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Maternal ethanol consumption reduces Se antioxidant function in placenta and liver of embryos and breastfeeding pups

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ABSTRACT

Aim: The fetal alcohol exposition during pregnancy leads to different disorders in offspring, related to the oxidative stress generated by alcohol. It is well-documented that there is an impairment of the antioxidant selenoprotein Glutathione peroxidase (GPx) activity in ethanol offspring during the embryo period, although no-one has described Selenium (Se) status. The aim is to analyze for the first time Se deposits in vivo and Se's biological implication in embryos and placenta after alcohol exposure and in offspring whose mothers continued to drink ethanol during lactation.

Materials and methods: Se deposits, GPx and glutathione reductase (GR) activity, lipid and protein oxidation and the expression of GPx1 were measured in placenta and liver of both embryos (E-19) and breastfeeding pups (L-21) in control and ethanol groups (20% v/v).

Key findings: Ethanol consumption decreased Se deposits, GPx activity and GPx1 expression, while increasing biomolecular oxidation in placenta and in the liver of E-19 and L-21. The GR/GPx ratio decreased in placenta and in E-19, together with an increase in lipid oxidation, while increased in the liver of L-21 pups with protein oxidation. Ethanol also decreased the GPx1 expression/GPx activity ratio in the liver of E-19 and L-21, indicating that alcohol decreases GPx activity by both depleting Se deposits and promoting GPx inactivation. In placenta GPx activity is proportional to the GPx1 expression found, so the ethanol affects GPx activity in offspring more than in dams.

Significance: Therefore, Se supplementation therapy in dams could contribute as an interesting antioxidant that prevents fetal alcohol syndrome.

1. Introduction

Ethanol is one of the most common human teratogen drugs consumed, leading to serious adverse outcomes in the fetus, such as intrauterine growth retardation (IUGR), craniofacial malformations, physical and mental retardation, and cardiac septal defects. This is known as fetal alcohol syndrome (FAS). Sometimes a lesser degree of deformations, described as fetal alcohol spectrum disorders (FASDs), could be associated with FAS. Some offspring, however, have no symptoms (resilience) or only present partial FAS-related symptoms [1]. These effects could be even worse if mothers continue consuming ethanol during breastfeeding [2].

The cellular mechanisms by which ethanol induces damage in utero are not well understood, but induction of oxidative stress is believed to be one important mechanism [3]. The fetal injury by ethanol could be explained by a direct mechanism through feto-toxicity from ethanol and/or acetaldehyde, which is intimately related to oxidative stress induction and/or an indirect mechanism through ethanol-induced placenta injury and selective fetal malnutrition [4]. Insufficient supplies of essential trace elements with antioxidant properties such as selenium (Se) could contribute to the oxidative stress generated by ethanol exposure. Oxidation of the placenta itself is central to the pathogenesis of many disorders during pregnancy [5]. It has been demonstrated that ethanol exposure during lactation via maternal milk also increases protein and lipid oxidation in the liver of breastfeeding pups, decreasing endogenous antioxidant enzymes activities and hepatic glutathione (GSH) levels [6].

During embryo development there is a complex interplay among proliferation, differentiation and apoptotic cell events. Despite the fact that a low amount of reactive oxygen species (ROS) acting as primary or secondary messengers to promote cell growth or death are vital for maintaining this interplay, an excess of ROS levels are related to

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protein, lipid and DNA oxidation, leading to cell dysfunctions. Fortunately, antioxidants can obviate these effects by modifying gene expression, transcription factor signaling, and cell cycle alterations [7]. One of the main cellular protective systems that plays an important role during embryo development is the selenium-dependent glutathione peroxidases (GPxs) family [8]. These enzymes reduce the pro-oxidative ROS hydroperoxides to harmless water and oxygen. GPx1 is regarded as this family's major antioxidant enzyme. It does, however, depend on Se levels; if Se decreases, GPx1 expression falls [9]. Despite the fact that studies with GPx1 knockout mice have revealed that these animals compensate mild oxidative stress [10], GPx4 knockout mice are non-viable [11]. GPx4 is the only selenoprotein which protects cellular membranes and mitochondria from ROS, with mitochondria being related to apoptosis [12].

There are few studies related to ethanol consumption and Se alteration during pregnancy, but there are a lot which describe an impairment of GPx activity and/or expression in FASD in different tissues during the embryonic period [13,14,15,16] and even in placenta [17,5]. Using a whole embryo culture system exposed to 1 μl/ml ethanol in vitro, [18] described that mRNA levels of cytosolic GPx1, GPx4 and SelP decreased. Recent in vivo studies have found that ethanol exposure during gestation and lactation profoundly alters Se homeostasis and its body distribution by direct action in the pups [19]. This Se imbalance is linked to lower antioxidant GPx activity in tissues and a higher OE [6]. GPx4 liver expression, however, increased [20].

The aim of this study is to analyze Se levels, GPx activity and GPx1 expression in liver of embryos and placenta after chronic alcohol exposure in vivo for the first time. Moreover, these results will be compared to those of liver in offspring at the end of lactation period whose mothers continued drinking ethanol during lactation.

2. Material and methods

2.1. Animals

Male (n = 8) and female (n = 16) Wistar rats weighing about 150–200 g (Centre of Animal Production and Experimentation, Vice-Rector's office for Scientific Research, University of Seville), were randomized into two groups: control (C) and alcohol (A). Drinking water (with or without ethanol) and diets were given ad libitum during the whole experimental period: induction (7 weeks), gestation (3 weeks) and lactation (3 weeks). The diet was prepared in the lab according to The Council of the Institute of Laboratory Animal Resources (ILAR, 1979) and contained 0.1 ppm of Selenium. After the induction period male and female rats were mated to obtain the 1st generation offspring for each group (1 male plus 2 females per cage). The presence of a copulatory plug in the rat cage was considered to be the day 0 of pregnancy. After reproduction, the pregnant rats were housed singly in individual ventilated cages and continued their alcoholic or control treatment until the end of the gestation period. Half of all dams from each group were sacrificed to 19 day gestation to obtain their embryos (E-19). The rest of mothers gave birth to offspring rats at 21 days of gestation; they continue with their treatments and their progeny in their own cages until the end of breastfeeding. Offspring at the end of breastfeeding (L-21) were used to carry out the rest of experiments. The animals were kept at an automatically controlled temperature (22–23 °C) and a 12-h light-dark cycle (9:00 to 21:00). Every day liquid and solid intake was measured. Animal care complied with the ethical Seville University approved and with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996).

2.2. Ethanol treatment

Chronic progressive ethanol treatment was administered to dams in tap water at increasing concentrations until a level of 20% v/v was reached in the induction period, this concentration was maintained during gestation and lactation [6]. Total kilocalories (kcal) was obtained by adding to the solid kcal the kcal derived from alcohol consumption. Solid kcal intake was calculated by multiplying the g of food ingested per day by 4.1 kcal, alcoholic kcal intake was determined by multiplying the ml of ethanol ingested per day by 7.1 kcal. Se intake was estimated by multiplying the g of food ingested per day by 0.1.

2.3. Samples

At the end of the first experimental period, on day 19 of gestation half of all dams were weighed and anesthetised with intraperitoneal 28% v/v urethane (0.5 ml/100 g of body weight). The abdomen was opened by a midline incision and whole livers were removed, debrided of adipose and connective tissue in ice-cold saline, weighed and stored to − 80 °C. The pregnant uterus was exposed via a mid-line incision and the anesthetized embryos were killed via spinal transaction. All fetuses (E19), their livers and their associated placentae were weighed and the samples were immediately stored at − 80 °C prior to biochemical determinations. At the end of the second experimental period, breast-feeding pups (L21) were anesthetised with intraperitoneal 28% v/v urethane (0.5 ml urethane/100 g of body weight). The abdomen was opened by a midline incision and whole livers were removed, debrided of adipose and connective tissue in ice-cold saline, weighed and stored at − 80 °C prior to biochemical determinations.

2.4. Gestation and lactating indexes

Female fertility index was calculated as (number of pregnancies / number of mating) × 100; Gestation index as (number of successful births / number of pregnancies rats) × 100; Live-born index as (number of pups born alive / number of pups born) × 100; and Lactation survival index as (number of total offspring − number of dead offspring / number of total offspring) × 100. Hepatic somatic index (HSI) was calculated as (liver weight / body weight) × 100 and placental efficiency (PE) as (fetal weight / placenta weight).

2.5. Selenium analysis

Se levels were determined by graphite-furnace atomic absorption spectrometry. Equipment: 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 are the same that we have used in the previous paper [21], with slight modifications in the mineralization step: ramp time and temperature were different between tissues depending on their matrix content. Samples: placenta and liver of E-19 and L-21 pups were weighed and digested in a sand bath heater (OVAN) with nitric acid during 72 h, and added perchloric acid and chloridric acid (6N).

2.6. Biochemical analysis

In order to measure the activity of GPx and GR as well as the oxidation of lipids and protein, liver tissue samples were homogenized in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA and 1 mM DTT) in an ice bath; the resulting supernatant was employed for biochemical assays. The degree of lipid peroxidation in the supernatant was evaluated by a colorimetric reaction at 535 nm with thiobarbituric acid (TBA) as described [22], the results were given as mol/mg protein. Proteins oxidation was measured according to a method based on the spectrophotometric detection of the reaction of 2.4-dinitrophenylhydrazine (DNPH) with protein carbonyl (PC) to form protein hydrazones [23]. The level of PC was calculated at the maximum