The effect of cadmium in combination with zinc and selenium on ovarian structure in Japanese quails

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Abstract

In this study the effect of single cadmium, cadmium + selenium and cadmium + zinc administration on the ovarian structure of Japanese quails was studied. The morphometric analysis of the relative volume of primary follicles detected the highest value in control group with a similar value in the group with administration of cadmium with selenium. Lower relative volume is reported in group with cadmium and zinc administration and the group with simple cadmium administration ($P<0.05$). The relative volume of growing follicles was very similar in all studied groups (11.33 – 15.35%), and the relative volume of stroma was very stable (82.59 – 86.45%).
In the evaluation of the number of follicles undergoing atresia significantly higher number of atretic primary follicles as well as growing follicles in the group with cadmium administration and cadmium with selenium administration in comparison with control group was found. In comparison of normal and atretic follicles we report the most negative effect on ovarian structure in group with cadmium administration. Selenium co–administration show protective effects but only the co–administration with zinc prevent significant cadmium ovarian alterations.

Key words: cadmium, zinc, selenium, ovary, Japanese quails

Introduction

Pollution of the environment and contamination of animals including game with cadmium is serious problem in most countries (Hecht et al., 1984). Cadmium is an environmental pollutant that has serious toxicity in humans and animals and causes Itai-Itai disease (Mochizuki et al., 2002), what induced proteinuria, glucosuria, and osteomalacia and/or osteoporosis (Friberg et al., 1986). Cadmium is known to be both extremely toxic and ubiquitous in natural environments. It occurs in almost all soils, surface waters and plants, and it is readily mobilised by human activities such as mining. Cadmium toxicity may be more common among natural populations of vertebrates than has been appreciated to date and that cadmium toxicity may often go undetected or unrecognised (Larison et al., 2000).
Quantifying the transfer of cadmium from foods to mammalian target organs is a key to estimating the health risk from this exposure; however, the bioaccumulation of cadmium is modified by many dietary components. Studies of dietary cadmium absorption would be simpler if it was known that cadmium added to foods as a soluble salt was as bioavailable as cadmium incorporated during growth of the food species (Chan et al., 2004).
In long – term chronic occupational exposure to cadmium, the kidneys are usually the most critically affected organs (WHO Study Group, 1980). The kidney is well known to be a major target organ of cadmium in animals and humans. During chronic exposure

the metal accumulates in renal cortex up to what appears to constitute a critical level at which the incidence of overt malfunction in a human population at risk begins to increase (Kjellstrom et al., 1984). Problems with cadmium were studied by many authors in human (Mueller et al., 1989; Ikeda et al., 1996), rabbits (Foulkers and Blanck, 1990; Massányi et al., 1995; Chan et al., 2004), rats (Krasny and Holbrook, 1977; De Wolf et al., 2004), wild animals (Świergosz et al., 1993; Doganoc and Gačnik, 1995; Larison et al., 2000; Jančová et al., 2002; Mochizuki et al., 2002; Massányi et al., 2003)
Cadmium exposure leads to renal tubular dysfunction. This is primarily a re-absorption defect in the proximal tubules and the critical effect of cadmium. There are also various effects on reproduction, causing follicular atresia in rabbit ovary (Massányi and Uhrín, 1996), degenerative alterations in testes (Massányi et al., 2002; Toman and Massányi, 2002) decreases spermatozoa motility (Lukáč et al., 2003; Massányi et al., 2004). Cadmium can be etiological factor in various pathological processes as higher blood pressure, arteriosclerosis, growth inhibition, alterations in central nervous system, hepatic dysfunction, bronchitis and teratogenic effects (Friberg et al., 1986; Oushi et al., 2000).
The aim of this study was to determine toxic effects of cadmium and also the effect of combined action of cadmium with zinc and selenium on the ovarian structure.

Material and methods

In this study Japanese quails 3 month old were used. After a week acclimatization animals were divided into groups. Control group (n=30) was fed with a feeding mixture with any additional substances. In cadmium (Cd; $CdCl_2$) group addition of cadmium representing 6 x multiplication of allowable limit in feeding mixture was added. In cadmium and selenium group (Cd+Se; Na_2SeO_3) the same amount of cadmium and addition of twice fold increased recommended concentration of selenium was administered. In the cadmium and zinc group (Cd+Zn; $ZnSO_4$) again the same cadmium concentration and addition of twice fold higher as recommended zinc concentration was added. The quails were fed with feeding mixture HYD 06 after from the beginning of egg production with HYD 10. All additional substances were added to the water and the samples were collected after 118 days of experiment.
Ovaries of the experimental (Cd, Cd+Zn, Cd+Se) and control animals were fixed in 10% formol, dehydrated in a graded series of ethanol and embedded in paraffin wax. Whole ovaries were sectioned on a microtome. The serial section 10 μm thick were stained with haematoxylin and eosin. From slides based on micromorphological criteria (Weibel et al., 1966; Massányi and Uhrín, 1996), the relative volume of primary follicles, growing follicles and stroma as well as the percentage of normal and atretic primary and growing follicles was evaluated with respect to each ovary.
To compare results the analysis of variance (mean, minimum, maximum, standard deviation) as well as F-test (PC program Excel) were applied.

Results

The sufrace of quail ovaries was covered by a single layer of epithelium. A substantial basement membrane separated the surface cells from the underlying ovarian tissue divided to the inner medulla and outer cortex, which consist of follicles and stroma.

An evaluation of the relative volume of primary follicles detected the highest value of this parameter in control group with a similar value in the group with administartion of cadmium with selenium. Lower relative volume was found in the group with cadmium and zinc administration and the group with simple cadmium administration ($P<0.05$). The relative volume of growing follicles was very similar in all studied groups (11.33 – 15.35%) with any significant differences. Also the relative volume of stroma was very stable (Table 1).
In the evaluation of the number of follicles undergoing degenerative alterations (atresia) we have found significantly higher number of atretic primary follicles in the group with cadmium administration and cadmium with selenium administration in comparison with control group. The same findings are reported for atretic growing follicles (Table 2).

Table 1.
Relative volume (%) of primary, growing follicles and stroma in ovary

	x	s.d.	CV	minimum	maximum
control					
primary follicles	**2.42**	1.12	46.18	0.99	4.12
growing follicles	**13.74**	2.92	21.22	10.84	17.62
stroma	**83.84**	3.20	3.82	80.31	88.17
Cd					
primary follicles	**0.79**+	0.36	46.21	0.49	1.35
growing follicles	**16.63**	8.49	51.09	5.99	29.60
stroma	**82.59**	8.79	10.65	69.05	93.41
Cd+Se					
primary follicles	**0.78**	0.31	39.42	0.59	1.13
growing follicles	**15.35**	5.28	34.41	9.82	20.34
stroma	**83.88**	5.53	6.59	78.53	89.57
Cd+Zn					
primary follicles	**2.23**	1.02	45.75	1.06	2.94
growing follicles	**11.33**	7.57	66.84	6.04	20.00
stroma	**86.45**	8.13	9.40	77.06	91.28

x – mean; s.d. – standard deviation; CV – coefficient of variation
+ – $P<0.05$ (control – Cd)

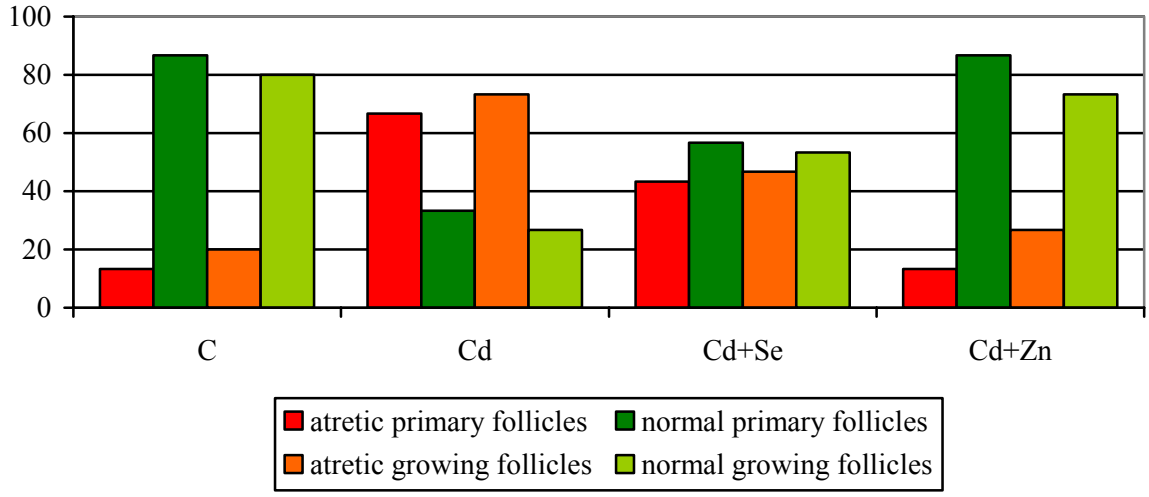
Table 2.
Relative number (%) of atretic follicles in ovary

	x	s.d.	CV	minimum	maximum
control					
primary follicles	**13.33**	11.55	86.60	0	20
growing follicles	**20.00**	10.00	50.00	10	30
Cd					
primary follicles	**66.67**+	15.28	22.91	50	80
growing follicles	**73.33**+	23.09	31.49	60	100
Cd+Se					
primary follicles	**43.33**	5.77	13.32	40	50
growing follicles	**46.67**	5.77	12.27	40	50
Cd+Zn					
primary follicles	**13.33**	11.55	86.60	0	20
growing follicles	**26.67**	5.77	21.65	20	30

primary follicles: + – $P<0.001$ (control – Cd); + – $P<0.01$ (Cd – Cd+Zn)
growing follicles: + – $P<0.01$ (control – Cd; Cd – Cd+Zn)

In comparison of normal and atretic follicles (Figure 1) we report the most negative effect on ovarian structure in group with cadmium administration. Selenium co–administration show protective effects but only the co–administration with zinc prevent significant cadmium ovarian alterations.

Figure 1.
The relative number of atretic and normal follicles in relation to studied elements



Discussion

It is generally known that cadmium is mainly accumulated in kidneys and liver of animals (Toman and Massányi, 1996; Massányi et al., 1995; Tataruch, 1994). Many authors present that organ of higher accumulation of cadmium is kidney as a detoxifying organ (Tataruch, 1994; Dip et al., 2001; Massányi et al., 2003; Linde et al., 2004).

Results of our study prove negative effects of cadmium on the ovarian structure. In previous study (Massányi and Uhrín, 1996) we have reported that on microspic level with regard to the number of follicles, the lowest number of primary follicles was found after i.p. administration of cadmium. Significantly lower numbers of follicles with less than two layers of granulosa cells were found. The number of atretic follicles was significantly higher in all groups with an administration of cadmium. Percentual content of growing follicles was significantly higher and of stroma significantly lower in the control group in comparison with all experimental groups receiving an administration of cadmium. In *in vitro* cultured porcine ovarian granulosa cells similar alterations were reported (Massányi et al., 2000). Cell membranes were disintegrated manifested by the occurrence of vacuoles in the cytoplasm. The vacuoles contained fibrillar or membranous material. The Golgi complex rarely remained intact. Increased number of lysosomes was detected. With increasing cadmium concentration the number of lipid droplets increased.

The stimulatory and inhibitory effects of cadmium on progesterone synthesis were recently investigated using the steroidogenically stable JC-410 porcine granulosa cells line, which was genetically modified with gene constructs containing the promoter

region of the cytochrome P450 side chain cleavage gene linked to a luciferase reporter gene (Henson and Chendrese, 2004).
Generally the effetcs of cadmium on reproductive parameters of various animal species are incompletely described. Most of studies describe accumulation of this toxic element in ovaries (Massányi et al., 1995, 1996). On the other hand, ultrastructural observation and specially quantification of toxic effect of cellular structures of various cells is inadequate. Exposure of human granulosa cells to cadmium resulted in morphological alterations in the monolayer depending on dose with longer exposure, cells began to separate from each other by contacting towards the centre and assuming a circular shape (Paksy et al., 1997).
Generally there are few data describing the effect of cadmium on granulosa cells (Henson and Chendrese, 2004; Smida et al., 2004; Vršanská et al., 2003; Drbohlav et al., 1998) with mainly monitoring and biochemical aspects of toxicity. In luteal cells only interference of cadmium with steroid biosynthesis in rat luteal cells *in vitro* was studied (Paksy et al., 1992).
The relation of cadmium to vascular disorders such as atherosclerosis in experimental animals was studied (Kaji, 2004). Cadmium destroys the monolayer of endothelial cells and the cytotoxicity is protected by zinc and copper without metallothionein induction. In addition, cadmium reduces endothelial fibrinolytic activity by induction of plasminogen activator inhibitor type 1 synthesis and by inhibition of tissue-type plasminogen activator, respectively. In vascular smooth muscle cells, cadmium can promote their proliferation and influence proteoglycan synthesis and fibrinolysis in different manners. Results indicate that cadmium have specific toxicity in the proliferation, fibrinolysis, and extracellular matrix formation of vascular endothelial and smooth muscle cells. Endotelial alteration of cadmium are described mainly in liver, causing an inflammatory processes that plays a major role in the secondary injury of the liver, and infiltration of neutrophils at the site of necrosis is a common observation (Mousa, 2004).

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