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Research Paper

Chronic aspartame intake causes changes in the trans-sulphuration pathway, glutathione depletion and liver damage in mice

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ABSTRACT

No-caloric sweeteners, such as aspartame, are widely used in various food and beverages to prevent the increasing rates of obesity and diabetes mellitus, acting as tools in helping control caloric intake. Aspartame is metabolized to phenylalanine, aspartic acid, and methanol. Our aim was to study the effect of chronic administration of aspartame on glutathione redox status and on the trans-sulphuration pathway in mouse liver. Mice were divided into three groups: control; treated daily with aspartame for 90 days; and treated with aspartame plus N-acetylcysteine (NAC). Chronic administration of aspartame increased plasma alanine aminotransferase (ALT) and aspartate aminotransferase activities and caused liver injury as well as marked decreased hepatic levels of reduced glutathione (GSH), oxidized glutathione (GSSG), γ-glutamylcysteine (γ-GC), and most metabolites of the trans-sulphuration pathway, such as cysteine, S-adenosylmethionine (SAM), and S-adenosylhomocysteine (SAH). Aspartame also triggered a decrease in mRNA and protein levels of the catalytic subunit of glutamate cysteine ligase (GCLc) and cystathionine γ-lyase, and in protein levels of methionine adenosyltransferase 1A and 2A. N-acetylcysteine prevented the aspartame-induced liver injury and the increase in plasma ALT activity as well as the decrease in GSH, γ-GC, cysteine, SAM and SAH levels and GCLc protein levels. In conclusion, chronic administration of aspartame caused marked hepatic GSH depletion, which should be ascribed to GCLc down-regulation and decreased cysteine levels. Aspartame triggered blockade of the trans-sulphuration pathway at two steps, cystathionine γ-lyase and methionine adenosyltransferases. NAC restored glutathione levels as well as the impairment of the trans-sulphuration pathway.

1. Introduction

Nowadays, no-caloric sweeteners are widely used to prevent the increasing rates of obesity and diabetes mellitus and to handle these patients, acting as critical tools in helping control caloric intake. Among them, aspartame stands out from all the others [1]. Aspartame is a dipeptide derivative (L-aspartyl L-phenylalanine methyl ester) that is used in a foods and beverages worldwide [2]. After its oral ingestion, aspartame is absorbed from the intestinal lumen and hydrolyzed to phenylalanine (50%) -the precursor for two neurotransmitters of the catecholamine family-; aspartic acid (40%) -an excitatory amino acid-; and methanol (10%) -which is oxidized to cytotoxic formaldehyde and formic acid- [3]. Although the Food and Drug Administration (FDA) approved aspartame consumption, its use has been controversial as it has been associated with several adverse effects as hyperglycemia [4,5], neurologic and behavioral disturbances [6] and hepatocellular lesions [7]. Most of them were ascribed to the generation of aspartame metabolites, particularly to methanol metabolites as formaldehyde and formate.

Methanol levels were found elevated after aspartame administration to humans [8] and rats [8–10]. However, there are some species differences in the metabolism of methanol because humans metabolize methanol to formaldehyde through alcohol dehydrogenase, whereas rodents use catalase, which also has antioxidative activity [11]. Formaldehyde is converted to formate through a similar mechanism in both species via formaldehyde dehydrogenase, which is a glutathione-dependent enzyme [12]. Then, formate in metabolized to carbon dioxide through a tetrahydrofolate-dependent pathway [13].

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Table 1
ALT and AST activities in plasma of aspartame-treated mice. Effect of NAC.

	Control	ASP	ASP+NAC
ALT (IU/L)	45.6 ± 3.5	78.4 ± 6.8*	56 ± 2.7#
AST (IU/L)	209 ± 46	304 ± 26*	284 ± 41

The number of mice per group was 6. Results are expressed as mean ± SD. The statistical difference is indicated as follows:
* $P < 0.05$ versus control.
# $P < 0.05$ versus ASP. ASP, aspartame; NAC, N-acetylcysteine.

Aspartame-derivative methanol has been linked to depletion of reduced glutathione [9,10]. Indeed, GSH depletion in brain, liver, and erythrocytes is a common feature of the long-term administration of aspartame [5,7,9,10,14,15]. The aim of this work was to determine the effect of chronic administration of aspartame on glutathione redox status and on the trans-sulphuration pathway in mouse liver.

2. Materials and methods

2.1. Animals

Male Swiss mice (30 ± 6 g b.w.) were obtained from Central Animal Facility of the Federal University of Santa Maria (Brazil). They were fed on a standard rodent chow (Supra, São Leopoldo, Brazil) and tap water *ad libitum*, in temperature- and humidity- controlled animal quarters under a 12-h light-dark cycle. The Ethics Committee of the Federal University of Santa Maria (Brazil) approved the study protocol (#001/2015).

2.2. Experimental protocol

Mice (n =18) were divided into three groups with six animals each one: control; aspartame (Sigma-Aldrich, St Louis, USA); and aspartame treated with N-acetylcysteine (NAC) (Sigma-Aldrich, St Louis, USA). Control group received vehicle (0.9% NaCl) by gavage for 90 days, whereas aspartame and aspartame treated with NAC groups received aspartame (80 mg/kg, 2.5 ml/kg, prepared in 0.9% NaCl solution). From day 60 until the 90 days immediately after administration of aspartame, the mice of the third group received NAC (163 mg/kg, pH 6.8–7.2) intraperitoneally, whereas the others received its vehicle intraperitoneally.

All treatments were prepared daily prior to administration. Prior to sacrifice, mice were anesthetized with isoflurane inhaled at 3%, blood was collected in heparinized tubes and subsequently the animals were sacrificed through exsanguination 3 h after the last treatment.

2.3. Assays

2.3.1. Alanine amino transferase and aspartate amino transferase activities

ALT and AST activities were determined in plasma using commercial kits (Labtest, Lagoa Santa, Brazil). Results were expressed as UI/L.

2.3.2. Histology

Liver samples were fixed 10% formaldehyde and embedded in paraffin. Next, 6 μm thick histological sections were cut and stained with hematoxilin-eosin to detect microarquitecture and morphological alterations.

2.3.3. Determination of sulfur-containing amino acids

Frozen liver samples were homogenized in 400 μl of phosphate saline buffer containing 11 mM N-ethyl maleimide (NEM). Perchloric acid (PCA) was then added to obtain a final concentration of 4% and centrifuged at 15,000*g* for 15 min at 4 °C. The concentrations of GSH, oxidized glutathione (GSSG), glutamylcysteine (γ-GC), cysteine, cystathionine, homocysteine, S-adenosyl homocysteine (SAH), S-adenosyl methionine (SAM) and methionine were determined in the supernatants by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). The chromatographic system consisted of a Micromass QuatroTM triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a Zspray electrospray ionization source operating in the positive ion mode with a LC-10A Shimadzu (Shimadzu, Kyoto, Japan) coupled to the MassLynx software 4.1 for data acquisition and processing. Samples were analyzed by reversed-phase HPLC with a C18 Mediterranea SEA column (Teknokroma, Barcelona, Spain) (5.060.21 cm) with 3 mm particle size. In all cases, 20 μl of the supernatant were injected onto the analytical column. The mobile phase consisted of the following gradient system (min/%A/%B) (A, 0.5% formic acid; B, isopropanol/acetonitrile 50/50; 0,5% formic acid): 5/100/0, 10/0/100, 15/0/100, 15.10/100/0, and 60/100/0. The flow rate was set at 0.2 ml/min. Positive ion electrospray tandem mass spectra were recorded with the electrospray capillary set at 3 keV and a source block temperature of 120 °C. Nitrogen was used as the drying and nebulizing gas at flow rates of 500 and 30 L/h, respectively. Argon at 1.5610−3 mbar was used as the collision gas for collision-induced dissociation. An assay based on LC-MS/MS with multiple reaction monitoring was developed using the transitions *m/z*, cone energy (V), collision energy (eV) and retention time (min) for each compound that represents favorable fragmentation pathways for these protonated molecules. Calibration curves were obtained using six-point (0.01–100 mmol/l) standards (purchased from Sigma-Aldrich, St Louis, USA) for each compound. The concentrations of metabolites were expressed as nmol/mg of protein.

2.3.4. RT-PCR

A small piece of liver was excised and immediately immersed in RNA-later solution (Ambion, Thermo Fisher Scientific, Waltham, USA) to stabilize the RNA. Total RNA was isolated using Trizol (Sigma-Aldrich, St Louis, USA). The cDNA for amplification in the PCR assay was constructed by reversion transcription reaction using Revertaid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, USA). Real time-PCR was performed using SYBR Green PCR Master Mix (Takara, Kusatsu, Japan) in an iQ5 real-time PCR detection

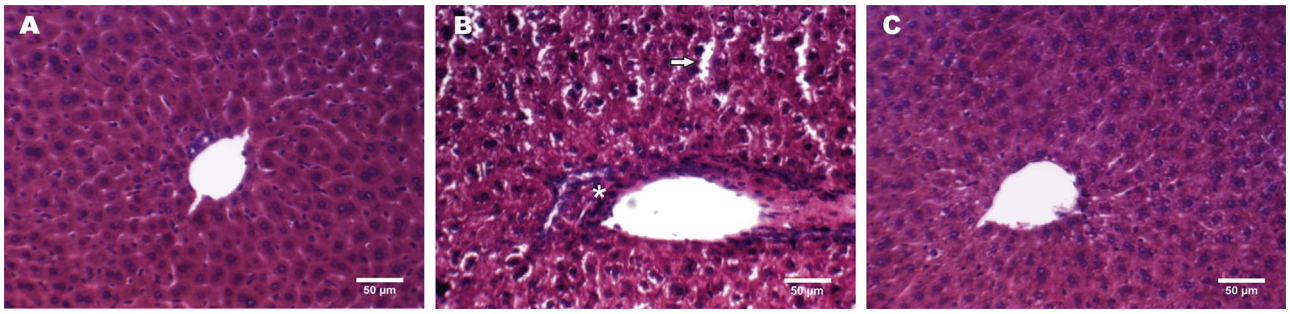

Fig. 1. Representative images of hematoxylin-eosin histological staining in liver of control (A), aspartame-treated mice (B) and mice treated with aspartame plus NAC (C). Leukocyte infiltration (asterisk), reduction in nuclear volume and degeneration of hepatocytes (arrows) are shown in aspartame-treated mice (B).