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Biological implications of selenium in adolescent rats exposed to binge drinking: Oxidative, immunologic and apoptotic balance

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ABSTRACT

Alcohol intermittent binge drinking (BD) during adolescence decreases the levels of selenium (Se), a trace element that plays a key biological role against oxidative damage in hepatocytes through different selenoproteins such as the antioxidant enzymes glutathione peroxidases (GPx1 and Gpx4) and selenoprotein P (SelP). In this context, it has been found that GPx4 has an essential antioxidant role in mitochondria modulating the apoptosis and NF-kB activation (a factor intimately related to apoptosis and immune function). To further investigate the effectiveness of selenium supplementation in oxidative balance, inflammation and apoptosis, the present study examined the protective effects of 0.4 ppm of dietary selenite administrated to adolescent rats exposed to BD. BD consumption depleted Se deposits in all the tissues studied. In liver, GPx1 activity and expression were decreased leading to protein and lipid hepatic oxidation. Moreover GPx4 and NF-kB expression were also decreased in liver, coinciding with an increase in caspase-3 expression. This hepatic profile caused general liver damage as shown the increased serum transaminases ratio AST/ALT. Proinflammatory serum citokines and chemocines were decreased. Se supplementation therapy used restored all these values, even AST levels.
These findings suggest for first time that Se supplementation is a good strategy against BD liver damage during adolescence, since it increases GPx1 and GPx4 expression and avoids NF-kB downregulation and caspase-3 upregulation, leading to a better oxidative, inflammatory and apoptotic liver profile. The therapy proposed could be considered to have a great biological efficacy and to be suitable for BD exposed teenagers in order to avoid future hepatic complications.

1. Introduction

Among teenagers alcohol is the most widely-used intoxicating drug (Lisdahl et al., 2013), with intermittent binge drinking (BD) being the pattern of alcohol consumption of greatest concern from both the clinical and public health perspectives (Johnston et al., 2011; Martinotti et al., 2016). However, despite the fact that acute alcohol exposure acts primarily by generating oxidative damage in liver (Ostrowska et al., 2004; Lu and Cederbaum, 2008) and the fact that it has recently been demonstrated that acute ethanol-induced hepatocyte apoptosis via the mitochondria pathway (Tian et al., 2016), the effects of BD intoxication on liver function in teenagers are not well characterized. Only recently, in the first clinical trial related to acute ethanol consumption during adolescence and liver function, have Binder et al. (2016) found that there might be an effect of a single event of alcohol intoxication on hepatocytes damage, since aspartate transaminase (AST) and alanine transaminase (ALT) levels increase, specifically the transaminase AST/ALT ratio, which reflects damage in hepatocytes. In fact, the increased index is considered as a specific marker of chronic alcoholic liver disease (Sorbi et al., 1999).

Selenium (Se) is an essential trace element which plays a key biological role, being the catalytic center of different selenoproteins, such as GPxs or the selenoprotein P (SelP), which are synthesized mainly in liver (Hoffmann et al., 2007). The GPx family members play different roles. Cytosolic GPx1 acts as an antioxidant, reducing free hydrogen peroxide to water, and it is also related to insulin resistance (Brigelius-Flohe, 2013), while GPx4 is the only GPx that can reduce hydroperoxides in lipoproteins, complex lipids and phospholipids of biomembranes (Brigelius-Flohe and Maiorino, 2013). It also plays an essential role in mitochondrial survival, decreasing their oxidation. This activity is very important, since oxidative stress in mitochondria induces the intrinsic pathway of apostotic through the release of cytocrhrome c (cyt. c) apoptogenic factors. Furthermore, it inhibits mitochondrial respiration and ATP production, induces mitochondrial permeability transition and inhibits mitochondrial respiration (Liang et al., 2009). GPx4 has also been implied in the modulation of the transcriptional factor NF-κB protein, a key player in inflammatory and immune responses, and a known master switch between life in death in hepatocyte

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modulating apoptosis (Wullaert et al., 2007; Greten et al., 2004; Nomura et al., 2000). The other hepatic selenoprotein studied, Sel P, plays a pivotal role in plasmatic Se transport (Hollenbach et al., 2008).

After BD exposure, a depletion of Se liver deposits has been described, together with a lower antioxidant response and GPx1 and GPx4 expression (Ojeda et al., 2015; Ojeda et al., 2016). This could be related to the lower expression of the transcriptional factor NF-κB in the liver (Nogales et al., 2014). In this context, Robin et al., 2005, have described that acute ethanol administration by gastric intubation decreases NF-kB, contributing to activate hepatic apoptosis in obese mice via caspase-3. It is known that acute alcohol increases Fas Ligand expression which activates the extrinsic pathway of apoptosis via caspase-8. Although this caspase is weak in hepatocytes, its action is amplified by mitochondrial activation, which activates the intrinsic pathway of apoptotis through the release of cyt. c into the cytosol (Tilg and Diejl, 2000; Galle et al., 1995; Feldmann et al., 2000). Recently Tian et al. (2016) have described that ethanol inhibits the protein SIRT1increasing p66Shc activation which in turn activates cyt. c and the apoptotic intrinsic pathway. Moreover SIRT1 down-regulation is also related to p53/p21 activation which activates Bax activity and the apoptotic mitochondria pathway (Stiuso et al., 2016). The mechanism by which ethanol inactivates SIRT1 is related to the oxidative stress and to the decreased of NAD + which this drug generates at high doses (French, 2016). Due to its involvement in mitochondrial survival, Gpx4 might be playing a role in this process.

Due to the above, the aim of this study is to discover whether Se supplementation therapy improves the functional hepatic AST/ALT ratio, oxidative stress in liver, NF-kB p65 and caspase-3 hepatic expression in BD adolescent rats supplemented with 0.4 ppm of selenite via the main hepatic selenoproteins GPx1, GPx4 and SelP.

2. Material and methods

2.1. Animals

Thirty-six adolescent male Wistar rats (Centre of Production and Animal experimentation, Vice-rector's Office for Scientific Research, University of Seville) were used in these experiments. Rats were received at 21 days old and housed in groups of two rats per cage for one week in order to acclimatize them to the housing conditions and handling. The experimental treatment was conducted over a 3–week period, beginning when the rats reached postnatal day (PND) 28 and ending at 47 days of age. This period corresponds to adolescence in Wistar rats (Spear, 2000). The animals were kept at an automatically controlled temperature (22–23 °C) and in a 12-hour light-dark cycle (09:00 to 21:00). Animal care procedures and experimental protocols were performed in accordance with EU regulations (Council Directive 86/609/EEC, November 24th 1986) and approved by the Ethics Committee of the University of Seville.

On PND 28, when the adolescent period began, rats were randomly assigned into four groups (n = 8/group) according to their treatments: control group (C): rats were given control diet and drinking water ad libitum, and on the corresponding days, an isotonic saline solution (SSF) intraperitoneally (i.p.); alcohol group (A): rats were given control diet and drinking water ad libitum, and on the corresponding days, an ethanol solution 20% (v/v) in isotonic saline (3 g/Kg/d) i.p.; control Se group (CSe): rats were given control diet and Se suplemented in drinking water ad libitum, and on the corresponding days an injection of SSF; and alcohol Se group (ASe): rats were given control diet and drinking water suplemented with Se ad libitum, and on the corresponding days, an alcohol solution 20% (v/v) in isotonic saline (3 g/Kg/d) i.p.

Standard pellet diet (2014 Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Laboratories, Barcelona, Spain) that contained 0.23 ppm of Se were available ad libitum in all the experimental groups. The Se supplemented groups (CSe and ASe) recieved 0.14 ppm of Se extra as anhydrous sodium selenite (Panreac, Barcelona, Spain) in drinking water during all experimental period.

2.2. Nutritional control

Body weight and the amount of food consumed by rats were monitored daily until the end of the experimental period. The amount of food and drinking water ingested every day were calculated by measuring these parameter every morning and the next day, the difference betwen them was the amount consumed. Knowing the Se concentration (ppm) in the diet and the drinking water, Se intake was calculated by multiplying by food and water ingested every day. All measurements were taken at 9:00 a.m. to avoid changes due to circadian rhythms.

2.3. Ethanol treatment

Alcohol-exposed groups (A and ASe) received a i.p. injection of alcohol (20% v/v) in SSF (3 g/Kg/d). Alcohol injections were given starting at 7:00 p.m., when the dark cycle began, for 3 consecutive days each week for 3 weeks. No i.p. injections were given during the remaining 4 days of each week (Nogales et al., 2014). Control groups (C and CSe) received an i.p. injection of an equal volume of SSF at the same time as the alcohol-exposed group's injections.

2.4. Samples

At the end of the experimental period the rats were fasted for 12 h and feces and urine samples were collected using individual metabolic cages. Then, 24 h after their last alcohol exposure or treatment with saline solution, adolescent rats were anesthetized with an i.p. injection of 28% w/v urethane (0.5 ml/100 g of body weight). The blood was obtained by heart puncture and collected in tubes. The serum was prepared using low-speed centrifugation for 15 min. at 1300 ×*g*. The abdomen was opened by a midline incision and whole livers were removed, debrided of adipose and connective tissue in ice-cold saline, weighed, frozen in liquid nitrogen and stored at −80 °C prior to biochemical determinations. Kidney, cerebellum and heart were also removed and stored at −80 °C.

2.5. Biochemistry parameter analysis

The serum levels of the transaminases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total bilirrubin, creatinine, alkaline phosphatase, glucose, cholesterol, tryglicerides, urea were measured with an automated analyzer (Technicon RA-1000, Bayer Diagnostics).

2.6. Selenium analysis

Serum, liver, kidney, cerebellum, heart, feces and urine Se levels were determined by graphite-furnace atomic absorption spectrometry. We used a PerkinElmer AAnalyst™ 800 high-performance atomic absorption spectrometer with WinLab32 for AA software, equipped with a Transversely Heated Graphite Furnace (THGA) with longitudinal Zeeman-effect background corrector and an AS-furnace autosampler (PerkinElmer, Ueberlingen, Germany). The sources of radiation were electrodeless Se discharge lamps (EDLs). The instrumental operating conditions and the reagents are the same that we have used in the previous paper Ojeda et al. (2009). Samples: serum samples were diluted five-fold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions and urine samples were diluted 1:2 v/v. After 72 h at 100 °C dry temperature, liver, kidney, cerebellum, heart and feces samples were digested in a sand bath heater (OVAN) for 72 h with nitric acid and added perchloric acid and chloridric acid (6 N).