Ind. J. Physiol. Pharmac., Volume 33, Number 4, 1989

METHYLMERCURY INDUCED BIOCHEMICAL AND HISTOCHEMICAL ALTERATIONS IN RAT TESTIS

AMAL ROY CHOWDHURY, SAVITA MAKHIJA AND KAURESH D. VACHHRAJANI

National Institute of Occupational Health, Ahmedabad - 380 016

(Received on March 3, 1989)

Abstract : The methylmercurry chloride (MMC) administered at doses of 5 and 10μg/kg over a period of 90 days to male rats caused enzymatic impairments in testicular tissue. The study at intervals of 15, 30, 60 and 90 days showed gradual diminution of testicular weight and gradual decrements in testicular protein and inhibition in testicular succinic dehydrogenase activity. Histochemical and biochemical studies revealed that testicular acid phosphatase activity was also inhibited at both the doses of MMC treatment. The inhibition of enzyme activity in testicular tissues after MMC treatment caused the impairment of both spermatogenesis and steroidogenesis in rats.

Key words : testicular tissues, protein, succinic dehydrogenase, acid phosphate, methylmercury chloride

INTRODUCTION

Earliar studies indicated the structural alterations in testicular tissues of different mammalian species exposed to mercury compounds (1). Moreover, morphological deformations and inhibition of spermatogenesis have been reported in methylmercury administered mice (2). Steroidogenic impairments in testicular tissues have also been confirmed after methylmercury treatment (3). Present study was undertaken with a view to note the alterations in different biochemical and histochemical parameters of the testicular tissues after methylmercury chloride treatment.

METHODS

Sixty five healthy male rats (Charles Foster strain) weighing 70±5 g were used in the present study. The rats were divided into three major groups. The animals were supplied with standard animal chow and water *ad libitum*. Body weight of the animals were recorded twice a week.

Methylmercury chloride (MMC) was dissolved in 10 mM Na_2CO_3 and $NaHCO_3$ buffer, pH 9.2. The control rats (group I n=25) were intraperitoneally administered vehicle while group II and group III rats (n=20 each) were treated intraperitoneally with 5 and 10 μg MMC/kg, daily, respectively, over a period of 90 days.

Five animals from each group were sacrificed on day 0 (only control) and immediately after 15, 30, 60

Reprint request : A Roy Chowdhury National Institute of Occupational Health, Meghani Nagar, Ahmedabad - 380 016, India.

Authors are grateful to ICMR, New Delhi and CSIR, New Delhi for the award of Research Fellowships

and 90 days of treatment The testes were cleanly dissected out and weighed. Five testes from each group in each duration were homogenized in ice cold water for biochemical estimations. Testicular proteins were estimated by Lowry et al (4). Succinic dehydrogenase (SDH) and acid phosphatase (ACPase) were estimated using sodium succinate and disodium phenyl phosphate, respectively, as substrates (5, 6).

Remaining five testes from each group on each duration of the experiment were immediately taken to the cryostatic chamber. 12μm fresh sections at $-20°C$ were cut, placed on coverslips and incubated at 37°C for 45 min in media containing sodium β-glycerophosphate as substrate (7). Substrate free media served as reaction control.

Statistical analysis of the data was performed by Student's 't' test.

RESULTS

Significant gradual decrease of testicular weight was noted on day 15 onwards in both the MMC administered groups (Table I).

Biochemical studies : In the control rats testicular protein, SDH and ACPase gradually increased till day 60 and thereafter the levels were maintained till day 90 (Table I) Testicular protein decreased significantly on day 15 and further gradual diminution was noted over aperiod of 90 days in both the MMC treated groups (Table I). Levels of SDH and ACPase also exhibited profound decrease on day 15 after MMC exposure at both the doses. Gradual significant decrease in SDH and ACPase was observed over a period of 90 days (Table I).

Histochemical studies : In the control testis, localization of ACPase was chiefly cytoplasmic. An intense activity was observed in the Sertoli cell-spermatid association (SSA) regions. The activity was also localized in the Leydig cells (Fig. 1a). After MMC treatment gradual decrease in ACPase activity was observed on day 15 onwards. However, on days 30 and 60 the activity in SSA region showed little increase as compared to the one observed on day 15 in the same group (Fig. 1b). Comparatively, less enzyme activity was observed on day 90 in the 10 μg MMC/kg dose group than in the

TABLE I : Effect of methylmercury chloride (MMC) on testicular weight, protein, succinic dehydrogenase and acid phosphatase.

Days	*Groups*	*Testicular weight* g (n=10)	*Protein* mg/g tissue (n=5)	*Succinic dehydrogenase* μg formazon/15 min (n=5)	*Acid phosphatase* μmole/litre (n=5)
Day 0	Control	0.383±0.040	74.10±0.820	216.10±4.400	76.40±0.930
Day 15	Control	0.720±0.030	98.30±1.820	263.40±3 040	137.80±2.210
	5 μgMMC/kg	0.600±0.040*	70.20±1.690*	176. 0±1.150**	82.20±0.870**
	10 μgMMC/kg	0.610±0.010*	53.00±0.150**	147. 7±1.750**	62.50±2.160**
Day 30	Control	1.200±0.030	108.30±3.430	211. 1±1.230	216.20±1.170
	5 μgMMC/kg	1.100±0.030**	65.30±2.150**	178.70±3.630**	72.30±2.800**
	10 μgMMC/kg	0.940±0.030**	50.00±1.890**	155.60±2.350**	60.40±0.806**
Day 60	Control	1.340±0.010	134.50±3.820	320.10±2.610	254.90±5.500
	5 μgMMC kg	1.120±0.003**	60.10±2.010**	184.30±0.760**	68.90±1.080**
	10 μgMMC/kg	1. 03±0.020**	42.00±2 540**	154.90±1.210**	57.10±1.370**
Day 90	Control	1.430±0 020	136.00±3.400	325.60±1.330	260.20±1.280
	5 μgMMC kg	1. 18±0.020**	54.20±1.90**	177 50±1.110**	60.90±1.160**
	10 μgMMC kg	1. 09±0.010**	30.60±1.72**	150.70±0 860**	48.30±1.390**

Each value is Mean±S.E

*P<0.05, **P<0 01

In each case significance is control Vs treated groups

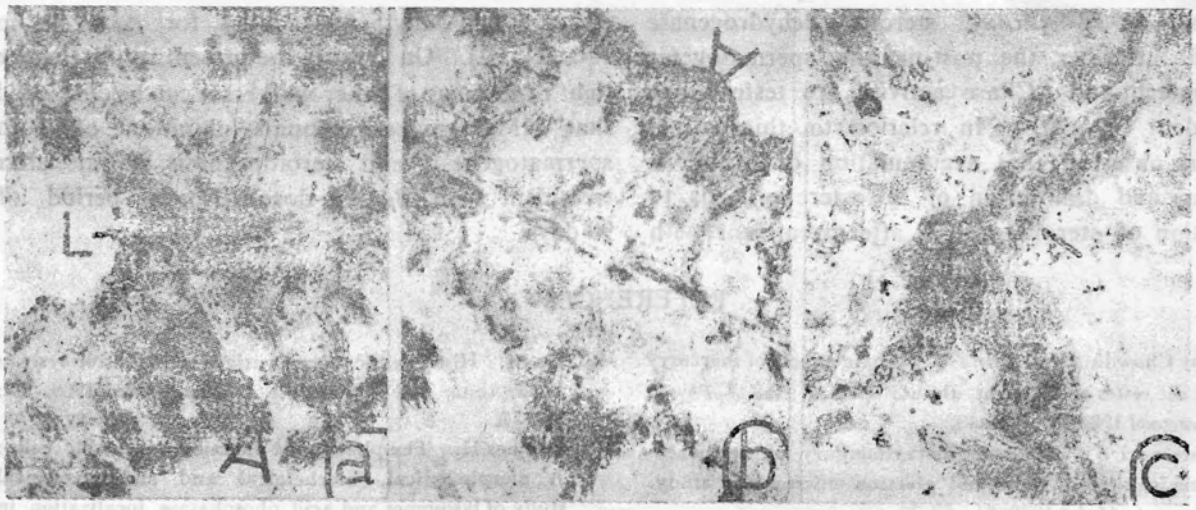

Fig. 1 : a) Intense activity of ACPase is localized in Sertoli cell spermatid association (SSA) regions (A) and in Leydig cells (L). Fine granular reaction is observed in basal part of the seminiferous tubule (1) ×250.

b) Diffused activity is localized in SSA region (A) after 5 μg MMC/kg treatment for 90 days ×250.

c) Inhibition of ACPase activity is clear on day 90 in the tubules (1) and Leydig cells (L), after 10 μg MMC/kg treatment ×250.

5 μg MMC/kg does group. In the seminiferous tubules scattered granular reaction was observed in the basal compartment near the peritubular membrane on the same day (Fig. 1c).

DISCUSSION

The manifestation of MMC toxicity was apparent due to potential loss of testicular weights alongwith concomitant diminution in testicular protein levels. Similar observations have been noted earlier after mercuric chloride exposure (8). Moreover, weight loss and decrease in protein levels are considered to be fundamental aspects of MMC toxicity (9, 10).

The cellular turnover rate is very high in testicular tissues which require high energy and SDH activity is an index for measuring the energy status of the cell (11). Histochemically SDH activity has been localized intensely in post meiotic spermatogenic cells (12). Therefore, graded inhibition of SDH activity in testicular tissues revealed low levels of energy production after MMC treatment. The process of steroidogenesis in testicular tissues has been mainly compartmentalized in Leydig cells. Burton and Meikle (3) demonstrated the alterations in the mitochondrial conversion of cholesterol to pregnenolone in testicular tissues of MMC administered rats. In corroboration with above data, inhibition of SDH activity by MMC could diminish the efficiency of mitochondria.

Acid phosphatase is intensely localized in postmeiotic spermatogenic cells and play a significant role in sperm formation (13, 14). Decline in the activity of ACPase indicated the cellular retardation in postmeiotic spermatogenic developments. Our other studies with mercuric chloride treatment demonstrated the postmeiotic spermatogenic inhibition (8). ACPase is an important lysosomal marker (15) and transport of testosterone is associated with lysosomal function (16). Inhibition of ACPase activity may lead to impairment of testosterone translocation; because mercury inhibited key enzyme of steroido-

genesis, Δ^5-3β-hydroxy steroid dehydrogenase (17, 18). Further, the post-meiotic spermatogenic development and ACPase activity are testosterone dependent (19, 20) In relation to this, other workers observed that accumulation of MMC in pituitary and diminution of testosterone leads to inhibition of steroidogenesis after treatment with 100μg MMC daily 5 days a week for 12 weeks in rats (21, 22). On viewing the present results in the light of findings of other workers it can be concluded that MMC causes profound inhibition of both spermatogenesis and steroidogenesis in rats after treatment with 10μg/kg dose over a period of 90 days.

REFERENCES

1. Roy Chowdhury A, Arora U. Toxic effects of mercurry on the testes of different animal species. *Ind J Physiol Pharmacol* 1982; 26 : 246-49.
2. Katsuragi S Effects of methylmercury on spermatogenesis in rats-A light and electron microscopic study. *Kumamoto Med J* 1978; 31 : 63-78.
3. Burton GV, Meikle AW. Acute and chronic methylmercury poisoning impairs rat adrenal and testicular functions. *J Toxicol Environ Hlth* 1980; 6 : 597-606.
4. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193 : 265-75.
5. Beatty CH, Basinger GM, Dully CC, Bocek RM. Comparison of red and white voluntary skeletal muscles of several species of primates. *J Histochem Cytochem* 1966; 14 : 590-600.
6. Belfield A, Goldberg DM. Revised assay for serum phosphatase activity using for amino antipyrin. *Enzyme* 1971; 12 : 561.
7. Lake BD. J Roy Micro Soc 1965; 85 : 73. c/f Pearse, AGE Histochemistry theoretical and applied J and A. Churchill, 1968. 573.
8. Roy Chowdhury A, Vachhrajani KD, Makhija S, Kashyap SK. Histomorphometric and biochemical changes in the testicular tissues of rats treated with mercuric chloride. *Biomed Biochem Acta* 1986; 45 : 949-56.
9. Berthoud HR, German RH, Weiss B. Food intake, body weight and brain histopathology in mice following chronic methylmercury treatment. *Toxicol Appl Pharmacol* 1976; 36 : 19-30.
10. Kuznetsou DA, Zavijalov NV, Govorkov AV, Sibileva TM. Methylmercury induced nonselective blocking of Phosphorylation processes as a possible cause of protein synthesis inhibition *in vitro* and *in vivo*. *Toxicol Lett* 1987; 36 : 153-60.
11. Walker DG, Selingman AM. Formalin fixation in the cytochemical demonstration of succinic dehydrogenase of succinic dehydrogenase of mithochondria. *J Biophys Biochem Cytol* 1961; 9 : 415-27.
12. Ito M. Histochemical observations of oxidative systems in the testis and epididymis. *Radiation Res* 1966; 28 : 266-77.
13. Chemes H. The phagocytic function of Sertoli cells : A morphological, biochemical and endocrinological study of lysosomes and acid phosphatase localization in rat testis. *Endocrinol* 1986; 119 : 1673-81.
14. Hilscher B, Passia DP, Hilscher W. Kinetics of the enzymatic pattern in the testis. I. Stage dependence enzymatic activity and its relation to cellular interaction in the testis of Wistar rat. *Andrologia* 1979; 11 : 169-81.
15. Dott HM, Lysosomes and lysosomal enzymes in reproduction. *Adv Reprod Physiol* 1973; 6 : 231-77.
16. Szego CM. The lysosomes as a mediator of hormone action in : Recent Progress in Hormone Research (R. O. Greep ed.) Academic Press, New York, 1974; 177-233.
17. Roy Chowdhury A, Vachhrajani KD, Chatterjee BB. Inhibition of 3β-hydroxy $-\Delta^5-$ steroid dehydrogenase in rat testicular tissues by mercuric chloride. *Toxicol Lett* 1985; 27 : 45-49.
18. Roy Chowdhury A, Vachhrajani KD, Kashyap SK. Inhibition of 3β-hydroxy $-\Delta^5-$ steroid dehydrogenase in rat testis by methylmercury chloride. *Adv Contracep Deliv Syst* 1987; 3 : 41-45.
19. Males JL, Turkington RW. Hormonal control of lysosomal enzymes during spermatogenesis in the rat. *Endocrinol* 1971; 88 : 579-88.
20. Steinberger E, Steinberger A. Hormonal control of testicular functions in mammals In : Handbook of Physiology, Endocrinology IV, Part 2, 1975, 325-45.
21. Freeman HC, Sangalang G, Uthe JF, Ronald K. Steroidogenesis in vitro in the Harp Seal (Pagophilus groenladicus) without and with methylmercury treatment in vivo. *Environ Physiol Biochem* 1975; 5 : 428-39.
22. Moller-Madsen B, Thorlacius-Ussing O. Accumulation of mercurry in the anterior pituitary of rats following oral or intraperitoneal administration of methylmercury. *Virchows Arch (Cell Pathol)* 1986; 51 : 303-11.