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Selenium tissue distribution changes after ethanol exposure during gestation and lactation: Selenite as a therapy

K. Jotty, M.L. Ojeda, F. Nogales, J.M. Rubio, M.L. Murillo, O. Carreras *

Department of Physiology and Zoology, Faculty of Pharmacy, Seville University, 41012 Seville, Spain

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ABSTRACT

Ethanol consumption affects maternal nutrition and antioxidant status together with the future health of their progeny. Selenium (Se) is a trace element with antioxidant activity; we will study the effect of ethanol in dams on Se bioavailability, antioxidant balance and gestational parameters. We also will study if a Se-supplemented diet (0.5 ppm) administered to ethanol-exposed dams avoids the undesirable effects provoked by ethanol. We have used four experimental groups: control (C); chronic ethanol (A); control + Se (CS) and chronic ethanol + Se (AS). Se levels in serum, urine, faeces, and several tissues were measured by graphite-furnace atomic absorption spectrometry. Serum glutathione peroxidase (GPx) activity was determined by spectrometry.

Se bioavailability is altered by ethanol, causing a decrease in Se retention, reducing Se levels in cortex, muscle, mammary gland and salivary gland while elevating Se values in heart, liver and spleen. On the other hand, Se supplementation increases some of these parameters. Serum GPx activity was decreased by ethanol, while a Se-supplemented diet restores these values to those found in controls. We have demonstrated that ethanol decreased Se retention in dams, affecting their tissues' Se deposits, decreasing GPx activity in serum, gestational parameters and the weight of their progeny. Selenite supplementation counteracts these decreasing effects, except in cortex.

1. Introduction

All essential trace elements required by offspring development are transferred from the dam via either the placenta or milk. Stress factors such as alcoholism or malnutrition during gestation and lactation in the dam can affect the developing foetus postnatally (Merlot et al., 2008). In this context, the hypothesis of "foetal programming" was described (Jones et al., 2007), asserting that tissues can be programmed *in utero* during critical periods of development with adverse consequences for their function in later life, especially if the foetus is undernourished (Fowden and Forhead, 2004).

Due to essential differences between intra-uterine and extra-uterine environment, the neonate is exposed to oxidative stress conditions generating reactive oxygen species (Przybylska et al., 2007). At this moment the nutritional quality of the milk received is decisive to diminish over-production of a free radical involved in the initiation of various diseases. Moreover, if an insufficient milk intake appears or if there is a disturbance in pups' intestinal permeability (both effects are provoked by ethanol exposure in pups (Murillo-Fuentes et al., 2007)), the low concentration of maternal immunoglobulins and nutrients may be transferred (Przybylska et al., 2007).

Selenium is an essential trace element of great importance to health due to its anti-inflammatory, chemo-preventive and antioxidant activity via different Selenoproteins (Rayman, 2000). The most abundant Selenoprotein is the antioxidant enzyme GPx. Maternal Se deficiency induces oxidative stress in the foetus, as measured by an increased generation of lipid peroxides on foetal liver, and impairs the development of the neonatal immune system.

It has been shown that prenatal Se supplementation provides an effective antioxidant system that is already in place at the time of birth and postnatal Se supplementation becomes the main determinant of progeny Se status after the first few days of progeny life (Pappas et al., 2008).

Ethanol exposure in pregnant mice disturbs embryogenesis by increased oxidative stress (Wentzel and Eriksson, 2006). We have recently reported that ethanol-exposed pups during gestation

Abbreviations: A, alcohol group; AS, alcohol + Se group; C, control group; CS, control + Se group; GI, gestational index; GPx, glutathione peroxidase; LSI, lactation survival index.

* Corresponding author. Address: Departamento de Fisiología y Zoología, Facultad de Farmacia. Universidad de Sevilla, C/ Tramontana s/n, 41012 Sevilla, Spain. Tel.: +34 954556518; fax: +34 954233765.
E-mail address: olimpia@us.es (O. Carreras).

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and lactation have altered their hepatic antioxidant activity, provoking a decrease in Se and GPx activity and an increase in carbonyl groups in protein. Administering Se together with alcohol to the pups' mothers balances the activities of scavenging enzymes and reduces peroxidation protein products, suggesting that Se could be effective in neutralising the oxidative damage of ethanol consumption during gestation and lactation in pups (Ojeda et al., 2009).

Our purpose is to study the absorption, balance, distribution and excretion of Se in dams exposed to ethanol during gestation and lactation and how these parameters could change with Se supplementation. In this context we will evaluate their Se bioavailability and Se's relationship with the status of their progeny. We will also study the activity of the antioxidant enzyme GPx in dams' serum.

2. Materials and methods

2.1. Animals

Male and female Wistar rats (*Centro de Producción y Experimentación Animal, Vicerrectorado de Investigación, Universidad de Sevilla*) weighing 150–200 g, were randomised into four groups: control (C), alcohol (A), alcohol + Se (AS) and control + Se (CS). Drinking water (with or without ethanol) and diet (supplemented or not) were given *ad libitum* during 8 weeks. Male ($n = 6$) and female ($n = 6$) rats were mated to obtain the 1st generation offspring for each group. After reproduction, pregnant rats were housed individually in plastic cages, and continued with their alcoholic treatment until the end of the lactation period, so alcohol was supplied to mothers for 14 weeks (induction, gestation and lactation periods). The day of parturition was designated as day 1 of lactation, the number of offspring being reduced to eight per mother at parturition, and day 21 designated as the final day of the lactation period. The experiments were performed on lactating dams at day 21 postpartum; their suckling pups also were used to measure Se levels in serum.

The animals were kept at an automatically controlled temperature (22–23 °C) and a 12-h light–dark cycle (9:00–21:00). Animal care complied with Seville University's ethical code and with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996).

2.2. Ethanol treatment

Chronic adult ethanol treatment (20%) has previously been described by our group (Ojeda et al., 2008).

2.3. Diets

The diets were prepared according to The Council of the Institute of Laboratory Animal Resources (ILAR, 1979). The supplemented diet contained 0.5 ppm of selenium vs. the control diet which contained 0.1 ppm. Se was supplemented as anhydrous sodium selenite (Panreac).

2.4. Se intake measurement

In order to know the amount of Se consumed by mothers, the food provided every day was weighed in the morning. The next morning we weighed the food again, the difference being the food consumed. Knowing Se ppm in each diet, we could calculate the amount of Se consumed.

2.5. Samples

At the end of the experimental period, the rats were fasted for 12 h and anaesthetized with intraperitoneal 28% w/v urethane (0.5 ml/100 g of body weight). The abdomen was opened by a midline incision and different organs were removed, debrided of adipose and connective tissue in ice-cold saline buffer, and weighed. Samples were immediately stored at −80 °C prior to biochemical determinations. Blood was collected by heart puncture and then centrifuged. Faeces and urine samples were collected using individual metabolic cages.

2.6. Selenium analysis

Se levels were determined by graphite-furnace atomic absorption spectrometry. *Equipment*: 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 AS-furnace autosampler (PerkinElmer, Ueberlingen, Germany). The source of radiation was a Se electrodeless discharge lamp (EDL). The instrumental operating conditions and the reagents were the same that we used in the previous paper Ojeda et al. (2009) with slight modifications in the mineralization step: ramp time and temperature were different between tissues depending on their matrix content. *Samples*: serum samples were diluted fivefold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions (Sohn et al., 1991) and urine samples were diluted 1:2 v/v. After 72 h at 100 °C dry temperature, faeces and different tissue samples were weighed and digested in a sand bath heater (OVAN) with nitric acid during 72 h, and added perchloric acid and chloridric acid (6 N) (Alferez et al., 2003). After washing in Milli-Q water + acetone + acetone + acetone + Milli-Q water, nails and hair were treated in the same manner as the rest of the tissues (Lorenzo Alonso et al., 2005).

2.7. Biochemical analysis

Serum samples were used to measure GPx activity. GPx activity was determined by spectrometry using Lawrence and Burk (1976)'s method which, based on GPx, which catalyses the oxidation of glutathione by hydrogen peroxide. The protein content of the samples was determined by the Lowry et al. (1951)'s method, using bovine serum albumin as the standard.

2.8. Index

Apparent Se absorption rate was calculated as $[(I - F)/I] \times 100$ and apparent Se balance as $I - (F + U)$, where I = Se intake, F = Se faecal excretion and U = Se urinary excretion. Gestational index (GI) was calculated as (no of successfully births/no of gestating rats) × 100 and lactation survival index (LSI) as (no of total offspring − no of dead offspring/no of total offspring) × 100.

2.9. Statistical analyses

The results are expressed as a mean ± SEM. The data were analysed using a statistical program (GraphPad InStat 3) by analysing the ANOVA parametric variance test followed by Tukey-Kramer tests. A p value of <0.05 was considered to be statistically significant.

3. Results

The alcohol group showed a significant decrease in selenium intake during induction ($p < 0.01$), gestation ($p < 0.01$) and lactation period ($p < 0.05$) respect the control groups (Table 1). The same relationship was found between supplemented rats ($p < 0.001$). Supplemented rats (AS and CS) showed significantly higher selenium intakes than non-supplemented ones during the whole experimental procedure ($p < 0.001$). This data supports our supplementation procedure, preventing any Se intake deficiency in the ethanol-supplemented group.

Chronic ethanol exposure changes the maternal Se excretion pattern and therefore A dams eliminated more Se via urine than C rats ($p < 0.05$) and less Se via faeces and hair ($p < 0.001$) (Table 1). In Se treated rats, Se supplementation to ethanol dams diminished Se excretion via faeces, urine and hair ($p < 0.001$). The control supplemented group eliminated more Se via urine and hair respect the rest of the groups ($p < 0.001$).

Ethanol exposure during gestation and lactation did not affect the apparent Se absorption rate, but it significantly decreased the apparent Se balance ($p < 0.001$). However, serum Se levels were similar in non-supplemented rats (Table 1). In supplemented dams the effect was similar, ethanol-receiving rats (AS) had similar apparent absorption and serum Se levels as CS, but significantly lower apparent balance values ($p < 0.001$). Se supplementation increases apparent Se absorption, serum Se levels and Se balance with respect to non-supplemented rats.

During the experimental procedure ethanol dams showed the lowest weight gain ($p < 0.01$ vs. C and $p < 0.001$ vs. AS) (Table 2). However, A rats had similar organ weights (g/g body weight (%)) to C rats. CS dams gained more weight during the experiment than C dams ($p < 0.05$) having higher heart and lung weight ($p < 0.05$). AS dams had the highest liver weights ($p < 0.05$), and they also showed lower pancreas weight than CS ($p < 0.05$).