HISTOLOGICAL STUDY ON THE POSTNATAL ALTERATIONS IN THE RAT KIDNEY

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ABSTRACT

INTRODUCTION: The aging kidney is characterized by numerous morphological changes in the nephrons and interstitium which are usually associated with failure of renal function. The aim of the present study is to describe and compare histological age-related changes in the kidney in three age groups of rats.

MATERIALS AND METHODS: Male Wistar rats aged 2, 4 and 6 months (n=3; per age group) were used in the present study. Alterations in kidney morphology were observed by light microscopic analysis using PAS reaction and Mallory’s trichrome staining.

RESULTS: We observed normal histological structure of the kidney in 2-month-old rats. We noted that renal corpuscles were relatively well preserved and proximal and distal tubules were visible as well-defined structures. No pathological changes in blood vessels and interstitial fibrosis were observed. As age advanced (4 and 6 months) we described periglomerular and periarteriolar sclerosis, wrinkling and thickening of glomerular and tubular basement membranes; interstitial fibrosis was generally expressed.

CONCLUSION: Renal aging is a multifactorial process characterized by an increased number of sclerotic glomeruli, tubular atrophy and interstitial fibrosis and is associated with declining renal function.

Keywords: kidney, age-related changes, rat

INTRODUCTION

Renal senescence is a multifactorial inevitable process where genetic background, gender, race, environmental factors and comorbid conditions have been discussed (1,2). As age advances, kidneys go through morphological changes, which are usually associated with a declining renal function – a limiting factor for the lifespan (3). The main alterations in the renal corpuscles include decreased proportion of intact glomeruli and increased prevalence of globally sclerotic glomeruli; hypertrophy of the glomeruli, expansion of the mesangial matrix, and thickening of the glomerular basement membrane (4,5). The percentage of age-related sclerotic glomeruli in the human population can be estimated by the formula: age/2-10, when age is beyond 40 years (6). As aging progresses the main tubulointerstitial alterations are characterized by tubular atrophy, dilatation of the lumen, thickening of the tubular basement membrane, and flattening of the covering epithelium (7). This is associated with an expansion of interstitial fibrosis and mononuclear infiltration (8). Intrarenal vascular changes also contribute to renal dysfunction which is a key component of advanced age (9). Arterial sclerosis leads to thickening of the walls and narrowing of the vascular lumen. The pathogenesis is a consequence to collagen deposition in the media and...
thickening of the intima (7,10,11). Fibro-intimal hyperplasia is characterized by intimal collagenous fibrosis and is primarily observed in interlobular arteries. The positive correlation between hyaline arteriolosclerosis and aging was first described by Moritz and Oldt (12). It is a common age-related vasculopathy, which is associated with deposition of proteins in the subendothelial space. Accumulation of hyaline can be demonstrated by Periodic acid-Schiff reaction, where it manifests itself in bright magenta, having a glassy texture (13). All these vascular changes cause cortical glomerulosclerosis with compensatory glomerular hypertrophy in the medulla, tubular atrophy and interstitial fibrosis (7). None of the described renal age-related morphological changes are specific and they can be a result of widespread diseases in older population such as diabetes and arterial hypertension (14,15).

The aim of the present study was to observe the postnatal morphological changes in the nephrons and renal interstitium in rats. The main task of our research was to describe and compare alterations in renal histology in three age groups of Wistar rats, aged 2, 4 and 6 months.

MATERIALS AND METHODS
Male Wistar rats, available at the Medical University of Sofia, aged 2, 4 and 6 months (n=3; per age group) were used for this study with the approval of the University Committee on Animal Resources. The rats were anesthetized intraperitoneally with Thiopental 40mg/kg b.w. The chest cavity was opened and transcardial perfusion was made with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The kidneys were quickly removed and fixed in 10% neutral buffered formalin. After routine paraffin embedding, 5μm thick sections were cut and stained with Mallory’s trichrome method and Periodic acid-Schiff reaction. For Mallory’s trichrome method and Periodic acid-Schiff (PAS) reaction. For Mallory’s trichrome stain, paraffin was removed, after which the slides were placed in 0.1% fuchsin for 1-2 minutes, washed and placed in 1% solution of phosphomolybdic acid for 3-5 minutes. After thorough rinsing, the slides were placed in a mixture of aniline blue, orange G and oxalic acid for 2 minutes, washed again and embedded in entellan. The PAS reaction involved a removal of the paraffin, followed by periodic acid immersion for 5-10 minutes and thorough rinsing with distilled water. The slides were then placed in Schiff’s reagent for 10-20 minutes. After that, they were rinsed for 5 minutes with lukewarm tap water. Finally, the slides were contrastained in haematoxylin for 1 minute, washed again and embedded in entellan. We analyzed and compared the morphological changes in nephrons and renal interstitium between three age groups of animals. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Institute of Health (NIH publication No. 86-23, revised 1996).

RESULTS
On histological slides from the kidneys of rats, we described changes observed throughout different periods of the postnatal development of the rats of the Wistar strain. We focused on the changes in the morphology of the renal corpuscles and the tubules, which form the nephron and we also described histological alterations noted in the blood vessels and the interstitial space. It was established that the morphological changes were most dynamic in the groups of 4- and 6-month-old animals.

We described a normal morphological structure of the kidneys in 2-month-old rats (Fig.1a; Fig.1b) during a light microscopic examination of Periodic acid-Schiff and Mallory’s trichrome stainings. The specific localization of the renal corpuscles classified nephrons into superficial, midcortical and juxtamedullary. Renal corpuscles were relatively well demarcated. We studied the glomerular capillary wall and described its structure, which featured endothelial cells, the glomerular capillary basement membrane and the visceral epithelial cells or podocytes. The podocytes represented the visceral layer of the Bowman’s capsule. The parietal layer of the Bowman’s capsule was covered by simple squamous epithelium. We did not observe any qualitative alterations in the structure of the glomerular and capsular basement membranes by PAS staining. The juxtaglomerular apparatus was formed by three types of cells – macula densa cells of the distal convoluted tubule, juxtaglomerular cells – specialized smooth muscle cells of the afferent arteriole, and extraglomerular mesangial cells. The lumen of proximal tubules was...
Histological Study on the Postnatal Alterations in the Rat Kidney

Fig. 1 a. Photomicrograph of the kidney stained by Mallory's trichrome method, age - 2 months. Magnification - x200
b. Photomicrograph of PAS reaction in the kidney, age - 2 months. Magnification - x400

Fig. 2 a. Photomicrograph of the kidney stained by Mallory's trichrome method, age - 4 months. Magnification - x200
b. Photomicrograph of PAS reaction in the kidney, age - 4 months. Magnification - x400

Fig. 3 a. Photomicrograph of the kidney stained by Mallory's trichrome method, age - 6 months. Magnification - x200
b. Photomicrograph of PAS reaction in the kidney, age - 6 months. Magnification - x400
not well defined as opposed to the lumen of distal tubules, which was a result of the prominent microvilli observed on the apical surface of the cellular membrane of the covering cuboidal epithelium. We did not notice an expansion of interstitial fibrosis or any pathological vascular changes in the observed glomerular capillaries, afferent and efferent arterioles, and larger blood vessels. We also described erythrocytes in their lumen which were stained red by Mallory’s trichrome staining.

As aging advanced (4- and 6-month-old rats) (Fig.2a; Fig.2b; Fig.3a; Fig.3b) we noted an increase in the number of sclerotic glomeruli, with evidence of more pronounced expression of interstitial fibrosis – periglomerular and peritubular fibrosis. The collagen fibers were stained blue by Mallory’s thricrome staining. We also observed thickening of the basement membranes of the glomeruli and Bowman’s capsules. The alterations of basement membranes were described by a qualitative analysis with PAS staining. The borders between the epithelial cells of the parietal layer of Bowman’s capsule were not clearly demarcated. We noted the presence of tubular changes, which were characterized by tubular atrophy, flattening of the covering epithelium, dilatation of the lumen and thickening of the tubular basement membrane. Pathological changes in blood vessels were characterized by intimal and medial hypertrophy, thickening of the walls and narrowing of the vascular lumen as a result of fibrointimal hyperplasia, arteriosclerosis and recruitment of smooth muscle cells. In some blood vessels hyaline arteriolosclerosis was demonstrated and stained bright magenta. The morphological structure of the glomerular capillary tufts was not clearly distinguished. We also found periarterial fibrosis, which was a result of increased accumulation of extracellular matrix proteins.

**DISCUSSION**

Age-related alterations in the kidney have been described in a wide range of species, including rats, mice and hamsters (16, 17, 18). A review of the pertinent literature suggests that age-related basement membrane thickening can be observed in senescent Wistar rats (19). This was also established by our comparative study. As aging advanced an increased expansion of renal fibrosis was observed. In the development of tubulointerstitial fibrosis, transition of tubular epithelial cells to mesenchymal cells, which in turn transform to fibroblasts migrating to the interstitial space, has been discussed (20). As aging progressed the number of sclerotic glomeruli and the size of the mesangial matrix increased, which was associated with a declining renal function. Morphological impairment of the Bowman’s capsule was observed – the borders between the simple squamous epithelial cells of its parietal layer were not clearly demarcated. Some studies suggest that glomerular sclerosis is primarily observed in the outer cortex (21,22,23). In 6-month-old rats, the observed tubular atrophy and dilatation, as well as interstitial fibrosis and thickening of the tubular basement membranes confirmed the data from several studies (24,25,26). Age-related vascular changes included fibrointimal hyperplasia and narrowing of the lumen of arteries – primarily in the interlobular arteries, arteriolosclerosis and deposition of hyaline, and periarteriolar fibrosis. These alterations were confirmed by other studies (24,29) and caused progressive obliteration of the vascular lumen. The main consequence is compromised renal blood flow, chronic renal dysfunction, and loss of renal mass (29,30).

In the present study, age-related renal morphological alterations (especially glomerular tufts, tubules, glomerular basement membrane) were demonstrated and described using Mallory’s trichrome staining and PAS reaction. A review of the pertinent literature showed insufficient data of such comparative analysis, especially with regard to age-related renal changes in young and adult rats. Furthermore, we did not establish significant histological alterations when we compared 2- and 4-month-old rats, but in 6-month-old rats, the renal changes were well represented. The results were compared with these observed in a human kidney and other mammalian species.

**CONCLUSION**

The knowledge of age-related changes in kidneys has important clinical significance, because they are associated with a declining renal function. Future studies of the mechanisms of aging may permit manipulation of the observed alterations and retardation of renal aging.
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