Studies of simultaneous exposure of lead and cadmium on Hypothalamic-Pituitary endocrine changes in relation to female reproductive system

Synopsis of the Thesis

Submitted to

Maharaja Sayajirao University of Baroda

For the degree of Ph. D.

(Doctor of Philosophy) in Biochemistry

By

Anil Kumar Pillai

CSIR Award # 9/114(112) 12KI/EMR-I

Department of Biochemistry

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Introduction

Heavy metals are present in the environment because of the application of modern technology in the industry and through the scientific and technical advances. They are taken by mammals through the food web and create a health risk for both humans and mammals. Field and laboratory studies indicated that bioaccumulation of heavy metals, occurs in primary and secondary consumers of the food web. Among them lead and cadmium have been shown to accumulate in various tissues such as the kidney and the liver (Daggett et al., 1998; El-Maraghi et al., 2001). More recently their accumulation in other organs such as the hypothalamus, pituitary or gonads were reported (Paksy et al., 1990; Lafuente et al., 1999a). The risk of cadmium exposure comes from its high persistence in the tissues as the mean half life of this metal is over 15 years in humans and over 200 days in rats. This accumulation leads to different disorders of the endocrine system (Paksy et al., 1989; Lafuente et al., 1999a). Many of the health effects of lead exposure such as developmental delay and decreased stature appear to be the result of neurotoxicity and consequent endocrine disruption.

One aspect of heavy metal associated endocrine disruption is reproductive dysfunction. Endocrine disruptions associated with environmental metal exposure of pregnant women and young children may also represent a significant public health problem. Results from some rat studies suggest that exposure to these heavy metals during pregnancy and the neonatal period may result in delayed sexual maturity, decreased sperm count, loss of masculine sex behavior, irregular estrous cycling, reduced numbers of corpora lutea, decreased volume of sexually dimorphic nucleus of the hypothalamus and irregular pulsatile release pattern of gonadotropins in adult offspring (Klein et al., 1994; Olsen et al., 1995; Ronis et al., 1998). Various investigators have suggested that the major site of action of these heavy metals on hypothalamic-pituitary-gonadal axis is at the level of the hypothalamus (Klein et al., 1994; Lafuente et al., 1999). This view was supported by observations that exposure to lead caused an impaired release of pituitary LH in response to hypothalamic challenge with the opiate antagonist naloxone, an enhanced release of LH in response to direct stimulation of the pituitary with luteinizing hormone releasing hormone, increased pituitary LH stores and increased

GnRH mRNA in the hypothalamus (Klein et al., 1994). However considerable evidence also exists for direct effects of lead at the level of gonads and on sex steroid/LH feedback loops.

Among the environmental endocrine disruptors, cadmium may play a role in the ateilogy of gonadal dysfunction (Olsen et al., 1995). It was shown that cadmium can affect the activity of the hypothalamic-pituitary-gonadal axis by acting at the hypothalamus (Das et al., 1993; Antonio et al., 1998), the pituitary (Lorenson et al., 1983; Cooper et al., 1987; Lafuente et al., 1999a) and the ovary (Paksy et al., 1990). Effect of lead and cadmium on hepatic tissue has also been reported (Hoffmann et al., 1972; Nigam et al., 1999; Patra et al., 1999). Since many reproductive functions are controlled by sex steroids, the possibility that changes in the synthesis or breakdown of these hormones may alter reproductive capacity in man and other animals exposed to these heavy metals cannot be excluded.

Despite the fact that toxic waste disposal sites and other exposure vectors common to lead and cadmium often involve both metals, most research on these two metals has dealt with each in isolation; that is, most investigations are designed to examine only lead or cadmium. On the other hand, populations in real life always have simultaneous multiple exposures, indicating the need for experimental work with combination of substances. Based on this the earlier dose dependent studies performed in our laboratory with low level isolated and combined exposure of lead and cadmium have shown that dose as low as 0.1 mg/kg body weight/day for 30 days can also affect δ-Aminolevulinic acid dehydratase (marker of lead toxicity) activity, differentially in isolated and combined state (Gupta et al., 1994). In light of this and the various deleterious effects of these metals on reproduction and endocrine function as cited above, it was worthwhile to study the simultaneous exposure of lead and cadmium on hypothalamus-pituitary axis function in relation with female reproductive system.

Objectives of the study

I. Dose dependent study on the effects of lead and cadmium in isolation and combination on hepatic, pituitary and hypothalamic steroid metabolism in non pregnant rats

II. To study the effects of lead and cadmium in isolation and combination in non pregnant rats on hypothalamic-pituitary axis function

III. To study the effects of lead and cadmium in isolation and combination in pregnant rats on

(a) reproductive performance

(b) hepatic estradiol metabolism

(c) hypothalamic-pituitary axis function

IV. The mechanism of action of lead and cadmium either alone or in combination on liver and hypothalamic-pituitary axis

Summary of the work done:

I. Dose dependent effect of lead and cadmium either alone or in combination on the hepatic, pituitary and hypothalamic steroid metabolism in non pregnant rats

Adult synchronized female rats were treated intraperitonially with lead acetate and cadmium acetate separately and in combination in dose dependent manner (0.025, 0.05 and 0.1 mg/ kg body wt/day) for 15 days. The estradiol metabolizing enzymes (17β-hydroxy steroid oxidoreductase and UDP glucoronyl transferase) activities decreased with increasing dose showing significant change compared to control. Also, significant decrease in cytochrome P450 (CYP450) content was found after the treatment. Displacement of zinc bound to metallothionein was more in cadmium treated rats compared to other groups. The protein, DNA and RNA content were found decreased in

all treated groups compared to control. Th microsomal cholesterol and lysosomal acid phosphatase levels were found increased after the metal treatment in a dose dependent manner. In all these parameters, treatment in combination of lead and cadmium showed intermediate results indicating some kind of competition between the two metals. But the histological studies showed that combined treatment causes more cytotoxic effect than cadmium and lead alone. These results indicated that metal cations tested did have a direct inhibitory effect on the metabolizing enzyme activities. Studies were also done on hypothalamic and pituitary steroid metabolising enzyme 3α hydroxy steroid dehydrogenase in both proestrous and estrous stages. The cadmium treated group showed significant decrease in enzyme activity at 0.05 and 0.10 doses compared to other groups. In 0.025 dose there was no change in the enzyme activity in any of the treatment groups. The effects observed were increased with increase in dose. There was no significant change observed between estrous and proestrous results. Thus the dose dependent study showed 0.05 mg/kg body wt. as the optimal dose which was then used for further experiments.

To understand whether effect on metabolising enzymes are direct effect or due to general hepatotoxicity, *in vitro* experiments with metal treatment with concentration equivalent to concentration reaching the tissue were performed. These results showed same order of inhibition which suggests direct effect of metals, these effects can be protected by pretreatment with GSH.

II. Effect of lead and cadmium either alone or in combination on hypothalamic-pituitary axis function in nonpregnant rats

Since hypothalamus is an important tissue which plays an important role in the modulation of pituitary hormone secretion it was of interest to know if the alterations in pituitary hormones by metal exposure are mediated by changes in the neurotransmitter levels at the hypothalamic level. Therefore the effects of lead and cadmium either alone or in combination on hypothalamic neurotransmitter content and plasma and pituitary levels of leutinizing hormone (LH) and follicle stimulating hormone (FSH) were studied. Adult female rats were treated intraperitonially with either lead acetate and cadmium acetate alone or in combination at a dose of 0.05 mg/kg daily for 15 days. Serotonin (5-HT) and norepinephrine (NE) levels decreased in individually and combined metal

treated groups whereas dopamine (DA) levels were decreased only in cadmium exposed group. The pituitary levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were decreased significantly in cadmium and combined treatment groups. In contrast, lead exposure failed to cause any change in serum LH and FSH levels whereas cadmium and combined treatments showed significant decrease in serum LH and FSH levels as compared to control. The accumulation of both metals increased in hypothalamus and pituitary after the treatment. These data suggest that the metal accumulation disrupts the regulatory mechanisms of the hypothalamic-pituitary axis where the effects produced by the combined treatment of metals are not additive. The pituitary protein and cholesterol levels were found decreased in all treated groups compared to control. Studies on the effect of the metals on the binding of GnRH on isolated pituitary membrane are under progress.

III. Effect of lead and cadmium either alone or in combination in pregnant rats

(a) ***reproductive performance***

Animals were also monitored for ovarian cycle by vaginal cytology. There was no change in estrous cyclicity in any of the metal exposed groups.

Treatment pattern

Pretreatment for 5 days	Mating	Treatment during gestation and lactation
Dose: 0.05 mg/kg body wt. per day s.c.		

There was no change in the reproductive cyclicity in any of the exposure groups. Frequency of pregnancy was equally distributed over all four exposure groups and no effect was observed on reproductive performance. The litter size, placental weights, pup weights, pup liver weights, maternal weights or maternal liver weights did not differ significantly. Growth of the progeny was monitored regularly.

(b) hepatic estradiol metabolism

Hepatic steroid metabolizing enzymes (17β-hydroxy steroid oxidoreductase and UDP glucoronyl transferase) were inhibited by the metal exposure. Both maternal and pups catabolising enzymes showed decrease in activity after the exposure. This was observed both in 0 day and 25 day post natal period. Displacement of zinc bound to metallothionein was more in cadmium treated rats compared to other groups. The protein, DNA and RNA levels were found decreased in all treated groups compared to control. Hepatic glycogen content was decreased in cadmium and combined treated groups in both lactating mother and 2υ p.n. pups. There was accumulation of lead and cadmium in the treated groups as compared to control. The accumulation of metals was also observed in fetal and 20 p.n. day pups. Zinc content was increased in cadmium and combined treated groups in pregnant mother whereas fetal liver showed decrease in the metal level in the above groups as compared to control.

(c) hypothalamic-pituitary axis function

Serotonin (5-HT) and norepinephrine (NE) levels decreased in individually and combined metal treated groups whereas dopamine (DA) levels were decreased only in cadmium exposed group. The accumulation of both metals increased in hypothalamus and pituitary after the treatment. Also the activity of steroid metabolising enzyme 3 hydroxy steroid dehydrogenase in hypothalamus and pituitary was decreased after the metal treatment with cadmium showing maximum inhibition.

IV. The mechanism of action of lead and cadmium either alone or in combination

Increasing evidence suggest that multifactorial mechanism might be involved in metal induced toxicity and it is suggested that one of the well known mechanisms is metal induced reactive oxygen species (ROS). Redox inactive metals such as lead and cadmium deplete cell's major antioxidants, particularly thiol containing antioxidants and enzymes. Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA. The toxic effects of free radicals upon membrane structure and functions have been reported such as changes in permeability, activity of enzymes,

channels, transport proteins, membrane fluidity and receptors.

Effects on hepatic, hypothalamic and pituitary antioxidant defense system

Female rats treated with i.p. injection of lead acetate and cadmium acetate alone and combination (0.05 mg/kg body weight daily for 15 days) showed decrease in glutathione (GSH) content as well as increase in lipid peroxidation (LPO) in cadmium and combined treated groups. Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities were decreased in lead, cadmium and combined metal treated groups. The catalase (CAT) activity was increased in cadmium and combined treated groups without any effect in lead treated group. The combined treatment resulted in intermediate effects in different antioxidative parameters studied indicating competition between the metals. Similar trend of changes in antioxidant parameters also observed in hypothalamus and pituitary.

In order to understand the interaction between the two metals in the production of free radicals, ***in vitro*** experiments were carried out using different induction systems as controls. The increase in the production of different free radicals such as superoxide, nitric oxide and hydroxyl ions by the metals either alone or in combination compared to the respective induction system was assessed. The metal salts in three different concentrations (0.01, 0.1 and 1.0 mM) were used in isolation and combination. There was significant increase in the production of superoxide and nitric oxide radicals in both isolation and combination groups in all the three doses compared to control system. Also increase in lipid peroxidation levels was observed after incubating the liver homogenate with metals salts.

Effects on pituitary membrane integrity

Since free radicals are known to affect membrane structure and function effects of metal treatment on pituitary membrane integrity were also monitored. It was seen that heavy metal treatment resulted in decreased membrane fluidity. Among the three groups cadmium treatment was showing more effect compared to other treatments and the combined treatment was showing intermediate values. Na^+K^+ATPase, a membrane bound enzyme, activity also was decreased significantly by cadmium and combined treatments. The Schiffs base and inorganic peroxide levels were increased after the metal exposure. Exposure to lead and cadmium caused accumulation of the metals in the pituitary and

lowered the membrane fluidity, which may affect membrane function and cause alterations in receptor binding and secretory mechanism(s) of pituitary hormones. These effects produced by the combined treatment of metals were intermediate.

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Research Papers Published/ Accepted:

- Title : **Effect of low level exposure of lead and cadmium on hepatic estradiol metabolism in female rats**

  Authors : **Anil Pillai**, Laxmipriya, Rawal A, Gupta S

  Journal : **Indian Journal of Experimental Biology** 40, 807-811 (2002).

- Title : **Effects of combined exposure to lead and cadmium on pituitary membrane of female rats**

Authors : **Anil Pillai**, Laxmipriya and Gupta S

Journal : **Archives of Toxicology** (in press)

- Title : **Effects of combined exposure to lead and cadmium on hypothalamic-pituitary axis function in proestrous rats**

  Authors : **Anil Pillai**, Laxmipriya and Gupta S

  Journal : **Food and Chemical Toxicology** (in press)

<u>Research Papers communicated:</u>

- Title : **Combined exposure of lead and cadmium on hepatic antioxidant system and lipid peroxidation**

  Authors : **Anil Pillai**, Laxmipriya and Gupta S

  Journal : **Journal of Trace Elements in Medicine and Biology**

- Title : **Dose dependent effect on simultaneous exposure of lead and cadmium on ovarian function in proestrous rats**

  Authors : Laxmipriya P.N., **Anilkumar Pillai** and Sarita Gupta

  Journal : **Bulletin of Environmental Contamination and Toxicology**

- Title : **Effect of simultaneous exposure of lead and cadmium on gonadotropin receptors of granulosa cells: an " *in vitro* " study**

  Authors : Laxmipriya P.N., **Anilkumar Pillai** and Sarita Gupta

  Journal : **Toxicology**

Table 6: Human exposure to various cadmium sources

Phosphate Fertilizers	41.3 %
Fossil fuel combustion	22.0 %
Iron & steel production	16.7 %
Natural sources	8.0 %
Non-ferrous metals	6.3 %
Cement production	2.5 %
Cadmium products	2.5 %
Incineration	1.0 %