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Light Microscopic and Immunohistochemical Study Revealed Sex-Dependent Kidney Morphology in DBA/2crSlc Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MAA and AY designed the study and wrote the protocol. Author MAA conducted the total research work with the technical assistance and guidance of author AY. Authors OI, MM and JA performed the statistical analysis. Author MAA wrote the first draft of the manuscript while author SS revised the text. Authors OI and JA did the bench work and literature search. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: Sex-dependent differences in kidney histology have been observed in different species of the laboratory animals. The present study was conducted to evaluate the sex and strain-dependent changes in DBA/2CrSlc mouse kidney morphology by using light microscopy and immunohistochemistry.
Methods: A total of 12 DBA/2CrSlc male and female mice of 2 months of age were used in this study. Mice were sacrificed by exsanguination under anesthesia using a mixture of Ketamine and Medetomidine. Both right and left kidneys were removed aseptically and central slices including hilum were cut perpendicular to the long axis of the organ and preserved in Zamboni solution. Paraffin blocks were made and tissue sections were stained with hematoxylin and eosin, and PAS stains to observe the general morphology of the kidney glomerulus. Immunohistochemistry was performed to detect renin positive

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sites, expression of Cyclooxygenase-2 (COX-2) and Nitric Oxide Synthase (nNOS). Number of renin, COX-2 and nNOS positive sites were counted and tabulated. The data were statistically analyzed for any significant differences between male and female mice.
Results: Our results reveal that the glomerular capsule of male mouse kidney was consisted of a single layer of simple cuboidal epithelium whereas it was a single layer of simple squamous epithelium in the female kidney. PAS-positive granules (small and giant granules) were observed in PST epithelium and collecting ducts in female kidney, but this feature was absent in male kidneys. Strong nNOS positive reaction for PST epithelium and collecting ducts was observed in female, but this character was absent in male kidney. The total number of glomeruli, renin, COX-2, and nNOS positive sites was comparatively higher in female kidneys then that in male. However, statistical analysis revealed no significant differences of the areas of renin, nNOS and COX-2-positive sites between the male and female kidneys (P<0.05).
Conclusion: Light microscopic and immunohistochemical study revealed sex-dependent histological morphology of the DBA/2CrSlc mouse kidney. DBA/2CrSlc female mouse kidney revealed renin, COX-2 and nNOS -positive reactions in the present study but male mice showed nNOS-negative reaction. The reason for nNOS-negative reaction in male is not clearly understood. It is suggested that this species can be experimentally used in the laboratory for investigating kidney function and related pathological studies.

Keywords: DBA/2CrSlc mouse; sex dependent; kidney morphology; immunohistochemistry.

1. INTRODUCTION

A through understanding of the complex structure of the mammalian kidney provides a basis for comprehending the multitude of functional characteristics of this organ in both healthy and disease states. Renal structural alterations are closely related to their functional changes, and renal histopathological analysis is indispensable to investigate kidney related diseases as well as kidney failure. To make a correct interpretation of histopathological observations in laboratory experiments, a clear understanding of normal histological features of laboratory animals is very important. The mouse has been used frequently in laboratory experiments, and there have been many reports regarding sexual dimorphism of the mouse kidney [1,2]. Yabuki et al. [3] reported that morphological features of the kidney differ based on species and strains differences. However, sex-and strain-dependent structural features have not been fully elucidated with the exception of very few reports described previously. The present work was undertaken to clarify sex differences in kidney morphology of DBA/2CrSlc mice, together with differences in the expression of three important enzymes, such as renin secreted from juxtaglomerular cells (JGCs), Cyclooxygenase-2 (COX-2), and neuronal nitric oxide synthase (nNOS) secreted from macula densa cells. Renin is a key enzyme of the renin-angiotensin (RA) system that controls blood pressure. Furthermore, renin secretion from JGCs is also regulated by the expression of COX-2 and nNOS from macula densa cells. Any alterations and or deviations of the functions of these three important enzymes will be the great concern for renal function. Therefore, the aim of the present study using the DBA/2CrSlc mouse was to asses the sex differences of kidney morphology together with the expression of these three proteins using immunohistochemistry.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twelve DBA/2CrSlc male and female mice of 2 months of age were used to investigate the sex dimorphism of kidney morphology by using different immunohistochemical techniques. The animals were purchased from CLEA Japan, Inc. (Tokyo, Japan) and acclimatized in our animal laboratory facilities, in the division of Laboratory Animal Science, Research Center for Life Science Resources, Kagoshima University for another 1 month. The animals were maintained under standard conditions of humidity, temperature and supplied autoclaved commercial diet (CE-2 from CLEA, Japan) and tap water ad *libitum*. The present investigation was carried out in accordance with the Guidelines for Animal Experimentation of the Faculties of Medicine and Agriculture, Kagoshima University.

Mice were maintained in a one-way airflow system (room temperature 22 ± 1ºC; humidity 55 ± 10%; light period 07:00-19:00; ventilation 12 cycle/h). Mice were sacrificed by exsanguination under anesthesia using a mixture of Ketamine (Sankyo, Tokyo, Japan) and Medetomidine (Domitor, Meiji Ltd., Tokyo, Japan) at the dose rate of interperitoneal injection of 0.002ml/g of body weight followed by cervical dislocation. Both right and left kidneys were removed aseptically and collected immediately after death of the animals. Central slices including hilum were cut perpendicular to the long axis of the organ and preserved in Zamboni solution. The specimens were kept at 4ºC for overnight. After several washes with phosphate buffer solution (0.1M, pH 7.4) the specimens were dehydrated with a graded series of ethanol and embedded in paraffin. The paraffin blocks were cut at 3 µm thickness and dried at 40ºC overnight. They were stained with hematoxylin and Eosin (H&E) and periodic acid Schiff (PAS) stains for general morphological studies. Immunohistochemical study was conducted for the detection of renin, COX-2, and nNOS. The materials and methods for immunohistochemical study were followed based on the protocol supplied by the company and according to the report published previously by Yabuki et al. [4]. A total count of glomeruli for both male and female mice was counted by using a light microscope connected with a digital camera (Nikon ECLIPSE E 600) and a computerized monitor (BUFFALO, Japan). Similarly, a total count of localization of renin, COX-2, and nNOS - positive sites was obtained and tabulated.

2.2 Data Collection

Data were statistically analyzed by using student's *t* test (StatView).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Light microscopy

Histological features of glomerular capsule in DBA/2CrSlc mouse kidney were found to be different between males and females. The parietal layer of the glomerular capsule in male mouse kidney consisted of a single layer of simple cuboidal epithelium, whereas it was a single layer of simple squamous epithelium in female (Fig. 1 A-B). Vacuoles of different size and shapes were observed in the proximal convoluted tubules (PCT) in male, but this histological feature was absent in female kidney (Fig. 1 C-D). PAS-positive brush border in

proximal straight tubules (PST) and collecting ducts in female revealed stronger staining intensity when compared with that of the male species (Fig. 2 A-B). PAS-positive granules (small and giant granules) were observed in PST epithelium and collecting ducts in female kidney, but this feature was absent in male. Strong nNOS-positive reaction for PST epithelium and collecting ducts was observed in female, but this histological character was absent in male kidney (Fig. 2 C-D).

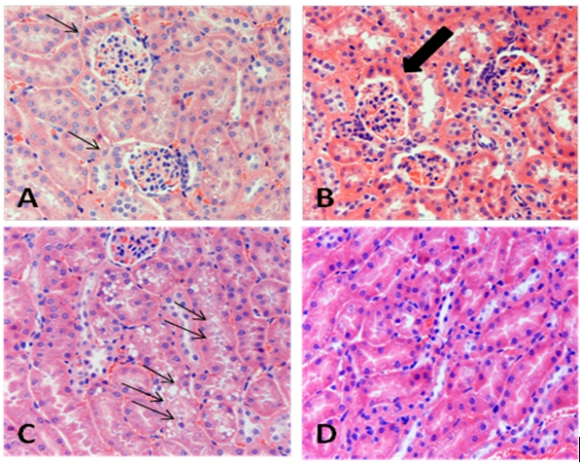

Fig. 1. Parietal layer of the glomerular capsule consisted of a single layer of simple cuboidal epithelium in DBA/2CrSlc male mice (Fig. 1A, small arrows), whereas in female mice it was a single layer of simple squamous epithelium (Fig. 1 B, big arrow). Vacuoles of different shapes and sizes appear in the PCT epithelium in males (Fig. 1C, small arrows), but this histological feature was absent in females (Fig. 1D). H/E; x 40

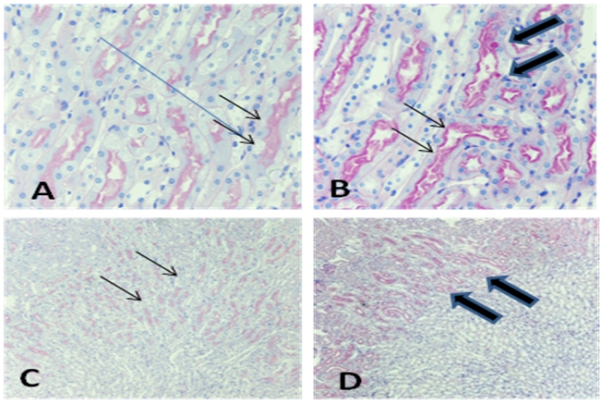

Fig. 2. PAS-positive brush border appears in the PST epithelium in male (Fig. 2A, small arrows). Strong reaction for PAS-positive brush border was observed in female (Fig. 2B). Small and giant granules were detected in PST epithelium in female (Fig. 2B, big arrows). PAS-positive brush border of PST epithelium in the outer medulla was observed in male (Fig. 2C, small arrows). Stronger staining for PAS-positive brush border of PST epithelium was prominent in the outer medulla of female mouse kidney (Fig. 2D, big arrows). PAS stain; x 40

3.1.2 Immunohistochemistry

Male mouse kidney revealed strong staining intensity for renin-positive site in the juxtaglomerular area. Although female kidney showed renin-positive site in the juxtaglomerular area, the reaction was comparatively weak (Fig. 3A-B). In male, nNOS-positive reaction for juxtaglomerular area was distinctly observed, but female kidney revealed comparatively weak reaction (Fig. 3 C-D) Female kidney showed strong reaction for nNOS-positive site in PST epithelium and cells lining the collecting duct, but male mouse kidney showed nNOS-negative reaction (Fig. 4 A-D). Although both male and female kidney shows COX-2-positive reaction in macula densa area but in female this reaction was observed comparatively weak.

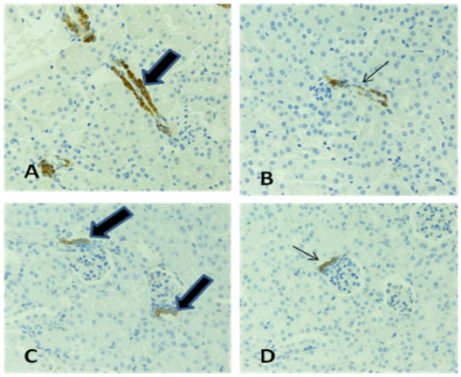

Fig. 3. Strong reaction for renin-positive site in Juxtaglomerular area was observed in male (Fig. 3A, big arrow). In female, reaction for renin-positive site was not prominent (Fig. 3B, small arrow). In male, nNOS-positive reaction was distinctly observed (Fig. 3C, big arrows) but, in female nNOS-positive reaction was not distinct (Fig. 3D, small arrow)

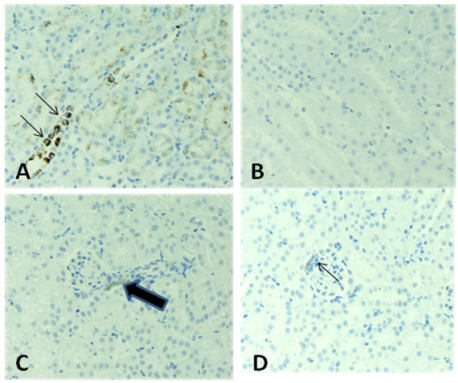

Fig. 4. Female kidney shows strong reaction for nNOS-positive site in PST epithelium and collecting ducts (Fig. 4A, big arrows), but male mouse kidney shows nNOS-negative reaction for PST and collecting ducts (Fig. 4B). Male and female kidney shows COX-2-positive reaction in macula densa area (Fig. 4C, D). In male, COX-2-positive reaction was observed prominent (Fig. 4C, big arrows). In female, COX-2-positive reaction was poorly detected (Fig. 4D, big arrow)

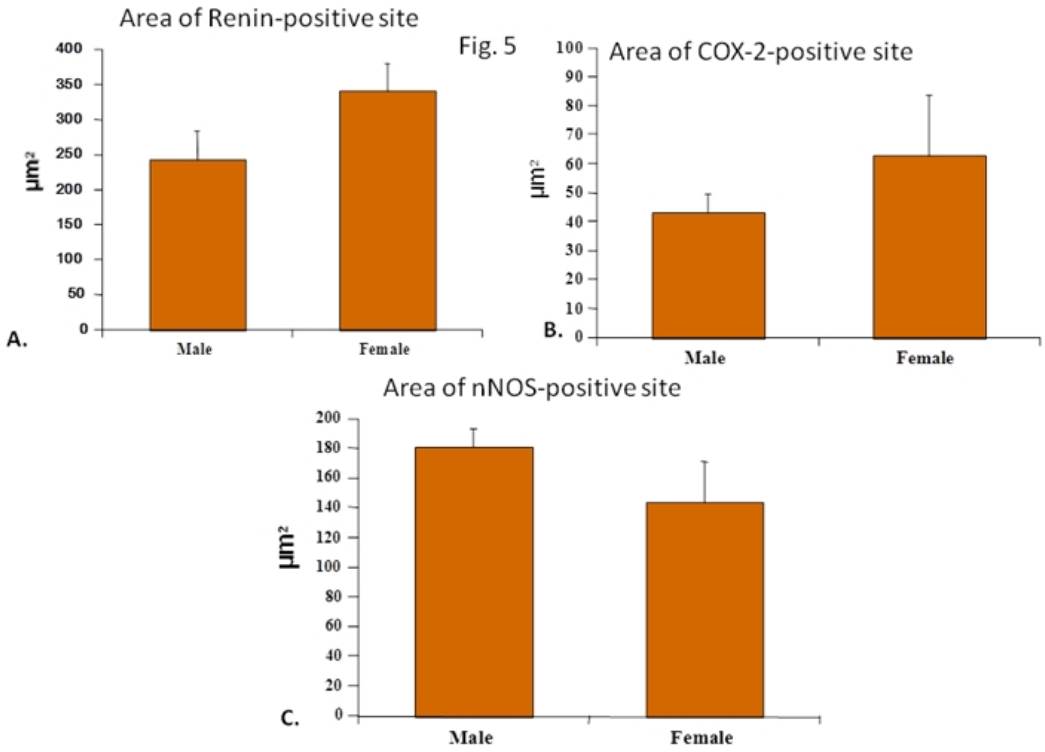

Fig. 5. Expression of renin, COX-2, and nNOS-positive sites in male and female mouse kidney shows no significant differences (*P*>0.05). Values represent Mean ± SE (n=16)

3.1.3 Significant test

Although variation for counting the number of glumeruli, renin, COX-2 and nNOS- positive sites was observed during light microscopy and immunohistochemical studies, but when the data was analyzed statistically the differences were not significant between male and female kidneys (*P*>0.05, Fig. 5).

3.2 Discussion

Sex-and strain-dependent differences in the morphology of glomerular capsule have been described in different laboratory animals [5,1,4,7]. DBA/2CrSlc mouse kidney also revealed sex-dependent kidney morphology in the present investigation. Parietal layer of the glomerular capsule in male mouse kidney consisted of a single layer of simple cuboidal epithelium, whereas it was single layer of simple squamous epithelium in female. This finding is consistent with the observations of Barberini et al. [1] and Yabuki et al. [4,3,6]. Vacuoles of different sizes and shapes were observed in the proximal convoluted tubules (PCT) in male, but this histological feature was not detected in female kidney. In our investigation, these vacuoles were not seen with PAS or HE stains and this is very similar to the observations described by Yabuki et al. [7,8]. Ultrastructurally these vacuoles were electron-dense multilamellar bodies and have been cytochemically identified as lysosomes. Similar structures have been demonstrated in the PCT of ICR, BALB/c, C57BL/6, C3H/Hen, DBA/2 mice, and Wistar rat kidney [5,1,2,9,10,11]. They also mentioned that these vacuolar

structures were especially remarkable in male DBA/2 mice and this is consistent with our present observation in DBA/2CrSlc male mouse kidney. Recent reports indicated that the renal proximal tubule plays an important role in the catabolism of low-density lipoprotein (LDL) and LDL receptor family is distributed throughout the renal proximal tubules [12,13,14,15]. It is assumed that, detection of vacuolar structures in PCT epithelium of male mouse kidney which was previously confirmed ultrastructurally as lysosomes is directly related with LDL metabolism [8]. Renal structures resembling vacuolar appearance of DBA/2 mice were reported in cat [16] and mastomys kidneys [17]. PAS-positive brush border of the proximal straight tubules (PST) and collecting ducts reveals stronger staining intensity than that of male, and these histological characters are very similar to the findings of Yabuki et al. [6] in DBA/2Cr mouse kidney. In the present study, we observed PAS-positive granules of different shapes and sizes (small and giant granules) in PST epithelium and collecting ducts in female mice kidney, but these cytological features were not detected in male. Yabuki et al. [12] first reported these PAS-positive granules in the PST of female ICR mouse kidney, and described these granules were prominent in female. Giant granules, larger than the nuclei in size, were detected in the proximal convoluted tubules of male DBA/2 mouse kidney and this granules were absent in female [6]. Our present observation was very much consistent with this report. Moreover, the number and size of these granules were subsequently found to differ based on the strains [3]. Strong nNOS-positive reaction for PST epithelium and collecting ducts was observed in DBA/2CrSlc female mouse kidney, but this histological character was absent in male. This finding was consistent with the observation reported by Yabuki et al. [6] in DBA/1 and DBA/2, mouse kidney. Arthur et al. [18] reported that nitric oxide (NO) synthase and its release play a vital role in the regulation of blood pressure and regional blood flow.

An increased number of glomeruli, renin, COX-2, nNOS- positive sites were observed in DBA/2CrSl female mouse when compared with that of the male, but when analyzed statistically the differences were not significant ($P>0.05$). In our present observation it has been revealed that the pattern of changes of the number of glomeruli, renin, COX-2 and nNOS-positive sites indicates towards significant between male and female mice. However, this speculation might be possible to prove if increased number of experimental mice was possible to include in this experiment.

Sex-and-strains dependent expression of renin, COX-2, and nNOS positive sites in the kidney was reported by many authors using different kinds of laboratory animals using light and electron microscopy as well as immunohistochemistry [5,1,2,9,10,4,3,6]. Our observations on kidney morphology in DBA/2Crslc mice are in partial agreement with the above studies.

4. CONCLUSION

Sex-dependent histological features of the DBA/2Crslc mouse kidney have been observed. The reasons for nNOS-positive reactions for the PST epithelium and collective ducts in female mouse kidney have not yet been clearly understood. A further study is needed to elucidate the reasons for these observations. DBA/2CrSlc mice revealed renin, COX-2, and nNOS positive reactions in the present study. It is suggested that this species can be used in the laboratory for investigating kidney function and related pathological studies.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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