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Age-Dependent Changes in the Kidney Morphology of Female DBA/2 Mice

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Abstract: A female DBA/2 mouse is characterized by the presence of abundant cytoplasmic granules in the renal tubules. In the present study, the morphometrics of kidneys from female DBA/2 mice at 5, 15, 18, 21, and 24 months of age were investigated to determine the age-dependent renal changes in this mouse strain. Glomerular and tubulointerstitial disease progressed with age, and the semiquantitative scores of these lesions showed significant increases. Granules were observed in the proximal straight tubules and no changes were observed in their localization, fine structure, and quantitative scores. It was concluded that the tubular cytoplasmic granules in the female DBA/2 mouse were not affected by age-dependent functional reduction of the kidney.

Key words: aging, DBA/2 strain, mouse kidney

In our recent studies, we have demonstrated strain and sex-based morphological varieties of mouse kidneys [6, 7]. In these studies, we investigated kidney morphology in male and female mice from five strains (ICR, BALB/c, C3H/He, C57BL/6, and DBA/2), and identified female-specific cytoplasmic granules in the proximal straight tubules (PSTs) [7]. Especially the female DBA/2 mouse is characterized by the presence of abundant granules, and they are of various sizes, are periodic acid-Schiff (PAS) positive, and are identified as multilammellar lysosomes owing to their ultrastructural and enzymatic features [7]. The expression of these granules is stimulated by estradiol and inhibited by testosterone [9]. A lectin-histochemical study identified various types of residual carbohydrates within the granules, and succinylated wheat germ agglutinin (s-WGA) lectin was recognized as a specific histochemical marker [8]. In order to clarify the functional importance of these structural features, the morphological and morphometrical changes in these granules need to evaluated under various physiological conditions. The effects of maturation have already been evaluated, and it has been found that the granules become prominent 70 days after birth [4]. However, the effects of aging remain unclear. Therefore, in the present study, we analyzed the age-dependent changes in the kidney morphology of female DBA/2 mice.

All experimentation in the present study proceeded in accordance with the Guidelines for Animal Experimentation of Kagoshima University, Kagoshima, Japan.

Female DBA/2CrSlc mice aged 5 (n=4), 15 (n=5), 18 (n=3), 21 (n=3), and 24 (n=3) months, were used in the present study. The mice were housed in an open system room with a one-way airflow system (temperature 22 ± 1°C; humidity 55 ± 10%; light period 07:00 to 19:00;

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ventilation 12 cycles/h), and were provided an autoclaved commercial diet (CE-2, 24.9% crude protein; CLEA Japan, Inc., Tokyo, Japan) and tap water *ad libitum*. All the mice were sacrificed by exanguination of the carotid arteries while they were under deep anesthesia induced by a mixture of ketamine (60 mg/kg; Sankyo, Inc., Tokyo, Japan) and medetomidine (0.3 mg/kg; Meiji, Inc., Tokyo, Japan). Central slices from the removed kidneys were fixed in 10% neutral buffered formalin and routinely embedded in paraffin. Sections (3 μm thick) were selected at a distance of every 30 μm and stained with PAS and Masson's trichrome (MT) stains. Lectin histochemistry was performed using an Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) and biotinylated s-WGA lectin (Vector Laboratories). Incubation with s-WGA lectin (8 μg/ml) was performed overnight at 4°C, and the reactivity was detected using a 3,3'-diaminobenzidine tetrahydrochloride H_2O_2 solution (DAB). The kidneys from 24-month-old mice were examined under an electron microscopy. Small pieces of the outer medullary tissues were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (CB) with 2% sucrose. After fixation with 1% osmium tetroxide in CB, the specimens were routinely embedded in Epok 812. Ultrathin sections were stained with uranyl acetate and modified Sato's lead stain [1] and observed under a transmission electron microscope (H-7000KU, Hitachi, Tokyo, Japan).

Randomized morphometric analyses were conducted using 5 sections from each animal. (1) Diameter of the renal corpuscles of the renal cortex: 5 cortical renal corpuscles with either the vascular pole or urinary pole were selected in each PAS-stained section and the widest distance of the cross-axis was measured. (2) The extent of glomerular lesion was evaluated by using a previously described semiquantative scoring system [5]. A minimum of 100 glomeruli per animal were examined in the MT-stained sections, and the severity of the sclerotic lesion in each glomerulus was graded from 0 to +4. The glomerular lesion score was evaluated for each animal. If, for example, 5 out of 150 glomeruli displayed a score of +1, 10 out of 150 demonstrated a score of +2, 15 out of 150 demonstrated a score of +3, and 5 out of 150 demonstrated a score of +4, the final score would be $[(1\times5/150)+(2\times10/150)+(3\times15/150)+(4\times5/150)]\times100=60.0$. (3) The extent of interstitial fibrosis was also evaluated by using a similar semiquantitative scoring system. A minimum of 20 non-overlapping cortical fields (MT stain; magnification ×200) per animal were observed and graded from 0 to +4 according the severity of fibrosis, and the fibrosis score was calculated by using the same formula described above. (4) The area of the s-WGA-positive region: A minimum of 10 non-overlapping digital photographs (magnification ×200) per animal were taken of the outer medullar zone (PSTs mainly run along this zone). The area of s-WGA-positive regions (brown color regions visualized by DAB reaction) was extracted, and the area of these regions was measured by using the image analysis software SigmaScan Pro 5.0 for Windows (Systat Software Inc., Point Richmond, CA, USA). Further, the ratio of the positive area to the entire area observed on the photographs was calculated for each animal. The results were expressed as the mean ± standard error (SE) and analyzed using the non-parametric Wilcoxon test ($P<0.05$). Statistical analysis was performed using JMP 5.1 for Windows (SAS Institute Inc., Carry, NC, USA).

In the histopathological observation, thickening of the glomerular basement membrane and expansion of the mesangial matrix within the glomeruli became severe with age. Within the tubulointerstitium, thickening of the tubular basement membrane, tubular atrophy, urinary casts, mononuclear cell infiltration and interstitial fibrosis became severe with age. The morphometric data are shown in Fig. 1. No significant age-dependent changes were detected in either wet kidney weight (Fig. 1A) or relative kidney weight (Fig. 1B). Although the diameters of the renal corpuscles showed no significant change (Fig. 1C), glomerular lesion scores continuously increased up to the age of 24 months, and this change was statistically significant (Fig. 1D). The interstitial fibrosis score increased up to 24 months of age, and this change was statistically significant (Fig. 1E). Cytoplasmic granules that were PAS-positive were observed in the PSTs of all the mice (Fig. 2A). The s-WGA lectin detected these granules with high specificity (Fig. 2B). The percentage of areas that were s-WGA lectin-positive areas showed no significant age-dependent changes (Fig. 1F). In the electron microscopic observation of the kid-

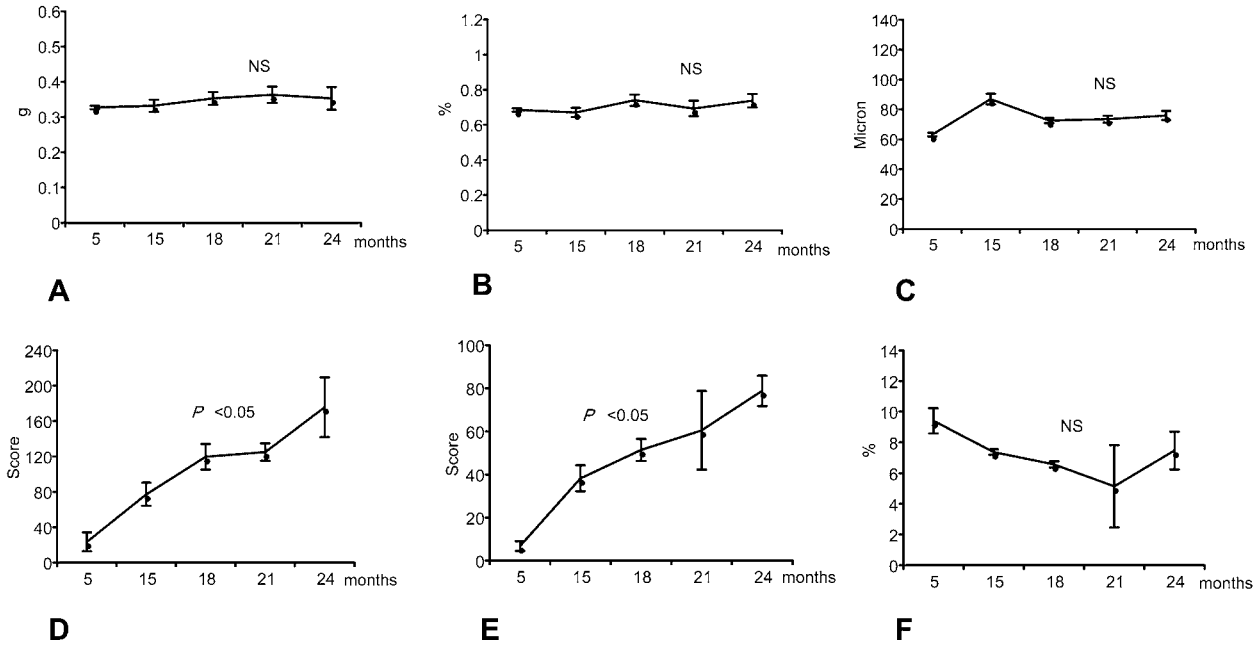

Fig. 1. Changes in kidney weight and morphometric parameters. A: Wet kidney weights (total kidney weights). B: Relative kidney weight (total kidney weight/body weight). C: Diameter of renal corpuscles. D: Score of glomerular lesion. E: Score of interstitial fibrosis. F: Percentage of s-WGA lectin-positive areas within the outer medullary zone. The value in each column represents the mean ± SEM. NS: Not significant. P<0.05: Age dependent significant change.

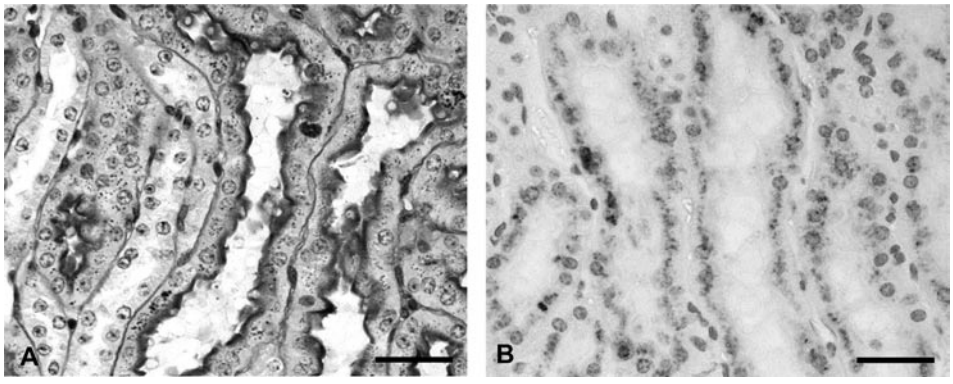

Fig. 2. Light micrographs of the renal outer medulla of a 24-month-old mouse. A: PAS stain. B: s-WGA lectin histochemistry. Variously sized PAS-positive granules were observed in the proximal straight tubular epithelium (panel A) and these granules were positively stained by s-WGA lectin histochemistry (panel B). Bars: 35 μm.

neys of 24-month-old mice, the cytoplasmic granules in the PSTs appeared as variously sized dense bodies consisting of multilamellar structures (Fig. 3). This ultrastructural feature was not different from those that were previously reported for the kidneys of the 3-month-old female DBA/2 mice [7].

The PAS-positive granules in the PSTs are a morphological feature of female mouse kidneys. The number and size of these granules vary remarkably between mouse strains and are especially abundant and large in the DBA/2 mice [7]. Although it is not clear whether the presence of these granules in the PSTs is beneficial or harmful to the renal function, the former may be concluded from the findings of the present study. If these

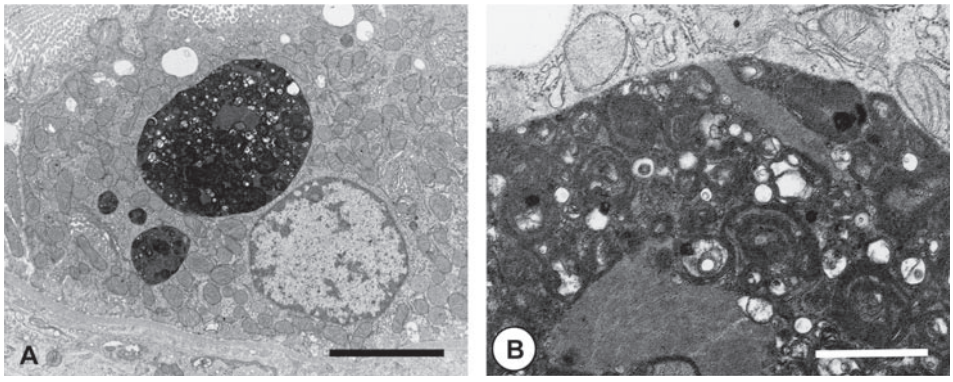

Fig. 3. Electron micrographs of a proximal straight tubular cell from the kidney of a 24-month-old mouse. A: Low magnification view. B: Higher magnification view of panel A. Variously sized dense bodies were seen in the cytoplasm (panel A), and included multilamellar fingerprint-like structures and vesicles (panel B). Bars: 5 μm (A) or 1 μm (B).

granules were harmful to the renal function, the kidneys of female DBA/2 mice would undergo severe damage with aging. However, the renal lesions observed in the present study were mild, with no renal or glomerular hypertrophy. In our recent study, age-dependent renal and glomerular hypertrophies were observed in the kidneys of female C57BL/6 mice [10]. However, the histopathological lesions in the glomeruli and tubulointerstitium were similar in these 2 mouse strains. In addition, female C57BL/6 was previously characterized as a mouse strain with inconspicuous granules in the PSTs [7].

The effects of sex hormones also support the hypothesis that cytoplasmic granules in the PSTs of female DBA/2 mice benefit the renal function. The expression of these granules is stimulated by estradiol and inhibited by testosterone [9]. A recent study suggested that estrogen prevents various types of renal injury, including age-dependent injury, whereas androgens cause adverse effects [3]. Such effects of sex hormones in renal injury are mediated through the renin-angiotensin system and nitric oxide (NO) synthesis system [3]. Estrogen exerts stimulatory effects on both NO synthase (NOS)-3 expression and NO generation [3]. Moreover, our recent immunohistochemical study demonstrated that NOS-1, and not NOS-3, is expressed in the granules in the PSTs of female DBA/2 mice [2]. These findings suggest that the granules in the PSTs of female DBA/2 mice serve as beneficial structures that prevent the progression of age-dependent renal injury; this effect might be mediated thorough NO generation via estrogen-induced NOS-1 expression in the granules.

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