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Localization of Methyl Mercuric Chloride in the Reproductive Tract of Male Mice

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ABSTRACT. Localization of mercury compounds was investigated in selective regions of the male reproductive tract using autometallography. The results demonstrated that mercury was observed in Sertoli and Leydig cells in testis, but not in the epithelial cells of rete testis and germ cells. In the efferent ductule, mercury compounds were observed in the cytoplasmic compartments of epithelial cells in the proximal and common regions, while they were observed in the supranuclear cytoplasmic compartments in the conus region. In the epididymis, the compounds were observed in the cytoplasmic compartments of narrow and basal cells, but not in the principal cells of the initial segment. In contrast, the compounds were evenly detected in the cytoplasmic compartments of principal cells in the caput. In the corpus and caudal epididymis, the compounds were observed in the basal region of principal cells. The data shows that mercury is differentially accumulated in the male reproductive tract in a region-specific manner.

Keywords: Mercury, Autometallography, Testis, Efferent ductule, Epididymis.

INTRODUCTION

Heavy metals including mercury are different from other toxic substances because they are not synthesized and destroyed in organisms. Moreover, they are not emitted into the environment but are accumulated in organisms (Li, 1981). Mercury exposure from the environmental pollution of air, soil, or water has been found to cause a variety of neurological symptoms including visual disturbances, ataxia, paresthesias, neurasthenia, hearing loss, dysarthria, muscle tremor, and movement disorders (Bakir *et al.*, 1973). Mercury compounds were autometallographically identified in the central nervous system (Møller-Madsen and Danscher, 1991; Schiønning and Møller-Madsen, 1992; Schiønning *et al.*, 1993) and kidneys (Nørgaard *et al.*, 1989; Stoltenberg and Danscher, 2000). The localization of mercury in testis was also investigated in rats (Danscher and Møller-Madsen, 1985; Stoltenberg and Danscher, 2000) and humans (Keck *et al.*, 1993). Ernst and Lauritsen (1991) reported that methyl mercuric chloride decreased the percentage of motile spermatozoa. Recently, Monsees *et al.* (2000) demonstrated that mercury caused cytotoxic effects on Sertoli cells of rats. These findings led us to investigate further ideas on the localization of mercuric compounds in the reproductive tract of male mice.

This study assessed and described detailed microscopic localization of mercuric chloride compounds in the testis, efferent ductules, and epididymis for a better understanding of mercury-induced histopathology. Experiments were performed in a mouse model system using a repeated injections of methyl mercuric chloride. Autometallography was followed by histological examination to qualify alterations in the levels of the mercuric deposits in selected regions of the male reproductive tract.

MATERIALS AND METHODS

Animals

Adult male ICR mice (35~39 g) were obtained from

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Korea Experimental Animal Center, Taejeon. Mice were individually housed in a controlled environment during all experimental procedures. Food and water were provided ad libitum and mice were maintained on a 12 hr light/dark cycle. On the day of the examination, injections were given in a quiet room to minimize stress. All animal use procedures were approved by the Institutional Animal Care and Use Committee and were performed in accordance with the provisions of the NIH Guide for the Care and Use of Laboratory Animals.

Preparation for Histopathology

Twenty mice were randomly divided into 2 groups, control and mercury treated. Each mouse in the treated group was administered a weekly subcutaneous injection (s.c.) of 1 mg methyl mercuric chloride dissolved in 0.1 ml 0.9% physiological saline for 20 days. The dosage of mercuric chloride was determined from preliminary studies conducted in this laboratory. Each control mouse was administered an equal volume of saline. On the day of examination, body weight was measured. Mice were then anesthetized with sodium pentobarbital (Choongwae Pharmaceutical Co., Seoul) and were intranscardially perfused with a fixative containing 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 30 rpm in a peristaltic pump for 20 min. Testes, efferent ductules, and epididymides were removed and stored in the same fixative at 4°C for further tissue processing. Testes were weighed and tissue samples were washed in 0.1 M phosphate buffer, pH 7.4, dehydrated in a series of ethanol, embedded in paraffin, and cut into sections of 6 μm thickness. Sections were mounted on glass slides, deparaffinized in xylene, hydrated in descending ethanols, washed in distilled water, and exposed to an autometallographic developer at 26°C for 2 hours.

Autometallography

A standardized procedure was followed as described previously (Danscher and Montagnese, 1994). Briefly, the development was processed in a light-tight box and the developer was made from the following components: (1) protective colloid, 1 kg of crystalline gum arabic resin was dissolved in 2 liters of distilled water and stirred on a magnetic stirrer for 3 days. (2) citrate buffer, 25.5 g citric acid and 23.5 g sodium citrate, mixed with a sufficient quantity of distilled water to make 100 ml. (3) reducing agent, 0.85 g hydroquinone dissolved in 15 ml distilled water at 45°C. (4) silver ion supply, 0.11 g silver lactate dissolved in 15 ml distilled water at 40°C, which was protected against light. Carefully mixed in the sequence of 60 ml of protective colloid, 10 ml of citrate buffer and 15 ml of reducing agent. And finally 15 ml of silver ion supply was added immediately prior to development. After completion of development, slides were washed in 40°C tap water for 45 min, cleaned in 2% Farmer solution for 10 sec followed by distilled water. Counterstaining was performed with 0.5% toluidine blue for 20 sec. After rinsing in distilled water, slides were dehydrated in a series of ethanols, cleared

Table 1. Body and testicular weights between control and mercury treated groups

Group	Body weight (g)	Testicular weight (g)
Control	38.6±1.45	0.129±0.007
Treated	36.5±1.62	0.123±0.022

*Weight was expressed as mean±S.D. (n=10).

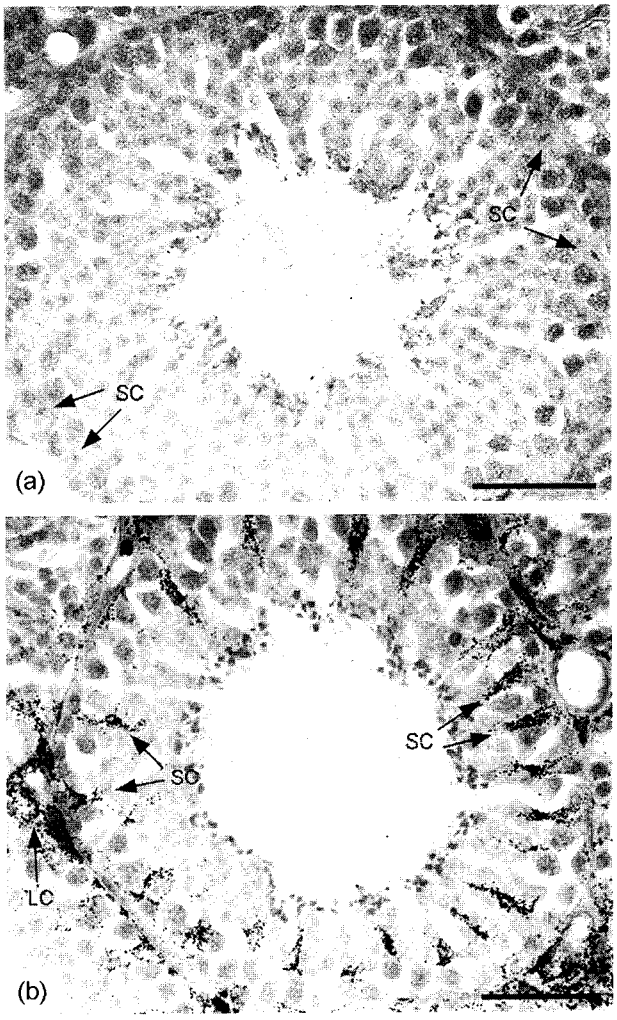

Fig. 1. Photomicrographs of testis obtained from control (a) and mercury treated (b) groups. Mercury deposits were homogeneously detected in Sertoli cells (SC) and Leydig cells (LC). Note that mercury deposits were more densely accumulated in Leydig cells than Sertoli cells. Bars represent 50 μm.

in xylene, and mounted with Permount (Fisher Scientific, New Jersey).

Statistics

Statistical significance of the body and testis weights between groups was determined using a one-way ANOVA on ranked data followed by Tukeys HSD test in SAS (Cary, NC). Statistically significant level was taken as $p<0.05$.

RESULTS

Body and Testicular Weights

We hypothesized that chronic administration of mer-

Fig. 2. Photomicrographs of the efferent ductules of control (a, c and e) and mercury treated (b, d and f) groups. (a) Proximal efferent ductules of control group. (b) Mercury deposits (arrowheads) were detected in the cytoplasmic compartments of epithelial cells in the proximal region. (c) Conus efferent ductules of control. (d) Mercury deposits (arrowheads) were predominantly detected in the supranuclear cytoplasms of epithelial cells in the conus region. (e) Common efferent ductules of control group. (f) Mercury deposits (arrowheads) were detected in the cytoplasmic compartments of epithelial cells in the common region. C, cilia; ED, efferent ductule; IS, initial segment; L, lumen. Bars represent 50 μm.

cury would lower body and testicular weight. However, our results showed that no significant difference was found in body and testicular weight between control and mercury treated groups (Table 1).

Localization of Methyl Mercuric Chloride in Testes

Mercury compounds were homogeneously observed in the long cytoplasmic compartments of Sertoli and Leydig cells (Fig. 1b) as compared with controls (Fig. 1a). Mercury compounds were not observed in germ cell line and epithelial cells of rete testis (data not shown).

Localization of Methyl Mercuric Chloride in Efferent Ductule

Mercury compounds were consistently observed in the epithelial cells of proximal (Fig. 2b), conus (Fig. 2d),

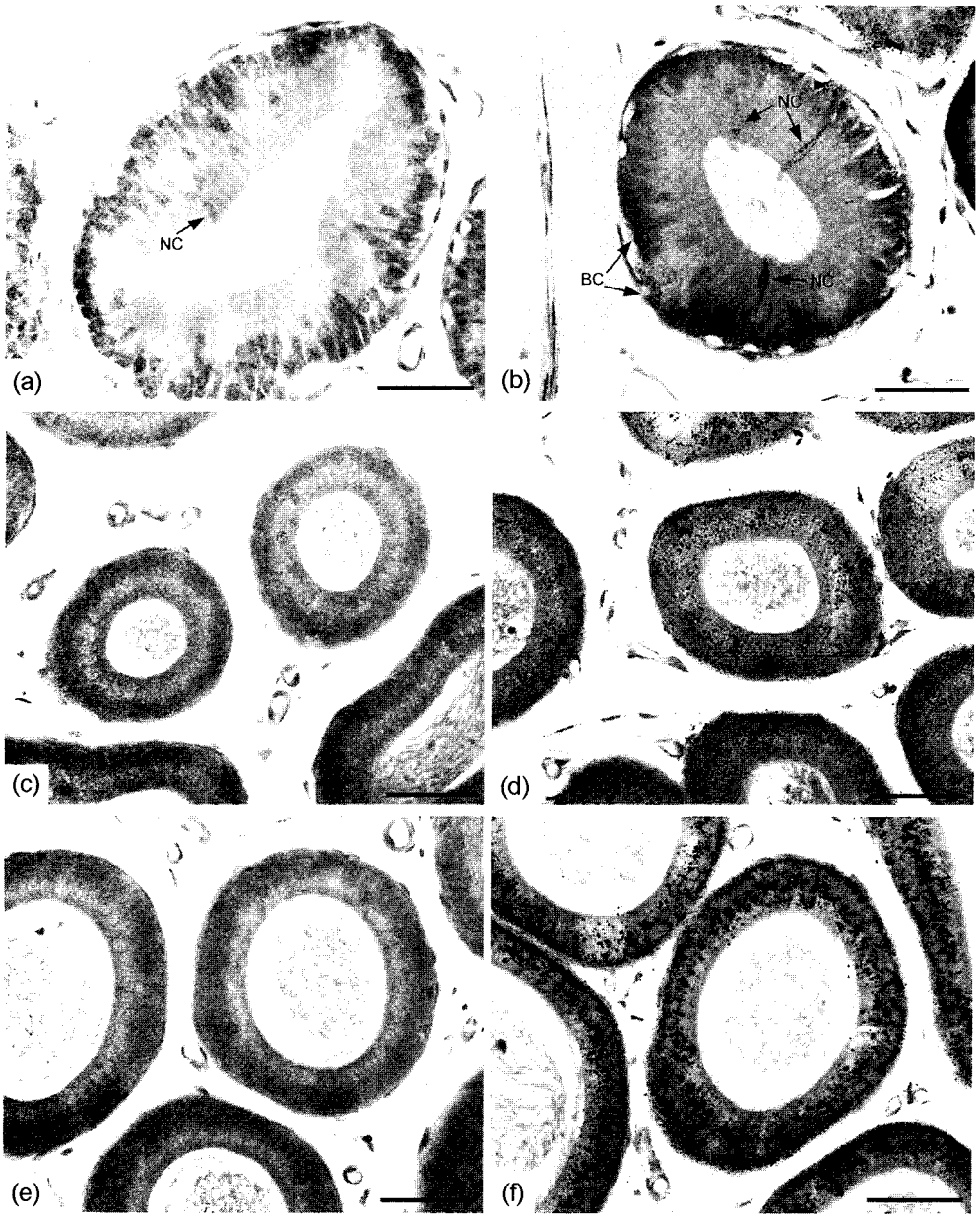

Fig. 3. Photomicrographs of the initial segment, proximal caput and distal caput of epididymis from control (a, c and e) and mercury treated (b, d and f) groups. (a) Initial segment of epididymis from control. (b) Mercury deposits were detected in narrow cells (NC) and basal cells (BC) in the initial segment. (c) Proximal caput of epididymis from control. (d) Mercury deposits were detected in the cytoplasmic compartments of epithelial cells in the proximal caput. (e) Distal caput of epididymis from control. (f) Mercury deposits were strongly detected in the supranuclear cytoplasms and basal area of epithelial cells in the distal caput. Bars represent 50 μm.

and common regions (Fig. 2f) of efferent ductule as compared with controls (Figs. 2a, 2c and 2e). As shown in figures (Fig. 2), the pattern of mercury compounds in the cytoplasmic compartments of the epithelial cells was different from the following regions: in proximal and common regions, mercury compounds were observed throughout the cytoplasms of the cells. In conus, mercury compounds were mainly detected in the supranuclear cytoplasms of the epithelial cells (Fig. 2d). However, mercury compounds were not detected in the cilia of ciliated cells and brush border microvilli of nonciliated cells (Fig. 2f).

Localization of Methyl Mercuric Chloride in Epididymis

The epididymis is composed of initial segment (Fig. 3a), proximal (Fig. 3c) and distal (Fig. 3e) caput, corpus (Fig. 4a) and caudal (Fig. 4c) regions. The initial segment of epididymis connected to efferent ductules is mainly composed of principal cells (Fig. 3a). Mercury compounds were not observed in the cytoplasmic compartments of the cells but were detected in narrow cells located in the upper half of principal cells in the initial segment (Fig. 3b). Mercury compounds were also observed in basal cells that reside on the basement of the epithelia of principal cells (Fig. 3b). As compared with the initial segment, mercury compounds were concentrated in the cytoplasmic compartments of the proximal caput (Fig. 3d). Intensive mercury compounds were also detected in the basal and upper cytoplasmic compartments of the distal caput (Fig. 3f). Mercury compounds in the distal caput were deposited to a greater degree than those in the proximal caput. In corpus and caudal regions, dominant mercury compounds were detected in the basal region of the cells (Figs. 4b and 4d). No mercury compounds were observed in the epithelia of vas deferens (data not shown).

DISCUSSION

Autometallography provides detailed localization of mercury deposits in animal tissues as demonstrated in nervous tissue (Møller-Madsen and Danscher, 1986),

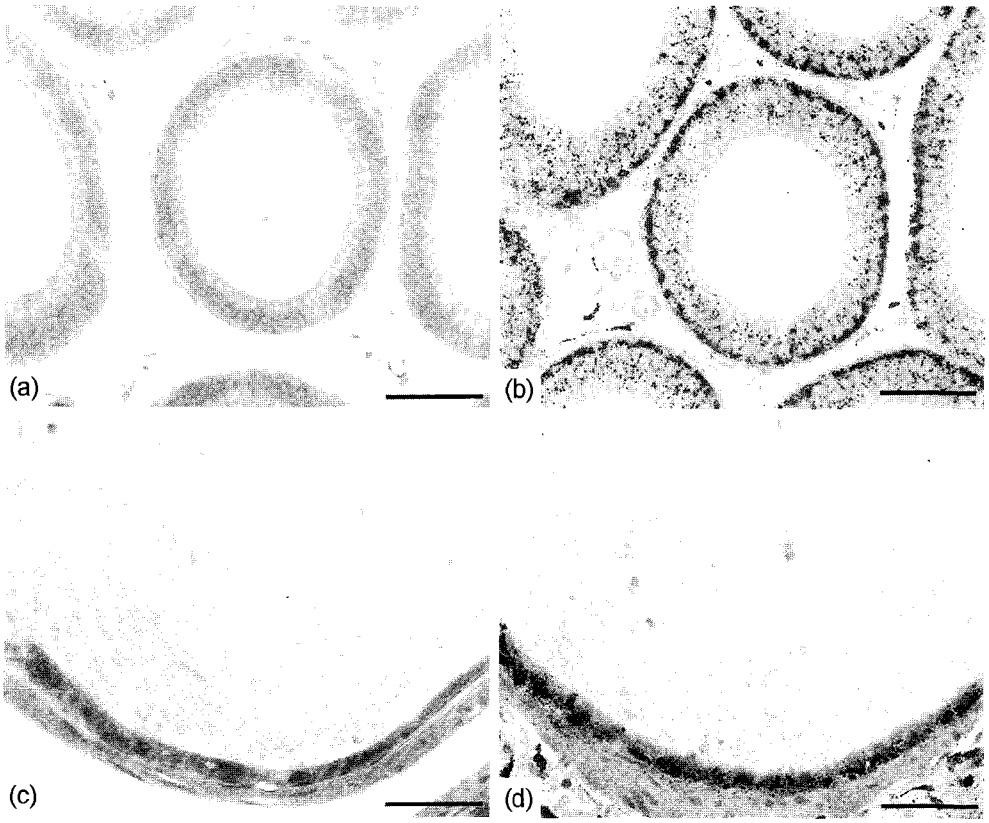

Fig. 4. Photomicrographs of the corpus and distal cauda of epididymis from control (a and c) and mercury treated (b and d) groups. (a) Corpus of epididymis from control. (b) Mercury deposits were strongly detected in the basal area of epithelial cells in the corpus region. (c) Distal cauda from control. (d) Mercury deposits were strongly detected in the basal area of epithelial cells in the caudal region. Bars represent 50 μm.

kidney (Stoltenberg and Danscher, 2000), and testis (Danscher and Møller-Madsen, 1985; Ernst *et al.*, 1991). The present data shows that morphological alterations were not found due to mercury accumulation throughout the male reproductive tract of mice. Mercury deposits were intensely found in the cytoplasmic compartments of Sertoli and Leydig cells of mice. Consistent with our observations, mercury was precipitated in the Sertoli and Leydig cells of rats (Ernst *et al.*, 1991) and human testis (Keck *et al.*, 1993). Sertoli and Leydig cells in rat testis are able to synthesize estrogen (Carreau *et al.*, 1999) and play an essential role in spermatogenesis (Monsees *et al.*, 2000). Mercury was found to cause progressive degeneration of Leydig cells of rats (Vachhrajani and Chowdhury, 1990). Therefore, these data suggest that estrogen production might be affected by methyl mercuric chloride accumulated in Sertoli and Leydig cells of mice.

Efferent ductules conduct spermatozoa from the rete testis to the initial segment of epididymis. In this study, mercury deposits were detected in the cytoplasms of proximal, conus, and common regions of efferent ductule epithelia. Mercury deposits were mainly detected in narrow and basal cells, but not in the principal cells of the initial segment. Predominant mercury deposits were observed in the epithelial cells of caput, corpus, and cauda regions, whereas fewer mercury deposits were detected in the narrow and basal cells of the epididymis. Moreover, mercury deposits were homogeneously distributed in the cytoplasm of epithelial cells in the caput, while they were mainly located in the basal area of caudal region of epididymis. With limited data from previous studies, these data suggest that epithelial cells of efferent ductule and epididymis have different functions in the capability of absorption and motility along with the tract. This speculation may be supported by the findings that sperm maturation depends on the sites of epididymis, such as initial segment, caput, corpus, and cauda (Tuener, 1991; Jones, 1998). In rat epididymis, the caudal region showed the highest capacity for sperm motility while the initial segment region showed relatively lower sperm maturation (Jones, 1998).

Taken together, this study demonstrated that mercury has minimal effects on the weight of the male reproductive tract, but is differentially accumulated in the tract in a region-specific manner. However, the effects of mercury accumulation on the male reproductive tract are presently unclear. It will be interesting to investigate this issue by examining whether spermatogenesis or sperm motility is affected by mercury in our future study.

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