THE EFFECT OF MOLYBDENUM ON OVARIAN FUNCTIONS OF RAT

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ABSTRACT

The objective of this thesis was to examine the effect of metals on the expression of regulators of apoptosis and secretory activity of ovarian cells of rats in vitro. The following substances were observed: growth factor IGF-I, steroid hormones progesterone and estradiol, regulators of apoptosis: Bcl-2, Bax and caspase-3. Growth factor IGF-I and steroid hormones progesterone and estradiol were detected by RIA method and intracellular peptides were detected by Western-imunoblotting. In our study all examined substances were affected by various concentrations of $[(NH4)_6.Mo_7O_{24}.4H_2O]$. The release of growth factor IGF-I was inhibited by all concentrations of selected Mo. The highest dose of Mo (500 $\mu g.ml^{-1}$) stimulated the most intense IGF-I secretion. In the case of the steroid hormone progesterone we did not achieve demonstrable ($p \geq 0,05$) results, but the secretion of P_4 had a decreasing tendency. The inhibitory effect of estradiol on the release of Mo was seen ($p \leq 0,05$) at concentrations of 90 $\mu g.ml^{-1}$, 170 $\mu g.ml^{-1}$, 500 $\mu g.ml^{-1}$, at a dose of 330 $\mu g.ml^{-1}$ results were not conclusive, but nevertheless, we detected a reduction in estradiol secretion. Inhibition of apoptosis marker expression of Bcl-2 has been observed due to the lowest concentrations (90 and 170 $\mu g.ml^{-1}$), increasing expression was observed in the experiment with the addition of 330 $\mu g.ml^{-1}$. At concentrations of 90, 170, 330 $\mu g.ml^{-1}$ was found to stimulate the expression of pro-apoptotic Bax peptide. Lower doses of Mo (90 and 170 $\mu g.ml^{-1}$) had no effect on the expression of caspase-3, release was not significantly ($p \leq 0,05$) stimulated concentrations of 330 and 500 $\mu g.ml^{-1}$. Data obtained from in vitro experiment indicate that Mo has an effect on endocrine and apoptotic processes in the ovaries of rats. The results of this work suggest that the effect of Mo on ovarian processes is dose-dependent. Compounds of this metal may be potential regulators of intracellular ovary processes, hormone secretion and expression of regulators of apoptosis.

Key words: ovaries, steroid hormones, regulators of apoptosis, molybdenum, rats

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INTRODUCTION

Molybdenum belongs to the Outline of the Periodic Table of Elements, is an essential trace element for the proper functioning of the plant and bacterial enzymes (eg nitrogenase). This element is also a cofactor for xanthine oxidase, sulfite oxidase, aldehyde oxidase, etc. in animal organisms. The fertility in females is affected by an amount of exogenous as well as endogenous factors, including the impact of heavy metals. High concentrations of these elements may negatively affect the endocrine system, which is closely linked to the system of reproduction. Ovaries, as organs rich in lipids, are places of accumulation of heavy metals in the female body. Thanks to experiments dealing with the regulation of reproduction, the area of reproductive biology and biotechnology can move forward by leaps and bounds. Among the main regulators we include hormones.

MATERIAL AND METHODS

Laboratory rats have been selected as a model organism (n = 10). Rat ovaries obtained post-mortem were stored individually in a thermos with a physiological solution at room temperature and have been treated up to 6 hours after death. Ovaries were washed with sterile culture medium DMEM/F12 (1:1 BioWhittaker™, Verviers, Belgium) and were cut into fragments of 2 mm in diameter. Fragments were then cultured in a sterile culture medium DMEM/F12 (1:1 BioWhittaker™, Verviers, Belgium), which were supplemented with 10% fetal calf serum (BioWhittaker™) and antibiotic - antimycotic (Sigma, St. Louis, MO, USA) without addition (control) or with the addition of ammonium molybdate tetrahydrate [$(NH_4)_6.Mo_7O_{24}.4H_2O$] in the following concentrations : group A (500 $\mu g.ml^{-1}$ ($(NH_4)_6.Mo_7O_{24}.4H_2O.ml^{-1}$), group B (330 $\mu g.ml^{-1}$ [$(NH_4)_6.Mo_7O_{24}.4H_2O$]), C (170 $\mu g.ml^{-1}$ [$(NH_4)_6.Mo_7O_{24}.4H_2O$]), group D (90 $\mu g.ml^{-1}$ [$(NH_4)_6.Mo_7O_{24}.4H_2O$]). Ovarian lysates were cultured (37 ° C, 5 % CO_2) without Mo (control) / with the addition of Mo (experimental groups) in the culture plates (1 $ml.culture^{-1}$). After 18 hours of culture was taken syringe medium from the culture plates and stored at 70 ° C until analysis.

RIA analysis and western imunoblotting

Steroid hormones progesterone and estradiol were analyzed by RIA method using hormone labeled with radioactive iodine ^{125}I. Concentrations of steroid hormones were determined by RIA in 25 to 100 ml incubation medium. These substances were attached using RIA kits (Immunotech SAS, Marseille Cedex, France) according to the manufacturer's instructions (Makarevich and Sirotkin, 1999). All of RIA kits were designed to be used with samples of culture medium.

Proteins were divided by gel electrophoresis and they were transfered on polyvinyldenfluoride membrane. After transfer, proteins were coloured by Ponceau S. Membrane was blocked by 15 minutes incubation in 3 % H_2O_2. Membrane was incubated one hour in solution of primary antibody with phosphate buffer (TTBS) and 1 % albumine bovine serum. Primary antibody was diluted follows: Bcl-2 (1:250), caspase-3 (1:500) and Bax (1:500). Unbounded antibodies were washed in TTBS 2x10 minutes. Membrane was incubated with secondary antibody against mice imunoglobulins joined with peroxidase. After incubation membrane was washed in TTBS 3x10 minutes and it was incubated 5 minutes in detection reagent Super Signal. After detection membrane was drained and exposed with Rtg film. Film was developed by FOMA LP-T developer (diluted 1:4) and universal fixer FOMAFIX from FOMA Bohemia s.r.o. (Hradec Kralové, ČR).

Statistical analysis

Analysis of substances in the incubation medium was performed in duplicate. Statistical differences between the control (no exposure $(NH_4)_6.Mo_7O_{24}.4H_2O$) and treatment groups, which were exposed to $(NH_4)_6.Mo_7O_{24}.4H_2O$ - A, B, C, D), were evaluated One Way ANOVA test using

statistical program Sigma Plot 11.0 (Jandel, Corte Madera, USA). Significance of differences between control and experimental groups was determined at $p < 0,05$.

RESULT AND DISCUSSION

Effect of molybdenum on the secretion of hormones

Effect of molybdenum to release IGF-I

In the revision the secretion of IGF -I rat ovarian cells is expressed without addition of ammonium molybdate tetrahydrate [$(NH_4)_6.Mo_7O_{24}.4H_2O$]. Ovary cells produced in the control group 4,16 ± 1,14 ng.ml^{-1} IGF -I compared to the experimental groups : A (2,60 ± 0,61 ng.ml^{-1}), B (2,58 ± 0,36 ng.ml^{-1}), C (1,79 ± 0,33 ng.ml^{-1}), D (0,77 ± 0,43 ng.ml^{-1}) with the addition of Mo. The release of IGF - I was significantly ($p < 0,05$) reduced the influence of all Mo concentrations used in our experiment.

Kolesárová et al. (2009b) indicate that molybdenum may have an effect on ovarian cells gilts. After the experimental administration of ammonium molybdate in doses of 0,33 mg.ml^{-1}, 0,5 mg.ml^{-1} and 1,0 mg.ml^{-1} release was inhibited by IGF-I . Another study, which looked at the influence of Mo on the release of IGF-I in layers, suggesting that the administration of 0,17 and 0,9 mg.ml^{-1} ammonium molybdate, conclusive ($p < 0,05$) differences between groups in the secretion IGF - I were recorded. Experimental group with the highest dose of Mo (0,33 mg.ml^{-1}) significantly inhibited the release of IGF-I (Kolesárová et al., 2009b), which is in line with our study. Bersényi et al. (2008) found that the addition of Mo to the feed of rabbits does not affect their growth. Rabbits were fed pellets, carrots, containing 39 mg Mo.kg^{-1} dry foods and dietary supplements that contained 40 mg Mo.kg^{-1}. During the 14 days conclusive changes were not found. The results of our work show the inhibitory effect of Mo on the secretion of growth factor IGF-I.

The release of progesterone

In the control group rat ovarian cells 108,47 ± 1,51 ng.ml^{-1} hormone progesterone was detected. By comparing the experimental groups (A, B, C, D) significant differences were found in the secretion of progesterone influence of molybdenum. Experimental group A produced 78,02 ± 18,87 ng.ml^{-1}, group B 67,62 ± 15,85 ng.ml^{-1}, group C 50,90 ± 8,58 ng.ml^{-1}, group D 46,99 ± 8,34 ng.ml^{-1}. The tendency of reduced secretion of progesterone by the effects of Mo has been reported, but these results were not conclusive ($p \geq 0,05$).

According to Kolesarova et al. (2009a), by adding 1,0 mg.ml^{-1} ammonium molybdate, progesterone release in granulosa cells gilts was stimulated, which is consistent with the authors' earlier work (Kolesárová et al., 2009b), who observed a similar trend secretion of progesterone influence of Mo compounds. The hens were induced with progesterone secretion by addition of 0,17 and 0,33 mg.ml^{-1}. Kolesárová et al. (2010) suggest that the release of progesterone ovarian cells gilts was affected by the addition of 1,0 mg. ml^{-1}, but other doses used had no effect, which is in line with our work. This finding points to the impact of higher doses of Mo on ovarian cells in animals. On the release of P_4 in the case of White Leghorn breed presence or absence of fetal calf serum in culture had no impact. Ovary cells that were cultured with or without the addition of addition of serum produced approximately equal amounts of progesterone (Sirotkin, 2010).

The release of estradiol¨

After administration of molybdenum were found between control and experimental groups A, C, D had significant differences ($p \leq 0,05$) in estradiol secretion. The control has fallen 223,85 ± 15,23 pg.ml^{-1}, in group A 36,04 ± 8,68 pg.ml^{-1}, B 177,69 ± 52,37 pg.ml^{-1}, C 54,48 ± 17,71 pg.ml^{-1}, D 47,31 ± 32,69 pg.ml^{-1} ammonium molybdate.

Regarding the secretory activity of ovarian cells of rats with a focus on estradiol, we recorded inhibition by Mo. Kolesárová et al. (2012) report that the release of estradiol in rats is affected by other additions, for example: bee pollen incorporated in feed. Female rats increasingly produced estradiol because of a feed mixture containing 5 kg of pollen per 1000 kg of feed. In the experimental group, which was fed with a mixture of lower pollen addition of 3 kg of pollen per 1000 kg of feed, a change was observed in estradiol secretion. Sirotkin (2010) states that the lack of fetal calf serum has an effect the release of estradiol. The differences between the impact of additions for secretion of estradiol and progesterone indicate different nutritional regulation of these hormones. Other authors state that the release of estradiol was inhibited by the addition of tetrathiomolybdate at 1-$\mu g.ml^{-1}$ first. The results suggest that the doses of Mo did not affect the secretion of steroid hormone progesterone, but inhibited the secretion of estradiol (Kendal et al., 2003).

The release of apoptic markers

Expression of Bcl-2 has been demonstrated in fraction 1 with molecular weight 26 K. Effect of addition $(NH_4)_{6.}Mo_7O_{24}.4H_2O$ were recorded changes in expression anti-apoptic peptid. In ovary cells were noticed inhibition of expression Bcl-2 peptid impact of $(NH_4)_{6.}Mo_7O_{24}.4H_2O$ by using lowest dose, increasing expression were recorded in group B. Most intensive ihibition was detected in groups C and D, using by lowest concentration Mo.

In ovary cells was detected change of expression pro-apoptic marker Bax, which has been demonstrated in fraction 1 with molecular weight 23 K. In this case was recorded only increasing expression of apoptic marker Bax impact of $(NH_4)_{6.}Mo_7O_{24}.4H_2O$. Most intensive expression was noticed by using lowest concentration Mo.

Expression of apoptic marker caspase-3 was detected in fraction 1 with molecular weight 34 K. Most intensive expression was noticed in groups A and B, where was used highest concentration of Mo, not modified expression was recorded in group D with lowest dose of Mo.

Expression of anti-apoptic peptid Bcl-2, pro-apoptic markers Bax and caspase-3 was affected with addition DON. Expression of Bcl-2 was decreased by using DON, while excretion pro-apoptic factors was decreased all of using doses (10 $ng.ml^{-1}$, 100 $ng.ml^{-1}$,1000 $ng.ml^{-1}$) DON (Kolesárová et al., 2011/11). Dose-depended way DON and apoptic regulators can develop apoptosis in human gastric carcinomes in cell line SGC-7901 a BGC-823 *in vitro.* Apoptic mechanisms can increase with production of homologic dimers Bax-Bax and can decrease with production dimers Bax-Bcl-2 (Liu et al., 2009). In previous studies was identified change of expression apoptic marker caspase-3 in granulose cell in gilts addition of Mo. Expression of caspase-3 was stimulated with addition Mo compound in doses: 0,09 a 1,0 $mg.ml^{-1}$ (Kolesárová et al., 2010).

CONCLUSIONS

Effect of molybdenum and mechanism of action on rat ovary cells, in conjunction with growth factor IGF-I, steroid hormones and apoptic markers are insufficiently investigated. Based on the findings of the presented work it is possible to believe that Mo affects the endocrine and apoptic processes in the rat ovary. Also, a potential regulator of folliculogenesis, steroidogenesis and apoptosis through intracellular regulators. Our knowledge indicates that the effect of Mo on ovarian process is dose-dependent. For a complete understanding of the mechanisms of action of Mo on ovarian processes will require further study and verification. Our work contributes only to clarify the mechanism of action of Mo in the female reproductive system.

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