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SALIVARY ALDEHYDE DEHYDROGENASE ACTIVITY – INFLUENCE OF DRUGS INTAKE, PRELIMINARY RESEARCH

JOANNA GIEBUŁTOWICZ[1*], PIOTR WROCZYŃSKI[1] and JACEK WIERZCHOWSKI[3]

[1]Department of Bioanalysis and Drugs Analysis, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha St., PL-02097 Warszawa, Poland
[2]Department of Biophysics, University of Warmia and Mazury, 4 Oczapowskiego St., PL-10719 Olsztyn, Poland

Abstract: The salivary aldehyde dehydrogenase (ALDH3A1) oxidizes mainly aromatic and long chain aliphatic aldehydes. This enzyme protects organisms from aldehydes originating from food and air pollution, and can also be an important factor in chemical carcinogenesis prevention. In the majority of saliva samples the ALDH3A1 is more than in 60% inactive due to sulfhydryl groups oxidation in the active site. Our previous studies showed that the ALDH3A1 activity (the total activity and the inactivation degree) is strongly variable during a day and within the healthy population. The aim of the present study was to describe the influence of drugs intake on ALDH3A1 activity. Hierarchical clustering grouped two dimensional data (the total ALDH activity, the inactivation degree) derived from a group of 124 subjects into four clusters. Clusters were analyzed by a correspondence analysis. The total ALDH3A1 activity and an inactivation degree vary in healthy subjects depending on many factors including cigarette smoking, alcohol and coffee consumption and age. This work demonstrates that treated hypertension and acute pain/infection as well as hormonal contraceptive drugs intake also significantly affect the salivary ALDH activity. The result is significant for food safety and nutrition research.

Keywords: aldehyde dehydrogenase, enzyme oxidation, saliva, non-opioid analgesics, hormonal contraceptive drugs, hypotensive drugs

The salivary ALDH3A1 (E.C. 1.2.1.3) is a homodimeric enzyme oxidizing mainly long-, medium-chain aliphatic and aromatic aldehydes (but not acetaldehyde) and utilizing both NAD^+ and $NADP^+$ as cofactors (1). It is involved in neutralization of a variety of aldehydes (especially aromatic) of plant origin as well as those formed during food processing or applied as food additives, and therefore may be directly related to protection and prevention of chemical carcinogenesis. Moreover, the ALDH3A1 protective role in oxidative stress is considered because of its relatively high affinity (Km = 45 μM) for 4-hydroxynonenal (4-HNE), whose concentration increases as a result of oxidative stress and lipid peroxidation. 4-HNE revealed cytotoxic effects such as RNA, DNA synthesis inhibition and cycle arrest. 4-HNE can also modify proteins by reacting with their residues like cysteine, lysine or histidine (2–5).

The ALDH3A1 expressed selectively in various tissues is responsible, along with ALDH1A1, for cellular resistance to oxazaphosphorine drugs (6).

We have shown recently that the salivary ALDH undergoes reversible oxidation in thiol-free media and the degree of such oxidation can be measured using fluorimetric two-assay method, utilizing fluorogenic naphthaldehyde substrates (7). This method profits from the fact that dithiols like dithiothreitol (DTT) or dithioerythritol are able to regenerate the previously oxidized enzyme within few minutes.

The total ALDH3A1 activity and the degree of inactivation vary with time and are highly variable in the healthy population (8). The total ALDH3A1 activity and an inactivation degree vary depending on cigarette smoking, alcohol and coffee consumption as well as an age and a diet. It is therefore interesting to check, if drug intake affects its activity as well.

EXPERIMENTAL

Chemicals

Syntheses of 6-methoxy-2-naphthaldehyde (MONAL-62) and the corresponding carboxylic

* Corresponding author: e-mail: jgiebultowicz@wum.edu.pl

acid were described previously (7); both compounds are now available from Sigma. Concentration of the stock solutions was determined spectrophotometrically by use of molar extinction coefficients 14400 for MONAL-62 and 7100 for the corresponding carboxylate. NAD^+, glutathione (GSH) and DTT were purchased from SIGMA (St. Louis, USA); all chemicals were of analytical grade. Water was purified with a Mili-Q filter system (Millipore Corp., USA).

Saliva collection

Saliva samples (n = 124) were collected directly to test tubes with 50 mM pyrophosphate buffer pH 8.1 with 0.5 mM EDTA and 1 mM GSH. Samples were collected between 8 and 9 a.m. after carefully washing the mouth with water, before the first meal and kept at 5°C. The activity was measured within 2–5 hour following the collection in the supernatant gently collected and stored in ice, obtained after centrifugation at 10,000 rpm. Saliva donation was preceded by filling in an anonymous interview indicating age, sex, intake of drugs, alcohol and coffee as well as cigarette smoking during the past week. People suffering from metabolic diseases or cancer were excluded from the analysis.

Methods

ALDH assay

Fluorimetric assays were run in 50 mM phosphate, pH 8.1, at 25°C, in the presence of 0.5 mM EDTA and either 0.5 mM DTT or 1 mM GSH. The assays utilize a highly fluorogenic naphthaldehyde substrate, 6-methoxy-2-naphthaldehyde (MONAL-62), reacting with NAD^+ as a co-substrate. The estimated apparent K_m values were 0.24 µM for MONAL-62 and 1.2 µM for NAD^+ (7).

Typical substrate concentrations were 5 µM for MONAL-62 and 100 µM for NAD^+. The saliva samples were diluted 20-fold with buffer, and an increase in fluorescence of the naphthoate was recorded for 6–10 minutes.

The purified reaction product (6-methoxy-2-naphthoate) at concentrations of 1–3 µM was used as an internal standard to obtain absolute reaction rates, which were calculated according to the formula:

$$V = \frac{dF}{dt} \cdot \frac{C_{st}}{F_{st}}$$

where dF/dt is the fluorescence slope, C_{st} is the concentration of standard and F_{st} is the fluorescence of standard. Specific activities were calculated as the ratio of the reaction rate to protein concentration, the latter determined by the Bradford method.

The degree of ALDH inactivation was determined by two consecutive assays (7). The activity was measured in the presence of 1 mM GSH ("actual activity"), which prevents further oxidation of enzyme and in the presence of 0.5 mM DTT ("total activity") – the thiol which reactivates an oxidized enzyme. The inactivation degree was calculated using the following formula:

$$I = \left(1 - \frac{V_{GSH}}{V_{DTT}}\right) \cdot 100\%,$$

where V_{GSH} and V_{DTT} are the reaction rates determined in the presence of 1 mM GSH and 0.5 mM DTT, respectively.

Fluorimetric assay was run on thermostated Shimadzu 5001PC spectrofluorimeter. Instrumental settings were as follows: excitation wavelength 310 nm, emission wavelength 360 nm; spectral bandwidths was 3–5 nm for excitation and 5 nm for emission.

Statistical analysis

We grouped individuals (n = 124) described with two dimensional date (the total ALDH3A1 activity and the inactivation degree) using Ward's

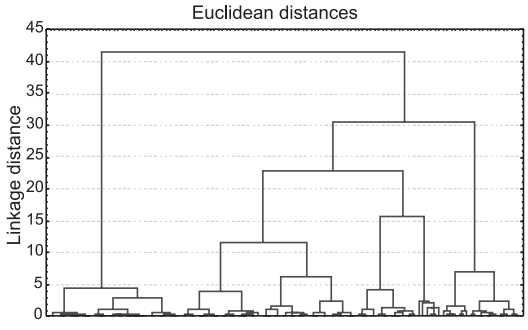

Figure 1. Hierarchical tree diagram (Ward's method) for 124 cases gruped by two dimensional variable (ALDH3A1 activity [U/g], inactivation degree [%])

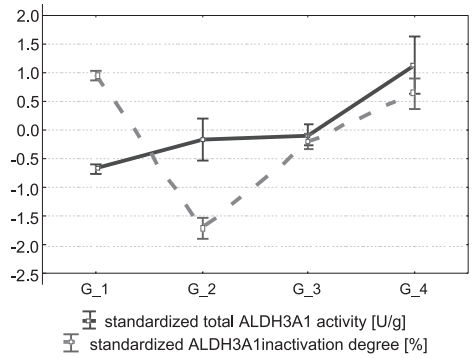

Figure 2. Standardized total ALDH3A1 [U/g] activity and it's inactivation degree [%] in four clusters

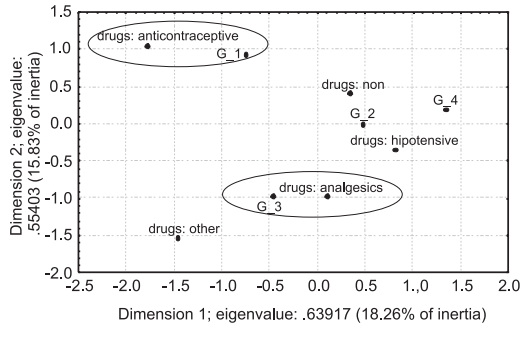

Figure 3. A correspondence map of drug intake by subjects from G1, G2, G3 and G4 group (dimension 1 i 2)

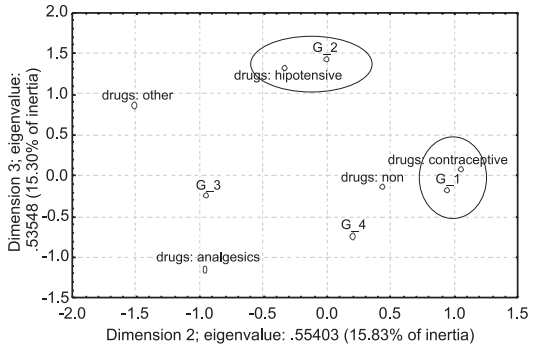

Figure 4. A correspondence map of drug intake by subjects from G1, G2, G3 and G4 group (dimension 2 i 3)

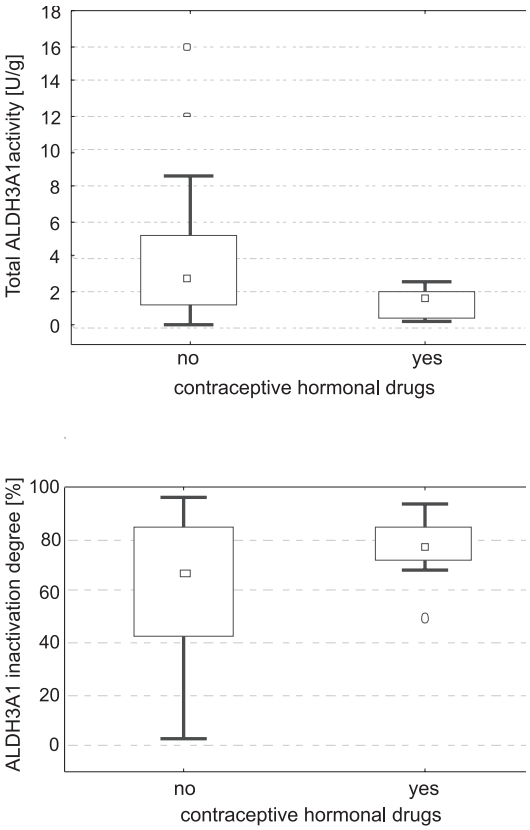

Figure 5. Median, quartile deviation, minimum and maximum of total ALDH3A1 activity [U/g] (left) and inactivation degree [%] (right) in group taking contraceptive hormonal drugs (n = 14) or not (n = 36)

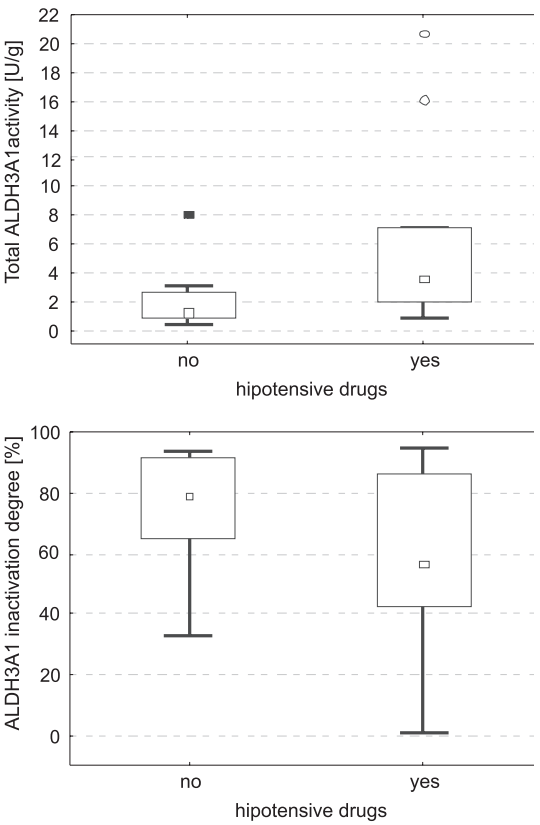

Figure 6. Median, quartile deviation, minimum and maximum of total ALDH3A1 activity [U/g] (left) and inactivation degree [%] (right) in group taking hypotensive drugs (n = 14) or not (n = 12

hierarchical clustering method (agglomerative method).

Considering the obtained results presented in Figure 1 we decided to create four clusters: G_1, G_2, G_3, G_4. Medians of standardized ALDH3A1 total activity and inactivation degree are presented in Figure 2. Cluster G_1 had lower than an average the total ALDH3A1 activity and the higher inactivation degree, whereas cluster G_2 had lower than an average the inactivation degree and an average the total ALDH3A1 activity. Cluster G_3 had an average the ALDH3A1 activity and the inactivation degree, whereas cluster G_4 had higher than average values of these variables.

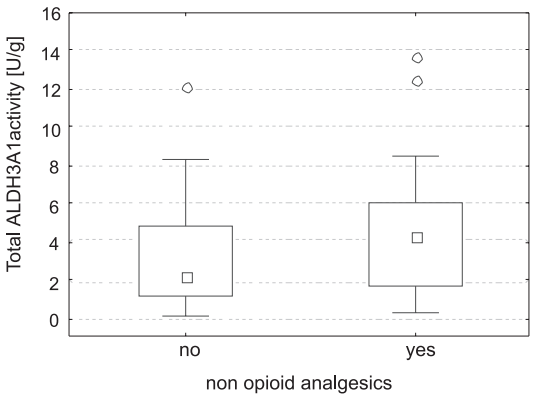

Figure 7: Median, quartile deviation, minimum and maximum of total ALDH3A1 activity [U/g] in group taking non-opioid analgesics (n = 21) or not (n = 61)

Using a correspondence analysis technique, we analyzed dependency between group affiliation and some quantitative variates such as a sort of drug intake. Only the most frequent ones were analyzed: hypotensive drugs (72% bisoprolol, 15% enalapril, 13% carvedilol and others), non-opioid analgesics (35% aspirin, 24% ibuprofen, 15% diclofenac, 15% paracetamol, 11% pyralgin and ketoprofen) and contraceptive hormonal drugs. Non-opioid analgesics were taken occasionally because of an acute pain (76%) or an infection (24%).

The results after limiting dimension to three, revealed that subjects taking contraceptive hormonal drugs are mainly in cluster G_1, those taking non-opioid analgesics are in G_3 , whereas those taking hypotensive are in cluster G_2 (see Figure 3 and 4). In the next stage of analysis, we selected all subjects taking these drugs, divided them into three groups (depending on a sort of drug) and created for them control groups of people of the same age, taking no drugs and having similar smoking and drinking habits (alcohol as well as coffee). We compared the total ALDH3A1 activity and the degree of its inactivation between the analyzed and the control groups using the unpaired Student *t*-test. In case of lack of normal distribution or homogeneity of variances in data the Mann Whitney U test was used.

RESULTS AND DISCUSSION

We have shown previously that the salivary ALDH3A1 activity (the total activity and the inactivation degree) is intra- and interpersonally highly variable, and additionally, high variability was recorded throughout the day time, with the highest activities observed between 8 and 10 a. m. The total ALDH3A1 activity decreases with age and increases with alcohol consumption and cigarette smoking, whereas the inactivation degree increases with age and coffee consumption (8). Having analyzed the obtained data, we observed some dependence between ALDH3A1 activity and some kinds of drug intake.

The subjects taking contraceptive hormonal drugs revealed the statistically significant lower total ALDH3A1 activity ($p = 0.037$, see Fig. 5) and the higher inactivation degree ($p = 0.043$, see Fig. 5 5). Moreover, the variability of the data in this group was much lower than in the control group. According to the literature, oral contraception can influence oxidative stress and significantly increases the lipid peroxidation and decreases plasma antioxidants (e.g. β-carotene, enzymes) (9–13). This phenomenon combined with lower „actual" activity of ALDH3A1, which also metabolizes toxic 4-HNE, could be the risk factor of cardiovascular diseases; 4-HNE resulting from lipid peroxidation.

Hypotensive drugs intake results in the higher total ALDH3A1 activity ($p = 0.008$, see Fig. 6) and the lower inactivation degree ($p = 0.070$ NS, see Fig. 6), whereas non-opioid analgesics intake results only in the higher ALDH3A1 activity ($p = 0.002$, see Fig. 7). Hypertension is correlated with lower antioxidants concentration and higher oxidative stress. Several β-blockers and angiotensin converting enzyme (ACE) inhibitors decrease oxidative stress. Despite this fact, some oxidative stress markers do not reach the level of healthy subjects (14, 15).

According to the literature, non-opioid analgesics (non steroidal anti-inflammatory drugs and paracetamol) induce oxidative stress, e.g., *via* the NADPH oxidase (the enzyme present also in salivary glands), induction of which results in enhanced superoxide ($O_2^{\cdot-}$) production and plasma concentrations of total peroxides. However, the oxidative stress could also be generated by acute pain or/and infection (16, 17).

The defence mechanism against oxidative stress, which we observed in smokers (to be published elsewhere), was related to the higher total ALDH3A1 activity and the lower inactivation degree. Similar results were obtained in case of treated hypertension and acute pain/infection treated with non-opioid analgesics. Reactive oxygen species and its reactive compounds/metabolites induce the expression of protective enzymes *via* an anti-oxidant response element (ARE) or electrophilic response element (EpRE) (18). ALDH3A1 transcription was reported to be also upregulated *via* EpRE, which is present in the 5'-upstream region of this isozyme (19).

However, we cannot exclude another type of upregulation of this isozyme transcription by means of hypotensive and non-opioid analgesics.

CONCLUSION

The ALDH3A1 in saliva is the first detoxification barrier against potentially toxic aldehydes derived from food and air pollution as well as those formed during oxidative stress. This enzyme activity is strongly variable and depends on drug intake and health condition. Preliminary research revealed that treated hypertension, non-opioid analgesics used in case of acute pain/infection and contraceptive hormonal drugs affects its activity. The knowledge about factors influencing the salivary ALDH3 activity seems to be important for food and drug safety as well as for nutrition research.

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