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ROLE OF VITAMIN E ON ANTIFOLLICULOGENESIS EFFECTS OF LEAD ACETATE ON DIAMETER OF FOLLICLES CONTAINING OVARIAN TISSUE OF SWISS ALBINO MICE

Durgesh Nandini Sharma & Lata Bhattacharya
Endocrinology and Physiology Unit, School of Studies in Zoology and Biotechnology,
Vikram University, Ujjain, M.P., India

Abstract

Lead is one of the oldest known and most widely studied occupational and environmental toxicant. Lead compounds are known to adversely affect the gonadal structure and functions, can cause alterations in fertility and impaired gamete function. The toxic effects of lead on adult female reproduction are decreased fertility, the inability to sustain pregnancy and reduced pregnancy outcomes. The objective of the present study was to examine the effect of oral administration of lead acetate (1.25 mg/kg) daily for 30 days on the folliculogenesis process in the mice ovary and the protective role of antioxidant vitamin E against the induced damage. Histomorphological studies of ovary exhibited remarkable damage in follicle development and reduction in follicle size.. The weight of ovary, uterus and fallopian tubules were significantly reduced in lead treated group. These changes were ameliorated with the administration of vitamin E.. The results of this study suggested that cotreatment with vitamin E has a protective role against lead induced ovarian damage in mice.

Key words: Antioxidant, Folliculogenesis, Histomorphological. Lead, Ovary, Swiss mice, vitamin.

Introduction

The female reproductive system and, therefore human fertility may be affected by exposure to environmental toxicants. In this regard, most attention has been paid to toxic environmental factors that cause ovarian toxicity (Hruska *et al.*, 2000). Epidemiological and animal studies have shown that trace metals such as lead, cadmium and mercury have the potential to disrupt ovarian function (Hoyer, 2005). Lead is a pervasive environmental pollutant whose mechanism of toxicity is currently being investigated. Reproductive consequences of lead exposure are widespread (Patrick, 2006), affecting almost all aspects of reproduction (Zheng, *et al.,* 2003). From high to low doses of lead exposure, there are different responses of lead including reduced fertility, spontaneous abortions, low birth weight, impairment in folliculogenesis, and even damage to the ovaries are also reported (Fortune, 2003). Animals have shown that low levels of lead accumulation in the ovaries could impede folliculogenesis (Lefevre, 2001). A low lead concentration in the mouse ovary caused dysfunction of folliculogenesis, with fewer primordial follicles and an increase in atretic antral follicles (Taupeau *et al.,* 2001). Oral administration of lead in high doses leads to reduction in the number of ovarian follicles revealing a strong correlation between blood lead level and atresia of ovarian follicles of albino mice (Shah *et al.,* 2008). It is reported that the female gamete physiology in vitro is modified by exposure to very low levels of lead (Avazeri *et al.,* 2006). Specific effects of lead on ovarian function have been observed in mice (Junaid *et al.,* 1997), rats and monkeys (Francks *et al.,* 1987). Longer and more variable menstrual cycles have been found in lead treated female Rhesus monkeys (Laughlin *et al.,* 1987).

Reproductive toxicity is the adverse effects of chemicals on gonadal structure and functions, alterations in fertility and impaired gamete function (Timbrell, 1995). The treatment of lead poisoning, especially at sub clinical level is equally important. Most of the chelating agents tend to have adverse side effects and the benefits are usually transitory, since, blood lead can be rapidly replaced from the bone store (Mahaffey *et al.,* 2000). Hence, metal chelation therapy has not been more successful to treat lead poisoning (Bondy, 1988). Oxidative damage associated with the presence of lead has been illustrated as one possible mechanism involved in lead toxicity (Adoneylo and Oteiza, 1999), which suggests that antioxidant might play a role in the treatment of lead poisoning (Gurer *et al.,* 2001).Animals have protective mechanism in the form of antioxidant nutrients, vitamins and several enzymes. Antioxidant may play an important role in abating some hazardous effects of lead. The body consists of an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals. Chow (1991) reported vitamin E and occupies an important and unique position in the overall antioxidant defense. The antioxidant function of vitamin E is closely related to the status of many dietary components. Antioxidative properties of vitamin E is believed to prevent reproductive disease associated with oxidative stress (Brigelius-Flohe *et al.* 2002). Vitamin E interacts with oxidizing radicals and terminates the chain reaction of lipid peroxidations (Jones *et al.,* 1995). The present study has been undertaken to assess the lead induced oxidative damage on folluculogenesis and their protection by antioxidant such as vitamin E using Swiss mice as an experimental model.

Materials and Methods

Animals

Inbreed, healthy female swiss albino mice (*Musmusculus*) in the age group of 5-6 weeks, with 22-28gm body weight were used for the experiment. Animals were maintained under standard laboratory condition and provided them

balance diet and water ad-libitum daily.

Treatments

Animals were divided into control, experimental and recovery groups. The control group was given vehicle only. The experimental groups were given lead acetate (1.25 mg/kg) daily for 30 days by gavage (0.2 ml/animal). The recovery groups received lead acetate (1.25 mg/kg) and Vitamin E (2 mg/kg) for the same period by the same route.

Measurements of body and organs weights

Animals were sacrificed after their respective treatments and their body weights were recorded together with individual organs weights.

Histopathological Techniques

The ovaries were fixed fixed in Bouin's fixative. Following routine procedure of dehydration and block preparation 5-6μm thick paraffin sections were cut with a rotary microtome and mounted on clear and albumenized slides. These slides were stained with haematoxylin and eosin and used for further observations.

Microscopic study

For microscopic analysis, sections were selected using a non-random 10% sampling. Numbers of ovarian follicles were counted in each 10th section of the ovary (Bolon *et al.,* 1997), so that each counted section was separated by a distance of approximately 50-60 μm from the next 10th section. Differential follicle counting and categorizing was performed by a blinded person. Ovarian follicles were classified on the basis of ovarian follicle morphology. Follicles that contained a single layer of squamous follicular cells were considered as primordial; the primary follicle contains an oocyte surrounded by a single layer of cuboidal follicular cells; the secondary follicle contains more than one layer of follicular cells around the oocyte and the antrum was not present; and the follicles containing scattered spaces or a distinct antrum were considered as antral (Britt *et al.,* 2000). For measuring the diameter of ovarian follicles in each developmental stage, 45 microscopic fields were randomly chosen in each mouse. Then, using an ocular micrometer of light microscopy (Olympus EH), at a magnification of ×10, the largest and smallest diameters of each ovarian follicle were measured and the mean was calculated.

Statistical analysis

The data in different groups were compared by one-way analysis of variance (ANOVA) and Tukey's test was used as a post hoc test. Differences were considered to be significant when $p<0.05$, $p<0.01$ and $p<0.001$.

Results

There were no significant difference in the body weight of mice in control and experimental groups. Ovarian weight significant decreases ($P<0.01$) in LA treated group compared to control while vit. E supplemented group show body weight near the control. Similarly the weights of fallopian tube and uterus were significantly reduced ($p<0.001$) as compared to control. In contrast, LA injected mice supplemented with Vit. E encountered significantly greater weight of fallopian tube and uterus than LA treated group (Table 1).

The histological examination of the control ovary showed normal structure of surface epithelium, and well developed stroma with cortical and medullary regions. Primary and secondary follicles showed compact appearance. Preantral and antral follicles were also apparent with a fluid filled cavity antrum present among the granulosa cells. While in lead treated group certain deformation in their histological structure were noted. In a few cases the primordial and primary follicles were highly damaged and were surrounded by atrophied follicular cells with karyohypertrophy and their diameter was significantly altered ($P<0.01$) with comparison to control. In some cases the granulosa cells gathered in the centre of the follicle and oocyte was not apparent. In most of the follicles the regular structure of granulosa cells altered. The degeneration in granulosa cells were apparent in all developing follicles. The large number of developing follicles exhibited degenerative changes characterized by pyknotic nuclei. Some of these were chumped together while others had big vacuoles. The diameters of all the developing follicles were reduced in comparison to control (Table 2). Supplementation of vitamin E daily to lead treated mice increase body and organs weights and partially protects ovary from oxidative stress. In addition, coadministration of vitamin E with lead resulted in maintenance of diameter of ovarian follicles at control levels and significantly lowers the morphologically deformed ovarian follicles population.

Discussion

The ovarian follicle is the functional unit of the ovary. It contains the oocyte that may eventually ovulate, undergo fertilization and form an embryo. It also provides the steroid and protein hormones required for maintenance of the ovarian cycle, the secondary sex characteristics and preparation of the uterus for implantation. In the present study lead toxicity induced histological alterations in the various components of the ovary and these changes were rebalanced with the administration of antioxidant vitamin E. The microscopical examination of the ovary in the present study revealed that there was apparent damage and reduction in the diameter of different developing follicles while the number of atretic follicles increases markedly. Taupeau *et al.,* (2001) reported that lead accumulation in low concentration in the ovary caused dysfunction of folliculogenesis, with fewer primordial follicles and an increase in atretic antral follicles. The coordinated development of follicles in the ovary is under the control of hormones and growth factors (Fortune, 2003). It has been demonstrated that LH stimulates follicular maturation and induces follicular atresia, recent study suggest that LH also stimulate early stages of follicular growth (Mori *et al.,* 2009). On the basis of the above hypothesis, our results

suggest that lead adversely affects the pituitary hypothalamus axis, and the balance of gonadotropin. Due to lead toxicity the equilibrium of gonadotropins in exposed females, alters and this leads to the alterations in the follicular growth. In the present study the above pattern of follicular growth and regression of atretic follicles were observed in control animals. Antral cavities with dead granulosa cells can be easily identified in ovaries of controls. But the dynamics of follicles were altered with the lead exposure.

The results of the follicle diameter, in the present study indicate that, ovaries of lead treated group showed decreased diameter of different developing follicles and congestion in stromal tissue.The weights of the ovary, fallopian tube and uterus were also reduced compared to controls. It was also observed that ovarian physiology and rate of ovulation might also altered in females exposed to lead, because corpora lutea was rarely seen in the treated females. Ercal *et al.* (1996) observed that chronic exposure to lead damaged primordial and medium follicles and arrested follicular development in Rhesus monkeys Taupeau *et al.* (2001) showed that even low doses of lead provoked an inhibition in folliculogenesis leading to dysfunction of this process.

In the present study combined treatment with lead and vitamin E led to improvement in most of lead induced apoptotic changes However, some vacuoles and empty spaces were observed among the developing follicles. This improvement might be secondary to antioxidant ability of vitamin E which attacks ROS and so antagonizes their harmful effects on the tissues (Chow, 2001). Also it has been found that lead toxicity is associated with decreased level of alpha tocopherol that explains the importance of the prophylactic use of the vitamin E in these conditions (Ergurhan *et al.,* 2008).The persistence of some degenerative and apoptotic changes in some follicles that appeared with pyknotic nuclei also the persistence of degenerated and vacuolated granulosa cells in animals treated with vitamin E and lead. This means that the protective role of vitamin E against lead induced follicular damage is dependent on the duration of lead exposure. The persistence of these changes in spite of the great neutralizing effect of vitamin E might result from the increased production of reactive oxygen species- by lead intoxication which overwhelms the capacity of intrinsic defense mechanisms in the cells. So this tocopherol enrichment is not sufficient to protect ovary from toxic effect of lead. On the other hand certain mechanisms might be implicated in the lead inducing testicular damage other than production of ROS. Lead could accumulate in cell nuclei associated with nuclear proteins and chromatin and change their structure (Quintanilla *et al.,* 2000) It has been found that lead induced DNA alterations might be irreversible (Acharya *et al.,* 2003). In agree with these finding, it was proved that vitamin E did not produce any appreciable effect on reduced glutathione (GSH) status and other related enzymes in animals after prolonged exposure to lead (Flora *et al.,* 2003).

In conclusion, the results of the present study suggested that oxidative stress is a major cause of lead induced follucogenesis damage in the ovarian tissue of mice. Using an antioxidant as vitamin E interferes with the reactive oxygen species production and improves lead toxicity.

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Annexure

Table 1: Body (g) and organs (mg) weights of control and experimental groups of female mice.

S.No.	Parameters	Control	Treated (LA) for 30 days.	Treated (LA+Vit.E) for 30 days
1.	Body Weight	36.8+0.96	39.6+0.45NS	38.2+0.87NS
2.	Ovary Weight	43.2+1.56	35.5+0.18**	41.00+1.51*
3.	Fallopian Tube Weight	44.00+1.97	29.6+2.8***	38.00+1.70**
4.	Uterus Weight	494.20+9.85	246.00+18.05***	531.00+13.36**

All values are expressed + SEM, Significant level, NS= Non significant, * = (P<0.05), ** = (P <0.01), * = (P<0.001)**

Table 2: Diameter (mμ) of different developing follicles in control and experimental groups of female mice.

S.No.	Parameters	Control	Treated (LA) for 30 days	Treated (LA+Vit.E) for 30 days
1.	Primordial Follicle	18.48+0.01	12.25+0.02**	17.45+0.05**
2.	Primary Follicle	40.65+0.08	30.45+0.01**	39.25+0.1**
3.	Secandary Follicle	168.25+2.24	140.32+2.10***	153.30+2.18**
4.	Antral Follicle	195.02+30.00	175.01+32.00***	190.01+24.10***

All values are expressed + SEM, Significant level, NS= Non significant, * = (P<0.05), ** = (P <0.01), * = (P<0.001)**