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Histological Study of Gender Differences in Accumulation of Silver Nanoparticles in Kidneys of Fischer 344 Rats

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The tissue distribution of silver (Ag) nanoparticles showed a dose-dependent accumulation of Ag in all the tissues examined, including testes, kidneys, liver, brain, lungs, and blood. However, a gender-related difference in the accumulation of Ag was noted in the kidneys, with a twofold higher concentration in female kidneys compared males after subacute exposure to Ag nanoparticles via inhalation or oral ingestion. To investigate the gender-specific accumulation of Ag nanoparticles in kidneys of Fischer 344 rats, detailed histopathological studies were conducted by Ag enhancement staining. Female rats showed a higher accumulation of Ag nanoparticles in all kidney regions, including cortex, outer medulla, and inner medulla. In particular, the glomerulus in the cortex contained a higher accumulation in females than males. The Ag nanoparticles were also preferentially accumulated in the basement membranes of the renal tubules in the cortex, middle and terminal parts of the inner medulla, and outer medulla. In addition, Ag nanoparticles were detected in the cytoplasm and nuclei of interstitial cells in the inner medulla of the kidney.

The absorption, distribution, metabolism, and excretion (ADME) of nanoparticles is an important research area when investigating the toxicity of nanomaterials. As such, the ADME of specific nanoparticles provides information on the fate of nanoparticles in a biological system, and enables predictive analyses of possible target tissues and affected organs after short- or long-term exposure to certain nanoparticles. Previously, Kim et al. (2008) reported gender differences in the accumulation of Ag nanoparticles in kidneys of Sprague-Dawley rats after subacute exposure. It is of interest that subacute inhalation of Ag nanoparticles did not produce gender differences with respect to accumulation in various tissues; however, kidneys were not analyzed (Ji et al., 2007). In a 90-d Ag nanoparticle inhalation study by KFDA (2007) a consistent preferential accumulation of Ag nanoparticles was noted in female kidneys. Measurements of the tissue concentrations of Ag revealed dose-dependent increases in the metal concentrations in all tissues tested, yet Ag concentrations in female kidneys were two- to threefold higher than in males for all the dose groups. However, the issue of gender differences as regards the accumulation of Ag nanoparticles is novel, as there have been no previous reports on gender differences in relation to accumulation of metals. Thus, the aim of this study was to examine histopathologically male and female kidneys using an enhanced Ag staining method as an initial approach to identify site-specific accumulation of metal.

MATERIALS AND METHODS

Silver Nanoparticles

Silver nanoparticles (52.7–70.9 nm, average 60 nm) were purchased from NAMATECH Co., Ltd (Korea), and were at least 99.98% pure. The vehicle, 0.5% aqueous carboxymethylcellulose (CMC), was obtained from Sigma (USA).

Animals and Conditions

Four-week-old male and female, specific-pathogen-free (SPF) Fischer 344 (F344) rats were purchased from OrientBio (Korea) and acclimated for 2 wk before starting the experiments. During the acclimation and experimental periods, the rats were housed in polycarbonate cages (maximum of 3 rats per cage) in a room with controlled temperature (23 ± 2°C) and humidity (55 ± 7 %) and a 12-h light/dark cycle. The rats were fed rodent food (Harlan Teklab, Plaster International Co., Korea) and filtered water ad libitum. At 5 wk, the rats were divided into 4 groups (10 rats in each group): vehicle control

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(0.5% carboxymethyl cellulose), low (30 mg CMC/kg/d), intermediate (125 mg CMC/kg/d), and high (500 mg CMC/kg/d). At 6 wk, the rats were exposed to Ag nanoparticles following OECD test guideline 408 (OECD, 1998), based on 90 d of repeated oral administration (dosing volumes were 10 ml/kg) using good laboratory practices (GLP).

General Histological Analysis

Following exposure, rats were anesthetized with ketamine hydrochloride; lungs and nasal cavity were removed and fixed in a 10% formalin solution containing neutral phosphate-buffered saline. The specimens were dehydrated in a graded ethanol series and embedded in paraffin. Sections were cut at a thickness of 3 to 4 μm, deparaffinized, and stained with hematoxylin and eosin (H&E) to study the histological structure.

Silver Enhancement Method

The paraffin-embedded tissue sections were deparaffinized with xylene and hydrated with grade alcohol. The tissues were then treated with 0.5% Triton X-100 in phosphate-buffered saline (PBS) and washed with PBS and deionized water several times. HQ SILVER (Nanoprobes, Yaphank, NY) using equal amounts of the three components (initiator, moderator, and activator) was prepared in the dark, mixed thoroughly, and the tissue sections were incubated with it for 15 min. After several washings with deionized water, the slides were stained with hematoxylin, dehydrated with grade alcohol, and mounted using Canada balsam.

RESULTS

Figure 1 shows the gender-differentiated Ag nanoparticle accumulation in the kidneys. Female rats showed a higher accumulation of Ag nanoparticles in all regions of the kidney, including the cortex, outer medulla, and inner medulla (Figure 1). When compared with the male specimens, the female glomerulus in the cortex displayed a higher accumulation (Figure 1, D and E vs. K and L), corresponding to concentration differences previously described by Kim et al. (2008). The outer stripe of the outer medulla (OSOM) also demonstrated a different Ag nanoparticle accumulation (Figure 1, F and G vs. M and N), along with the inner medulla (IM), including the middle portion of the inner medulla (IMm) and terminal portion of the inner medulla (IMt) (Figure 1, I and J vs. P and Q). The difference was even more evident at higher magnification of kidneys (Figure 2). The basement membrane of the glomerulus in the cortex (Figure 2, A and B vs. H and I) and renal tubules in the outer medulla in the females (Figure 2, C and D vs. J and K) showed a higher

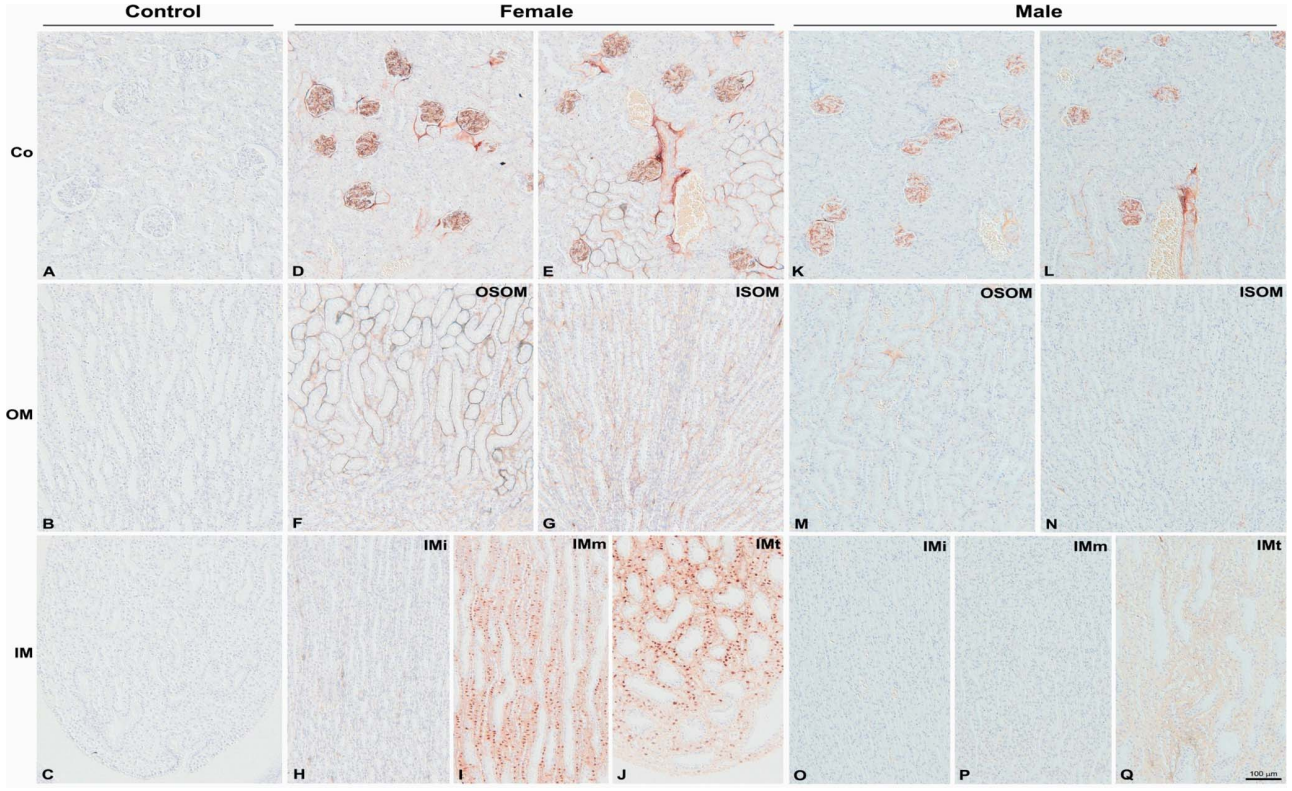

FIG. 1. Low magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 100 μm.

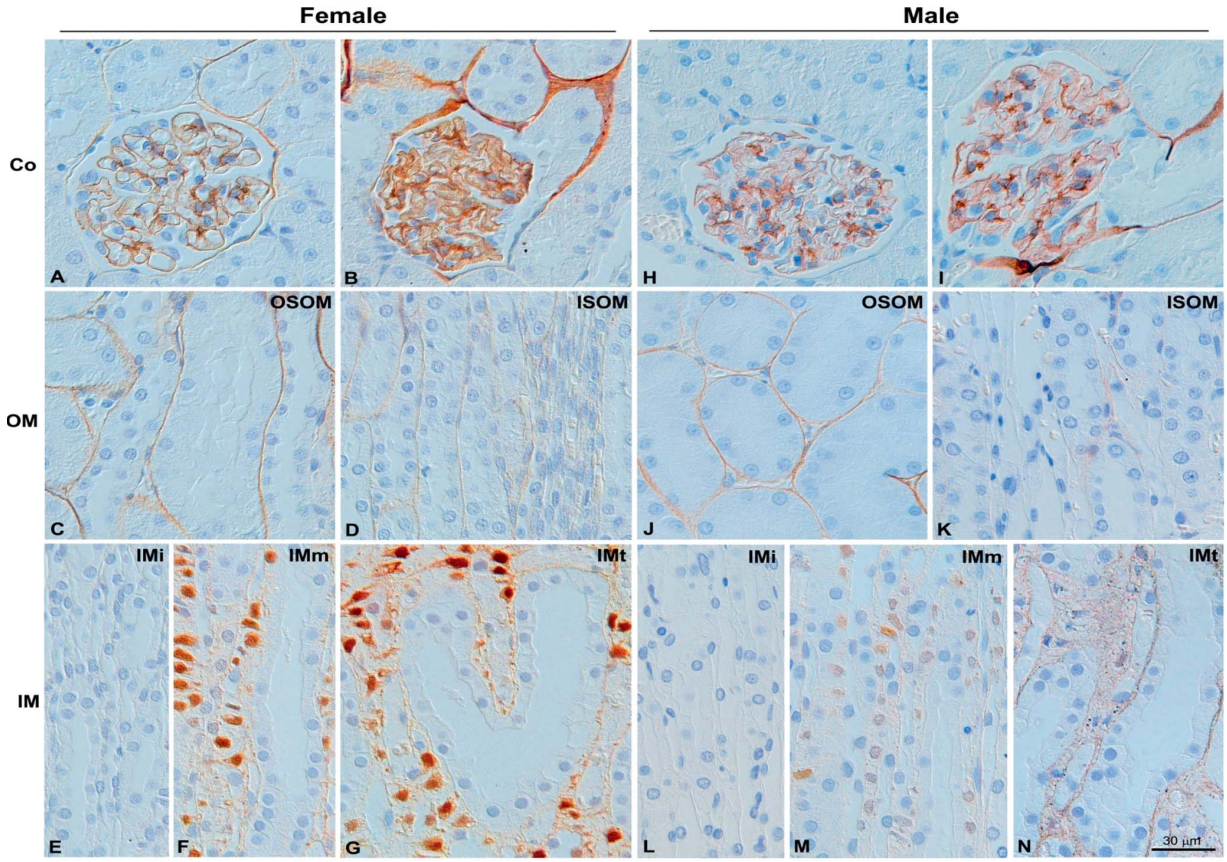

FIG. 2. High magnification of kidneys. Co, cortex; OM, outer medulla; OSOM, outer stripe of outer medulla; ISOM, inner stripe of outer medulla; IM, inner medulla; IMi, initial part of inner medulla; IMm, middle part of inner medulla; IMt, terminal part of inner medulla. The bar indicates 30 µm.

accumulation of Ag nanoparticles. The nucleus of the interstitial cells in the inner medulla also displayed a strong positive response (Figure 2F and G). In addition, the basement membrane of the inner medullary collecting ducts showed a higher accumulation of Ag nanoparticles in females when compared with those of males (Figure 2, F and G vs. M and N).

Most of the Ag nanoparticles appeared to be located in the basement membrane. The urinary bladder also showed a preferential deposition of Ag nanoparticles in the basement membrane of the transitional epithelium (Figure 3).

The capsule and medulla demonstrated a strong positive response to Ag staining in the adrenal gland (Figure 4, C and D

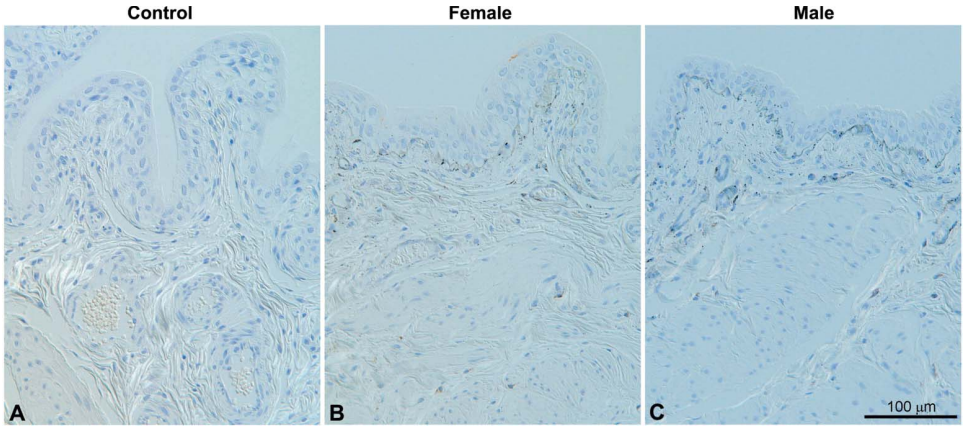

FIG. 3. Urinary bladder. The bar indicates 100 µm.

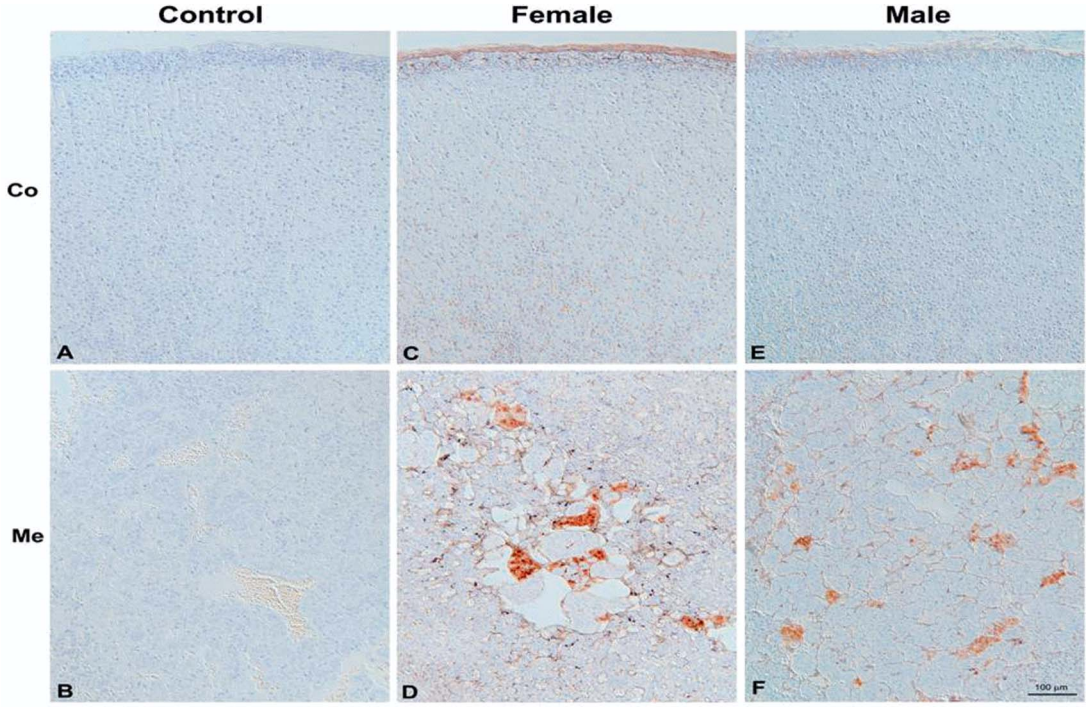

FIG. 4. Adrenal gland. The bar indicates 100 μm.

vs. E and F). Further, the zona glomerulosa (ZG) (Figure 5, E vs. H) and zona reticularis (ZR) (Figure 5, E vs. I) in the cortex showed strong positive reactions, with Ag nanoparticles likely distributed in the capillary basement membrane in females. The cytoplasm and nuclei in some of the medulla cells also displayed positive reactions to Ag nanoparticles (Figure 5, F and J).

DISCUSSION

This study attempted to identify histological differences in the preferential accumulation of Ag nanoparticles in female versus male kidneys. Kim et al. (2008) noted that the kidneys were the only organ exhibiting gender differences in Ag nanoparticle accumulation. Gender differences in phenobarbital metabolism or nephrotoxicity and in developmental immunotoxicity to lead were reported (Peng et al., 2001; Hong et al., 2001; Bunn et al., 2001). Although there have been no previous reports on gender differences with respect to tissue accumulation of Ag or other metals, the histopathological results in this study clearly demonstrated a gender difference in Ag nanoparticle accumulation in kidneys, urinary bladder, and adrenal glands. The preferential accumulation sites were mainly the basement membrane of the glomerulus and renal tubules, along with the adrenal capsule and cortex.

A gender difference in hepatic cadmium (Cd) accumulation was already reported, where females were found to accumulate a significantly higher concentration of Cd in liver than male rats after a subcutaneous injection of metal (Blazka et al., 1988). The renal metallothinein (MT) levels in the females were also significantly higher than those in male rats (Blazka & Shaikh, 1991a); while estradiol pretreatment induced renal MT formation, this hormone did not affect renal Cd accumulation (Blazka & Shaikh, 1991b). Furthermore, no gender differences in the MT levels were found in young animals, whereas weaned females had significantly more hepatic MT than the corresponding males (Blazka et al., 1988). In squirrelfish, the females store large amounts of zinc (Zn) in the liver and ovaries when compared to males, and this gender difference in Zn storage is dependent on two major zinc-binding proteins: MT and a female-specific zinc-binding protein (FsZnP). The hepatocytes of female squirrelfish possess a high capacity to transport Zn across the membrane. In addition, estrogen may trigger a redistribution of Zn from liver to ovaries (Hogstrand et al., 1996).

The gender difference in the expression of OCT (organic cation transporters) in the kidneys is responsible for gender differences in organic cation transport activity of the basolateral membrane of the renal tubular cells (Urakami et al., 1998). OCT from different species are multispecific facilitative transporters that mediate the translocation of small organic cations, such as tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium (MPP), N1-methylnicotinamide (NMN), choline, and dopamine, and these transporters depend upon a transmembrane potential difference. The expression levels of the OCT mRNA and protein are significantly higher in male than in

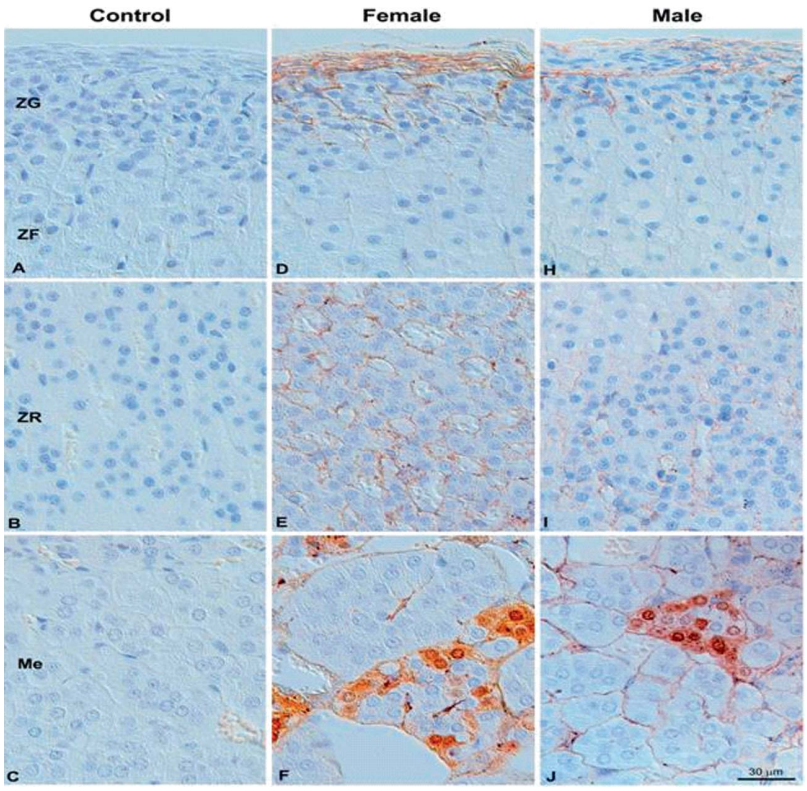

FIG. 5. Adrenal gland with high magnification. ZG, zona glomerulosa; ZF, zona fasciculate; ZR, zona reticularis; Me, medulla. The bar indicates 30 μm.

female kidneys (Urakami et al., 1999). However, since silver is an inorganic metal, it does not use OCTs.

Another possible explanation for the observed gender differences in Ag accumulation in kidneys may be due to hormonal regulation. The kidneys are a target organ for several hormones, such as thyroid hormones and testosterone, with important physiological and pathological effects on the systemic and renal functions. For example, since the renal secretion of organic anions is higher in males than in females, most xenobiotics excreted by organisms through this pathway are eliminated more rapidly by males than by females (Reyes et al., 1998). Testosterone also has a stimulatory effect on several renal cellular functions, such as secretion (Kleinman et al., 1966; Reyes et al., 1998), whereas female sexual hormones do not exhibit any apparent effect on secretion (Bräunlich et al., 1993).

Some transporter or binding protein molecules, such as MT and FsZnP, may be involved in gender differences in Ag accumulation in rat kidneys. In addition, gender differences in the basement structure of female kidneys also need to be further investigated. For example, the anatomical difference in the basement membrane between genders needs to be studied by electron microscopy. The effect of hormones on the clearance or accumulation of Ag nanoparticles may also be an important regulator for gender differences. Thus, further studies are currently underway to examine the influence of hormones on gender difference in Ag nanoparticle accumulation using ovariectomized female and castrated male rats.

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