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Salivary alcohol dehydrogenase in non-smoking and smoking alcohol-dependent persons

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ABSTRACT

Increasing attention to the importance of saliva testing is not surprising because smoking and alcohol drinking act synergistically on oral tissues, and their metabolite levels, e.g., acetaldehyde, are much higher in saliva than in blood. The activity of salivary alcohol dehydrogenase (ADH) comes from oral microbiota, mucosa, and salivary glands. The purpose of this study was to investigate the involvement of ADH in the oral health pathology of smoking (AS) and non-smoking (ANS) alcohol-dependent males. The results indicated that the AS group had a more significant and longer duration (until the 30th day of alcohol abstinence) decrease in ADH activity and output than the ANS group (until the 15th day of alcohol abstinence) compared to controls (social drinkers; C). The decreased salivary flow (SF) in alcoholics was observed longer in the ANS group (until the 30th day of alcohol abstinence), whereas in the AS group SF normalized at the 15th day, probably due to the irritating effect of tobacco smoke on the oral mucosa. Because saliva was centrifuged to remove cells and debris (including microbial cells), the detected salivary ADH activity was derived from salivary glands and/or oral mucosa. A more profound and longer decrease in ADH activity/output in smoking than non-smoking alcoholics was likely due to the damaged salivary glands and/or oral mucosa, caused by the synergistic effect of alcohol drinking and smoking. The lower values of salivary ADH in smoking than non-smoking alcoholics might also be partly due to the reversed/inhibited ADH reaction by high levels of accumulated acetaldehyde.

Introduction

During ethanol consumption, ethanol is absorbed from the gastrointestinal mucosa, including oral, gastric, and duodenal mucosa. The ethanol is then circulated by the blood to other tissues, such as the mucus membranes of the upper gastrointestinal tract and salivary glands (Seitz & Stickel, 2007). Ethanol diffuses rapidly into the whole water phase of the oral cavity tissues. Immediately after drinking, its concentration in saliva is temporarily higher than in plasma. In saliva, the level of acetaldehyde, the main ethanol metabolite, exceeds 10 to 100 times the blood level (Waszkiewicz, Chojnowska, et al., 2013). In smokers, salivary acetaldehyde levels were found to be twice as high as in non-smokers. Although tobacco smoke contains high concentrations of acetaldehyde itself, chronic smoking increases *in vivo* acetaldehyde production from ethanol by about 100%, even after a moderate dose of alcohol (Salaspuro, 2012; Salaspuro & Salaspuro, 2004). Because smoking modifies the oral flora to produce higher concentrations of acetaldehyde from ethanol, the concomitant habits of smoking and alcohol drinking have a synergistic – even 7-fold – carcinogenic effect of acetaldehyde on the upper digestive tract (Salaspuro, 2012). Aside from the main toxic compound/metabolite – acetaldehyde – reactive oxygen species (ROS) generated during drinking and smoking, as well as non-oxidative metabolites of ethanol (e.g., fatty acid ethyl esters; FAEEs), might be involved in the oral cavity tissue damage (Waszkiewicz, Chojnowska, et al., 2013; Waszkiewicz, Szajda, Kępka, Szulc, & Zwierz, 2011). The deleterious effects of alcohol drinking and smoking on oral tissues (e.g., salivary flow and saliva composition changes, oral injury, atrophy, hyperproliferation, or cancer) is also due to ethanol itself (acts as a solvent for other toxins/carcinogens, ethanol-water competition

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mechanism) and tobacco smoke toxins (up to 3000 toxic substances such as nicotine, nitrosamines, carbon monoxide, and other aldehydes) (Chang, Straif, & Guha, 2012; Waszkiewicz, Chojnowska, et al., 2013; Waszkiewicz, Zalewska-Szajda, et al., 2013).

Cytochrome proteins and mRNA (e.g., CYP1A2, CYP3A4) have been found in human salivary gland parenchyma, and CYP2E1 induction has been observed in the oropharyngeal mucosa of alcoholics. Therefore, cytochrome enzymes may be potentially responsible for the alcohol- and smoking-related tissue toxicity, e.g., by ROS and acetaldehyde metabolites (Asakage et al., 2007; Seitz & Stickel, 2007; Waszkiewicz, Chojnowska, et al., 2013). It is also known that microbes (bacteria and yeast) representing oral flora are responsible for most of the acetaldehyde present in the saliva of alcohol drinkers (Seitz & Stickel, 2007). Many microbe species possess alcohol dehydrogenase (ADH) activity, being able to oxidize ethanol to acetaldehyde. The salivary ADH activity comes also from the oral mucosa and salivary glands (Maier, Born, Veith, Adler, & Seitz, 1986; Waszkiewicz, Chojnowska, et al., 2013). Smoking can shift oral microflora toward colonization with yeast and gram-positive bacteria, which create more acetaldehyde due to a high ADH activity (Salaspuro & Salaspuro, 2004). Although most of the acetaldehyde is locally formed in the oral cavity by microbial oxidation, acetaldehyde may also be produced by the mucosal ADH (ADH4, previously called ADH7) of the aerodigestive tract including oral mucosa, which is active upon exposure to a locally high dose of ethanol (Asakage et al., 2007; Jelski & Szmitkowski, 2008). Human oral and esophageal mucosa have been shown to possess high ADH activity but low aldehyde dehydrogenase ($ALDH_2$) activity, which additionally favors acetaldehyde accumulation in the saliva while drinking (Chang et al., 2012; Salaspuro, 2012). Moreover, it has been suggested that ALDH may be inhibited by smoking, leading to less efficient acetaldehyde metabolism (Helander & Curvall, 1991; Salaspuro & Salaspuro, 2004). Acetaldehyde sources (other than mucosal, bacterial, and systemic) include salivary glands, dietary components, and alcoholic beverages *per se* (Visapää et al., 2004; Yokoyama et al., 2002). It is notable that ADH may also participate in the oxidation of retinol (epithelial differentiation influence) and other alcohols and aldehydes, lipid peroxidation (4-hydroxynonenal substrate), and glutathione metabolism (Jelski & Szmitkowski, 2008; Seitz & Stickel, 2007).

Up to now, we have not encountered reports on ADH values in the saliva of alcohol-dependent persons. We therefore investigated the activity of salivary ADH in non-smoking and smoking alcohol-dependent persons, in comparison to the control social drinkers, to check the involvement of ADH in the oral health pathology of alcoholics.

Materials and methods

Participants

Thirty-five males participated in the study: the control group ("C") consisted of 10 healthy social drinking volunteers (mean age ± SD; 41 ± 9) with no history of alcohol abuse or smoking, 10 alcohol-dependent non-smoking individuals ("ANS"; age 47 ± 7), and 15 alcohol-dependent smoking individuals ("AS"; age 44 ± 10). The duration of chronic alcohol drinking ranged from 3 to 69 days (mean ~27) in AS and 3–14 days (mean ~9) in ANS group. During drinking, AS individuals ingested an average of 265 g (75–700 g) of alcohol per day and ANS individuals drank an average of 155 g (100–250 g) of alcohol per day. Alcohol-dependent individuals met the criteria for alcohol and nicotine dependence (AS) or only for alcohol dependence (ANS), according to the ICD-10 and DSM-IV criteria. The average time of dependence was 16 ± 8 years for alcohol and 20 ± 9 years for smoking in the AS group, and 11 ± 8 years of alcohol dependence in the ANS group. AS participants smoked 17 ± 8 cigarettes daily.

Ethical issues

Informed written consent was obtained from each human subject after explanation of the nature, purpose, and potential risks of the study. The study was approved in accordance with the ethical standards of the responsible committee on human experimentation (the local Bioethical Committee) and with the Helsinki Declaration of 1975, as revised in 1983.

Data and sample collection

Material on the first day of alcohol abstinence was collected in the Unit of Treatment of Alcohol Withdrawal Symptoms and on the 15th and 30th day of therapy in the Unit of Alcohol Dependence Therapy. A check-up of the oral cavity in all participants was done by one qualified dentist in artificial light, using a dental mirror and a probe. Check-up of alcoholics was done in Choroszcz Hospital and check-up of healthy social drinking volunteers was done in the Department of Paedodontics. Following the WHO criteria, the oral hygiene index, simplified (OHI–S; scores plaque index from 0 to 3), was utilized to determine the state of oral hygiene. The level of dental caries was determined using the DMFT index (D: decayed, carious tooth; M: missing tooth because of caries; FT: filled tooth). Gingival status was assessed using gingival index (GI) and papilla bleeding index (PBI). The GI assessing the prevalence and severity of gingivitis scores the marginal and interproximal tissues from 0 to 3. The PBI evaluates the patient's gingival condition, based upon the actual bleeding tendency of the gingival papillae, which was recorded from 0 to 4. The subjects were instructed to refrain from smoking, food, and beverages, except water, for 2 h before saliva collection. During saliva collection, the patient was seated on a chair and protected from gustatory and other stimulation. The subject was advised to rinse his mouth several times with deionized (distilled) water and then to relax for 5 min. The unstimulated whole saliva was collected under the control of one dentist by passive spitting into a container immersed in crushed ice (under standardized conditions; Dawes, 1987; Navazesh, Christensen, & Brightman, 1992). The volume of each sample was measured with a pipette calibrated in 0.1 mL units. All samples (3 mL) of the residual whole saliva were collected between 8:00 and 9:00 A.M. to minimize the influence of circadian rhythms, and centrifuged (3000 × g; 20 min; 4 °C) to remove cells and debris. The supernatants, in 200 μL portions, were frozen and kept at −80 °C until analyzed. Salivary flow (SF) was calculated by dividing the volume of saliva by the time of its collection.

Analytical methods

The total salivary ADH activity was estimated by the photometric method with p-nitrosodimethylaniline (NDMA) as a substrate (Jelski, Zalewski, & Szmitkowski, 2008; Skurský, Kovár, & Stachová, 1979). The reduction of NDMA was monitored at 440 nm on a Shimadzu UV/VIS 1202 spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany). The output (mU/min) of ADH was calculated by multiplying enzyme activity (mU/L) by the salivary flow (mL/min).

Statistical analysis

The results were analyzed with Statistica 10.0 Statsoft (StatSoft, Cracov, Poland). All data were tested for normal distribution. Results were expressed as median (IQR or minimum–maximum).