetical.

Unique Characteristics of ALDH2 Gene Mutation

Finding a specific carcinogenic agent is a key factor in cancer prevention. To that aim a single point mutation in the *ALDH2* gene proves conclusively the causal role of lo-

cal ACH, especially in alcohol-related upper digestive tract and particularly in oropharyngeal cancer [3, 5]. Mutation results in deficient activity of the main ACH metabolizing low K_M mitochondrial ALDH2 enzyme. As a result, ALDH2-deficients are exposed via saliva to about 2 and via gastric juice to 5–6 times higher local ACH concentrations than ALDH2-actives each time they drink alcohol [6, 7]. The enhanced ACH exposure in alcohol drinkers is associated with markedly and dose-dependently increased risk for oral, pharyngeal, esophageal, and gastric cancer [6, 8, 9].

The incidence of *ALDH2* gene mutation (1:13) is more frequent than that of familial hypercholesterolemia (1:500). Today its carrier frequency is about 600 million people of East-Asian descent. Because this human ACH exposure model is randomized by nature, it lacks the bias caused by confounding factors hampering most epidemiological studies on alcohol-related cancer. Smoking, diet, under reporting, drinking habits, use of different types of alcoholic beverages, oral hygiene, HPV, and BMI can be assumed to be evenly distributed among ALDH2-deficient and ALDH2-active alcohol drinkers. Any other group 1 carcinogen ($n = 120$) has equally strong gene-epidemiologic and gene-biochemical evidence for its carcinogenicity in humans. With regard to ACH, data based on animal toxicology is problematic due to poor study quality, problems in animal models, and relevance of the endpoint (cancer linkage) in translation to humans [6]. Consequently, animal and DNA-based carcinogenicity data on ACH can be considered to be of secondary importance as compared to the specific human data provided by the single point mutation in the *ALDH2* gene.

Oropharynx and Salivary ACH

Oropharynx provides an ideal target organ for the quantitative estimation of local ACH exposure via saliva in humans for 3 reasons: (1) oropharyngeal mucosa lacks low K_M ALDH-enzymes and is therefore unable to eliminate from either ethanol-formed or free ACH present in many alcoholic beverages [10]; (2) oral microbes have a very low or no ALDH activity, and therefore their capacity to eliminate local ACH is minimal [11]; (3) there is adequate research data on salivary ACH levels in the presence of ethanol and also on alcohol-related oropharyngeal cancer risk in ALDH2-active and ALDH2-deficient alcohol drinkers [6].

Instant and Long-Term Local ACH Exposure via Saliva

Oropharyngeal mucosa is exposed to ACH via saliva in 2 phases (Fig. 1). The most prominent local exposure takes place immediately after sipping alcohol [3, 12, 13]. Ethanol is distributed to oral mucosal surfaces and saliva

Fig. 1. Instant and long-term salivary ACH formation from ethanol after a dose of alcohol. Gray columns, microbial ACH formation; black column, excess ACH in ALDH2-deficients compared to that of ALDH2-actives.

Table 1. Approximation of instant local ACH exposure via saliva after one dose of 40% alcohol ingested in 3 sips at 5-min intervals in relation to the risk for oropharyngeal cancer

Exposure model to local ACH	Salivary ACH, mg/L	Exposure time, min	AUC, mg/L × min/day	OR for oropharyngeal cancer [17, 18]
Instant				
3 × first 5 min	6.2	15	93	
1 × next 5 min	4.4	5	22	
Instant total, <i>n</i> (%)			115 (68)	
Long term				
20–80 min	0.88	80	53 (32)	
Total, <i>n</i> (%)			168 (100)	1.17–1.21

Adapted from Nieminen and Salaspuro [3] and Linderborg et al. [12].

instantly after each sip of an alcoholic beverage. Five milliliters of 40% ethanol results in peak salivary ethanol levels of 900 mM (4.3%) within 30 s and, stay elevated >21 mM (1‰) for at least 10 min [12, 13]. This is associated with instant and mainly microbial ACH formation from ethanol in saliva in mutagenic concentrations (mean 150 μM, peak 260 μM), which lasts up to 15–20 min [12, 13]. The *ALDH2* genotype has no effect on the instant ACH exposure via saliva, indicating that neither mucosal nor salivary gland *ALDH2* enzymes play any significant role in this metabolic process [13].

Long-term exposure represents ACH formed from ethanol that is diffused back to saliva from blood after the distribution phase of alcohol to the water phase of the human body, that is, within about 30 min after the last sip of alcohol (Fig. 1). Depending on the total amount of alcohol ingested, the long-term phase lasts for hours, that is, for as long as alcohol stays in the blood circulation and saliva [14]. In *ALDH2*-actives, the mean salivary ACH

concentration during the long-term phase is about 25 μM, but depending on the individual characteristics of oral microflora the peak levels may go up to over 100 μM [14]. In contrast to instant ACH exposure, the mean salivary ACH during the long-term phase is about double (approx. 53 μM) in *ALDH2*-deficients compared to *ALDH2*-actives (Fig. 1) [6, 15]. The additional ACH during the long-term phase of ACH exposure is presumably derived to the oral cavity from the salivary glands [15, 16].

Estimation of Local ACH Exposure via Saliva after One Dose of Alcohol

Monitoring salivary ACH levels after drinking alcohol provides unique means for the quantitative approximation of ACH exposure via saliva in the oropharynx. One dose of alcohol (10 g of ethanol) daily is associated with a significant risk (RR 1.17–1.21; approx. 20% increase) for oropharyngeal cancer [17, 18]. After one dose of alcohol the total local ACH exposure via saliva can be calculated

Table 2. Approximation of the excess long-term ACH exposure via saliva of ALDH2-deficient moderate and heavy drinkers as compared to that of ALDH2-actives and in relation to the risk for oropharyngeal cancer

Exposure model	Excess salivary ACH, mg/L ¹	Exposure time, min ²	AUC, mg/L × min/day	OR for oropharyngeal cancer [8, 20]
ALDH2-deficiency model				
Moderate drinkers (33 g/ethanol/day)	1.1	283	311	1.7–2.6
Heavy drinkers (77 g/ethanol/day)	1.1	660	726	3.6–7.3

Adapted from Nieminen and Salaspuro [3] and Lachenmeier and Salaspuro [6].

¹ Long-term excess ACH exposure via saliva (salivary ACH [mg/L] in ALDH2-deficients minus that in ALDH2-actives).

² Exposure time to local ACH via saliva is based on the elimination rate of ethanol (7 g/h).

Fig. 2. Effect of alcohol on the approximated local ACH exposure (mg/L × min/day) in relation to increased oropharyngeal cancer risk (OR/RR). ACH¹ (white circle), mean instant ACH exposure via saliva after one dose of 40% alcohol (Table 1); excess ACH² (gray and black circles); mean long-term exposure to excess salivary ACH in ALDH2-deficients compared to that of ALDH2-actives (Table 2).

by approximating the area under the curve during the instant and long-term phase of ACH exposure (Table 1). Without the presence of alcohol or tobacco smoke, salivary ACH levels are under the detection limit [14, 19]. If one dose of strong alcohol is assumed to be ingested in 3 sips at 5-min intervals, the mean instant ACH exposure represents about 70% of the total ACH exposure (Table 1). High concentrations of free ACH present in some alcoholic beverages results in a short, 1- to 2-min peak (up to approx. 1 mM) in the salivary ACH concentration, which can be calculated to represent a maximum of 10% of the total local ACH exposure [3, 12].

Estimation of Excess Local ACH Exposure via Saliva in ALDH2-Deficients Compared to ALDH2-Actives

According to one meta-analysis and one well-performed study, ALDH2 deficiency is associated with a 1.68- to 2.61-fold oropharyngeal cancer risk in moderate drinkers and 3.57- to 7.28-fold risk in heavy drinkers [8, 20]. Based on 5 studies with consistent findings, the long-term salivary ACH concentration has a mean

increase of 2.1-fold in ALDH2-deficient subjects compared with ALDH2-actives for as long as alcohol stays in the human body after its intake [6]. On the basis of the normal alcohol elimination rate (7 g/h), the long-term exposure time to an elevated ACH level in saliva is 283 min in moderate and 660 min in heavy drinkers. By using these numbers, the total excess ACH exposure of the oropharyngeal mucosa of ALDH2-deficients compared to that of ALDH2-actives can be approximated as described in Table 2. In summary, oropharyngeal cancer risk derived from epidemiological studies appears to correlate dose dependently with the local ACH exposure caused by one dose of alcohol per day and also with the excess local ACH exposure due to the deficient ALDH2 enzyme (Fig. 2). This gene-epidemiologic and gene-biochemical evidence proves conclusively the causal role of local ACH in the pathogenesis of oropharyngeal cancer. Furthermore, the model provides a unique way for the quantitative estimation of local ACH exposure in the oropharynx in relation to alcohol-related cancer risk in alcohol drinkers.

Table 3. Major factors affecting salivary ACH concentrations

Factor	Effect	Implications
Individual characteristics of oral microbes	30-fold range in the ability of oral bacteria to produce ACH from ethanol in vitro	High variety in salivary ACH levels after alcohol ingestion
High variation in K_M values of microbial ADHs	Number of microbes producing ACH from ethanol increases in rising salivary ethanol concentrations	High salivary ACH levels (up to 260 μM) during the instant phase of ACH exposure
Alcohol concentration	Higher salivary ethanol concentration associates with higher ACH formation both in vitro and in vivo	High salivary ACH concentrations especially during the instant phase of ACH exposure
Smoking	ACH of tobacco smoke dissolves into saliva during active smoking	Results in about 7-fold increase in total ACH exposure via saliva and explains, together with microbial changes, the synergistic effect of smoking and drinking on oropharyngeal cancer risk
Smoking and heavy drinking	Both increase microbial ACH production in saliva by 50%; when combined the increase is 100%	
Poor oral hygiene	Increases in vitro and in vivo salivary ACH production from ethanol	Poor oral hygiene is a risk factor for oropharyngeal cancer
High ACH concentration in alcoholic beverages	Results in a short 30-s – 2-min peak in salivary ACH	Depending on ACH of beverage and number of sips may represent 0–10% of total ACH exposure
ALDH2 deficiency	ALDH2 deficiency results in 2.1-fold local long-term ACH exposure after alcohol drinking as compared to that of ALDH2-actives	Provides a unique human model for local ACH exposure via saliva

Dominant Role of Oral Microbes in Salivary ACH Production from Ethanol

Convincing evidence supports the major role of oral microbes in the formation of salivary ACH from ethanol [3]. The oropharyngeal microbiome consists of over 700 bacterial species and a rich fungal flora. Many microbes representing normal oral flora are high ACH producers. These include, for instance, many *Streptococcus* and *Neisseria* genuses, some *Candida albicans* and some non-*C. albicans* species. Characteristically, oral microbes represent a variety of alcohol dehydrogenase (ADH) enzymes, which under aerobic conditions are able to produce marked amounts of ACH from ethanol both in vitro and in vivo. On the contrary, oral microbes show no or very low ALDH activity [11]. These microbial characteristics result in the linear accumulation of ACH in mutagenic concentrations in saliva in the presence of increasing levels of ethanol. The key role of normal oral microflora in salivary ACH production from ethanol is supported by the fact that ACH formation is reduced by half after a 3-day use of chlorhexidine containing antiseptic mouthwash [14]. This associates with a significant decrease in baseline aerobic and anaerobic bacterial counts of saliva.

4-methylpyrazole (4-MP) is a potent inhibitor of human ADH enzyme. However, much higher 4-MP concentrations are required for the inhibition of microbial

ADH enzymes in saliva [14]. A single dose of 4-MP before ethanol ingestion reduces the ethanol elimination rate, the flushing reaction, and both blood and salivary ACH levels in ALDH2-deficient subjects, but not in subjects with the normal ALDH2 genotype [16]. These results suggest that the role of oral mucosal and glandular ADHs in salivary ACH production from ethanol is minimal in subjects with normally active ALDH2 enzyme and that among them salivary ACH production is mainly of microbial origin. On the other hand, the findings support the conclusion that the excess long-term ACH exposure in ALDH2-deficient alcohol drinkers is derived from salivary glands [15, 16].

Major Factors Affecting Salivary ACH Levels

Individual characteristics of thousands of different microbial strains play a key role in the regulation of salivary ACH concentrations in the presence of ethanol (Table 3). Based on 100 saliva samples of healthy volunteers, a 30-fold range in the ability of oral microbes to produce ACH from ethanol has been demonstrated [21]. This explains at least in part the high individual variation in salivary ACH concentrations after alcohol ingestion. Particularly high salivary ACH levels are found immediately after sipping alcohol, that is, during the instant phase of local ACH exposure, when salivary ethanol levels are highest (Fig. 1). This can

also be explained to be at least in part secondary to the high variation in K_M values of different microbial ADH enzymes. Consequently, the number of microbes producing ACH from ethanol increases in rising salivary ethanol concentrations. A higher salivary ethanol concentration associated with higher ACH formation in saliva has been demonstrated both in vitro and in vivo [7, 12–14].

Tobacco and alcohol have a synergistic effect on the risk for oropharyngeal cancer [22]. ACH of tobacco smoke dissolves into saliva during active smoking, resulting in a mean salivary ACH concentration of 260 μM [19]. Furthermore, chronic smoking and heavy drinking modify the oral microflora to produce higher ACH levels from ethanol in vivo and in vitro [19, 23]. Consequently, smokers with active smoking during ethanol challenge are exposed to 7 times higher ACH levels via saliva than non-smokers [19]. Poor oral hygiene is an independent risk factor for oropharyngeal cancer. On the other hand, it significantly increases in vitro salivary ACH production from ethanol [24]. Some strong fruit-based alcoholic beverages, such as calvados and grappa, may contain very high ACH concentrations. However, due to the fast elimination of ACH immediately after sipping this type of beverage, the additional free ACH of the drink contributes only marginally (max. 10%) to the total ACH exposure via saliva [3, 12]. ALDH2 deficiency results in a 2.1-fold local long-term ACH exposure after drinking alcohol as compared to that of ALDH2-actives. Therefore, the single point mutation in the *ALDH2* gene provides a unique human model for local ACH exposure via saliva.

Low Alcohol Levels in Food and Beverages: Significant Epidemiological Bias

Microbial fermentation has been used for thousands of years for food preservation. Although lactic acid is the most common end product of food fermentation processes, many microbes are able to also produce ethanol and ACH under prevailing anaerobic conditions. Ethanol concentration levels ranging from 0.5‰ to 2.5% or even higher have been measured. These products include, for instance, homemade mead and beer, kefir, mursik milk, soy sauce, kimchi, pickled food, and vinegar [25–28]. Alcohol is often also added to marinades, fondues, salads, and sushi, but the ethanol concentration of the end product served is not known. Furthermore, it is poorly recognized that alcohol is not totally evaporated during cooking. Depending on the cooking procedure, alcohol concentrations ranging from 0.06 to 4.21% can still be measured after boiling [29].

The evidence on the carcinogenicity of alcohol and ACH in the oropharynx has so far been based on epidemiological studies in which alcohol consumption has been assessed by various types of questionnaires including only

official alcoholic beverages (wine, beer, spirits) containing 3–50% ethanol. However, there is no systematic information on the ethanol and ACH concentrations of various widely used food products produced or preserved by fermentation. The same also concerns beverages containing lower concentrations of ethanol than official alcoholic drinks. Neither is it known how much and how often these types of foodstuffs or beverages have been used. This so far unknown part of local exposure of the oropharynx and other parts of the upper digestive tract to ACH derived from microbial oxidation of ethanol forms an obvious bias in alcohol-related upper GI tract cancer epidemiology.

Conclusions

Normal human saliva does not contain measurable levels of ACH. However, alcohol ingestion results within seconds in a concentration-dependent accumulation of ACH in saliva, which continues up to 10–15 min after each sip of alcoholic beverage. This instant ACH exposure phase is followed by a long-term phase derived from alcohol diffused back to saliva from blood circulation. Microbes representing normal oral flora play a major role in salivary ACH formation from ethanol. The carcinogenicity of local ACH is based on a unique gene-epidemiologic and gene-biochemical human model concerning millions of East-Asian alcohol drinkers. A single point mutation in the *ALDH2* gene results in the doubling of local ACH exposure via saliva during the long-term phase of ACH exposure after drinking alcohol, which associates with dose-dependently increasing oropharyngeal cancer risk. This provides conclusive evidence for the causal relationship between local ACH exposure and oropharyngeal cancer. It should be noted that the instant microbial ethanol metabolism results in mutagenic ACH concentrations in saliva also in lower ethanol concentrations ranging from 0.5‰ to 2.5%, i.e., in those levels that are commonly measured in many food products and beverages produced by fermentation. Therefore, ethanol present in many “low-alcoholic beverages” and foods forms an unrecognized bias in upper digestive tract cancer epidemiology.

Statement of Ethics

No ethical considerations are relevant to this review article.

Disclosure Statement

The author has no conflicts of interest to declare.

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