Acta Histochemica 117 (2015) 712–717

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Acrylamide alters glycogen content and enzyme activities in the liver of juvenile rat

Renata Kovac*, Vesna Rajkovic, Ivana Koledin, Milica Matavulj

Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Vojvodina 21000, Serbia

ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form
17 September 2015
Accepted 21 September 2015

Keywords:
Acrylamide
Immature liver
Glycogen
Liver enzymes

ABSTRACT

Acrylamide (AA) is spontaneously formed in carbohydrate-rich food during high-temperature processing. It is neurotoxic and potentially cancer causing chemical. Its harmful effects on the liver, especially in a young organism, are still to be elucidated. The study aimed to examine main liver histology, its glycogen content and enzyme activities in juvenile rats treated with 25 or 50 mg/kg bw of AA for 3 weeks. Liver samples were fixed in formalin, routinely processed for paraffin embedding, sectioning and histochemical staining. Examination of haematoxylin and eosin (H&E)-stained sections showed an increase in the volume of hepatocytes, their nuclei and cytoplasm in both AA-treated groups compared to the control. In Periodic acid-Schiff (PAS)-stained sections in low-dose group was noticed glycogen reduction, while in high-dose group was present its accumulation compared to the control, respectively. Serum analysis showed increased activity of aspartate aminotransferase (AST), and decreased activity of alanine aminotransferase (ALT) in both AA-treated groups, while the activity of alkaline phosphatase (ALP) was increased in low-dose, but decreased in high-dose group compared to the control, respectively. Present results suggest a prominent hepatotoxic potential of AA which might alter the microstructural features and functional status in hepatocytes of immature liver.

1. Introduction

Acrylamide (AA) (CASR No. 79-06-1) is highly reactive, water-soluble monomer which is considered as toxic and potentially cancer causing chemical to humans. Adverse health effects regarding AA and its more reactive metabolite glycidamide (GA) were detected in experimental animals, and included neurotoxicity (Burek et al., 1980; Tyl et al., 2000; Lehning et al., 2003; LoPachin et al., 2003), genotoxicity (Paulsson et al., 2003; Maniere et al., 2005; Yang et al., 2005), and carcinogenicity (Carere, 2006; Hogervorst et al., 2010). Well-documented human epidemiological studies claim that AA has neurotoxic effects (Garland and Patterson, 1967; He et al., 1989) and it is probably carcinogenic to humans (IARC, 1994). So far, genotoxicity and carcinogenicity are considered as potential human health risks only on the basis of animal studies (Tyl et al., 2000).

General population can be mainly exposed to AA through consumption of carbohydrate-rich food processed at high temperatures (above 120 °C). AA is formed spontaneously during heat-induced non-enzymatic reaction, also known as the Maillard browning reaction, between reducing sugars (glucose and fructose), and free amino acids (mainly asparagine) (Tareke et al., 2002). Groceries such as: coffee, chocolate, almonds, French fries, crackers, potato chips, cereal and bread typically contain the highest levels of AA (Tareke et al., 2002). In addition to that, the presence of AA is detected in used frying oil and it is also a component of cigarette smoke (JECFA, 2005; Hays and Aylward, 2008). The average AA intake for the general population and high consumers is estimated to be approximately 1 and 4 μg/kg per body weight a day (μg/kg bw/day), respectively (JECFA, 2005). More importantly, it is anticipated that the children have 2–3 times higher AA intake than the adults (FAO/WHO, 2002; Dybing et al., 2005; JECFA, 2005). Even more, AA is contaminant of baby food and infant formulas (Erkekoğlu and Baydar, 2010). The later consequently emphasized a concern for the health of a younger human subpopulations regarding dietary AA exposure. The whole spectrum of changes and consequences caused by AA intake, especially in young, developing organism are still poorly understood.

Having in mind that AA metabolism takes place in a liver, the study aimed to investigate the main histological and biochemical

* Corresponding author at: Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovica 2, 21000 Novi Sad, Serbia.
E-mail addresses: renata.kovac@dbe.uns.ac.rs (R. Kovac), vesna.rajkovic@dbe.uns.ac.rs (V. Rajkovic), ivana.koledin@dbe.uns.ac.rs (I. Koledin), milica.matavulj@dbe.uns.ac.rs (M. Matavulj).

http://dx.doi.org/10.1016/j.acthis.2015.09.004
0065-1281/

changes in the liver of juvenile rat following subchronic AA intoxication. Studies related to the liver suggest that both AA and its metabolite, GA, may cause hepatotoxicity and carcinogenicity in rats (Yousef and El-Demerdash, 2006). In that prospect, examining the structural and functional events that take place in the immature liver following AA intoxication are essential to adequately protect a young organism from AA-related health risks. The general liver architecture, glycogen content as well as the serum activities of hepatic enzymes might be good initial parameters for the assessment of the structural and functional alterations in the immature liver after AA consumption.

2. Materials and methods

2.1. Animals

Experiment was performed on 30 peripubertal male Wistar rats aged 23 postnatal days at the beginning of the study. After being weighted, animals were randomly assigned into three experimental groups, with 10 animals per group. During the experiment, animals were maintained under a constant temperature of $22 \pm 01\,^{\circ}C$ and controlled photoperiod (12 h light, 12 h dark), had free access to a standard granulated food and drinking water.

All procedures were performed strictly according to the generally accepted international rules and regulations on animal experiments. The investigation was carried out with the permission of the Ethical Committee on Animal Experiments of the University in Novi Sad, Serbia (License No. IV-2010-01) and in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health.

2.2. Experimental design

Acrylamide (AA) (>99.5% pure) was purchased from Sigma–Aldrich Chemie GmbH, Germany, dissolved in distilled water and orally administered to rats via gavage. Two groups received 25 or 50 mg/kg bw of AA, respectively, 5 days a week, during 3 weeks, while the third group was the control and received distilled water in the same way. The doses applied in this study were chosen according to the previously reported studies involving subchronic exposure of rats to AA (El-Bohi et al., 2011; Rawi et al., 2012). Animals were anesthetized by ether inhalation and decapitated, in the morning hours between 8.30 and 9.00 h, 24 h after the last treatment. The liver tissue was sampled from the middle lobe for histological analysis, and the blood samples were collected for biochemical analysis.

2.3. Histology

Liver samples were fixed in 10% buffered neutral formalin for 24 h, routinely processed for paraffin embedding and cut into 5-μm thick serial sections for subsequent histochemical staining. The main liver histology was analyzed on sections stained with haematoxylin and eosin (H&E). Periodic acid-Schiff (PAS) reaction was used to visualize a presence and distribution of carbohydrates, particularly glycogen, in the liver cells. The intensity of purple colour in PAS-stained liver sections corresponds to the amount of glycogen in hepatocytes. Histological analysis was performed on PAS-stained liver sections from centrilobular to periportal region of liver lobule on every fourth serial section per animal using the Reichert light microscope.

2.4. Stereological analysis

In order to quantitatively examine the volume of hepatocytes and liver sinusoids, H&E-stained sections were subjected to a point-counting stereological analysis using the multipurpose stereological grid with 42 points (M42) (Weibel, 1979) placed in the ocular of a Reichert light microscope under the total magnification of 400×. Stereological analysis was performed on 20 randomly selected fields of vision per section on every fourth serial section per animal. Evaluated stereological parameters included the volume density of hepatocytes (Vvh), their nuclei (Vvh_n) and cytoplasm (Vvh_c) as well as the volume density of liver sinusoids (Vvs).

2.5. Serum preparation and analysis

Blood samples were collected and subsequently centrifuged at 1.800 rpm for 10 min at 4 °C. Serum samples were further analyzed by the Institute for the Application of Nuclear Energy, University of Belgrade, Serbia, according to the ISO 9001 standard. The serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) were analyzed in order to assess the liver functions as well as the concentration of total serum proteins.

2.6. Animal body weight

The body weight of rats was monitored throughout the entire experimental procedure.

2.7. Statistical analysis

The Kruskal–Wallis ANOVA test followed by multiple comparisons of mean ranks for all groups was applied to compare the differences in analyzed stereological parameters as well as in the body weights among the experimental groups. The one-way ANOVA followed by the Bonferroni's post hoc test was applied to compare the differences in biochemical parameters among the experimental groups. The p values less than 0.05 were considered significant.

3. Results and discussion

Histological examination of H&E-stained liver sections showed a normal liver architecture, with no marked morphological changes in both AA-treated groups when compared to the control. Nevertheless, stereological analysis detected some level of changes regarding the microstructural features of hepatocytes in the treated groups. Namely, the volume density of hepatocytes, their nuclei and cytoplasm (Vvh, Vvh_n, Vvh_c, respectively) were dose-dependently increased in both AA-treated groups compared to the control. The increase in Vvh and Vvh_c proved to be statistically significant in both AA-treated groups compared to the control (both at $p < 0.001$) (Table 1). Meantime, the volume density of liver sinusoids (Vvs) in both AA-treated groups was significantly decreased in a dose-dependent manner compared to the control (at $p < 0.001$) (Table 1).

Increased volume of liver parenchyma detected in rats from both AA-treated groups suggests a cell damage caused by AA intoxication, while decreased volume of liver sinusoids present may point to a reduced lobular circulation. According to Smith and Orrenius (1984), the cellular damage caused by toxic substances is mostly accompanied by an increase in cell membrane permeability. AA-induced structural changes in the liver may be caused by oxidative stress and perturbation of lipid and protein metabolism of a developing rat (Allam et al., 2010). Also, AA may induce a depletion of glutathione (GSH) levels in liver parenchymal cells which potentially may result in oxidative stress, leading to a loss of cell viability, apoptosis or necrosis (Tong et al., 2004). Veenapani et al. (2010) suggested that, following acute exposure to AA, the hepatocytes are able to get damaged from 24 mg of AA onwards, while Rawi et al. (2012) reported degeneration and even apoptosis in some