Chapter 7 Summary

Summary

Reproductive effects of environmental toxicants in general and heavy metals in particular; have been an issue of great concern since decades. Most of the earlier studies on male reproductive system mainly dealt with testicular toxicity and reproductive performance. However, not much emphasis was on complex communication system and regulatory function of HPG axis. Various environmental pollutants are considered as endocrine disrupters including heavy metals like lead and cadmium, which can alter endocrine function by altering synthesis, breakdown of hormones and modifying functions of hormone receptors. Few issues of concern are learning more about interactions of multiple pollutants even at low level and their mechanism of action. Hence, in the present study effect of lead and cadmium in isolation and combination were evaluated on HPT axis and further biochemical, molecular and cellular mechanism of toxicity was studied. Epidemiological studies reported that environmental exposure to lead and cadmium has association with several diseases. So, in present study demographic study has been carried out to understand association of environmental pollutants with benign prostatic hyperplasia (BPH).

Adult male rats were treated with lead and cadmium either alone or in combination (0.025 mg/kg body wt/15 days) and was evaluated for their steroid metabolizing enzymes in hypothalamus, pituitary (3α-hydroxy steroid dehydrogenase), testis (3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase) and liver (17β-hydroxy steroid oxidoreductase and UDP glucoronyl transferase). Various steroid metabolizing enzymes in hypothalamus, pituitary, testis and liver exhibited maximum effect in cadmium exposed group and simultaneous exposure did not demonstrate any additive effect rather exhibited least effect. Amongst all tissues testis is found to be most sensitive to lead and cadmium and this was also correlated with accumulation of metals and variation in metallothionine protein content. This 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).

Since the control of testicular function by hypothalamic-pituitary axis is regulated and by neurotransmitters and neuromodulator, it was of interest to know whether alterations observed in steroid metabolism by lead and cadmium alone and in combination are due to the change in hypothalamic neurotransmitter content. A significant decrease has been observed in hypothalamic norepinephrine (NE) and dopamine (DA) levels with lead and cadmium (individual) metal exposed groups. 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 (Kasprzak and Poirier, 1985), thereby decreasing release of neurotransmitters (Cooper and Manalis, 1983; Nation *et al.*, 1989) and decrease in LHRH level followed by gonadotropin depletion resulting in decrease testosterone levels (Paksy *et al.*, 1989). The results obtained in our study is in accordance with the above report, as both serum and testicular levels of testosterone were decreased in lead, cadmium and combined metal exposed groups. Thus, the metal accumulation disrupts the regulatory mechanisms of the hypothalamic-pituitary-testicular axis which further affected infertility parameters (testicular and epididymal sperm count, sperm motility) in all metal treated groups.

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-testicular axis function. Even though 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 well elucidated. Therefore, present study was further carried out to understand biochemical, molecular and cellular mechanism of lead and cadmium induced toxicity.

One of the possible biochemical mechanisms for the metal induced effects is oxidative stress. Our current study has clearly demonstrated depletion in GSH content, antioxidant enzyme activities (SOD, Catalase, GPx, GST, GR, G6PDH), elevation in lipid peroxydation in all metal exposed rats. Thus, metals (Pb,Cd)

modulate redox status; their by causing oxidative stress. This could be one of the mechanism by which metals cause toxicity.

Further, in vitro exposure of metals demonstrate lower extent of inhibition on both 3β-HSD and 17-β-HSD enzyme activities, compared to *in vivo* exposure suggesting that hypothalamus and pituitary are the master regulators, and play important role.

It is clear from above experiments that metals (Pb and Cd alone and in combination) affects activities of steroidogenic enzymes. Therefore, it was of great interest to determine whether lead and cadmium cause any effect on transcription of key steroidogenic regulatory enzymes. Hence, mRNA expression analysis using RT-PCR was carried out. Results revealed that mRNA levels of genes responsible for cholesterol transport and steroidogenesis including steroid acute regulatory protein (StAR), was decreased significantly compared to control. Also, mRNA levels of CYP11a, 3β-HSD and 17β-HSD was also demonstrated a decrease. The pattern of inhibition obtained was similar to that of enzyme activities. The results clearly indicate that metals interfere both at transcriptional as well as at biochemical level. The decrease in mRNA expression of StAR, CYP11a, 3β-HSD and 17β-HSD in Pb and Cd exposed animals could be due to the replacement of zinc ion by Cd/Pb from essential transcription factor which further resulted in an increase in the mRNA levels of MT I and MT II. As it is known that increase in free Zn activates the MTF-1 a regulator of metallothionine transcription (Zhang et al., 2003).

Further, it was of great interest to study effects of Pb and Cd at cellular level using leydig cells as the model. *In vitro* experiments were performed with the concentration of lead and cadmium reaching the tissues after *in vivo* exposure for 15 days (Pb, Cd and Pb+Cd at a final concentration of 3 µM, 0.5 µM and 2 µM + 0.015 µM respectively). Results on isolated and purified rat leydig cells with Pb and Cd exposure (*in vitro*) clearly demonstrated altered gonadotropin receptor (LHR and FSHR) binding, suppressed key steroidogenic enzyme activities (17β hydroxy steroid dehydrogenase and 3β hydroxy steroid

dehydrogenase activity) and decreased testosterone secretion in all experimental groups. Possible reasons for decreased binding could be co-operation of metal ions and receptor, binding to amino acids like cysteine residues of the receptors or interferences of metal with the stability of hormone-receptor complex. We found significant decrease in both 3β-HSD and 17β-HSD enzyme activities in leydig cells exposed to Pb and Cd in isolation and in combination. Immediate decrease in the activity of both 3β-HSD and 17β-HSD obtained on exposure to Pb and Cd or both can be explained by the binding of metal/s directly to the amino acids present at the active site of the enzyme. Both Pb and Cd can bind to -SH groups of cysteine residue present at the NAD binding domain (Persson et al., 1991) and thereby altering the structure of the substrate binding site and affecting enzyme function significantly. Cellular study signifies the decrease in gonadotropin binding as well as direct effect on enzyme inhibition, which lead to decreased hormonal secretion. Histopathological observation of testis and liver for cytotoxic changes demonstrated marked alteration in liver histology mainly in cadmium exposed group.

Study was further extended to correlate impact of lead and cadmium in real life situation on human health. Hence, epidemiological study was carried out to understand the association of environmental pollutants (Pb and Cd) with the incidence of BPH in patients of Western India. Our study showed that cadmium accumulation has a positive correlation with ACP the known biochemical markers and negative correlation with Qmax a known clinical marker. Also, positive family history and age, has been recognized as potential factors in severity of BPH with decrease in Qmax. Decrease in GSH, antioxidant enzyme activities and increase in LPO levels, ultimately reflects ROS mediated effects in the pathogenesis of BPH, which can be correlated with higher Cd content and lower Qmax in smokers compared to non-smokers along with increased lipid peroxidation and altered antioxidant enzymes activities. Pb content shows significant positive correlation only with lipid peroxidation. The result thus suggests that imbalance of pro-oxidant and antioxidant status due to Pb and Cd accumulation may be another factor responsible for the progression

of BPH.In our study highest correlation was observed with Cd and not with lead, supporting the estrogen mimicking role of Cd for BPH pathogenesis apart from oxidative stress.

Present study as clearly indicated that Pb and Cd affect all the steroidogenic proteins, both at expression and at activity level resulting in altered HPT axis function. In, conclusion Pb and Cd exposure at low level affects reproductive function leading to male fertility. Demographic study also suggests that environmental contaminants are responsible for progression of BPH.

All the effects are correlated to accumulation of metals. The significant accumulation of both metals was noted in all tissue. Cd seems to be more toxic than Pb. Although protective mechanism exists; Cd at sub-clinical level is a potent eco-toxicant than lead. Combined metal exposed groups demonstrated least effects suggesting competition between the two metals for binding site when the metals are present at same concentration. Therefore the effects depend on the dose and time of exposure and mechanism of interaction of the toxicants. Such sub clinical toxicity is alarming situation for more deleterious effects on human health with nutritional, physical, mental and emotional stresses.

References

Bo Zhang, Oleg Georgiev, Michael Hagmann, C¸agatay Gu¨nes, Mirjam Cramer, Peter Faller, Milan Vasa´k, and Walter Schaffner Activity of Metal-Responsive Transcription Factor 1 by Toxic Heavy Metals and H_2O_2 In Vitro Is Modulated by Metallothionein Molecular And Cellular Biology. 2003, 23, 8471-8485.

Bratton GR, Hiney J.K, Dees WL. Lead (Pb) alters the norepinephrine induced secretion of luteinizing hormone releasing hormone from median eminence of adult male rats in vitro. Life Science 1994, 55, 563-571.

Cooper GP, Manalis RS. Influence of heavy metals on synaptic transmission, A review. Neurotoxicology 1983, 4, 69-84.

Hirning LD, Fox AP, McCleskey EW, Olivera BM, Thayer SA, Miller RJ, Tsien RW.Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from sympathetic neurons. Science 1988, 239, 57-60.

Kasperczyk A, Kasperczyk, S, Dziwisz M, Birkuer E, Walecko C, Winiarska H, et al. Lead and cadmium concentration in human semen. Ginekol Pol 2002, 73, 449-453.

Nation JR, Frye, GD, Von Stultz J, Bratton GR. Effects of combined lead and cadmium exposure, Changes in schedule-controlled responding and in dopamine, serotonin, and their metabolites. Behav Neuroendocr 1989, 103, 1108–1114.

Paksy K, Varga B, Horvath E, Tatrai E, Ungvary G. Acute effects of cadmium on preovulatory serum FSH, LH and prolactin levels and on ovulation and ovarian hormone secretion in oestrus rats. Reprod Toxicol 1989, 3, 241–247.

Persson B, Krook M, Jornall H. Characteristics of short chain dehydrogenases and related enzymes. European J Biochem. 1991, 200, 537-543.

Quig D, Cysteine metabolism and metal toxicity. Altern Med Rev. 1998, 3, 262-270.

Conclusion

- Co exposure of lead (Pb) and cadmium (Cd) in isolation as well as in combination at dose of 0.025 mg/kg body weight for 15 days of exposure caused a modulation in steroid status. Both metal toxicants inhibited key steroidogenic enzyme-3β-HSD, 17β-HSD in testis, 3α-HSD in hypothalamus in pituitary and 17β-HSOR in liver. Maximum inhibition of all above key enzymes was obtained in Cd exposed group while least inhibition was seen in coexposed group as compared to control. These effects obtained were well correlated with accumulation of metals in respective tissues.
- Accumulation of metals and inhibition of steroid metabolizing enzymes significantly affected, reproductive function parameters in Cd exposed animals as compared to control; however no structural change in testis was observed in all metal treated groups. This alteration in reproductive function can be correlated with change in testosterone level, sperm count, motility and alteration in semen biochemical parameters, which is evident in all metal groups.
- To understand the mechanism for such observed modulation of steroid status, and animals were treated with same dose and time regime. The metal toxicants caused an alteration in redox status leading to increase in lipid peroxidation and decrease in all anti-oxidant enzyme activities and sulphydryl status. Also, molecular studies demonstrate that expression of all key steroidogenic enzymes and protein (StAR, CYP 11A, 3β-HSD, 17β-HSD) were decreased in all metal treated groups. However, significant inhibition of MT-I and MT-II was exhibited in Cd exposed animals.
- To explore the direct toxic effect on the testicular leydig cells, *"in vitro"* experiments were performed. Both decreased gonadotropin binding and steroidogenic activity was well correlated with decreased testosterone levels in all metal treated groups. However, effects of *"in vitro"* exposure on steriodogenic enzyme were significantly less as compared to *"in vivo"* exposure suggesting role of entire hypothalamic-pituitary-testicular-hepatic (HPTH) axis in causing such deleterious effects.

- As male reproductive system function also depends on the functioning of accessory male organs, it was interesting to study the association of metal pollutant with benign prostate hyperplasia. All patients demonstrated change in biochemical markers, which is especially well correlated with Cd status. Also, all patients exhibited an alteration in redox status with increase in heavy metal concentration. Patient's history suggests that smoking and genetic predisposition also plays an important role in etiology of BPH.

Current study demonstrates the association between metal concentration and endocrine disruptions at various target sites (HPTH axis). It is evident that both molecular and biochemical reproductive events are affected upon sub-clinical metal exposure. It is also very clear that metals in combination have antagonistic effect on HPTH axis thereby showing least effect which is due to competition between metals.

Thus, lifestyle modifying factors like smoking, environmental pollutants does play an important risk factor in maintenance of male fertility. Hence, our study emphasizes that sub-clinical toxicity pose a threat in maintenance of male reproductive function and future progeny.

... ... *Beginning of journey*

.....The submission of this thesis is not an end of my educational voyage. Rather, it is the beginning to understand the philosophy of this universe through the eyes of 'Biochemistry'. Swami Vivekananda has stated "Education is the manifestation of the perfection already in man". In the want of this 'perfection' in the area of Biochemistry this research was taken by me. This work taught me not only research methodology but also precious lessons of my life like perseverance, tolerance, expression of ideas, skills, co-operation and much more.