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RESEARCH COMMUNICATIONS

Effects of molybdenum on sperm quality and testis oxidative stress

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Abstract

In order to investigate the effects of molybdenum (Mo) on sperm parameters and testicular oxidative stress, the ICR strain of adult mice were exposed to different doses of molybdenum for a sub-acute toxicity test. Compared to the control, our results showed that the sperm parameters, including the epididymis index, sperm motility, sperm count, and morphology, increased by a moderate dose of Mo (25 mg/L), but were negatively affected at high doses (≥ 100 mg/L). In addition, the changes of sperm paramet SOD) activities yde (MDA) levels in ing the testicula

Key

Introduction

A variety of endocrine disrupting chemicals have been released into the environment in the rapid industrial progress, which may exert adverse health effects in human and animals [Carlsen et al. 1992; Khurana et al. 2000; Friedmann 2002]. Molybdenum (Mo) is an essential trace element in animals and humans. It has been identified as part of the active sites of over 50 enzymes, and may promote normal cell function possibly by catalyzing a variety of hydroxylation, oxygen atom transfer and other oxidation-reduction reactions [Hille et al. 1998]. Molybdenum is also an endocrine disruptor and has been widely present and detected in our food and water [Underwood 1981; Mills and Davis 1987; Kargar et al. 2011; Yu et al. 2011]. In addition, Mo is broadly used in industrial production, such as metallurgical processes, the manufacture of electronic products, glass, ceramics, lubricants, catalysts, pigments and nano materials [Pandey and Singh 2002; CDC 2005; Braydich-Stolle et al. 2005; Ema et al. 2010]. Furthermore, Mo is also an environmental pollutant discharged from uranium processing, combustion processing, contact lens solutions, and the color additives in cosmetics [ACGIH 1995]. This wide distribution greatly increases the risk of animals and humans exposed to the high level of Mo in the environment. For example, molybdenum concentrations have risen to 0.2 mg/L in areas near mining sites, however, the WHO recommends a maximum level of molybdenum in drinking water of 0.07 mg/L [WHO 1993].

Excessive amounts of Mo can induce reproductive toxicity, especially for male animals and humans [Thomas and Moss 1951; Sharma et al. 2004; Bersényi et al. 2008]. In rats, ingestion of a high dose of Mo caused decreased sperm motility, count, morphologic abnormalities, epididymis weight decline, and testis histopathologic changes [Pandey and Singh 2002, Lyubimov et al. 2004.], and fertility [Wirth and Mijal 2010]. A reduction in the germ cells and mature spermatocytes in rabbits has been observed [Bersényi et al. 2008] as well as a decline in sperm quality and morphology in humans [Meeker et al. 2008]. H of male animals

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Seminal plasma contains three main enzymatic antioxidants: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Spermatozoa possess primarily enzymatic antioxidants, with SOD being the most predominant [Makker et al. 2009]. Superoxide dismutase in conjugation with CAT and GPx scavenge both intracellular and extracellular superoxide radicals and inhibit lipid peroxidation [Agarwal and Prabhakaran 2005]. Malondialdehyde (MDA) is one of the byproducts of lipid peroxidation that indirectly reflects the level of peroxidation and the degree of cell injury [Sharma et al. 2004]. This byproduct has been used in various biochemical assays to monitor the degree of oxidative damage sustained by spermatozoa [Aitken et al. 1989; Aitken and Fisher 1994]. Oxidative stress can be evaluated by detecting the activities of SOD and GPx, as well as the MDA level in the tissue. Up to now, there are no data available for the oxidative stress of testicular tissue caused by Mo on mice.

This study has been undertaken to evaluate the effect of orally administered Mo on the sperm parameters of the epididymis index, sperm motility, count, and morphology changes. The oxidative stress of testis was considered as a function of the levels of MDA, SOD, and GPx in mice.

Results and Discusssion

The epididymis index, sperm motility, and count are summarized in Table 1. The change in morphology at a range of Mo concentrations is shown in Figure 1. Molybdenum at 25 mg/L improved the sperm quality ($P < 0.01$), but Mo ≥100 mg/L negatively affected the sperm quality ($P < 0.05$); Mo at 12.5 and 50 mg/L did not affect sperm quality significantly.

Figure 1. Photomicrographs of the commonly observed abnormal sperms from the 100 mg/L mo eading); C) pin head cteristic curvatur hed.

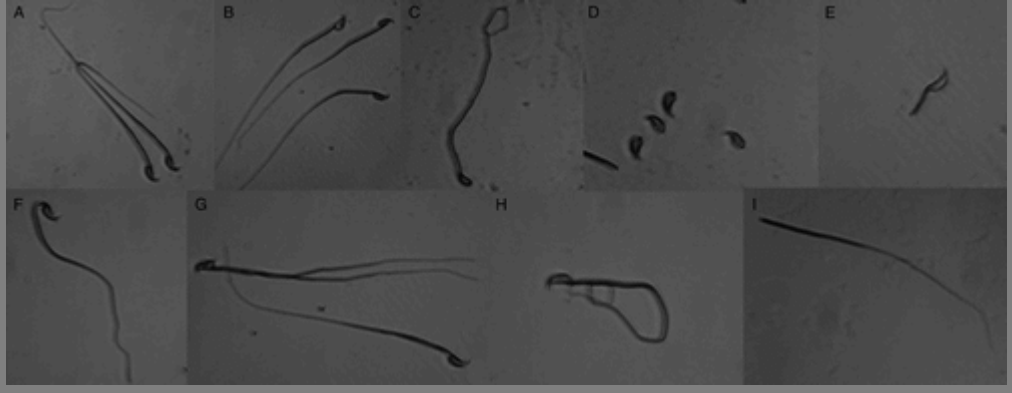

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Table 1. Effects of molybdenum treatments on sperm parameters in mice.

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As shown in Table 2, compared to control, Mo at ≥100 mg/L decreased the activities of SOD and GPx, yet the content of MDA significantly increased. At 25 mg/L Mo markedly improved the activities of SOD and GPx, but did not change MDA. At a concentration that ranged from 12.5 and 50 mg/L only GPx activity decreased significantly. The level of SOD and MDA did not markedly change.

Table 2. Effects of molybdenum treatments on the SOD, GPx, and MDA levels of testes in mice.

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The results presented in this study show that the changes of sperm quality are accompa us oral doses of eased within the 25 m nd oxidative dam

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oxidative stress as shown by a significant decrease in the activity of both SOD and GPx, and a considerable increase in MDA in testicular tissue (Table 2). The effects of Mo on reproductive improvement have been described in several in vitro studies. Our previous study showed that at 5 μg/ml Mo is likely to improve the development of mouse embryos cultured in vitro [Bi et al. 2012]. In contrast, Braydich-Stolle et al. [2005] observed that in vitro 5 μg/ml and 10 μg/ml of Mo nano-particles seem to promote plasma membrane leakage of mouse spermatogonial stem cell lines. Molybdenum at ≥100 mg/L negatively impacted sperm quality and increased the oxidative damage in testicular tissue. There is little information in the literature on the in vivo effect of Mo on male mouse reproductive parameters. However, similar phenomena have been observed in other animals and humans. Pandey and Singh [2002] reported a dose-dependent degeneration of testicular morphology and function with declining sperm concentration, motility, normal morphology, and epididymides in rats after oral administration of sodium molybdate at a dose level of ≥ 30 mg/kg body weight. Similarly, Lyubimov et al. [2004] observed that a significant reduction of epididymal weight, sperm count, motility, morphologic abnormalities, and histopathologic changes in testis and epididymis occurred in the rats treated by tetrathiomolybdate at 12 mg/kg/day for 2 months. Bersényi et al. [2008] revealed a reduction in the number of germ cells and mature spermatocytes in the testes, and an appearance of a large number of syncytial giant cells and degenerated cells among the spermatogenic cells in the seminiferous tubules of rabbits fed carrots containing 39 mg Mo/kg dry matter as compared to animals given uncontaminated samples. Meeker et al. [2008] found dose-dependent trends between Mo and declined sperm quality and morphology in humans. In addition, mice exposed to ≥100 mg/L Mo in the present study, like the report in bull calves fed by high dietary intakes of Mo [Thomas and Moss 1951], exhibited a complete lack of libido, and sterility. This probably reflects the response to marked damage of the interstitial cells and germinal epithelium with impaired spermatogenesis in the testes. The resu the epididyn d through oxidative Px level, and an ative dam dicating that a hi increased oxidative significant decrease in the

oxygen and hydrogen peroxide to protect the structures and functions of cell membranes from the interference and damage by peroxides is apparent [Ola-Mudathir et al. 2008; Portugal-Cohen et al. 2010]. Similar phenomena were observed in rabbits. Bersényi et al. [2008] observed that high dietary Mo (39 mg Mo/kg dry matter) can generate free radicals or reactive intermediates, resulting in altering MDA and GPx activity.

In conclusion, molybdenum affects sperm quality through regulating the testicular oxidative stress in a complex manner. Male reproductive parameters apparently improved at moderate doses (25 mg/L), but were significantly repressed at high doses (≥ 100 mg/L). The change in the levels of SOD, GPx, and MDA indicate that the dual functions of Mo on sperm quality are likely to be mediated through oxidative stress in testicular tissue.

Materials and Methods

Chemicals

Unless otherwise stated, all components used in the present study were procured from Sigma–Aldrich Corp. (St. Louis, MO, USA).

Animals

All of the following studies were approved by the Animal Care and Use Committee of Henan University of Science and Technology. The ICR strain adult (3 to 4 weeks of age) male mice weighing 30-35 g were used for the acute toxicity experiments. All mice used in this study were maintained under Good Laboratory Practice (GLP) conditions. The mice had free access to drinking distilled water and commercial standard pellet diet.

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Collection of testes and epididymides

The testicular tissues of mice from each group were used for determination of SOD, GPx, and MDA, and epididymides for collecting the sperm. The mice were sacrificed by cervical dislocation on day 14 of the experiment. Testes and epididymides were quickly removed and weighed. The epididymides were placed into 37°C preheated saline, the cauda epididymis was lacerated for incubation of sperm. The testes were put in 4°C precooled saline in a refrigerator, then transferred into −20°C before homogenate preparation.

Evaluation of sperm parameters

Semen samples were collected after incubation for 30 min, and semen analysis was conducted following the World Health Organization protocol [WHO 1999]. Sperm concentration (million sperm per milliliter), percent motile sperm, and sperm morphology were investigated in this study. The concentration of immobilized sperm was determined on a hemacytometer. Sperm motility was evaluated within 1 hr after collection. Percent motile was the sum of the percentages with rapid linear progression (3 to ≥ 4) and slow linear progression (≥ 2). Sperm morphology (percent normal forms) was determined using air-dried smears stained with a modified Wright-Giemsa stain. At least 200 sperm in four different areas of the slide were evaluated according to Kruger's strict criteria [Kruger et al. 1988].

Detection of MDA, SOD, and GPx levels in testes

The testicular tissue stored at −20°C were homogenized at 4°C after adding pre-cooled 0.9% saline in the ratio of 1:9. When testicular tissues were disrupted, the homogenate was centrifuged at 3,000 × g for 10 min at 4°C. The supernatant was used for the assay of SOD, GPx, and MDA according to the instructions for these kits (Nanjing Jiancheng Bioengir

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Five r pressed as the r y single variable etween experim t $P < 0.01$.

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Author contributions: Conceived and designed the experiments: F-JL, Z-JY, X-WZ, Y-LZ; Performed the experiments: X-WZ, QQ, YB; Analyzed the data: X-LC, L-JJ, X-GM; Contributed reagents/materials/analysis tools: RS; Wrote the manuscript: F-JL, X-WZ, Y-LZ, RS.

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