

Serum and urinary β -glucuronidase in acute alcohol intoxication: a pilot study

Waszkiewicz N.^{1*}, Zalewska-Szajda B.², Buras A.¹, Szulc A.¹, Zwierz K.³, Ładny JR.⁴, Szajda SD.⁴

¹ Department of Psychiatry, Medical University of Białystok, Poland

² Department of Imaging Diagnostics, Medical University of Białystok, Children Hospital, Poland

³ Medical College of the Universal Education Society, Łomża, Poland

⁴ Department of Emergency Medicine and Disasters, Medical University of Białystok, Poland

ABSTRACT

Introduction: Beta-glucuronidase (GLU) is a member of the lysosomal glycosidase family that catalyzes hydrolysis of β -D-glucuronic acid residues from the non-reducing end of glycosaminoglycans. Increased activities of GLU have been earlier reported in the serum of alcohol-dependent patients after a chronic heavy drinking period but not after acute intoxication (called binge drinking). The accelerating binge drinking phenomenon is an alarming public health issue that requires better prevention.

Purpose: To determine the activity serum and urinary GLU, after an acute, single, and a large dose of alcohol intoxication.

Materials and methods: The serum and urine of eight healthy binge drinkers were collected before binge drinking, and 2 and five days after the

drinking session. The activity of GLU was determined by the colorimetric method.

Results: There was a tendency to decrease in the serum GLU activity two days after acute alcohol intoxication (binge drinking), which was followed by the significant increase in the GLU activity five days after drinking. The urinary activity of GLU was not changed after intoxication.

Conclusion: Alcohol-induced imbalance in the serum GLU activity might be associated with alcohol-induced liver hypoxia and subsequent reperfusion, and can be detected even five days after the drinking session.

Key words: β -glucuronidase, acute alcohol intoxication, binge drinking, serum, urine, aminotransferase

*Corresponding author:

Department of Psychiatry
Medical University of Białystok
16-070 Choroszcz, Poland
Tel./ Fax: +48857193977
Email: napwas@wp.pl (Napoleon Waszkiewicz)

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INTRODUCTION

Binge drinking problem has received considerable medical and media attention in recent years. Acute alcohol intoxication, known as a binge drinking, occurs on a third of drinking occasions [1]. An increasing number of working young adults use alcohol as a recreational drug, tending to concentrate their drinking at weekends. It was estimated that 38.5% males and 8.5% females in Poland's binge drink. It was also found that significantly more men than women reported more than five binge episodes during the past three months (11.3% versus 1.9%) [2]. Binge drinking increases the risk for numerous acute adverse health and social events including alcohol poisoning, acute myocardial infarction, injuries, accidents, suicide, interpersonal violence, etc [3-5]. Therefore, the binge drinking problem is an alarming public health issue that requires better prevention. Binge drinking is characterized by the consumption of alcohol leading to intoxication (drinking to get drunk), often measured as having more than four (women) or five (men) number of drinks on one occasion (1 standard drink contains 10 g of ethanol), or bringing blood alcohol concentration (BAC) above 0.08 gram percent in about 2 hrs. Other binge measures include drinking over half the "sensible" number of units per week (1–14 units per week for women and 1–21 for men), or double the recommended daily guidelines in one session [5-8].

The lysosomal exoglycosidase, β -glucuronidase (GLU), is involved in the hydrolysis of β -D-glucuronic acid residues from the non-reducing end of glycosaminoglycans [7, 8]. Earlier studies found the increased activity of GLU in the plasma of alcohol-dependent patients after a heavy drinking period [9], and in the saliva of healthy persons after acute alcohol intoxication [7]. Generally, serum exoglycosidases such as β -hexosaminidase, α -fucosidase, and α -mannosidase, are increased in the serum of patients suffering from alcohol dependence, which seem to be due to the chronic liver damage [5, 7, 10-12]. In persons addicted to alcohol, increased activity of lysosomal exoglycosidases was found also in the saliva [13].

Changes in the metabolism of liver glycoconjugates (glycoproteins, glycolipids, and proteoglycans) during alcohol drinking, are ascribed to the alterations in the synthesis, transport, glycosylation, secretion, degradation, and elimination processes, which occur also in the extracellular matrix and serum [8]. The alcohol-induced pathomechanism of the liver damage is complicated by the fact that the effect of alcohol intoxication on the glycoconjugate metabolism depends not only on the duration of ethanol exposure, but demonstrates also dose-sensitivity [3, 8]. In the liver tissue, damages involve the action

of: ethanol (ethanol-water competition mechanism), acetaldehyde, reactive oxygen species (ROS), and nonoxidative metabolites of alcohol — fatty acid ethyl esters (FAEEs) [1, 3, 8].

The aim of the study was to examine, whether the single acute alcohol intoxication can change the activity of serum or urinary β -glucuronidase in healthy persons.

MATERIALS AND METHODS

Participants and procedure

Eight healthy male volunteers aged 27.5 [27, 28] (median [Q1, Q3]) (range 22 to 31 years), who anticipated heavy drinking on Friday evening, took part in the study. The body weight was 74.5 [72, 85.5] kg (range 71 to 98 kgs). Prior to the experiment, all volunteers were verified clinically to be in good general health. None of the participants were taking medication. All of them have a history of social drinking or occasional drinking, but they did not meet any alcohol abuse criteria. All men were infrequent binge drinkers (reported bingeing 1–11 times yearly and/or 1–2 episodes in the past month), who had abstained from alcoholic beverages and drugs for 10 days, before the experiment. The participants stayed at home during the drinking session, under the supervision of sober friends and a physician, who helped verify quantities and the time when drinking stopped. During the alcohol session (7 p.m. to 1 a.m.), participants drank 120–160 g of ethanol (12–16 standard drinks) as 40% vodka (2.1 g/kg of body weight; ranging from 1.42 to 2.5 g/kg), together with light meals and fruit juice (excluding grapefruit juice). Such amounts of alcohol are common in spirit-drinking countries, including Poland, provoking a tolerable but severe intoxication [5]. The study was approved by the local Bioethical Committee of the Medical University of Bialystok, Poland. Informed written consent was obtained from all participants after the explanation of the nature, purpose and potential risks of the study. The subjects were deprived of food and beverages, except water, for 2 h before sample collection. The blood and urine samples were collected (before drinking -BD, on second day-after drinking, and on fifth day after drinking), and then centrifuged to remove cells. The supernatants were divided into 200- μ L portions, frozen and kept, until analyzed.

β -glucuronidase assay

The activity of GLU in supernatants of serum and urine, was determined in duplicates by the colorimetric determination of p-nitrophenol

released from p-nitrophenyl- β -D-glucopyranoside (Sigma, USA) [14].

Aminotransferases assay

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to standard laboratory techniques (EMAPOL reagents, Poland).

Statistical analysis

Statistical analysis was performed using Statistica 10.0 PL (Statsoft, Tulsa, OK, USA) . Changes of the GLU activity across time were analysed by Friedman's analysis of variance (ANOVA) and Kendall's concordance. For comparison between aminotransferases levels, Wilcoxon matched pair test was used. Spearman's rank correlation coefficient was used to measure the statistical dependence between two variables. Statistical significance was defined as $p < 0.05$.

RESULTS

There was a statistically significant effect of binge drinking on the serum GLU activity ($p < 0.001^{***}$). After the binge drinking session, the activity of serum GLU (pkat/ml) had tendency to decrease at 2nd day (105.5 [73, 155]), when compared to the preconsumption activity (177.5 [167, 181]) ($p = 0.093$). At 5th day, the activity of serum GLU (200 [186, 211]) increased, when compared to the 2nd day ($p = 0.0004^{***}$) (Fig. 1.).

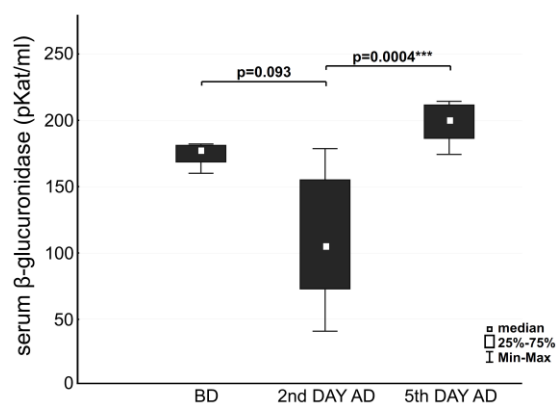


Figure 1. The activity of serum β -glucuronidase before drinking (BD), on second day after drinking (2nd DAY AD), and on fifth day after drinking (5th DAY AD). There was a statistically significant effect of binge drinking on the serum GLU activity ($p = 0.0006^{***}$).

There was no statistical significant difference between preconsumption day and 5th day thereafter ($p = 0.247$). In the urine, the activity of GLU before drinking, at 2nd and at 5th day after

drinking, were respectively 163 [126, 180], 138 [106, 160], 149 [139, 162] (pKat/ml). No significant changes in the urinary activity of GLU after the binge drinking session were found. ($p = 0.553$) (Fig. 2.).

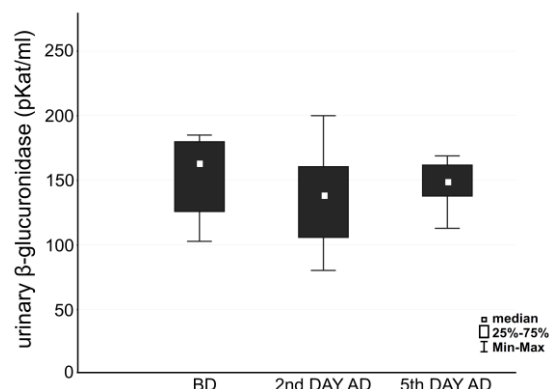


Figure 2. The activity of urinary β -glucuronidase before drinking (BD), on second day after drinking (2nd DAY AD), and on fifth day after drinking (5th DAY AD). There was no statistically significant effect of binge drinking on the urinary GLU activity ($p = 0.247$).

The AST activity was significantly lower at fifth day after binge drinking session than at second day ($p = 0.027^*$) (Tab. 1).

Table 1. The effect of binge drinking on the serum activity of aspartate (AST) and alanine (ALT) aminotransferases at second day after drinking (2nd DAY), and at fifth day after drinking (5th DAY 2nd DAY to 5th DAY AD

Variable	2nd DAY	5th DAY	<i>p</i> Value
AST	30 [24, 37]	23 [19, 32]	0.027*
ALT	36 [28, 46]	36 [33, 45]	0.398

Values are expressed as median [Q1, Q3]; $n = 8$; p Value < 0.05 considered statistically significant p Value:

There was no significant difference in the ALT activity between second and fifth day after the binge drinking session ($p = 0.398$).

There were no any correlations between amounts of alcohol and: serum and urinary GLU activity, serum AST and ALT, at any time point after drinking.

DISCUSSION

The intestinal mucosal surface, damaged by the alcohol and its metabolites, becomes more permeable than a normally protected mucosa for the gut endotoxin (LPS-lipopolysaccharide) [1]. LPS by the receptor CD14, activates Kupffer cells to produce prostaglandin D2 and E2 (PGD2, PGE2), reactive nitrogen and oxygen species (RNS, ROS), endothelin-1 (ET-1), tumor necrosis factor- α (TNF- α), and interleukin 1 and 6 (IL-1, IL-6), resulting in the hypermetabolic liver state [1, 8]. It is known that the chronic alcohol-induced increase in the exoglycosidase activity (e.g. GLU) in the serum may be due to the lysosomal membrane permeability of the liver cells and leakage of the enzyme to the cell, intercellular space, and subsequently to the body fluid such as the serum and finally to the urine [3, 5, 8]. Other potential mechanisms of the increased serum GLU activity may include impaired glycosylation and trafficking of the enzyme to the organelles, leakage from damaged cells, from the activated Kupffer cells, or due to the decreased clearance of lysosomal enzymes from the blood [1, 8].

In our study, we observed a tendency to the temporal decrease in the activity of serum GLU at second day-after drinking, which was followed by the increase in the GLU activity at fifth day after drinking (Fig. 1.). At second day, the decrease in the serum GLU activity may be due to the exhaustion of GLU or its decreased synthesis in the temporarily hyperactivated/damaged liver [1, 8]. As GLU is considered to be an indicator of the neutrophil activity/influx and a marker for primary granule release by these cells as well as an acute alcohol intoxication (binge drinking) results in the temporal specific defects in the innate immunity (e.g. it decreases the activity of neutrophils) [7, 15], the decreased serum GLU activity might also be due to the temporal alcohol-induced neutrophil defect. As alcohol intoxication increases diuresis and urinary clearance of some enzymes [16], the reduced GLU activity might be the effect of its increased clearance. The exhaustion of GLU in the liver may potentially be compensated later by the adequate (over)production of the enzyme in the reperfused liver [8]. Ethanol metabolism by the hepatocytes tends to increase oxygen uptake, resulting in the significant hypoxia of the perivenous hepatocytes and release of the prostaglandin E2 and stimulation of the hepatocyte metabolic activity, which contributes to the onset of hypoxia [17]. After the binge drinking-induced temporal severe hypoxia, there was found a hepatic reperfusion injury and a second peak of the ROS release [17, 18]. Hence the subsequent reperfusion-induced liver cell damage may contribute to the additional hydrolase releasing including GLU [8], which may explain the increased GLU activity, five

days after drinking in our study, when compared to the 2nd day. Furthermore, further lipopolysaccharide-induced activation of the Kupffer cells, may contribute to the late liver dysfunction and to the increased synthesis of the lysosomal enzymes such as GLU [8]. As the decreased clearance of lysosomal enzymes from the blood (in hepatic reperfusion injury), has as well been proposed to be the explanation for the increased activity of exoglycosidases in the blood [8], the return of GLU activity to the preconsumption activity in our study, might as well be the result of reperfusion or returned function of neutrophils.

Since AST is generally a better and more specific marker of the alcohol-induced liver damage, and its increased activity was noted earlier in acute alcohol intoxication [6], the observed by us normalization of the serum GLU activity, at fifth day-after drinking, was likely due to the liver dysfunction throughout reperfusion. The GLU activity in the urine did not change significantly after the binge-drinking session (Fig. 2), which might be due to the not-disturbed renal function and balanced clearance of the GLU.

Although there are some limitations of the present study including a small binge drinking group, the comparison of the alcohol preconsumption to the postconsumption GLU activity was based on the results obtained from the same persons, which gives the reliability of our findings. Changes in the serum GLU activity may be potentially applicable to the binge-drinking prevention. Binge drinking may precede the sequence of events leading to alcoholism [19]. Thus, it is important to detect binge drinking as early as possible and to prevent these events. As binge drinking levels and prevention are well accepted as part of a primary healthcare physician's daily work, targeting new guidelines for people who "binge" together with feedback given based on the laboratory changes may potentially enhance the binge-drinking prevention strategy [19]. However, the usefulness of serum GLU activity as a laboratory feedback needs confirmatory further research, based on a relatively large sample to be sufficiently representative of a vast population.

CONCLUSIONS

1. A single acute alcohol intoxication (binge drinking) changes the serum activity of β -glucuronidase, which may be due to the alcohol-induced liver dysfunction.
2. Changes in the serum GLU activity can be detectable even five days after the binge-drinking session.

Conflicts of interest

We declare that we have no conflicts of interest.

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