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Key role of local acetaldehyde in upper GI tract carcinogenesis

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ABSTRACT

Ethanol is neither genotoxic nor mutagenic. Its first metabolite acetaldehyde, however, is a powerful local carcinogen. Point mutation in *ALDH2* gene proves the causal relationship between acetaldehyde and upper digestive tract cancer in humans. Salivary acetaldehyde concentration and exposure time are the two major and quantifiable factors regulating the degree of local acetaldehyde exposure in the ideal target organ, oropharynx. Instant microbial acetaldehyde formation from alcohol represents >70% of total ethanol associated acetaldehyde exposure in the mouth. In the oropharynx and achlorhydric stomach acetaldehyde is not metabolized to safe products, instead in the presence of alcohol it accumulates in saliva and gastric juice in mutagenic concentrations. A common denominator in alcohol, tobacco and food associated upper digestive tract carcinogenesis is acetaldehyde. Epidemiological studies on upper GI tract cancer are biased, since they miss information on acetaldehyde exposure derived from alcohol and acetaldehyde present in 'non-alcoholic' beverages and food.

The worldwide annual incidence of upper GI tract cancers is close to 2 million and age standardized rate represents one fourth of all cancers [1]. The poor prognosis of these cancers underlines the importance of preventive measures. A key to cancer prevention is the identification of specific carcinogenic compounds, recognition of risk groups and early detection of precancerous conditions. Alcohol and tobacco are major risk factors for oral, pharyngeal and oesophageal cancer with a synergistic effect on their incidence [2,3]. They are also independent risk factors for stomach cancer [4,5]. A common carcinogenic denominator in alcohol, tobacco and food associated upper GI tract cancer is acetaldehyde, which in the presence of ethanol accumulates in the saliva and gastric juice. Acetaldehyde is also the most abundant carcinogen of tobacco smoke, which dissolves into saliva during smoking [6,7].

The ethanol molecule is neither genotoxic nor mutagenic [8,9]. Acetaldehyde is a group 1 carcinogen to humans, when associated with the consumption of alcoholic beverages [10]. Group 1 classification concerns acetaldehyde formed from ethanol by microbial and mucosal oxidation and when present in alcoholic beverages.

Acetaldehyde formation from ethanol starts instantly in saliva after the sipping of alcohol and continues for as long as ethanol is present in blood [11,12]. Solid genetic epidemiologic and biochemical evidence based on a point mutation in the *ALDH2* gene proves the causal relationship between local acetaldehyde exposure and upper GI tract cancer and provides a unique human model for the quantitative assessment of the carcinogenicity of acetaldehyde in man [13].

Exposure of upper GI tract mucosa to carcinogenic acetaldehyde is cumulative and can be markedly reduced both at population and individual level. Knowing the key factors regulating local acetaldehyde concentration in the upper digestive tract is thus of essential importance for health care workers, regulatory authorities, food and tobacco industry and not least consumers.

1. Acetaldehyde

1.1. Main characteristics

Acetaldehyde is a small molecule with orange aroma and low

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boiling point. It is soluble in water and lipids and passes therefore easily through the cell membranes. Acetaldehyde is widely present in our everyday environment. Its characteristics are described in Table 1. With regards to upper GI tract cancer, most important is the local *in vivo* oxidation of ethanol to acetaldehyde in reactions catalyzed by microbial alcohol dehydrogenase (ADH) enzymes [11,12]. This results in salivary and gastric juice acetaldehyde concentrations, which may exceed markedly its mutagenic level (40–100 μM, 1.8–4.4 mg/l) and recommended upper limit (5 mg/l) for cosmetic products [14,15]. Acetaldehyde is also a key metabolite in the alcoholic fermentation pathway from glucose to ethanol. Therefore, acetaldehyde is present in mutagenic concentrations in many alcoholic beverages and foodstuffs produced or preserved by fermentation (Table 2) [16–18]. Potentially risky acetaldehyde levels may exist also in some fruits and the substance may be added to certain foods as a flavouring compound [18].

1.2. Genotoxicity, mutagenicity and carcinogenicity

Acetaldehyde is genotoxic, mutagenic and carcinogenic *in vitro* and *in vivo* [9,10]. It causes DNA-protein crosslinks, DNA strand breaks, DNA adducts, sister chromatid exchanges, chromosomal aberrations, and micronuclei in eukaryotic cells. Although many of these effects have been produced in rather high acetaldehyde concentrations, there is strong evidence for the generation of specific mutagenic DNA adducts and induction of micronuclei in mammalian cells also at acetaldehyde concentrations realistically achievable from alcoholic beverage consumption [9,10,19].

Oral ingestion of alcohol produces dose dependent mutagenic acetaldehyde-DNA damage in the oral cavity of humans within 2–4 h (Fig. 1) [19]. In the alcohol sipping model used in that study salivary acetaldehyde can be assumed to have been about 150 μM (6.6 mg/l) for the first 40 min (Fig. 1) [11,20]. This profound instant effect of alcohol on salivary acetaldehyde is most obviously due to the dose dependent *in vitro* and *in vivo* capacity of oral microbes to produce acetaldehyde in increasing ethanol concentrations [11,20–22]. Within the following 30 min alcohol is distributed evenly to the body water including saliva resulting in rapid decrease in salivary acetaldehyde concentration to mean 20–30 μM for as long as alcohol stays in the blood (Fig. 1) [12]. These findings suggest that the *in vivo* mutagenic effect of acetaldehyde on human oral cells is exponential as indicated also by earlier *in vitro* findings on acetaldehyde-DNA adducts [23]. The formation of mutagenic acetaldehyde-DNA adducts in oral mucosa caused by local acetaldehyde formation from ethanol has been confirmed in rhesus monkeys exposed to alcohol over their lifetimes [24].

Table 1
Acetaldehyde (ethanal).

- CH3 - CHO, orange aroma, boiling point 20.2^OC, soluble in water and lipids
- Widely present in our everyday environment
  - Microbial formation from ethanol
  - Key metabolite in alcoholic fermentation
  - Most abundant carcinogenic compound of tobacco smoke
- Forms carcinogenic acetaldehyde-DNA adducts
- Genotoxic, mutagenic and carcinogenic even in physiologically relevant concentrations
- Common denominator for all known risk factors of upper GI tract cancer
- Causal relationship between acetaldehyde and upper GI tract cancer is based on a single mutation in *ALDH2* gene resulting in a profound effect on local acetaldehyde exposure after alcohol drinking

Table 2
Acetaldehyde concentrations (mg/l) in some widely used alcoholic beverages, foodstuffs and fruits [16–18]. Mutagenic concentration: 40–100 μM (1.8 – 4 mg/l).

Beverage/foodstuffs	n	Ranges (mg/l)
Beer (Germany)	364	0–1435
Beer (Italy)	12	3.6–15.1
Wine (Europe)	213	0–211
Wine (Italy)	60	18–477
Grappa (Italy)	13	23–1850
Calvados	27	9–67
Yogurt (Germany)[a]	23	2.4–17.4
Fruit juice[a]	4	0.8–19.1
Apples[a]	8	0.3–2.4
Bananas[a]	8	1.9–18.3

[a] If not containing any ethanol, the local exposure time of upper GI tract to acetaldehyde can be assumed to be considerably lower than that of ethanol containing beverages and food.

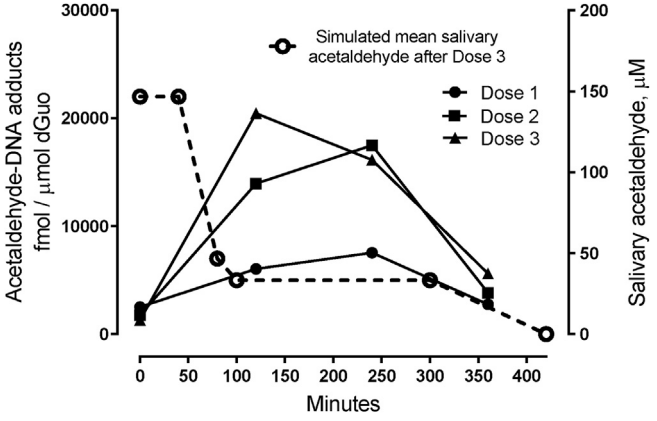

Fig. 1. Mutagenic acetaldehyde-DNA adducts in mouthwash samples of healthy human volunteers after ingestion of alcohol in relation to simulated salivary acetaldehyde levels. Adapted from Refs. [11,12,19,20]. Alcohol doses 1, 2 and 3 were aimed to reach 0.3, 0.5 and 0.7‰ blood alcohol levels, respectively. In alcohol sipping model used (Dose 3: diluted vodka at 5 min intervals), salivary acetaldehyde concentration can be assumed to have been about 150 μM for the first 40 min (max. 260 μM). Within the following 30 min there is a rapid decrease in salivary acetaldehyde to 20–30 μM for 350 min. Left y-axis: N^2-ethylidene-dGuo (fmol/μmol dGuo). Right y-axis: salivary acetaldehyde (μM).

In cultured human buccal epithelial cells, mutagenic acetaldehyde-DNA adducts are formed *in vitro* in a dose-dependent manner at acetaldehyde concentrations that are relatively nontoxic to the cells [25]. Furthermore, aldehyde dehydrogenase 2 (ALDH2)-deficient alcoholics have significantly higher blood levels of acetaldehyde-DNA adducts than ALDH2-actives [26,27]. Alcohol treated ALDH2-deficient mice show increased acetaldehyde-DNA adduct levels in the liver, stomach and oesophagus [28–30]. Another mutagenic acetaldehyde-DNA adduct (1,N^2-propanodeoxyguanosine) increases exponentially in 100–500 μM acetaldehyde concentrations in the presence of physiological polyamine concentrations [23]. Polyamine synthesis is tightly related to cellular proliferation, with the highest levels being found in rapidly dividing cells, which is characteristic for the regenerating oral and oesophageal mucosa [31].

According to the International Agency for Research on Cancer (IARC) acetaldehyde is carcinogenic in experimental animals [10]. Inhaled acetaldehyde produces nasal carcinomas in rats and laryngeal carcinomas in hamsters [32,33]. Life time administration of acetaldehyde in drinking-water to rats resulted in increased

number of pancreatic adenomas, lymphomas, leukaemias, uterine and mammary gland adenocarcinomas, and head osteosarcomas [34]. However, no obvious dose-response relationship was observed in this animal model.

2. ALDH2-deficiency - a unique human cancer model for acetaldehyde

2.1. Background

A single point mutation in *ALDH2* gene results in deficient activity of the main acetaldehyde metabolizing low Km mitochondrial enzyme (ALDH2). Consequently, ALDH2-deficient subjects are exposed in the presence of alcohol via saliva 2 to 3 times and via gastric juice 5 to 6 times higher acetaldehyde concentrations than those with the active ALDH2-enzyme [35–39]. Parallel to increased local acetaldehyde exposure, the risk of ALDH2-deficient alcohol drinkers for oral, pharyngeal, oesophageal and gastric cancer is many-fold compared to alcohol consuming individuals with the active ALDH2-enzyme [40–44].

The atypical ALDH2*487Lys allele is probably derived from ancient Pai-Yuei tribe, which was widely distributed along the southeast coast of China up to Yunnan Province and the northern part of Southeast Asia 2000–3000 years ago [45]. Today its carrier frequency is about 600 million people with Eastern Asian descents [45,46]. Thus, ALDH2-deficiency is the most prevalent genetic health risk in the world, passing in frequency that of familial hypercholesterolemia (FH). The prevalence of FH is 1:200–250, but that of ALDH2-deficiency 1:13 [45–47]. Comparable human cancer model based on a single gene mutation is not available for any other of the IARC group 1 human carcinogens.

2.2. Quantitative assessment of the carcinogenicity of acetaldehyde in human oropharynx

From one dose of alcohol (10 g ethanol) the human body is able to produce potentially 217 000 μmoles of acetaldehyde. However, hepatic aldehyde dehydrogenase enzymes oxidize acetaldehyde formed from ethanol so effectively that after a moderate dose of alcohol blood acetaldehyde levels are not detectable in individuals with normal ALDH2 enzyme, and in ALDH2-deficients they are only slightly elevated (≤10 μM) [35–37]. In sharp contrast to the liver, oral mucosa lacks low Km aldehyde dehydrogenase enzymes and is thus unable to eliminate acetaldehyde formed microbially from ethanol [48]. Therefore, alcohol drinking results in mutagenic salivary acetaldehyde concentrations in both ALDH2-active and -deficient individuals [11,12,35–37,39]. The capacity of oral microbes to eliminate acetaldehyde is also low, and in this characteristic, there is no evidence for any differences between ALDH2-deficients and ALDH2-actives [49].

Human oropharynx provides an ideal target organ for the quantitative assessment of the carcinogenicity of acetaldehyde in alcohol drinking ALDH2-deficients compared to ALDH2-actives for many exceptional reasons (Table 3). Based on five studies with uniform results salivary acetaldehyde concentration is mean 1.0 mg/l higher in ALDH2-deficients than in ALDH2-actives for as long as alcohol stays in the human body after its intake [13]. According to alcohol elimination rate (7 g/h) the increased exposure time to salivary acetaldehyde caused by deficient ALDH2 enzyme is about 283 min in moderate and 660 min in heavy drinkers [13]. By multiplying the difference in salivary acetaldehyde concentration (1 mg/l) between ALDH2-deficients and ALDH2-actives with the exposure time, the total additional exposure of oropharynx to acetaldehyde is 283 mg/l/283 min in moderate and 660 mg/l/660 min in heavy drinkers, respectively (Fig. 2). Based on one meta-analysis and one well done study (total n = 1189 cases and 3239 controls) the ALDH2-deficiency is associated with 1.68–2.61 -fold oropharyngeal cancer risk in moderate and 3.57–7.28 -fold risk in heavy drinkers (Fig. 2) [13,40,42]. Oropharyngeal cancer risk of ALDH2-deficient alcohol drinkers compared to that of ALDH2-actives correlates thus dose-dependently with the additional acetaldehyde exposure caused by deficient ALDH2 enzyme (Fig. 2).

Markedly increased risk for oesophageal and gastric cancer in ALDH2-deficient alcohol drinkers compared to that of ALDH2-actives indicates that similar dose dependent association between local acetaldehyde exposure and cancer risk might exist also with regard to these organs [13,41,43,44]. However, so far there is no data available on local acetaldehyde concentrations in the

Table 3
Human oropharynx as an ideal target organ for the quantitative assessment of the carcinogenicity of acetaldehyde in alcohol drinking ALDH2-deficients compared to ALDH2-actives [13].

- Ethanol molecule is not carcinogenic
- Acetaldehyde associated with alcohol consumption is Group 1 carcinogen
- Zero acetaldehyde levels in saliva without the presence of ethanol or tobacco
- Oral mucosa lacks aldehyde dehydrogenase enzymes
- Low or zero capacity of oral microflora to eliminate acetaldehyde
- In ALDH2-deficients additional acetaldehyde is secreted from the salivary glands
- Adequate data on salivary acetaldehyde in ALDH2-decicients vs. ALDH2-actives
- Adequate epidemiological data on *ALDH2* polymorphisms and oropharyngeal cancer
- No differences between ALDH2-deficients and ALDH2-actives:
  - In the capacity of oral microflora to produce acetaldehyde from ethanol
  - In the capacity of oral microflora to eliminate acetaldehyde
  - In the mean unstimulated saliva flow rate (0.5 ml/min)
  - In the exposure time to salivary acetaldehyde being equal to the rate of ethanol elimination (7 g/h)
  - In confounding factors hampering most epidemiological studies on alcohol related cancer
    - smoking, diet, oral hygiene, human papilloma virus (HPV), use of different beverages, varying drinking habits, BMI and under reporting

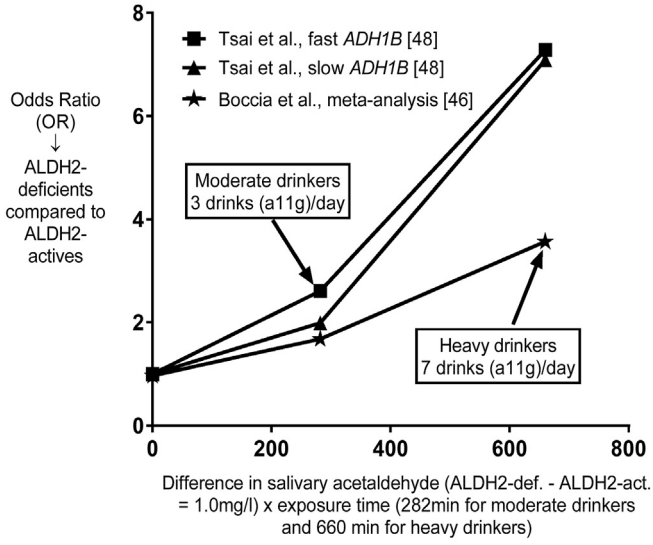

Fig. 2. Oropharyngeal cancer risk (OR) of ALDH2-deficients compared to ALDH2-actives in relation to the additional exposure of oropharyngeal mucosa to acetaldehyde due to the deficient ALDH2 enzyme. Adapted from the Tables 1–5 of reference [13].

oesophagus after alcohol intake, and the data on gastric juice acetaldehyde concentrations are based only on one study, in which alcohol was administered intragastrically [38].

In conclusion, a single point mutation in *ALDH2* gene has "randomized" millions of alcohol consumers to about two times higher exposure to carcinogenic acetaldehyde via saliva every time when they are drinking alcohol and for as long as alcohol stays in their blood circulation. Since the model is not biased by any significant confounding factors, ALDH2-deficiency provides a natural and long-term human model for the quantitative assessment of the carcinogenicity of acetaldehyde in humans. The model shows that salivary acetaldehyde concentration and exposure time after alcohol intake are the two major and quantifiable factors regulating the degree of local acetaldehyde exposure in the oropharynx.

2.3. Practical implications

For any genotoxic and carcinogenic compound, there is an increased risk for cancer even at low exposure levels. Therefore, controlling exposure levels to ALARP (As Low as Reasonably Practicable) should be an overriding principle concerning also acetaldehyde [50–52]. However, acetaldehyde still has a 'generally recognized as safe' (GRAS) status validated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1998 and the Japan Flavour and Fragrance Materials Association (JFFMA) in 2015 [53,54]. However, the decision tree for the safety assessment of flavouring substances applied by JECFA, JFFMA, the Flavour and Extract Manufactures Association (FEMA) Expert Panel, and the European Food Safety Authority (EFSA) is based on a critical error concerning acetaldehyde's metabolism. The WHO model used by these authorities, predicts that acetaldehyde undergoes complete metabolism to safe products via acetate [53–55]. This is not, however, the case with acetaldehyde, which may accumulate in mutagenic concentrations in saliva and gastric juice in the presence of even very low ethanol concentrations (10.5 mM, 0.5‰) [11,12,35–39].

3. Salivary acetaldehyde

Normal human saliva does not contain measurable levels of acetaldehyde and its unstimulated flow rate ranges from 144 to 2880 ml/day [12,56]. However, acetaldehyde present in saliva in association with drinking, smoking or eating is distributed evenly to the water phase of saliva and is transported after each swallowing to the mucosal surfaces of the whole upper digestive tract. Therefore, all factors having an effect on salivary acetaldehyde concentration or saliva flow rate is of importance with regard to the exposure of the upper digestive tract to carcinogenic acetaldehyde.

3.1. Instant and long-term exposure

Alcohol is distributed to oral mucosal surfaces and saliva instantly after the ingestion of 40% alcohol solution resulting in up to 900 mM (4.3%) salivary ethanol concentration at 30 s [11,20]. Microbial acetaldehyde formation from ethanol, instant acetaldehyde exposure, starts subsequently within a few seconds after alcohol intake and continues for 5–10 min after each sip of alcohol [11,20]. On the other hand, long-term exposure represents acetaldehyde formed from ethanol that is diffused to saliva from blood for as long as alcohol is present in the human body [12].

One dose of alcohol (10 g ethanol) daily is associated with a significant relative risk (RR) of 1.29 (20% increase) for oropharyngeal cancer [57]. Assuming that the dose is taken in three swallows at 5 min intervals, oropharyngeal mucosa is exposed to an average 150 μM (6.6 mg/l) concentration of acetaldehyde for the first 15 min after alcohol ingestion [11,20]. Thereafter local acetaldehyde exposure is rapidly levelling off to about 60 μM (2.6 mg/l) for the following 10 min and goes further down to about 20 μM (0.88 mg/l) for the subsequent 55 min [12]. Area under the curve from zero-time point to 25 min is thus 126 mg/l/25 min representing up to 72% of the total (174 mg/l/80 min) acetaldehyde exposure caused by one dose of alcohol.

High levels of acetaldehyde in spirits from Central European countries and France (Normandy) have been linked to the particularly high rate of alcohol related upper digestive tract cancers in these geographical areas [58,59]. This hypothesis is supported by biochemical findings showing that sipping of alcoholic beverages containing 'free' acetaldehyde from 470 to over 15500 μM results in a short, 1–2 min' peak (up to ≥1000 μM) in salivary acetaldehyde concentration [11,37,60]. Thus, high concentration of 'free' acetaldehyde present in an alcoholic beverage contributes significantly to the total instant acetaldehyde exposure of the upper GI tract.

3.2. Major role of oral microflora

Individual characteristics of oral microbiota play a pivotal role in the regulation of acetaldehyde concentration in the oral cavity and saliva after alcohol intake [12,21,22,61,62]. This is underlined by a highly significant correlation ($r > 0.9$) between *in vivo* and *in vitro* salivary acetaldehyde production in 0.5–1.0‰ ethanol concentrations and by about 50% decrease in salivary acetaldehyde production from alcohol after a 3-day use of an antiseptic mouthwash (chlorhexidine, without ethanol), which associated with a marked decrease in baseline salivary aerobic and anaerobic bacterial counts [12].

Some neisseria and viridans group streptococci representing bacteria in normal oral microflora possess particularly high alcohol dehydrogenase (ADH) activity and are able to produce *in vitro* dose dependent acetaldehyde in increasing ethanol concentrations from 11 mM to 1500 mM (0.5‰ - 7%) [21,22]. The finding indicates varying and rather high Km values for bacterial ADHs and explains why *in vivo* salivary acetaldehyde concentrations are highest (up to 258 μM) immediately after alcohol ingestion resulting in over 1.4% (300 mM) ethanol concentrations in saliva [11,20]. Also, many Candida species representing yeasts of normal oral microflora are characterized by marked capacity to produce acetaldehyde from ethanol *in vitro* [61,63–65]. Under low oxygen tension Candida albicans isolates are able to produce high levels of acetaldehyde from glucose, too [66].

3.3. Role of mucosa, salivary glands and gene polymorphism

Human oral mucosa has very low ADH activity, 1/10th of that in the oesophagus and 1/16th of that in the liver [48,67]. Therefore, the contribution of mucosal ADH to acetaldehyde production from ethanol in saliva is presumably minimal as suggested also by studies on 4-methylpyrazole that is an effective inhibitor of the ADH of somatic cells [36]. Buccal mucosa expresses also ethanol metabolizing cytochrome CYP2E1 [68]. Its possible role in the regulation of acetaldehyde level in saliva in the presence of ethanol, however, is not known. Most importantly, oral mucosa is not able to eliminate acetaldehyde because of the lack of both cytoplasmic and mitochondrial low Km ALDH enzymes [48]. This characteristic apparently plays a decisive role in the accumulation of acetaldehyde in saliva after alcohol drinking.

ALDH2 genotype has no effect on salivary acetaldehyde without the presence of ethanol in the systemic blood circulation [20]. This is in accordance with earlier findings indicating that ethanol is metabolized to acetaldehyde in the salivary glands [35,36]. However, due to the deficient activity of salivary gland mitochondrial ALDH2 enzyme in ALDH2-deficient individuals, the additional acetaldehyde is secreted from the glands to saliva.

The ADH enzyme encoded by the *ADH1C*1* allele metabolizes ethanol to acetaldehyde 2.5 times faster than that encoded by the *ADH1C*2* allele. In heavy alcohol drinkers, this polymorphism is associated with increased risk for upper aerodigestive tract cancer, and in homozygotes, in higher salivary acetaldehyde concentrations following alcohol ingestion than in volunteers heterozygous for *ADH1C* or homozygous for *ADH1C*2* [69]. Among Japanese alcoholics the ADH1B*2 allele encoding fast ethanol-metabolizing ADH enzyme does not have significant effect on salivary acetaldehyde levels in the presence of ethanol as compared to those with the slow-metabolizing *ADH1B*1/*1* genotype [39]. However, slow ADH appears to be associated with the prolonged presence of ethanol in blood and saliva and thus also with extended exposure time of the upper digestive tract mucosa to microbially from ethanol formed acetaldehyde [39].

3.4. Role of tobacco smoking and oral hygiene

Acetaldehyde is the most abundant genotoxic carcinogen of tobacco smoke [6]. It is also present in smoking cessation products such as electronic cigarettes [70]. As a water-soluble compound, acetaldehyde dissolves readily in the saliva during smoking and is by that means distributed to the mucosal surfaces of the upper GI tract [7]. During smoking, salivary acetaldehyde increases promptly to mean 261 μM (11.5 mg/l) level and declines after smoking of about 5 min rapidly back to the basal zero level [7]. The area under the salivary acetaldehyde curve for 1 cigarette is thus 58 mg/l/5 min and for 4 cigarettes 230 mg/l/20 min/day. In never alcohol drinkers 3–5 cigarettes/day associates with 2.01-fold (OR) for head and neck cancer [2].

Alcohol, tobacco and poor oral hygiene are established risk factors for upper digestive tract cancer [2,3,5,42,71]. Smoking and heavy drinking independently increase *in vitro* salivary acetaldehyde production from ethanol by 60–75% and their combined effect is about 100% [72]. Poor dental status is associated with infrequent oral hygiene habits and dental visits, and increases *in vitro* salivary acetaldehyde production from ethanol by 100% [73,74]. Chronic smoking increases also *in vivo* acetaldehyde production in saliva from ethanol by about 100% after a moderate dose of alcohol [7].

3.5. L-cysteine – salivary acetaldehyde

Exposure of the oropharynx to acetaldehyde derived either from alcohol or tobacco can be markedly decreased or almost totally abolished by using special medical devices releasing slowly L-cysteine [75,76]. L-cysteine is a semi-essential, natural and safe sulphur-containing amino acid that binds non-enzymatically to acetaldehyde forming inactive 2-methyltiazolidine-4-carboxylicacid (MTCA). A buccal tablet containing slow-release L-cysteine (100 mg) decreases the exposure of oropharyngeal mucosa to acetaldehyde by 60% for 5.3 h after ingestion of a moderate dose (0.8 g/kg) of alcohol [75]. A lozenge containing 5 or 2.5 mg of L-cysteine eliminates acetaldehyde from saliva during smoking by 100 and 96%, respectively [76].

4. Acetaldehyde and oesophageal cancer

Alcohol and tobacco have a synergistic effect on the risk for squamous cell oesophageal cancer [3,77]. Other established risk factors of oesophageal cancer are ALDH2-deficiency in alcohol drinkers, poor oral health, preserved and fermented food, chronic oral candidosis and gastric hyposecretion [41,71,78–81]. A common denominator of all of these cancer hazards is an enhanced exposure of the oesophageal mucosa to carcinogenic acetaldehyde. However, alcohol and smoking appear not to be associated with oesophagogastric adenocarcinoma as discussed in detail in the other review of this issue [82].

The regulation of acetaldehyde concentration in the oesophagus after alcohol intake is much more complicated and far less understood than that in the oropharynx. Alcohol dehydrogenase (ADH) activity of oesophageal mucosa is 7–12 times higher than in the tongue and gingiva [48,83]. Furthermore, chronic alcohol consumption induces cytochrome P-450 2E1 (CYP2E1) in the oesophageal mucosa [84]. Both of these enzyme systems are able to produce acetaldehyde from ethanol, which may contribute significantly to acetaldehyde delivered to the oesophagus from the oropharynx with saliva.

In contrast to the oral cavity, oesophageal mucosa has some aldehyde dehydrogenase enzyme activity, 1/35th of that in the liver [67,83]. Its role in the local detoxification of acetaldehyde in the oesophagus is not known. Anyway, ALDH2-deficiency increases markedly and dose-dependently the risk for oesophageal cancer among alcohol drinkers, which indicates that oesophageal mucosa contributes significantly to the exposure of the oesophagus to carcinogenic acetaldehyde [41].

The normal human oesophagus is colonized with a resident bacterial flora of its own, which has similarities to that of the oral mucosa [85]. The most frequent inhabitants of the oesophagus are streptococci, with an occurrence rate in brush samples and biopsies of 95–98% [85]. How much these microbes contribute to the exposure of the oesophageal mucosa to carcinogenic acetaldehyde derived from ethanol present either in alcoholic beverages or food is not known.

5. Acetaldehyde and gastric cancer

Gastric cancer is the third leading cause of cancer death in both sexes worldwide, representing 8.8% of the total cancer mortality [1,86]. The six-month survival rate is 65% when diagnosed in the early stages and less than 15% in those diagnosed in the advanced stage. The five-year survival rate ranges only from 5 to 10%, underlining the importance of preventive measures [86]. *Helicobacter pylori*, atrophic gastritis, tobacco smoking, alcohol drinking, ALDH2-deficiency associated with alcohol drinking, pickled foods, fermented soy food, some fermented dairy products and acid suppressive drugs are established risk factors for stomach cancer [4,5,43,44,86–91]. A common denominator behind these gastric cancer hazards appears again to be carcinogenic acetaldehyde.

5.1. Major role of microbes and gastric mucosa

The normal acidic human stomach is free of microbes. However, ADH containing and acetaldehyde producing bacteria and yeasts can survive and even proliferate in the gastric contents of patients with achlorhydric atrophic gastritis [92,93]. In patients with hypo- or achlorhydric stomach bacterial overgrowth has been shown to be associated with the local formation of ethanol (up to 27 mM = 1.3‰) and minor levels (max. 15.7 μM) of acetaldehyde

from glucose [94,95]. In the presence of alcohol, the intragastric acetaldehyde production in patients with hypo- or achlorhydric stomach secondary either to atrophic gastritis or the use of acid suppressive drugs is increased to mutagenic levels [38,96–98]. Also, many Helicobacter pylori strains possess ADH activity and are able to produce acetaldehyde from ethanol under microaerobic conditions at least *in vitro* [99].

Gastric mucosal ethanol and acetaldehyde metabolizing enzymes are responsible for a significant gastric first-pass metabolism of alcohol. Stomach mucosa possesses ADH activity representing 1/3rd of that in the esophagus and 1/12th of that in the liver [100,101]. In contrast to oropharynx, gastric mucosa contains also ALDH enzymes with 2.2 times higher activity than in the oesophagus and 1/8th of that in the liver [100,101]. Furthermore, gastric mucosa expresses some cytochrome P4502E1 activity [102]. However, the contribution of these various ethanol metabolizing systems to the regulation of gastric juice acetaldehyde levels in the presence of alcohol is not so far known.

In individuals with normal acidic stomach and active ALDH2 enzyme intragastric alcohol infusion (0.5 g/kg) causes a slight increase from zero level to mean peak of 10.4 μM in the acetaldehyde concentration of gastric juice [38]. However, in ALDH2-deficients a profound elevation to mutagenic acetaldehyde concentrations (mean peak 47.1 μM) is seen [38]. Highest intragastric acetaldehyde levels (mean 63.9 μM, range 32.0–96.7 μM) have been demonstrated in ALDH2-deficient subjects after 7day's treatment with an acid suppressive drug (rabeprazole 10 mg b.i.d.) and intragastric alcohol infusion [38]. The alcohol-induced marked increase in gastric juice acetaldehyde concentrations of hypo- and achlorhydric patients, especially among ALDH2-deficient subjects, provides strong evidence for the local carcinogenic potential of acetaldehyde in gastric carcinogenesis.

5.2. L-cysteine – gastric juice acetaldehyde

Local exposure of the gastric mucosa to carcinogenic acetaldehyde can be mitigated by slow-release L-cysteine formulations. After intragastric alcohol installation to patients with atrophic gastritis 60–70% of carcinogenic acetaldehyde is eliminated to inactive MTCA after ingestion of two slowly L-cysteine releasing capsules (2 × 100 mg) [97,98]. With slow-release L-cysteine peak acetaldehyde concentrations of gastric juice remained in all patients under the level of mutagenicity and L-cysteine stayed in the stomach for up to 3 h. The area under the curve (AUC) for MTCA was 11-fold higher than that for acetaldehyde, which indicates significant gastric first-pass metabolism of ethanol mediated by microbes and mucosa [98]. With placebo markedly elevated acetaldehyde levels were found also at low intragastric ethanol concentrations corresponding to the alcohol levels of many 'non-alcoholic' beverages and foodstuffs containing anyway percentage concentrations of alcohol [38,97,98].

Slow-release L-cysteine eliminated 60–70% of carcinogenic acetaldehyde also from the gastric juice of both ALDH2-active and ALDH2-deficent subjects treated with proton pump inhibitor (PPI) [38]. Nondependent changes in gastric juice and salivary acetaldehyde levels caused by ALDH2 deficiency, PPI treatment, and intragastric L-cysteine indicate that the gastric juice acetaldehyde concentration is locally regulated by gastric mucosal ADH- and ALDH2-enzymes and by oral microbes colonizing the achlorhydric stomach secondary to PPI treatment [38].

Since acetaldehyde formed locally from alcohol in the upper GI tract is a mutagenic and genotoxic carcinogen in humans, randomized and placebo controlled clinical trials aimed for the prevention of upper GI tract cancers with slowly L-cysteine releasing formulations will be questionable because of ethical reasons. Therefore, the actual effectiveness of slow-release L-cysteine formulations in cancer prevention remains to be evaluated in prospective intervention studies with established risk groups for stomach cancer, in which controlling acetaldehyde exposure levels to ALARP (As Low As Reasonably Practicable) should be an overriding principle.

6. Acetaldehyde derived from food – significant epidemiological bias

Microbial formation of acetaldehyde from ethanol present in 'non-alcoholic beverages', fermented foodstuffs or added to the dish during food preparation forms currently a significant epidemiological bias in upper GI tract carcinogenesis. Microbial fermentation has been used for thousands of years to prepare alcoholic beverages and for the preservation of food. Lactic acid is the most common end product of food fermentation reactions. Many microbes are, however, able to produce also ethanol from sugar under anaerobic conditions. Therefore, beverages and foodstuffs containing 0.05–2.5% (10.5–525 mM) or even higher concentrations of alcohol are widely used in everyday life. Good examples are home-made mead and beer, vinegar, kefir, mursik milk, soya sauce, kimchi, pickled food and even some packed bakery products [78,103,104]. Several epidemiological studies have shown that fermented food products are established risk factors for upper digestive tract cancer [78,79,88–90].

Excessive use of salt and intragastric formation of potentially carcinogenic N-nitroso compounds have been suggested to explain the increased gastric cancer risk associating with the use of these products. However, salt is neither mutagenic nor carcinogenic, but an essential additive in food fermentation and preservation. On the other hand, most recent cohort studies have failed to provide conclusive evidence with regard to the role N-nitroso compounds in upper GI tract carcinogenesis [105]. Acetaldehyde associated with the use of alcoholic beverages is, however, a group 1 carcinogen to humans and there is no evidence that beverages and foodstuffs containing ethanol up to 2.5% and above and/or mutagenic concentrations of acetaldehyde is less carcinogenic than acetaldehyde derived from official alcoholic beverages.

Moreover, alcoholic beverages and ethanol containing sauces and dressings are frequently used as an essential part of a meal. Examples are salads, sushi, Marsala, marinating, cooking, flaming and fondue. In contrast to general belief, alcohol is not evaporated out of food during cooking. Instead 40–85% of alcohol is retained in the dish after food preparation resulting in 0.06–4.21% (13–884 mM) ethanol concentrations in the end product [106]. However, an ethanol level of 10.5–21 mM (0.5–1‰) is more than enough for the production of mutagenic concentrations of acetaldehyde both *in vitro* and *in vivo* in the saliva and gastric juice of individuals with hypo- or achlorhydric stomach [11,12,20,35–39,95–98]. Depending on eating habits and number of teeth, these products may expose oropharyngeal and oesophageal mucosa to mutagenic acetaldehyde concentrations for ten to 30 min daily and gastric mucosa for several hours.

In developed countries the decreased incidence of Helicobacter pylori infection, atrophic gastritis and smoking and the increased availability of refrigerators and improved oral health have undoubtedly had a great decreasing effect both on the incidence of upper GI tract cancer and as well on the local acetaldehyde exposure in the upper digestive tract. However, the carcinogenic impact of changing drinking habits and varying concentrations of alcohol and acetaldehyde in both alcoholic and 'non-alcoholic' beverages and food remains unanswered until this information is made available for researches, health care workers, regulatory authorities and consumers.

Practice points

- A point mutation in *ALDH2* gene proves the causal relationship between acetaldehyde and upper GI tract cancer in humans and provides a novel method for the quantitative assessment of the carcinogenicity of acetaldehyde.
- Local acetaldehyde is a common denominator in alcohol, tobacco and food associated upper GI tract cancer.
- Microbial acetaldehyde formation starts instantly in saliva and gastric juice of achlorhydric stomach after alcohol ingestion and continues for as long as ethanol is present in the human body.
- Microbial formation of acetaldehyde from ethanol present in 'non-alcoholic' beverages, fermented food or added to the dish during food preparation forms a significant epidemiologic bias in upper GI tract carcinogenesis.
- Exposure of upper GI tract mucosa to acetaldehyde is cumulative and can be markedly reduced both at population and individual level.
  - Quit from smoking (including electronic cigarettes), reduce your alcohol consumption, take care of good oral hygiene, avoid alcoholic beverages containing high levels of 'free' acetaldehyde, avoid ethanol and acetaldehyde containing 'non-alcoholic' beverages and food, and consider use of slow-release L-cysteine in established risk groups.

Research Agenda

- Further studies on the role of alcohol, acetaldehyde and microbes present in 'non-alcoholic' beverages and food in the regulation of local acetaldehyde concentration in the upper GI tract.
- Further studies on the possible role of food and tobacco derived acetaldehyde in the formation of mutagenic acetaldehyde-DNA adducts in the upper digestive tract mucosa.
- Studies on the effect of saliva flow rate on the exposure of upper digestive tract mucosa to acetaldehyde.
- Studies on the possible colonization of hypo- or achlorhydric stomach by acetaldehyde from ethanol producing microbes used in the fermentation of alcoholic beverages and food or as probiotics.
- Intervention studies, in which the exposure of upper GI tract mucosa to carcinogenic acetaldehyde derived from ethanol and/or acetaldehyde present in alcoholic and 'non-alcoholic' beverages, food and tobacco is minimized to ALARP (As Low As Reasonably Practicable).

Statement of coi

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