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Histopathological Changes in the Testis of the Sprague Dawley Rat Following Orally Administered Manganese

T. P. Ponnapakkam, G. H. Sam, M. B. Iszard

College of Pharmacy, Xavier University of Louisiana, New Orleans, LA 70125, USA

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Manganese is an essential element and is a cofactor for a number of enzymatic reactions. While manganese is beneficial or essential at low intake levels, inhalation or oral exposure to high levels are toxic and causes adverse effects. A survey of literature indicated numerous reports have been published on manganese toxicity in previous years. Manganese has been shown to induce biochemical changes (Singh et al. 1974), cytological changes (Dikshith 1978), neurochemical alterations (Deskin 1980), hematological changes (Carter et al. 1980), reproductive alterations (Laskey 1982, 1985) and histopathological changes in rats (Katira and Bawa 1993).

Despite numerous published papers, many questions regarding manganese toxicity remain unanswered. The need to better understand the effect of manganese is becoming more important due to the increased release of manganese into the environment. For example, recently, an organic manganese containing compound, methylpentadienyl manganese tricarbonyl (MMT) received approval for addition to unleaded gasoline to replace tetraethyl lead. Combustion of MMT will increase environmental manganese levels, thereby increasing human exposures to manganese. There is insufficient scientific evidence to assess the public health risk of MMT use (Frumkin and Soloman 1997, Lyznicki et al. 1999). Manganese is also found at hazardous waste sites. It has been found in at least 603 of 1467 National Priorities List sites identified by the Environmental Protection Agency (ATSDR 1997). Manganese acetate is used in textile dyeing, paint, varnish, fertilizer, food packing, feed additives and as an oxidation catalyst. Review of the literature revealed that most of the past research on manganese toxicity was conducted with different manganese compounds such as manganese chloride, manganese carbonate, manganese sulfate, and manganese oxide. There is limited research (Smyth et al. 1969, Komura et al. 1992) on manganese acetate toxicity. Furthermore, there is lack of information on the dose-effect relationship of manganese in laboratory animals.

Our study investigated the effects of varying doses of manganese acetate on the histopathology of testis of Sprague-Dawley rats. The results of this study will contribute novel information to the scientific literature on the adverse effects of oral exposure to manganese in rats.

Correspondence to: M. Iszard

The oral route, which is common to humans and animals, estimates the most probable route of manganese exposure next to inhalation.

MATERIALS AND METHODS

Manganese (II) acetate tetrahydrate, $(CH_3C0_2)_2Mn_4H_20$), FW 245.09, MP> 300 °C, density 1.559, was obtained from Aldrich Chemical Company (Milwaukee, WI). Manganese acetate was chosen as the test compound because of its limited availability in published literature. Manganese acetate (Mn) solution was prepared fresh in distilled water every day prior to dosing. Male SD rats (150-175g), approximately six weeks old were obtained from Harlan-Sprague Dawley Company (Indianapolis, IN). The animals were housed two per cage in stainless steel, wire-bottom cages. The animals were fed Purina Rodent chow and filtered tap water, ad libitum. After a two-week acclimation period with 12h light / dark cycle, at 23± 3°C and a relative humidity of 30-70%, animals were randomly distributed into 5 groups of 12 rats in each group. The group consisted of a vehicle control group (distilled water) and four manganese dosage groups of 306, 612, 1225, and 1838 mg/kg dissolved in distilled water. The manganese concentrations were determined based on the mortality and clinical signs of toxicity in the initial dose range studies. Animals were dosed daily for 63 days at approximately the same time of day by oral gavage. At the end of 63 days, 50% of the animals were sacrificed and the remaining 50% were maintained for two weeks without the administration of manganese. Dosing volume was based on body weight of rats. The animals were weighed every other week and the dose was adjusted accordingly. The experimental design of predosing for 63 days assures epidydymal sperm had been exposed to manganese through their differentiation from spermatogonia. The animals were humanely sacrificed by CO_2 inhalation and necropsied. Reproductive organs were isolated weighed and fixed for histopathological studies. The organs consisted of testis, epididymis, ductus deferens, prostate, seminal vesicle and coagulation gland.

Samples of all reproductive organs were placed in Bouin's solution for approximately 15 hours, rinsed in 70% alcohol and transferred to 10% neutral buffered formalin (NBF). Other organs were fixed in 10% NBF for histopathology. After fixation, the tissues were routinely processed on an automatic tissue processor and embedded in paraffin wax, sectioned at 5 μm and stained with hematoxylin and eosin (HE).

RESULTS AND DISCUSSION

Our primary reproductive endpoints included segmental degeneration of germinal epithelium within seminiferous tubules and also to characterize loss of developing spermatozoa and spermatids. Histopathological examination confirmed testicular degeneration in treated rats of 612 (33%), 1225 (50%), and 1838 (33%) mg Mn/kg groups. The most prominent abnormality observed in male animals was testicular degeneration. Affected animals had mild to moderate segmental degeneration of germinal epithelium within seminiferous tubules. This change

was characterized by loss of developing spermatozoa and spermatids, vacuolation and swelling of Sertoli cells, degeneration of Sertoli cells, and widening of the tubular luminal diameter. Binucleate and multinucleate cells were observed within tubular lumina and degenerate epithelium. Many degenerated tubules also had a decreased cross-sectional diameter. The basement membrane of affected tubules appeared intact, but occasionally had a wavy appearance. Often tubules had relatively normal areas adjacent to affected areas. These changes, collectively, were consistent with segmental tubular degeneration. This change was present in higher numbers of treated animals (12 out of 24 total) and changes were noted in only one tubule of one control rat out of 6 rats (1 out of 6 rats).

Mild perivascular epididymitis was present in three rats, one control rat and two treated. The control rat also had seminiferous tubule degeneration, as described above. The other two rats affected with epididymitis were in the 306 mg/kg and 1838-mg/kg groups. Those with epididymidal changes also had accompanying testicular degeneration or prostatitis, but not both. Focal mild prostatitis was present in three treated animals. Prostatitis was not seen in control animals. Prostatic changes included focal to locally extensive suppurative inflammation within tubules and interstitium. This change was present mainly in the two high-dose groups without correlation to testicular lesions. One rat in the high dose group, 1838 mg/kg of Mn, had a lesion consistent with a sperm granuloma. This mass consisted of a large collection of spermatozoa encased in a thick sheath of macrophages and multinucleate giant cells surrounded by several layers of dense fibrous connective tissue. No evidence of malignancy was observed. This same animal also had interstitial epididymitis and focal prostatitis with glandular dilation. No significant lesions were observed in the coagulation glands, seminal vesicles or ductus deferens. Our results also reveal that the change induced in the testis tissue appears reversible on cessation of manganese exposure, as the incidences of testicular degeneration were lower in this group.

Histopathological observations revealed degeneration of seminiferous tubules, epididymis and prostatitis. Various investigators reported similar results although routes of exposure to manganese varied. Katira and Bawa (1993), reported disturbed spermatogenesis and degeneration in seminiferous tubules.

Chandra (1971) reported a cellular change induced by manganese in the rat testis. Intratracheal instillation studies in rabbits demonstrated that a single high dose of 158 mg/kg, as MnO_2 could cause severe degenerative changes in the seminiferous tubules and lead to sterility. (Chandra et al. 1973, Seth et al. 1973). Testicular changes have been demonstrated in the rat after intravenous administration of permanganate at 50 mg/kg and in the rabbit after administration of manganese dichloride at 3.5 mg/kg (WHO, 1981). Testicular damage was produced in rats by subcutaneous administration of manganese chloride (3 mg/kg) for a period of three weeks. Singh et al. 1974) reported that in rats manganese sulphate injection for 25 days produced degeneration in seminiferous epithelium, depleted number of spermatids and no spermatocytes in the seminiferous tubules. In humans,

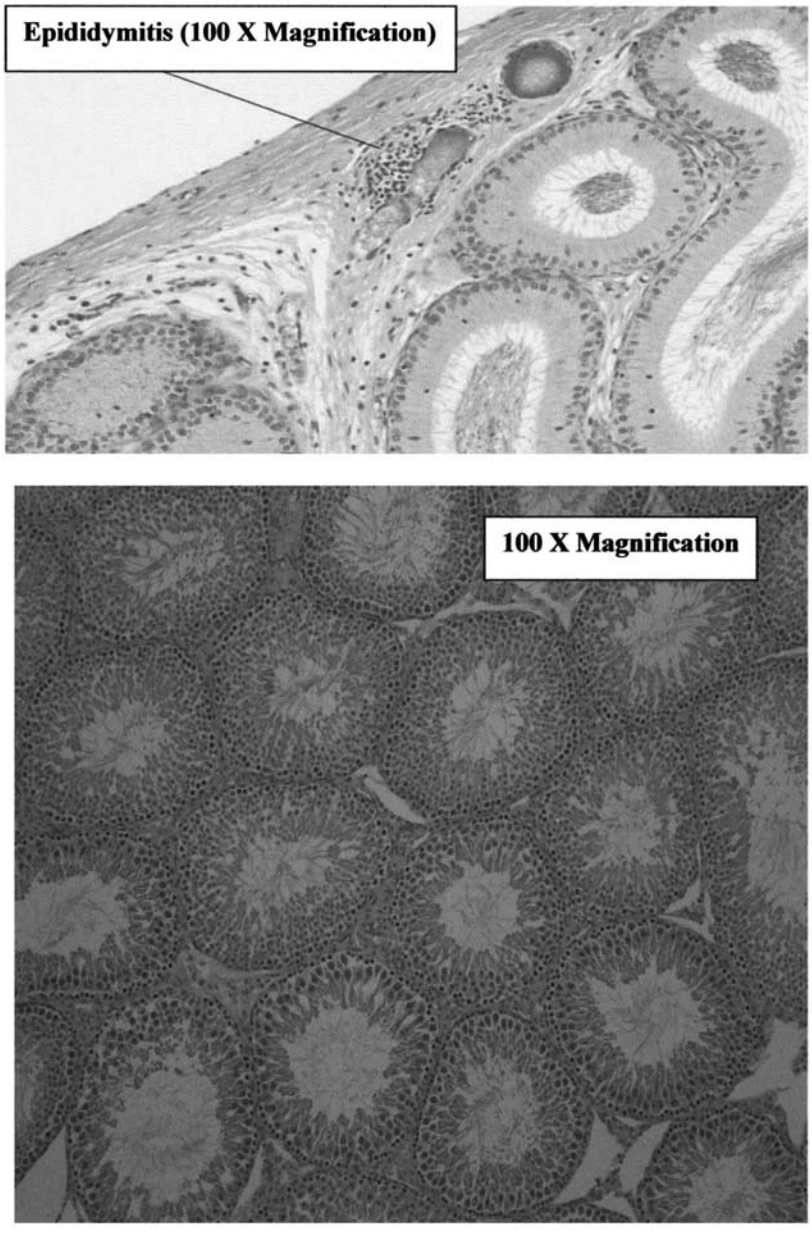

Figure 1. Top -Treated Rat, high dose group, Perivascular and interstitial epididymitis (Black line) (100 X Magnification). Bottom - Control Rat, Normal Epididymis (100 X Magnification)

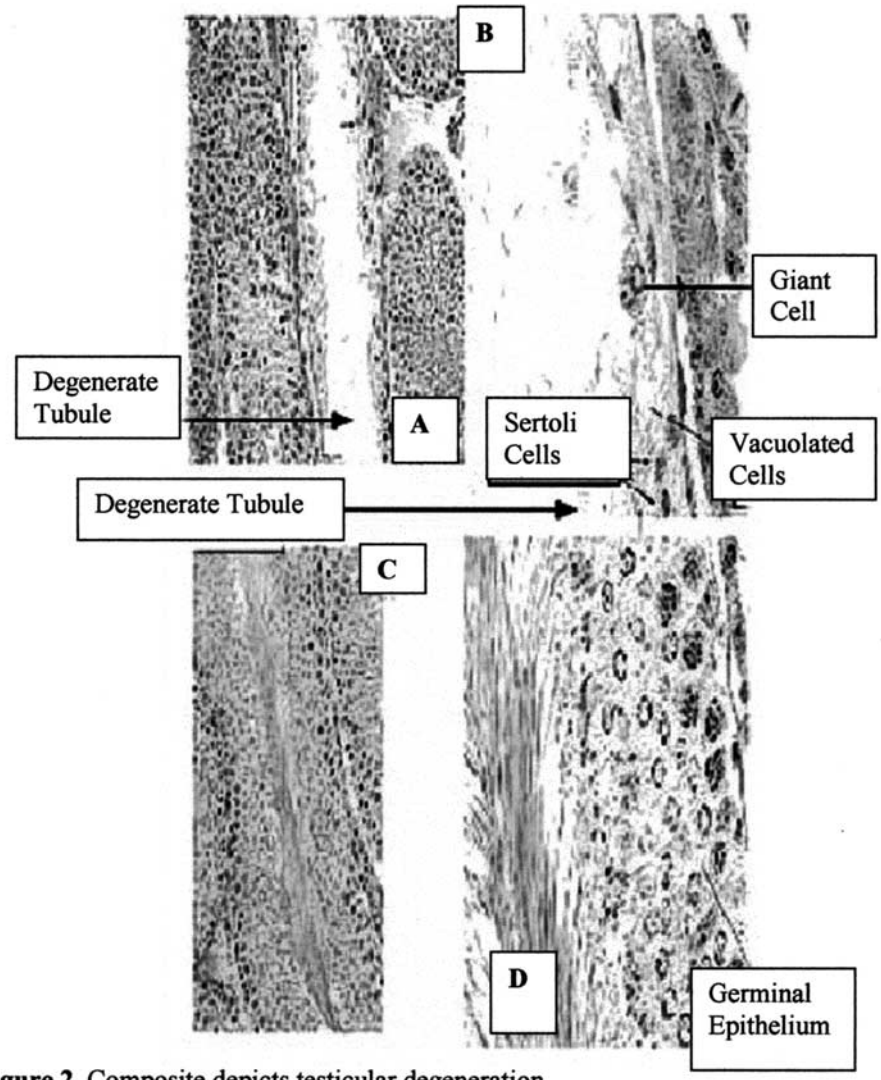

Figure 2. Composite depicts testicular degeneration
A: Degenerate seminiferous tubules (100X Magnification)
B: Closer view of degenerate seminiferous tubule (400X) from photo A
C: Normal seminiferous tubules (100X)
D: Closer view of normal seminiferous tubules (400X)

reproductive effects such as impotence and loss of libido have been noted in male workers exposed to high levels of manganese by inhalation (ATSDR 1997).

The mechanism by which manganese affects the testis is not clear, but several theories exist. Manganese may cause alteration in gonadotrophic hormone synthesis from the pituitary gland causing altered testosterone synthesis from Leydig cells, resulting in the observed changes in male reproductive organs (Laskey et al. 1985). It is also possible that manganese can act at the hypothalamic level rather than the pituitary. Direct toxicity to the tubular germ cells and Sertoli cells may also be a factor in testicular damage

Our studies demonstrated that the male reproductive system is a target of Mn exposure. However, there were no observed dose response relationships with the increasing manganese doses in the treated rats. Further studies are needed to more clearly elucidate the underlying mechanism of reproductive organ toxicity.

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