Drug and Alcohol Dependence 129 (2013) 33–40

Contents lists available at SciVerse ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Salivary hexosaminidase in smoking alcoholics with bad periodontal and dental states

Napoleon Waszkiewicz[a,*], Sylwia Chojnowska[b], Anna Zalewska[c], Krzysztof Zwierz[d], Agata Szulc[a], Sławomir Dariusz Szajda[e]

[a] Department of Psychiatry, Medical University of Białystok, ul. Plac Brodowicza 1, 16-070 Choroszcz, Poland
[b] Medical Institute, College of Computer Science and Business Administration, ul. Akademicka 14, 18-400 Łomża, Poland
[c] Department of Paedodontics, Medical University of Białystok, ul. Waszyngtona 15a, 15-269 Białystok, Poland
[d] Medical College of the Universal Education Society, ul. Adama Mickiewicza 59, 18-400 Łomża, Poland
[e] Department of Emergency Medicine and Disasters, Medical University of Białystok, ul. Szpitalna 37, 15-295 Białystok, Poland

ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised form 8 September 2012
Accepted 9 September 2012
Available online 29 September 2012

Keywords:
Alcohol
Ethanol
Smoking
Saliva
β-Hexosaminidase
Marker

ABSTRACT

Background: A sensitive alcohol marker, β-hexosaminidase (HEX), in the saliva of alcoholics, is investigated for the first time.
Methods: The activity, specific-activity and output of total HEX and its isoenzymes HEX A and HEX B were measured in the saliva of healthy controls (C), alcohol-dependent non-smokers (ANS), and alcohol-dependent smokers (AS).
Results: We observed a significantly increased activity/specific-activity and output of HEX A in the ANS and AS groups, due to the inflammatory state of the oral-cavity/salivary-glands. Significantly increased activity of HEX A contributed to an increase in the salivary activity of the total HEX in the ANS group. A significant decrease in the activity/specific-activity of HEX B in AS seemed to be due to HEX B inactivation by cigarette smoke. We noticed a tendency for deteriorated dental state (lower decayed-missing-filled-teeth index – DMFT), worse periodontal state (higher gingival index – GI and papilla-bleeding index – PBI) in AS, and worse periodontal state (higher GI) in ANS, as compared to the controls. We found no differences in the salivary protein concentrations between all groups and decreased salivary flow in both alcoholic groups as compared to the controls. In alcoholics, the area under the curve (AUC) for HEX A activity/specific-activity was significantly greater than for HEX and HEX B. The salivary HEX A activity/specific-activity had good/excellent sensitivity and specificity in smoking and non-smoking alcoholics, whereas salivary HEX and HEX B had poor/fair sensitivity and specificity.
Conclusions: Salivary HEX A may be helpful in the diagnosis of chronic alcohol intoxication, even in smokers.

1. Introduction

Alcohol and tobacco are the most abundantly consumed noxious compounds worldwide (Seitz and Stickel, 2007). Approximately 80% of alcoholics smoke cigarettes (Romberger and Grant, 2004). Ethanol diffuses rapidly into saliva and oral tissues, and immediately after drinking, salivary concentration of ethanol is temporarily much higher than in plasma; whereas the level of ethanol metabolite–acetaldehyde in saliva exceeds 10–100 times the blood level (Waszkiewicz et al., 2008a, 2011a). Acetaldehyde found in alcoholics who smoke comes from ingested ethanol and tobacco smoke. Besides acetaldehyde, tobacco smoke is a source of oxidative stress, and contains up to 3000 toxic substances, such as nicotine, nitrosamines, carbon monoxide, and other aldehydes, that may damage the oral tissues (Ryder, 2007). Moreover, reactive oxygen species (ROS) generated during drinking and smoking, as well as non-oxidative metabolites of ethanol (e.g., fatty acid ethyl esters; FAEEs) and the ethanol–water competition mechanism, might be involved in the resulting damage of oral tissue (Waszkiewicz et al., 2011a,b, 2012a).

N-acetyl-β-hexosaminidase (hexosaminidase; HEX) is a lysosomal exoglycosidase releasing *N*-acetylhexosamines from glycoconjugates (Waszkiewicz et al., 2011b). The HEX A isoenzyme in body fluids reflects enzyme loss during cell turnover, secretory activity of cells, activates mitogenesis factors (e.g., protein kinase), and is a physiologically significant inflammatory mediator secreted together with HEX B into the extracellular space by mast cells, basophils, macrophages, eosinophils, and neutrophils. Because HEX

* Corresponding author. Tel.: +48 85 7193977; fax: +48 85 7193977.
E-mail addresses: napoleonwas@yahoo.com, napwas@wp.pl (N. Waszkiewicz).

0376-8716/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.drugalcdep.2012.09.008

B is closely connected with the lysosomal membrane, its increase in body fluids is an early manifestation of an impaired membrane function, cellular damage and injury progression (Lew et al., 1999; Waszkiewicz et al., 2008b).

The increased activity of serum and urinary HEX has been reported in alcohol-dependent patients after chronic drinking, and in saliva, serum and urine after a heavy single binge drinking session (Waszkiewicz et al., 2008b). HEX B in serum and HEX in urine, are very sensitive markers of alcohol abuse (Waszkiewicz et al., 2011b). HEX levels fall rapidly to normal following 7–10 days of abstinence (Waszkiewicz et al., 2010; Waszkiewicz and Szulc, 2010). The increased activity of HEX in smokers was found in the serum of postmenopausal women (Hultberg et al., 1994), the urine of healthy persons (Hultberg et al., 1992) at the time of arsenic, cadmium and lead exposure coincidence (EL-Safty et al., 2004; Milnerowicz et al., 2008), or in the saliva of smoking diabetic patients (Knas et al., 2006).

Saliva has been proposed for the detection of alcohol abuse, e.g., by the determination of salivary aminotransferases and gamma-glutamyl-transferase (Shivashankara et al., 2011), ethanol (Tu et al., 1992), methanol (Heberlein et al., 2010), diethylene and ethylene glycol (Shin et al., 2008), or sialic acid (Pönniö et al., 1999). To assess exposure to tobacco smoke, measurements of salivary concentrations of nicotine and its metabolite, -cotinine, as well as thiocyanate, have been proposed (Galanti, 1997; Soo-Quee Koh and Choon-Huat Koh, 2007; Tricker, 2006).

Increasing attention to the importance of saliva testing is due to the need for a noninvasive and easy method of assessing drug-intoxicated patients. This trend is not surprising as saliva collecting is easy, without the risk of needle-stick injuries, devoid of stress, with possible self-collection after instruction. Saliva contains a wide array of components that are very sensitive to toxic substances and reflects a real-time level of biomarkers (Soo-Quee Koh and Choon-Huat Koh, 2007). Therefore, the aim of this study was to investigate the effect of chronic alcohol intoxication and smoking on the activity of total salivary HEX, HEX A and HEX B.

2. Materials and methods

2.1. Participants

Eighty-five individuals participated in the study: 40 alcohol-dependent smoking individuals (AS; 8 females, 32 males) aged 21–64 years (43 ± 9.4; mean ± SD), and 10 alcohol-dependent non-smoking individuals (ANS; 10 males) aged 22–56 years (44 ± 8.9), recruited from the alcohol detoxification unit. The chronic alcohol intoxication ranged from 3 to 90 days (mean ~23) in AS and 3–14 days (mean ~6) in ANS group. During intoxication, AS individuals drank an average 231 (100–700) g and ANS – 162 (100–250) g of alcohol per day. Alcohol-dependent individuals met the criteria for the alcohol and nicotine (AS) or only for alcohol dependence (ANS), according to the ICD-10 and DSM-IV criteria. The average time of dependence was 13 ± 7 years for the alcohol and 21 ± 8 years for the smoking in the AS group, and 11 ± 7 years for the alcohol in the ANS group. AS participants smoked 18 ± 6 cigarettes daily. The control group consisted of 35 healthy social drinkers (C group; 12 females and 13 males) aged 30–53 years (41 ± 6.9) with no history of alcohol abuse or smoking.

2.2. Procedure

2.2.1. Ethical issues. The study was approved by the local Bioethical Committee and was 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.

2.2.2. Data and sample collection. Material in the Detoxification Unit was collected on the second day of abstinence, via an examination with dental mirror and probe by a qualified dentist in artificial light. Following the WHO criteria, the level of dental caries was determined using the DMFT index (D – decayed, carious tooth; M – missing tooth because of caries, FT – filled tooth) (Waszkiewicz et al., 2008a). Gingival status was assessed using the gingival index (GI; Loe and Silness, 1963) and papilla bleeding index (PBI; Saxer and Muhlemann, 1975). 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 DMFT, GI, and PBI indices are presented in Table 1. The subjects were instructed to refrain from all smoking, food and beverages, except water, for 2 hours before saliva collection. All samples (3 ml) of residual whole saliva were collected in plastic tubes placed on ice by the spitting method, under standardized conditions (Dawes, 1987; Navazesh et al., 1992), between 8:00 and 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 collection.

2.3. Analytical methods

Activities of HEX, HEX A and HEX B in supernatants of the saliva were determined by the method of Marciniak et al. (2006) with 4-nitrophenyl-β-D-*N*-acetylglucosaminopyranoside (Sigma, St. Louis, MO, USA) as a substrate. HEX α- and β-subunits compose heat-labile HEX A (αβ) and heat-stable HEX B (ββ). Heat-stable HEX B was measured after selective heat denaturation of thermolabile HEX A. HEX A was calculated from the difference between the total HEX and HEX B activity. Measurements of *p*-nitrophenol released by the HEX activities were carried out at 405 nm using the microplate reader Elx800. The activities of HEX, HEX A and HEX B were assayed in duplicate, and the means were used as final values.

The salivary protein concentrations (Pc) were duplicated by the bicinchoninic acid BCA method (PIERCE BCA Protein Assay Kit).

HEX activity is a measure of the quantity of active enzyme per 1 milliliter (pKat/ml). The specific activity of HEX is the activity of an enzyme per microgram of total protein (pKat/μg).

2.4. Statistical analysis

Statistical analysis was performed with the Statistica version 10.0 (Statsoft, Cracov, Poland). Normality estimations, using a Kolmogorov–Smirnov test, for the activity, specific activity and output of HEX, HEX A and HEX B, presented normal values (in all of three groups) only for the activity/specific activity of HEX. Nonparametric methods are most appropriate when the sample sizes are small (e.g., ANS group). Therefore, the comparisons between groups were made using the Kruskal–Wallis analysis and differences located using Mann–Whitney "*U*" test. Spearman's rank correlation coefficient was used to measure the statistical dependence between two variables in alcohol-dependent persons. A receiver performed characteristic (ROC) analysis, calculations of specificity, sensitivity and area under the ROC curve (AUC). Statistical significance was assumed at $p < 0.05$.

3. Results

3.1. Oral cavity and salivary parameters

No significant change in the salivary protein content (Pc) between controls and ANS and AS as well as between ANS and AS groups was found (Table 1).

The salivary flow (SF) was similar in ANS and AS, but significantly less in ANS and AS than in the controls ($p < 0.001$). In alcohol-dependent persons, there were significant inverse correlations between SF and: activity of HEX ($r = -0.422$, $p = 0.010$), HEX A ($r = -0.467$, $p = 0.004$) and salivary protein content ($r = -0.471$, $p = 0.003$); whereas no significant correlation was found between SF and activity of HEX B ($r = -0.280$, $p = 0.097$).

The DMFT index (decayed, missing, filled teeth) had the tendency to increase in AS comparing to C group ($p = 0.065$). There was similar DMFT in ANS and C, as well as in ANS and AS. The DMFT correlated positively with GI ($r = 0.418$, $p = 0.002$) and PBI ($r = 0.339$, $p = 0.013$). We found positive correlations between DMFT and activity ($r = 0.304$, $p = 0.028$) and the output ($r = 0.284$, $p = 0.040$) of HEX A (Fig. 1).

The gingival index (GI) was similar in ANS and AS, but significantly higher in ANS and AS than in C ($p = 0.004$ and $p < 0.001$, respectively). The GI in alcoholics correlated positively with salivary protein concentration ($r = 0.483$, $p = 0.042$), HEX A activity ($r = 0.439$, $p = 0.002$), HEX A specific-activity ($r = 0.474$, $p = 0.002$), HEX A output ($r = 0.526$, $p < 0.001$) and output of HEX B ($r = 0.330$, $p = 0.028$).

The papilla bleeding index (PBI) was similar in ANS and C, ANS and AS, but significantly higher in AS, comparing to the controls