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Toxicity of methimazole on femoral bone in suckling rats: Alleviation by selenium

Ibtissem Ben Amara [a], Afef Troudi [a,1], Nejla Soudani [a,1], Fadhel Guermazi [b], Najiba Zeghal [a,*]

[a] Animal Physiology Laboratory, Sfax Faculty of Science, BP 1171, 3000 Sfax, Tunisia
[b] Nuclear Medicine Service, CHU Habib Bourguiba of Sfax, 3029 Sfax, Tunisia

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ABSTRACT

Aims: Selenium has a pharmacological properties and it is well considered as an antioxidant. The present study investigated the potential ability of selenium, used as a nutritional supplement, to alleviate bone impairments in suckling rats whose mothers were treated with methimazole, an antithyroid drug.
Main methods: Female Wistar rats were randomly divided into four groups of six each: group I served as control which received standard diet; group II were rendered hypothyroid by administration of methimazole (250 mg L^{-1} in their drinking water); group III received both methimazole (250 mg L^{-1} in their drinking water) and selenium (0.5 mg kg^{-1} of diet); group IV received 0.5 Na_2SeO_3 mg kg^{-1} of diet. Treatments were started from the 14th day of pregnancy until day 14 after delivery.
Key findings: Methimazole treatment decreased femur length and weight in 14-day-old rats, when compared to controls. Femur antioxidant enzyme activities, superoxide dismutase, catalase and glutathione peroxidase decreased. Lipid peroxidation recorded an increase revealed by high femur malondialdehyde levels. Methimazole also caused a significant decrease in calcium and phosphorus levels in bone. Yet, in plasma and urine, they increased and decreased inversely. Besides, plasma total tartrate-resistant acid phosphatase was enhanced, while total alkaline phosphatase was reduced. Co-administration of selenium through diet improved the biochemical parameters cited above. Nevertheless, distorted histoarchitecture revealed in hypothyroid rat femur was alleviated by Se treatment.
Significance: The present study suggests that selenium is an important protective element that may be used as a dietary supplement protecting against bone impairments.

1. Introduction

Bone is a specialized connective tissue, which forms the framework of the body. Various physiological conditions can adversely affect femoral bone metabolism. For instance, food deprivation (Fetoui et al., 2006), iodine and/or selenium (Se) deficiency (Nekrasova et al., 2006; Moreno-Reyes et al., 2001, 2006; Ren et al., 2007), pollutants such as fluoride (Bouaziz et al., 2004), pesticides (Mahjoubi Samet et al., 2005), goitrogenic agents like thiocyanate (Ghorbel et al., 2008) and antithyroid drugs (Pahuja and De Luca, 1982) affects bone maturation. These compounds are able to interfere, directly or indirectly, with the synthesis of thyroid hormones which fundamentally determine the development and growth of many organs, including the bone. In fact, thyroid hormones are necessary for skeleton maturation (Lioté and Orcel, 2000), the linear growth, maintenance of bone mass and calcium-phosphorus homeostasis (Bassett and Williams, 2003). However, a decreased thyroid function in youth retards growth and delays skeletal maturation and increases fracture risk (Harvey et al., 2002). Moreover, thyroid impairment in fetuses/neonates can occur after treatment of their pregnant and breast-feeding dams with antithyroid drugs like methimazole (MMI), widely used to manage hyperthyroidism associated with Grave's disease (Zakrzewski, 2008). Yet, MMI reduces the risk of this pathology in dams (Mandel and Cooper, 2001) but is transmitted through placenta or milk (Marchant et al., 1977; Marchant and Alexander, 1972), thus exposing progeny to a risk of hypothyroidism (Dussault and Ruel, 1987). Consequently, MMI represents a pathogenesis factor by inducing hypothyroidism and bone disorders in fetuses and neonates.

Several studies have demonstrated that MMI, associated with oxidative stress and cellular damage, is the consequence of both increased production of free radical and reduced capacity of the antioxidative defence system (Das and Chainy, 2004; Sarandol et al., 2005). Free radicals production is believed to induce bone related diseases by suppressing bone formation and stimulating bone resorption. Indeed, antioxidants deficiency has a negative impact on bone mass (Ramajayam et al., 2007). So, there is growing evidence that oxidative stress contributes to bone damage, whereas antioxidants may prevent the undesired oxidative dam-

* Corresponding author at: Animal Physiology Laboratory, Life Sciences Department, Sfax Faculty of Science, BP1171, 3000 Sfax, Tunisia. Tel.: +216 74 274 600; fax: +216 74 274 437.
E-mail addresses: naj_zgh@yahoo.fr, najiba.zeghal@tunet.tn (N. Zeghal).
[1] These authors contributed equally to this work.

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age induced by reactive oxygen species in the bone tissue (Brzoska et al., 2009). In fact, organisms are equipped with several lines of antioxidant defence against oxidative damage. The defence lines act either as non-enzymatic antioxidant defences, such as vitamins C and E, or as enzymatic antioxidant defences, such as scavenger enzymes (SOD, GPx) (Halliwell and Gutteridge, 2007). Accordingly, Ramajayam et al. (2007) have reported a protective role of antioxidants (vitamins C and E) against oxidative damage in bone tissue. However, selenium-containing molecules may be better nucleophiles (and therefore antioxidants) than classical antioxidants (Arteel and Sies, 2001). In fact, selenium, an essential element in almost all biological systems, has pharmacological properties and it is well considered as an antioxidant (Meotti et al., 2004). Several selenoproteins are expressed in bone tissue and are important in bone metabolism (Ebert and Jakob, 2007). In general, oral supplementation is a better option for administration of antioxidants as therapeutic molecules (Subudhi et al., 2009). Selenium, an essential trace element of fundamental importance for animals and humans, is obtained from dietary sources including meat, fish and eggs which contribute as the major part of dietary Se in several countries such as Greece, Portugal and Japan (Pappa et al., 2006; Ventura et al., 2007; Haratake et al., 2007). In this respect, Combs (2001) indicates that an adequate adult diet should have at least 40 μg/day of Se to support the maximum expression of seleno-enzymes.

Inorganic Se compounds, used throughout the experiment, are generally provided as a nutritional source (Rock et al., 2001), being easily absorbed by duodenum (Ognjanovic et al., 2008). Selenite compounds, according to Anan et al. (2009), are effectively incorporated into placenta during pregnancy and transferred to pups during lactation.

In recent reports, Se supplementation has been proved to have protective effects against some pathological states such as MMI-induced cerebrum and cerebellum impairment (Ben Amara et al., 2009), cancer (Steinbrenner and Sies, 2009), diabetes (Kiersztan et al., 2007), Kashin-Beck osteoarthropathy (Zou et al., 2009), atherosclerosis and possibly osteoporosis (Ebert and Jakob, 2007). Based on these facts, we hypothesize that Se supplementation could be a useful method to protect against bone impairment, thus allowing further exploration of its protective potential. This could be therefore a step forward in the protection of human fetuses/neonates against hypothyroidism and its adverse effects, including bone impairment.

To our knowledge, there are no studies carried out on suckling rats describing methimazole-induced oxidative stress and bone disorders during late pregnancy and early postnatal periods. Besides, the protective role of selenium on methimazole-induced femur toxicity has not yet been investigated. So, we first assessed the effects of MMI, an antithyroid drug, on bone toxicity and maturity of suckling rats and, subsequently, the ability of Se supplementation to improve and protect bone maturation and development in those rats whose dams were treated with MMI.

2. Materials and methods

2.1. Chemicals

Sodium selenite (Na_2SeO_3), methimazole ($C_4H_6N_2S$), glutathione (oxidized and reduced), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and thiobarbituric acid (TBA) were purchased from Sigma (St. Louis; MO, USA). All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

2.2. Animals and diet

Male and female Wistar rats weighing 180 ± 10 g were purchased from the Central Pharmacy (SIPHAT, Tunisia). They were kept in polypropylene cages in normal housing conditions at ambient temperature 22 ± 3 °C with a 12-h light/dark cycle and a minimum relative humidity of 40%. Rats were fed a commercial rodent diet purchased from Industrial Society of Nutrients (SICO, Sfax, Tunisia). Diet iodine content (0.720 μg iodine g^{-1} of diet) was determined in basal diet, after acid mineralization, using the catalytic method of Sandell and Kolthoff (1937). The concentration of Se in standard diet (0.17 mg kg^{-1} of diet) was also determined by us, after acid mineralization, by the electrothermic atomic absorption spectrometry (ET-AAS) technique previously followed for food samples by Kumpulainen et al. (1983) and described by Ekholm et al. (2007). Briefly, 0.5 g of diet was digested in a mixed acid of HNO_3, $HClO_4$ and H_2SO_4. Se was reduced to Se IV with 3 M HCl, chelated with ammonium pyrrolidine dithiocarbamate and extracted into methylisobutylketone for the determination. Measurements were performed on a Perkin-Elmer 5100/Zeeman Atomic Absorption Spectrometer with a 196-nm wavelength.

After a one-week acclimatization in the laboratory conditions, pairs of male and virgin female rats were kept overnight in each cage. Pregnant female rats were inspected daily by the presence of the vaginal plug which indicated day zero of pregnancy.

2.3. Experimental procedure

Twenty-four pregnant females were randomly divided into four groups of six each. The first group served as a control (0.17 mg Na_2SeO_3 kg^{-1} of diet); the second group (MMI) received in their drinking water 250 mg L^{-1} of methimazole $C_4H_6N_2S$ (dissolved in distilled water); animals of the third group MMI + Se were treated orally with methimazole (250 mg L^{-1} in their drinking water) and 0.5 mg kg^{-1} of Se added to their diet as Na_2SeO_3 (mixed with pellet diet); the fourth group (Se) received 0.5 mg Na_2SeO_3 kg^{-1} of diet. Treatments were started from the 14th day of pregnancy until the 14th day postnatal.

Hence, our treatment groups were as follows:

Group 1: sodium selenite (0.17 mg kg^{-1} of diet; negative control).
Group 2: methimazole (250 mg L^{-1} in their drinking water).
Group 3: methimazole (250 mg L^{-1} in their drinking water) + sodium selenite (0.5 mg kg^{-1} of diet).
Group 4: sodium selenite (0.5 mg kg^{-1} of diet; positive control).

The dose of methimazole (250 mg L^{-1} of drinking water) and the beginning of the treatment (the 14th day of pregnancy) were chosen according to Schwartz et al. (1997), since MMI-induced hypothyroidism without lethal effects. The Se dose (0.5 mg kg^{-1} of diet) used in our experiments and in other findings gave high protection against hypothyroidism (Golstein et al., 1988; Ben Amara et al., 2009) and stress conditions (Ognjanovic et al., 2008; Ben Amara et al., 2009; Soudani et al., 2010).

The rats were allowed to deliver spontaneously 3 weeks after coitus. In the first day postnatal, the number of pups born and their sex were recorded. Each litter was culled to eight pups for each mother (four males and four females if possible) as it has been shown that this procedure maximizes the lactation performance (Fishbeck and Rasmussen, 1987). MMI, Se and iodine quantities ingested by lactating rats were determined daily after measuring drinking water and food consumption, respectively (Table 1).

The experimental procedures were carried out according to the general guidelines on the use of living animals in scientific investigations (Council of European Communities, 1986) and approved by the Ethics Committee of Sciences Faculty of Sfax and