Chapter VII

Summary

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Reproductive and developmental effects of environmental contaminants have become an issue of great concern in the recent times. Previous studies on female reproductive system mainly dealt with ovarian toxicity, reproductive performance and effects during gestation and lactation, both on mother and fetus. Not much emphasis has been given to understand their role as endocrine disruptors. Moreover, the earlier studies have not considered the role of simultaneous exposure to toxicants, which is very important in light of the fact that general population is exposed to multiple toxicants. Alterations of the endocrine system can be complex, and not necessarily limited to a particular organ or molecular mechanism. For instance, they can alter: (1) hormone production at its endocrine source; (2) the release of stimulatory or inhibitory hormones from the pituitary or hypothalamus and (3) hepatic biotransformation of hormones. All of these mechanisms must be examined if contaminant-induced endocrine disruption is suspected. In the present study the effect of lead and cadmium, two well-known reproductive endocrine disruptors, alone and in combination on neuroendocrine and hepatic steroid metabolism alongwith hypothalamic-pituitary axis function have been evaluated. In addition, the mechanism of action of both the metals in liver and pituitary was also studied.

Adult female rats were treated with lead and cadmium either alone or in combination in dose dependent (0.025, 0.05 and 0.10 mg/kg body wt.) and time dependent manner (15, 30 and 45 days) and evaluated for steroid metabolizing enzymes in hypothalamus, pituitary (3α-hydroxy steroid dehydrogenase) and liver (17β-hydroxy steroid oxidoreductase and UDP glucoronyl transferase). All the metal exposed groups exhibited significant inhibition in the activity of steroid metabolizing enzymes in dose and

maximum inhibitory effects in cadmium, minimum in lead and intermediate effects in the combined metal exposed groups. It has been observed that the inhibition in the enzyme activities could be due to the binding of these metals with the –SH groups present at the active site of the above enzymes (Quig, 1998). The metal concentration was increased with increase in dose and resulted in displacement of zinc bound to metallothionein fraction from liver and decrease in cytochrome P450 content. An established deficiency of essential metal in the enzyme pool and the increased ability of toxic metals to compete for the binding sites of the high molecular weight proteins may explain inhibitory effects on the enzyme activities (Onosako and Cherian, 1981). Although the cadmium exposure was causing maximum inhibitory effects at the enzyme level, the histological studies showed that combined treatment causes more cytotoxic effect than cadmium and lead alone which indicate that these metal cations have a direct inhibitory effect on the metabolizing enzyme activities. There was no significant change observed between estrous and proestrous results.

From dose and time dependent studies 0.05 mg/kg body wt. and 15 days exposure were selected as the optimum dosage for further experiments. Since steroid metabolism is under the control of hypothalamic-pituitary axis, it was of interest to know whether alterations observed in steroid metabolism by lead and cadmium alone and in combination are due to the changes at neuroendocrine level. A significant decrease has been observed in hypothalamic serotonin (5-HT) and norepinephrine (NE) levels and individual and combined metal treated groups with decrease in dopamine (DA) levels only in cadmium exposed group. As reported earlier lead and cadmium can alter either calcium mobilization (Hirning *et al.*, 1988; Bratton *et al.*, 1994) or calcium influx through membrane channel

(Cooper *et al.*, 1987; Kasprzak and Poirier, 1985; Poirier *et al.*, 1983), thereby decreasing release of neurotransmitters (Cooper and Manalis, 1983; Nation *et al.*, 1989) followed by decrease in LHRH level resulting in gonadotropin depletion (Lorenson *et al.*, 1983; Paksy *et al.*, 1989). The results of our study agree with this, as both LH and FSH levels were decreased in lead, cadmium and combined metal exposed groups. As these changes were accompanied by increase in metal concentration in both hypothalamus and pituitary it can be suggested that the metal accumulation disrupts the regulatory mechanisms of the hypothalamic-pituitary axis.

Thus it is very well demonstrated that lead and cadmium in isolation and combination act as endocrine disruptors by affecting neurotransmitters, gonadotropins and hepatic biotransformation of gonadal steroids in non-pregnant rats. Pregnancy and lactation are physiological conditions, which are regulated by proper balance of hormonal milieu. Hence further study has been carried out with respect to reproductive performance, pregnancy and lactation with lead and cadmium exposure in isolation and combination with 0.05 mg/kg body wt. subcutaneously from 5 days preconception till the completion of gestation and lactation. No effect was observed in the reproductive performance such as percentage of successful pregnancy, litter size, number of resorption, and number of dead pups or birth weights. This could be due to the superinduction of hepatic metallothionein (MT) mRNA by continued metal exposure (Hazelhoff Roelfzema et al., 1988) and reduction in the bioavailability of highly toxic metal ions like free lead and cadmium. As the metal administration is continued during pregnancy and lactation, the heavy metals getting more accumulated in the liver compared to non-pregnant rats can displace the zinc ions present in MT. Since cadmium has high affinity to zinc, it resulted in more decrease in

Zn level in the lactating animals. The displaced Zn ions are transferred through blood as Cd, Zn-MT to kidney. During pregnancy placenta is an important link between mother and fetus. It is a known fact that placenta is impermeable to cadmium and sequester cadmium ions as Cd-MT (Lucis et al., 1972; Arizono et al., 1981), which results in less toxic effects to the fetus. In case of pups (21 days old) hepatic cadmium concentration was found more; the mechanism for this could be due to its transport through maternal milk during lactation. Also, Zn gets displaced during lactation from maternal liver. However, cadmium exposed neonates still showed less amount of Zn, this could be due to the competition of Cd and Zn for transport. As lead is known to cross the placental barrier and is not easily excreted, it gets accumulated in fetal liver throughout gestation and lactation.

The steroid metabolism was affected both at hepatic as well as hypothalamo-hypophyseal level after the metal exposure. The hepatic 17β-hydroxy steroid oxidoreductase activity was inhibited more in lactating animals as compared to non-pregnant and pregnant (20 days gestation) animals, which indicates that with increase in concentration of the metals in liver the enzyme activity is inhibited more. We could not observe any significant change in the activity of UDPG-Transferase among pregnant and non-pregnant animals. The hypothalamic as well as pituitary steroid metabolizing enzyme activity was inhibited more in pregnant animals as compared to the non-pregnant and lactating animals. The metal induced alterations in the hypothalamic neurotransmitter levels were seen in pregnant rats also, but no significant change observed among the non-pregnant and lactating animals. Metal intoxication of mothers during gestation and lactation caused decrease of hepatic glycogen content.

From the above discussion it becomes clear that both lead and cadmium either alone or in combination are able to inhibit the steroid metabolism and cause alterations in hypothalamic-pituitary axis function. Eventhough it is known that both these metals have high affinity to sulfhydryl groups, the mechanism of action of these metals when present together is not known. One of the possible mechanisms for the metal induced effects is oxidative stress. Because fatty acid chain length and unsaturation are associated with membrane susceptibility to peroxidation (Lawton and Donaldson, 1991); metal induced arachidonic acid elongation might be responsible for the enhanced lipid peroxidation as observed in the pituitary membrane after metal exposure. By causing lateral phase separation and/or by increasing lipid peroxidation rates, the metal could affect membrane-related processes as has been demonstrated in the present study like the activity of membrane enzyme, Na^+K^+ATPase and fluidity. Eventhough both vitamin E and Mn ions have showed beneficial role on metal stress, only Mn ions could completely restore the metal induced decrease in MnSOD activity and increase in TBARS to control value. When both the metals are used in combination, there seems to be competition between the two metals leading to intermediate effects. These results indicate that both lead and cadmium could bind –SH groups, inactivating the antioxidant defense system and thus promote generation of free radicals.

Conclusion

- Lead and cadmium in isolation and combination resulted in dose and time dependent inhibition on hepatic, pituitary and hypothalamic steroid metabolizing enzyme activities. Accumulation of metal increased with dose and time and caused zinc displacement from metallothionein protein and also decreases in cytochrome P450 levels.
- 0.05 mg/kg body wt. for 15 days exposure time was selected as optimum dose. Decrease in hypothalamic neurotransmitters, pituitary gonadotropin levels were observed in non-pregnant, pregnant and lactating animals with not much variation between the three different physiological states.
- Estrous cyclicity, reproductive performance, frequency of pregnancy, litter size and maternal, fetal and neonatal weights did not demonstrate any change at 0.05 mg/kg body wt. subcutaneously from 5 days preconception till the completion of gestation and lactation. However, the steroid metabolizing enzyme activities were inhibited in pregnant and lactating mothers as well as in fetus and neonates.
- To understand the mechanism of such biochemical changes *in vivo* and *in vitro* experiments were performed in isolation and combination with lead and cadmium, which resulted in depletion in GSH and antioxidant enzyme activities. Elevation in lipid peroxidation, change in membrane fluidity and membrane bound enzyme, $Na^{+}K^{+}$ATPase indicating the role of oxidative stress.

Chemical mediators like neurotransmitters and hormones are major regulators of various body functions. In the present study, it has been demonstrated

systematically that lead and cadmium in isolation and combination inhibit neurotransmitters at hypothalamic level, gonadotropins at the pituitary level and biotransformation of gonadal steroids during non-pregnant, pregnant and lactating periods of life, implying the fact that toxicants in the environment also must be causing similar kind of deleterious effects at each and every level of human body. It is not necessary that metals (toxicants) when present together always mediate additive response. Therefore the effects depend on the dose and time of exposure and mechanism of interaction of the toxicants. Also the present study could not demonstrate any toxicity in relation to disruption of estrous cyclicity or reproductive, gestational and lactational performance. Such subclinical toxicity is alarming situation for more deleterious effects on human health with nutritional, physical, mental and emotional stresses.