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Salivary immune proteins monitoring can help detection of binge and chronic alcohol drinkers: Preliminary findings

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ABSTRACT

Background: We compared effects of binge and chronic alcohol drinking on oral health and salivary immunity proteins.
Methods: The study involved males: 13 healthy social-drinking (C), 10 alcohol-dependent after chronic alcohol-intoxication (A), and 8 binge-drinkers after a single binge-drinking session (B). We compared periodontal/dental state and salivary immune proteins (lactoferrin –Lf, lysozyme –Lz, oral peroxidase –OPO, immunoglobulin A –IgA) in all groups.
Results: Group A had worse dental and periodontal states than group C and B. Group B had a lower OPO activity and Lz concentration, and a higher IgA concentration in comparison to group C. Group A had a lower OPO activity than group C. Group B had a lower Lz and a higher LF and IgA outputs than C. Group A had a lower IgA output and a strong tendency of Lf and Lz outputs to be lower than in group C. Positive correlations were found between alcohol amounts and OPO and Lf output in group A, with no such correlations in group B. Only IgA concentration in group B and OPO activity in group A have potential to be markers that help to differentiate binge from chronic alcohol drinking, and OPO activity had better accuracy than IgA.
Conclusion: Binge alcohol consumption resulted in specific disturbances in salivary innate immunity (Lz), whereas chronic drinking led to disturbances in both adaptive and innate immunity (IgA, Lz and Lf). There is potential applicability of raised salivary IgA concentration and especially OPO activity in binge and chronic drinking detection and differential-diagnosis.

1. Introduction

It is known that alcohol abuse is linked to host susceptibility to infectious diseases such as pneumonia, hepatitis C, and tuberculosis (Nelson and Kolls 2002; Waszkiewicz et al., 2015). Usually, acute alcohol consumption in binge drinkers (drinking acutely more than 5 units of alcohol per day) results in a specific disturbance in innate immunity, whereas chronic alcohol consumption in alcohol dependent persons leads to disturbances in both the innate and the adaptive immunity. Acute and chronic alcohol intoxication typically decrease phagocytosis, but may also induce pathological immune response with acute phase proteins and increase in the level of immunoglobulins (Waszkiewicz et al., 2012a,b,c,d).

About 52% of the global adult population consumes alcohol; alcohol-dependence affects 2–4% of population, whereas acute alcohol drinking (binge-drinking) is noted in about 16–18% of drinkers (WHO, 2014; Waszkiewicz et al., 2015). Early screening of binge-drinking is therefore especially important, as it is a much more common problem than chronic-drinking and may precede the sequence of events leading to alcohol addiction (Waszkiewicz et al., 2013a).

The oral mucosal surface is the portal of entry for the majority of pathogens; therefore, human salivary proteins participate in the protection of oral tissues, upper digestive and respiratory tracts against pathogens (Waszkiewicz et al., 2008a). Ethanol through ethanol-water competition mechanism and various alcohol metabolites, such as acetaldehyde, reactive oxygen species (ROS), fatty acid ethyl esters (FAEEs) or alcohol congeners such as methanol, etc. destroy various tissues of human body, including oral tissues and associated salivary glands (Waszkiewicz et al., 2011, 2012e, 2014a,b).

Chronic alcohol drinking was found to affect: oral mucosa

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(epithelial atrophy, hyperregeneration, dysplasia/keratosis), salivary glands (fat accumulation, acinar cell swelling, atrophy/reduction in weight and protein, diffuse mononuclear infiltration), and saliva (reduced salivary flow, sodium, bicarbonate, chlorine concentrations, salivary glyco-proteins levels eg. amylase) (Riedel et al., 2003; Waszkiewicz et al., 2014a). The reduced salivary flow in alcohol abusers may lead to inflammatory states of the oral cavity, periodontal diseases, or infections (Waszkiewicz et al., 2013b). Worse dental and oral hygiene was also observed in alcohol-addicted persons (Waszkiewicz et al., 2013b).

Acute alcohol drinking in moderate doses was found to stimulate parotid salivary flow rate, whereas higher doses transitionally (few hours) reduced salivary secretion and secretory protein synthesis, decreased concentration or activity of amylase, lysozyme (Lz), oral peroxidase (OPO), electrolytes, and increased late immunoglobuline A (IgA) concentration (Martin and Pangborn, 1971; Enberg et al., 2001; Waszkiewicz et al., 2008a). Acute alcohol-induced local and transient oral mucosa damage or disseminated ulcerations were observed in the animal studies (Waszkiewicz et al., 2008a).

There is no study that compares salivary effects of binge drinking and chronic alcohol drinking (alcohol dependence) of those dependent on alcohol with healthy controls. Until now, only the effect of chronic alcohol drinking together with smoking on salivary proteins concentration/output was shown (Lz, OPO, lactoferrin –Lf, IgA) (Waszkiewicz et al., 2012a,b,c,d); whereas no study checked the effects of binge drinking on the output of salivary immune proteins. Literature describes different and specific effects of acute and chronic alcohol drinking on a human tissue immunity (mostly blood and internal organs): acute binge drinking affects mostly innate immunity and chronic binge drinking affects both innate and adaptive immunity. Therefore, the aim of this study is: a) to investigate whether acute and chronic alcohol drinking have different effects on the salivary immune proteins (Lf, Lz, IgA and OPO) and b) to determine if some of these changes may be helpful in diagnosis of acute and chronic alcohol use or in their differential diagnosis.

2. Materials and methods

2.1. Participants

Thirty one non-smoking individuals (25–45 years of age) were involved: 10 alcohol-dependent males after chronic alcohol intoxication (A; aged 25–45 years, all met ICD-10 and DSM-IV criteria for alcohol-dependence) recruited from the Unit of Treatment of Alcohol Withdrawal Symptoms in Choroszcz, 8 infrequent binge-drinking healthy males after a single binge-drinking session (B; 25–39 years, reported bingeing 1–11 times/year and/or 1–2 episodes/past-month) after a single binge-drinking session, and 13 healthy social-drinking males (C; 30–45 years; usually drink not more than 2–3 units of alcohol per day; 1 unit = 10 g of pure ethanol). The chronic alcohol drinking period before hospital admission ranged from 5 to 17 days [12 (5)], during intoxication these subjects drank from 100 to 250 g of pure alcohol per day [180 (60)]. Binge drinkers consumed in a row (4–6 h) from 140 to 180 g of pure alcohol [160 (20)].

2.2. Procedures

2.2.1. Ethical issues

The study was approved by the local Bioethical Committee and conducted in accordance with the Helsinki Declaration. Informed written consent was obtained from all the subjects after explanation of the nature, purpose, and potential risks of the study.

2.3. Oral cavity check

A check-up of the oral cavity was done at the Unit of Treatment of Alcohol Withdrawal Symptoms (group A) and in a dental clinic (group B), by the same qualified dentist in artificial light, by using a dental mirror and a probe. The dental state was determined according to the WHO criteria, using the DMFT index, expressed as the total number of teeth (T) that were decayed (D), missing (M), or filled (F) in patients, and scored from 0 to 28 or 32, depending on whether the third molars are included in the scoring. Gingival status was assessed using gingival index (GI) and papilla bleeding index (PBI). GI scores the marginal and interproximal tissues separately on the basis of 0–3. The criteria were, in general: normal gingiva (0); mild inflammation (1) – slight change in color and slight edema but no bleeding on probing; moderate inflammation (2) – redness, edema and glazing, bleeding on probing; severe inflammation (3) – marked redness and edema, ulceration with tendency to spontaneous bleeding. The intensity of any bleeding in PBI was recorded as: no bleeding (0); a single discreet bleeding point (1); several isolated bleeding points or a single line of blood appears (2); the interdental triangle fills with blood shortly after probing (3); profuse bleeding occurs after probing (4), blood flows immediately into the marginal sulcus. Good periodontal and dental health were interpreted as follows: < 1 for PBI and GI, and < 20 for DMFT (Waszkiewicz et al., 2008a, 2013b).

2.4. Data and sample collection

The subjects were instructed to refrain from smoking, food and beverages, except water, for 2 h before saliva collection. All samples (3 mL) of the residual/resting whole saliva were collected with plastic tubes placed on ice by the spitting method, under standardized conditions (Dawes 1987; Navazesh et al., 1992), between 8:00 to 9:00 am to minimize the influence of the 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.

2.5. Analytical methods

Oral peroxidase (OPO) activity was measured by colorimetric method, according to Mansson-Rahemtulla et al. (1986). Salivary lysozyme concentration was measured by radial immunodiffusion (Human NL Nanorid plate; The Binding Site, Birmingham, UK) as described by Mancini et al. (1965). The concentrations of lactoferrin and sIgA were assessed by ELISA (Bioxytech Lactof EIA; Oxis Health Products, Portland, OR, USA; Human sIgA Elisa; Immunodiagnostic, AG, Bensheim, Germany) (Hamelinck et al., 2005; Waszkiewicz et al., 2008a). The salivary protein content (Sp) was determined by the bicinchoninic acid method (BCA) (BCA Protein Assay Kit; PIERCE, Rockford, IL, USA), with bovine albumin as a standard. All analyses were performed in duplicate.

The output of salivary proteins (IU/min or µg/min) was calculated by multiplying enzyme activity (IU/L) or concentration (mg/L) by the salivary flow (mL/min), respectively.

2.6. Statistical analysis

Statistical analysis was performed with Statistica version 10 (Statsoft, Cracov, Poland). All data were tested for normal distribution. Nonparametric methods were most appropriate when the sample sizes are small and results were expressed as median (then results were expressed as median [IQR or minimum–maximum]). Comparisons in the oral cavity parameters between groups C, B, and A were made using the Kruskal-Wallis analysis and differences were located using the Mann-Whitney "U" test. Spearman's rank correlation coefficient was used to measure the statistical dependence between variables. A receiver operating characteristic (ROC) analysis, calculations of specificity, sensitivity and area under the ROC curve (AUC) were performed using

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