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Salivary lysozyme in smoking alcohol-dependent persons

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Abstract: The purpose of this study was to evaluate the effect of chronic alcohol intoxication and smoking on the concentration and output of salivary lysozyme. Thirty seven men participated in the study, including 17 male smoking alcohol-dependent patients after chronic alcohol intoxication (AS), and 20 control non-smoking male social drinkers (CNS) with no history of alcohol abuse or smoking. The level of lysozyme was assessed by the radial immunodiffusion method. Significantly lower lysozyme output in the AS group compared to the CNS group was found. Moreover, gingival index was significantly higher in AS than in the CNS group. It appeared that the reduced salivary lysozyme output was more likely the result of ethanol action than smoking. In conclusion, persons addicted to alcohol and nicotine have a poorer periodontal status than non-smoking social drinkers, which may partially be due to the diminished protective effects of lysozyme present in the saliva. *(Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 4, 609–612)*

Key words: lysozyme, saliva, alcohol dependence, smoking, gingival index

Introduction

Lysozyme (muramidase or N-acetylmuramide glycanhydrolase) is a cationic protein participating in innate immunity. The highest lysozyme concentrations have been recorded in tears, gastric juice and mothers' milk, as well as in saliva, sweat and bronchial secretions [1–3]. Lysozyme is synthesised in secretory cells, mainly in the submandibular and sublingual salivary glands. Gingival crevical fluid and the monocyte-macrophage system are also sources of lysozyme, which is known to be an important element of the anti-bacterial defence mechanism. The antibacterial action of lysozyme is multilateral, but mainly depends on destruction of the bacterial cell wall due to the hydrolysis of glycosidic bonds that connect N-acetylmuramic acid with N-acetylglucosamine [1, 2]. After hydrolysis of the heteropolysaccharide chains of murein, the

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cell wall of bacteria is more susceptible to the lysis in the salivary hypo-osmotic environment [4]. The cooperation of cationic lysozyme with monovalent anions such as thiocyanates, chlorides, iodides, bromides, fluorides, nitrates, bicarbonates and proteases, destabilises the microbial cell membrane by the activation of endogenous bacterial autolysins [5]. The lytic mechanism of lysozyme action may also involve its association with the microbial nucleic acids that leads to subsequent mutations or disintegration of pathogens' DNA [1, 2]. Lysozyme can also inhibit bacterial glucose uptake and the production of acids [6] and can aggregate oral bacteria, inhibiting their adhesion and colonisation on the oral mucosa and teeth [5]. An inverse correlation has been found between the activity of lysozyme and the accumulation of dental plaques [5].

As much as 3.8% of global deaths, 4.6% of diseases and injuries, and 30% of health care costs, are attributable to alcohol abuse. About 2% of the world population is alcohol-dependent [7]. Ethanol is directly toxic to the mucosa of the oral cavity, mouth, throat, oesophagus and stomach. Acute local mucosa damage is reversible and proportional to the quantity and concentration of alcohol. Ethanol immediately diffuses into the saliva and oral tissues, reaching even higher concentrations than in plasma [8–10]. Acetaldehyde and reactive oxygen species derived from alcohol and tobacco smoke are widely known factors of tissue damage and carcinogenesis. Besides acetaldehyde, tobacco smoke contains up to 3,000 different toxic substances such as nicotine, nitrosamines, carbon monoxide, etc. [11].

We [3] and others [12] have previously reported, in acutely intoxicated and alcohol-addicted persons, lower levels of respectively salivary and serum lysozyme compared to the preconsumption level and to that of social drinkers (respectively). The aim of this study was to determine, for the first time, the effect of chronic alcohol consumption and cigarette smoking on the concentration and output of salivary lysozyme.

Material and methods

A cohort of 37 persons took part in the study, consisting of 17 male alcohol-dependent smoking patients (AS; alcohol + smoking) admitted to the Detoxification Unit of the Psychiatric Hospital in Choroszcz, Poland, after chronic alcohol intoxication (mean age: 42 years; range: 26–55; 100–700 g/day of alcohol; 10–20 cigarettes/day) and 20 control male social drinkers (CNS; control non-smokers) with no history of alcohol abuse or smoking (mean age: 42 years; range: 30–53). The AS group individuals met criteria for alcohol and nicotine dependence according to ICD-10 (the average time of dependence was 15 ± 7 years for alcohol and 20 ± 8 years for smoking; mean ± SD). The length of alcohol intoxication ranged from three to 90 days (mean ~30). An interview about smoking habit was conducted during a dental examination. Material from persons admitted to the Detoxification Unit was collected on the second day of the abstinence period. The study was approved by the Bioethical Committee of the Medical University of Bialystok. Informed written consent was obtained from all the participants after explanation of the nature, purpose, and potential risks of the study.

A check-up of the oral cavity was done by one qualified dentist, following the World Health Organisation criteria. The DMFT, GI, and PBI indices of our subjects were: in alcohol-dependent subjects 19.5 ± 5.7, 0.99 ± 0.76, and 0.57 ± ± 0.27, respectively, and in controls 18.8 ± 5.70, 0.30 ± ± 0.47, and 0.35 ± 0.48, respectively. The subjects were instructed to refrain from smoking, food and beverages, except water, for two hours before saliva was collected. All salivary samples (3 ml of resting whole saliva) were collected to plastic tubes placed on ice by the spitting method, under standardised conditions, between 8:00 and 9:00 am, to minimise the influence of circadian rhythms. The samples were centrifuged at 3,000 × g for 20 minutes at 4°C, to remove cells and debris.

The salivary lysozyme concentration was determined by radial immunodiffusion (Human 'NL' Nanorid plate, No GT073.3, Binding Site Ltd., Birmingham, UK) according to the manufacturer's instructions. The precipitation ring diameters were measured using Digital Rid Plate Reader (Binding Site Ltd., Birmingham, UK).

Statistical analysis was performed with Statistica version 8.0 (Statsoft, Krakow, Poland). Results are expressed as means ± SD. The differences between groups were evaluated using a Mann-Whitney U test. Spearman's rank correlation coefficient was used to measure the statistical dependence between two variables. Statistical significance was assumed at $p < 0.05$.

Results and discussion

We found no significant difference between the concentration of lysozyme in the saliva of the AS group (56 ± 97 mg/L) and the CNS group (44 ± 22 mg/L) ($p = 0.131$). Salivary lysozyme output was significantly lower in AS (9.6 ± 14.2 μg/min) compared to the CNS group (16.5 ± 6.4 μg/min) ($p = 0.002$) (Figure 1). We also found that the gingival index (GI) was significantly higher in AS than in the CNS group ($p = 0.003$) (Table 1). There were no significant differences in PBI and DMFT indices between the AS and CNS groups. We also did not find any significant correlations between the amount and length of alco-

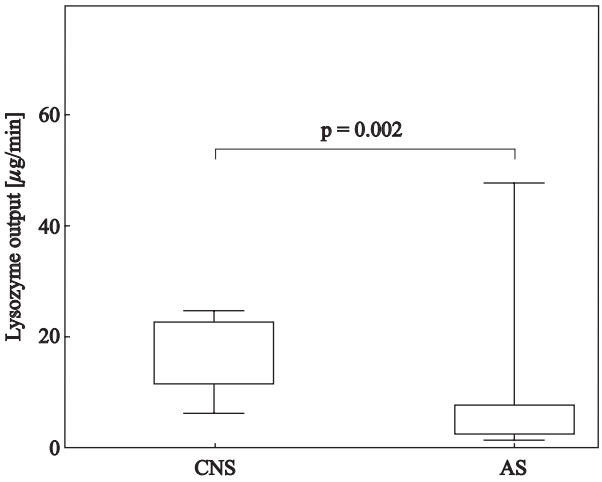

Figure 1. Lysozyme output in control non-smokers (CNS) and smoking alcohol-dependent persons (AS)

Table 1. Effect of chronic ethanol intoxication and smoking (AS) on the DMFT (decayed, missing or filled teeth), GI (gingival) and PBI (papilla bleeding) indices compared to control non-smokers (CNS)

Variable	CNS	AS
DMFT	18.8 ± 5.7	19.5 ± 5.7
GI	0.30 ± 0.47	0.99 ± 0.76**
PBI	0.35 ± 0.48	0.57 ± 0.27

Values are expressed as the mean ± SD. **statistically significant between CNS and AS groups, $p<0.01$

hol consumption as well as cigarette smoking, and the salivary concentration as well as secretion of the lysozyme. Also, there were no significant correlations between salivary lysozyme output/concentration and SF. However, we found a significant inverse correlation between lysozyme output and DMFT index ($r^2 = -0.44$, $p = 0.026$).

There is known to be an association between heavy alcohol drinking and poor oral hygiene, as dental and oral hygiene are often neglected by persons addicted to alcohol [11]. In earlier studies, we and others found that salivary flow (SF) was significantly lower in smoking alcohol-dependent persons than in controls [8, 13]. It is also known that in alcohol abusers, reduced salivary flow (SF) may lead to oral cavity inflammation, infection and periodontal disease [14].

Generally, about 80% of alcohol-dependent persons smoke cigarettes [11]. Smoking is usually associated with poor oral hygiene and a heavier course of periodontitis than non-smoking [11]. Similarly to alcohol, cigarette smoke is a source of oxidative stress and increased concentrations of acetaldehyde in the oral cavity [8–11]. In alcohol abusers and smokers, there have been reported disturbed function of the salivary glands with concomitant decrease in the secretion of salivary proteins, impairment in the immune mechanisms such as phagocytosis, chemotaxis, increased production of peroxides, and impaired function and proliferation of B and T lymphocytes and production of immunoglobulins [3, 8, 15]. People who chew tobacco have decreased levels of lysozyme and lactoferrin and increased secretion of immunoglobulin A in their saliva [9, 10], which indicates reduced innate and activated acquired immunity of the saliva. Persons addicted to alcohol have been reported to have lower output of lactoferrin (non-smokers) [10] and immunoglobulin A (smokers) [9], which, together with the reduced output of lysozyme in our study, indicated suppression of immunity mediators present in the saliva. Generally, both alcohol abuse and cigarette smoking produce a synergistic increase in the concentration of acetaldehyde in the saliva [9]. In our research, the lack of correlation between the amount of alcohol/cigarettes and duration of alcohol intoxication/smoking with lysozyme concentration and output, as well as with GI, PBI and DMFT values, suggests an indirect damaging effect of alcohol and smoking on tissues and innate immunity of the oral cavity. It is known that smoking alone increases temporarily resting and stimulated salivary flow, which is due to the irritating effect of tobacco smoke on the oral mucosa [11]. Therefore, the reduction of the salivary flow found by us in earlier studies resulted from alcohol intoxication, rather than smoking [8, 9, 11].

It is known that the processes of macrophage mobilisation and subsequent bacteria inactivation are substantially impaired by acute and chronic alcohol abuse [16]. As about 25% of proteins secreted by macrophages consist of lysozyme, the lysozyme level may characterise the activity of the monocyte/macrophage system [12]. Lower levels of lysozyme in alcohol abusing patients than in controls, and thus a greater reduction in the activity of the monocytes/ /macrophage system (compared to the neutrophil system), has been reported after chronic alcohol intoxication in alcohol-addicted persons [12]. Acute exposure to ethanol or its primary metabolite acetaldehyde has been shown to inhibit lysozyme activity and the release of lysozyme from monocytes, whereas activity of neutrophils, characterised by the secretion of such markers as β-glucuronidase and lactate dehydrogenase, was unchanged [17, 18]. In our study, the reduction in lysozyme output after chronic alcohol intoxication is partially explained by the available literature [17, 18]. It has been reported that ethanol can

inhibit the accumulation of lysozyme in the cells (also in salivary duct cells) and its subsequent release [17], and that dehydration caused by ethanol can lead to lysozyme denaturation [19, 20]. Also acetaldehyde, the main and highly reactive metabolite of ethanol, can inactivate the lysozyme by modification of the indole and amide groups of aminoacids, or guanidine residues of lysozyme's arginine [17]. The ethanol-induced inhibition of calcium pump, or the increase in the intracellular concentration of adenylyl cyclase, may also be taken into consideration as mechanisms of reduced lysozyme output [18].

In conclusion, chronic ethanol intoxication in smokers reduces salivary lysozyme output, probably as the result of ethanol action rather than smoking. Persons addicted to alcohol and nicotine show deteriorated periodontal status compared to controls, which partially may be due to the diminished protective effects of lysozyme present in the saliva.

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