Pathological and functional significance of aging mouse kidneys: clinical implications to reduce the risk of hyper- or hypokalemia in the elderly

Itsuro Kazama

School of Nursing, Miyagi University, Miyagi, Japan

Elderly patients are prone to develop hyper- or hypokalemia, since they are susceptible to drugs or diets that affect the urinary or fecal potassium (K⁺) excretion. In aging mouse kidneys, in addition to glomerulosclerosis, proximal tubular atrophy, and atherosclerosis in renal arterioles, there was diffuse tubulointerstitial fibrosis with a number of inflammatory leukocytes infiltrating into the cortical interstitium. Since these pathological features greatly influence renal K⁺ handling, slowing the progression of kidney aging would fundamentally reduce the risk of developing hyper- or hypokalemia. Immunohistochemistry demonstrated the overexpression of K⁺ channels (Kv1.3) in leukocytes within the cortical interstitium, which was strongly associated with “chronic inflammation” in aging kidneys and the subsequent progression of renal fibrosis. In our basic studies, antihypertensive drugs (benidipine, nifedipine, verapamil, diltiazem) and anticholesterol drugs (lovastatin, simvastatin, pravastatin) strongly suppressed the leukocyte Kv1.3 channels and thus exerted anti-inflammatory effects. Given such pharmacological properties of these drugs, they may also be useful in slowing the progression of tubulointerstitial fibrosis in aging kidneys and reducing the risk of hyper- or hypokalemia in elderly patients.

Keywords: Aging, Anti-hypertensive drugs, Fibrosis, Hyperkalemia, Hypokalemia, Inflammation, Kv1.3 potassium channel

Introduction

Elderly patients are prone to develop hyper- or hypokalemia, the common electrolyte disorder caused by the disturbance of potassium (K⁺) homeostasis [1,2]. Patients with hyper- or hypokalemia are usually asymptomatic or only present with mild neuromuscular symptoms. However, severe cases can be life-threatening, since the typical electrocardiogram abnormalities, such as the widening of QRS complexes and the prolongation of QT intervals, occasionally evolve into fatal cardiac arrhythmias or sudden cardiac arrest [3,4]. Under a physiological condition, about 90% of K⁺ ions absorbed from the diet are excreted into the urine, while the remaining 10% are excreted into the feces [5]. Therefore, in elderly patients, the complication of renal insufficiency or chronic constipation, which decreases urinary or fecal K⁺ elimination, often causes hyperkalemia [6,7]. Additionally, the use of drugs that impair the urinary K⁺ excretion or promote its transcellular shift, such as angiotensin-converting enzyme inhibitors, nonsteroidal anti-inflammatory drugs, and beta adrenergic blockers, also cause hyperkalemia [8,9]. On the other hand, hypokalemia is frequently observed in elderly patients with a decreased dietary K⁺ intake or those taking drugs that promote uri-
nary K⁺ excretion, such as loop diuretics and herbal medications [10,11]. Further, in elderly patients, age-related physiological changes, including the decline of renal function, hemodynamic changes, and hormonal imbalance, would also influence their K⁺ homeostasis [12].

Main texts

Using experimental animal models, previous studies revealed the pathological features of aging kidneys [13–15]. However, they have not been examined concerning the functional relevance to K⁺ homeostasis. In the present study, to reveal the pathological and functional significance of aging kidneys, we carefully examined the histopathological features of 21-month-old mouse kidneys and compared to those of 8-week- or 10-month-old mice (Fig. 1). Consistent with previous findings obtained from humans or experimental animals [15,16], sections of kidneys from 8-week- and 10-month-old mice showed relatively normal glomeruli and tubulointerstitium in both cortex and papilla (a, b, d, and e in Fig. 1A). However, in those from 21-month-old mice, most of the glomeruli were sclerotic and there were a number of inflammatory leukocytes infiltrating into the expanded interstitium (c in Fig. 1A). Both proximal tubular cells in the cortex and collecting duct cells in the papilla were flattened as a result of tubular atrophy (c and f in Fig. 1A). In periodic acid-Schiff (PAS) staining, compared to the glomeruli from 8-week-old mice (a in Fig. 1B), those from 10-month-old mice were hypertrophic and partially sclerotic (b in Fig. 1B). In 21-month-old mouse kidneys, most of the glomeruli were characterized by more massive deposition of PAS-positive material (c in Fig. 1B), indicating the progression of glomerulosclerosis. In the cortex of 21-month-old mouse kidneys, Masson’s trichrome-staining demonstrated diffuse fibrosis within the expanded interstitium (f in Fig. 1B), which was not observed in those of 8-week- or 10-month-old mice (d and e in Fig. 1B). In renal arterioles of 21-month-old mouse kidneys (f in Fig. 1B), vascular smooth muscle cells proliferated and the layers of elastic fibers thickened (arrowhead), indicating the progression of atherosclerosis.

Inflammatory leukocytes, such as lymphocytes and macrophages, predominantly express delayed rectifier K⁺-channels (Kv1.3) in their plasma membranes, and the channels play pivotal roles in the activation and proliferation of the cells [17]. In our previous animal studies, the overexpression of Kv1.3 channels in leukocytes was strongly associated with their in situ proliferation in kidneys with tubulointerstitial fibrosis [18,19]. In the present study, consistent with our previous findings [18,19], immunohistochemistry for Kv1.3 (1:50; Biozzi, Inc.) demonstrated positive staining in the cytoplasm of intact or relatively intact proximal tubular cells in 8-week- and 10-month-old mouse kidneys (a, b in Fig. 1C). In those of 21-month-old mice, the expression of Kv1.3 was additionally observed in the cytoplasm of interstitial leukocytes (arrowheads), each of which was overexpressed within the cells (c in Fig. 1C). By producing the driving force for the calcium influx, Kv1.3 channels in leukocytes initiate the calcium signaling required for cell proliferation [17]. Therefore, as previously shown [18,20], the membrane hyperpolarization caused by the overexpression of the channels directly stimulates the cell cycle progression, and thus facilitates the proliferation of leukocytes (Fig. 2). Additionally, since inflammatory cytokines produced by the leukocytes stimulate the activity of fibroblasts to produce collagen [21], the increased number of leukocytes in the cortical interstitium of aging kidneys was thought to be responsible for the progression of renal fibrosis (Fig. 2).

In renal collecting ducts, other K⁺ channels, such as the renal outer medullary K⁺ channels (ROMK or Kir1.1) and large-conductance calcium-activated potassium channels (BK or Kca1.1), are abundantly expressed in the apical membrane of principal cells [5,22]. These channels, which are directly stimulated by aldosterone, are primarily responsible for the urinary excretion of K⁺ ions and the maintenance of K⁺ homeostasis (Fig. 2). From our results, since the collecting duct cells were atrophic in aging kidneys (f in Fig. 1A), such-induced functional disturbance of these K⁺ channels may affect the K⁺ homeostasis. Additionally, the pathological changes observed in the glomeruli and cortical interstitium of aging kidneys (Fig. 1) would also greatly influence the K⁺ homeostasis by the following mechanisms (Fig. 2). First, since glomerulosclerosis reduces the surface area available for the blood filtration [23], glomerular filtration rate declines and accumulates unfiltered K⁺ ions in the serum, causing hyperkalemia. Second, the progression of tubulointerstitial fibrosis facilitates hyperkalemia, since it deteriorates renal insufficiency by reducing the total nephron number within the kidneys [13,23]. This consequently
Figure 1. Histological features and the expression of Kv1.3 in aging mouse kidneys. (A) Hematoxylin and eosin (H&E) staining of cortex (a–c) and papilla (d–f) in 8-week-old (a, d), 10-month-old (b, e), and 21-month-old (c, f) mouse kidneys. Sections of kidneys from 8-week- and 10-month-old mice showed relatively normal glomeruli and tubulointerstitium in both cortex and papilla. However, in those from 21-month-old mice, most of the glomeruli were sclerotic and there were a number of inflammatory leukocytes infiltrating into the expanded interstitium. Both proximal tubular cells in the cortex and collecting duct cells in the papilla were flattened as a result of tubular atrophy (magnification, ×20). (B) Periodic acid-Schiff (PAS) staining of glomeruli (a–c; magnification, ×60) and Masson’s trichrome-staining of cortex (d–f; magnification, ×20) in 8-week-old (a, d), 10-month-old (b, e), and 21-month-old (c, f) mouse kidneys. Compared to the glomeruli from 8-week-old mice, those from 10-month-old mice were hypertrophic and partially sclerotic. In 21-month-old mouse kidneys, most of the glomeruli were characterized by more massive deposition of PAS-positive material, indicating the progression of glomerulosclerosis. In the cortex of 21-month-old mouse kidneys, Masson’s trichrome-staining demonstrated diffuse fibrosis within the expanded interstitium, which was not observed in those of 8-week- or 10-month-old mice. In renal arterioles of 21-month-old mouse kidneys, vascular smooth muscle cells proliferated and the layers of elastic fibers thickened (arrowhead), indicating the progression of atherosclerosis. (C) Immunohistochemistry using antibody for Kv1.3 (brown) in 8-week-old (a), 10-month-old (b), and 21-month-old (c) mouse kidneys. There was a positive staining in the cytoplasm of intact or relatively intact proximal tubular cells in 8-week- and 10-month-old mouse kidneys. In those of 21-month-old mice, the expression of Kv1.3 was additionally observed in the cytoplasm of interstitial leukocytes (arrowheads), each of which was overexpressed within the cells (magnification, ×60).
causes glomerular hyperfiltration in the residual nephrons, leading to glomerular hypertrophy and further progression of glomerulosclerosis [13] (Fig. 2). Third, these structural changes in the aging kidneys also reduce the renin synthesis from the juxtaglomerular apparatuses [24] and suppress the activity of renin-angiotensin-aldosterone system (RAAS) (Fig. 2). This functionally impairs the urinary K⁺ excretion from the collecting ducts and facilitates hyperkalemia [5,22]. On the other hand, the destruction of proximal tubules, which reabsorb the bulk of filtered K⁺ through paracellular diffusion [22], increases urinary K⁺ excretion (Fig. 2) and causes hypokalemia. Additionally, the atherosclerosis-induced renal artery stenosis causes hypoperfusion of glomerular capillaries [25] and thus stimulates the renin production from the residual nephrons [26] (Fig. 2). This activates RAAS and increases urinary K⁺ excretion from the collecting ducts and facilitates hypokalemia.

Due to these predisposing conditions that influence K⁺ homeostasis (Fig. 2), elderly patients are susceptible to drugs or diets that affect the urinary or fecal K⁺ excretion [1,2,6–9]. Therefore, to prevent the development of hyper- or hypokalemia, elderly patients have to be cautious about taking medications or dietary supplements. Additionally, concerning the mechanisms by which K⁺ homeostasis is disturbed (Fig. 2), slowing the progression of kidney aging would fundamentally reduce the risk of developing hyper- or hypokalemia. In addition to smoking, high protein intake, obesity, and the complications of cardiovascular diseases, systemic hypertension greatly contributes to the progression of glomerulosclerosis and atherosclerosis in aging kidneys [27]. Therefore, in elderly patients, the use of antihypertensive drugs should be initially recommended to lower the risk of hyper- or hypokalemia (Fig. 2). Recently, several studies additionally demonstrated the involve-

![Figure 2](image_url)

**Figure 2. Pathological features of aging kidneys and the mechanisms by which hyper- or hypokalemia develops.** Glomerulosclerosis reduces the surface area available for blood filtration and accumulates unfiltered K⁺ ions in the serum, causing hyperkalemia. Overexpression of Kv1.3 channels is responsible for leukocyte proliferation and the progression of tubulointerstitial fibrosis. The fibrosis facilitates hyperkalemia and causes further progression of glomerulosclerosis. On the other hand, the destruction of proximal tubules increases urinary K⁺ excretion and causes hypokalemia. Additionally, the changes in renin synthesis and the activity of renin-angiotensin-aldosterone system affect the urinary K⁺ excretion, causing hyper- or hypokalemia. In addition to antihypertensive drugs that slow the progression of glomerulosclerosis and atherosclerosis, the use of anticholesterol drugs that strongly suppress the leukocyte Kv1.3 channels may also be useful in slowing the progression of tubulointerstitial fibrosis and reducing the risk of hyper- or hypokalemia in elderly patients.
ment of “chronic inflammation” in the pathogenesis of kidney aging [28,29]. In the present study, as we previously demonstrated in rat kidneys with renal insufficiency [18,19], the overexpression of Kv1.3 channels in leukocytes was strongly associated with chronic inflammation and the subsequent progression of renal fibrosis (Fig. 2). In our previous studies, in addition to margatoxin, a selective Kv1.3 channel blocker [17], benidipine, an antihypertensive drug that also inhibits the channel [30,31], actually suppressed the activity of leukocytes and slowed the progression of renal fibrosis [19]. In our following patch-clamp studies, in addition to benidipine, other antihypertensive drugs, such as nifedipine, verapamil, diltiazem, and anticholesterol drugs, such as lovastatin, simvastatin, pravastatin, also strongly suppressed the Kv1.3 channels in leukocytes and thus exerted anti-inflammatory effects [32–34]. Given the pharmacological properties of these drugs, they may also be useful in slowing the progression of tubulointerstitial fibrosis in aging kidneys (Fig. 2) and reducing the risk of hyper- or hypokalemia in elderly patients.

The study was performed in accordance with the guide for the care and use of laboratory animals of Miyagi University, which included ethical considerations. The protocols for the use of the animals were approved by the Animal Care and Use Committee of Miyagi University (No. 2024-03).

**Conclusion**

In aging mouse kidneys, in addition to glomerulosclerosis, proximal tubular atrophy, and atherosclerosis in renal arterioles, there was diffuse tubulointerstitial fibrosis with a number of inflammatory leukocytes infiltrating into the cortical interstitium. Immunohistochemistry demonstrated the overexpression of Kv1.3 channels in leukocytes within the cortical interstitium, which was strongly associated with “chronic inflammation” in aging kidneys and the subsequent progression of renal fibrosis. Given the anti-inflammatory properties of the channel inhibitors, they may also be useful in slowing the progression of tubulointerstitial fibrosis in aging kidneys and reducing the risk of hyper- or hypokalemia in the elderly.

**Conflicts of interest**

The author has no conflicts of interest to declare.

**Funding**

This work was supported by the Miyagi Kidney Foundation Grant, the Special Research Grant from Miyagi University and the Salt Science Research Foundation (No. 2218) and the Tojuro Iijima Foundation for Food Science and Technology to the Author.

**Data sharing statement**

The data presented in this study are available from the corresponding author upon reasonable request.

**ORCID**

Itsuro Kazama, https://orcid.org/0000-0001-8487-647X

**References**

7. Mari A, Mahamid M, Amara H, Baker FA, Yaccob A. Chronic constipation in the elderly patient: updates in evaluation and