Blood Vessels and the Aging Kidney

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Key Words

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Abstract

Aging is associated with a degenerative effect on many organs including the kidney. Blood vessels play a key role in the progression of renal damage in aging, with reductions in glomerular filtration rate and renal blood flow. Therefore, there is considerable interest in the haemodynamic and molecular mechanisms that may be responsible for alterations in the vascular system in aging. In this review, we will describe the evidence that aging is accompanied by alterations in vascular tone and angiogenesis alongside renal damage. The contributions of mediators such as nitric oxide, angiotensin II and vascular endothelial growth factor will also be discussed.

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Aging is a natural process that occurs in all species and has a degenerative effect on many organs. Interestingly for nephrologists, one of the most prominent organs affected by aging is the kidney. As the majority of patients treated clinically for end-stage renal failure are from the elderly population, it is important for us to understand the histological, functional and molecular changes that occur in the aging kidney.

Renal Histological and Functional Changes in Aging

As we get older our kidneys become progressively smaller amounting to a 10% decrease in renal mass per decade after the age of 50. Histologically, both in aging human and rat renal biopsies focal and segmental glomerulosclerosis develop which is associated with mesangial matrix expansion, increased basement membrane thickening and a loss of capillary loops. In the tubulointerstitial areas, many tubules become dilated and atrophic. This is accompanied by an increased degree of interstitial fibrosis and an influx of infiltrating mononuclear cells [1]. There is an increase in cellular senescence with shortening of telomeres [2] accompanied by alterations in senescence genes including cell cycle regulators such as p16^{INK4a} in human kidneys [3] and lipofuscin and senescence-associated β -galactosidase in rodents [4]. Functionally, creatinine clearance is decreased by 30–40% in individuals over the age of 80 and there is an increased prevalence of microalbuminuria. Sodium balance is also altered in the aging kidney, resulting in both defective conservation in the presence of sodium restriction and defective excretion in the setting of an increased sodium load. The tubules fail to concentrate urine and nocturnal polyuria is common [reviewed in 1].

Alterations in blood vessels also contribute to the renal damage in aging. Functionally both glomerular filtration rate (GFR) and renal blood flow decline with increasing age. Structurally, the majority of renal preglomerular arterioles become thicker due to the recruitment of vascular smooth muscle cells and increased synthesis of extra-cel-

lular matrix proteins [1] and this often is accompanied with hyalinosis. Shunts develop between the afferent and efferent arterioles in the juxtamedullary area of the kidney, effectively causing blood flow to bypass the glomeruli [5]. Evidence from aging rats indicates that there is also a focal loss of peritubular and glomerular capillaries of the kidney [6, 7]. Finally, there is a positive correlation between blood vessel stiffness and advancing age as determined by Mitchell and colleagues [8] in patients from the Framingham Heart Study who were free of clinical cardiovascular disease, hypertension, diabetes, smoking, dyslipidaemia and obesity. These observations have led to studies investigating the haemodynamic and molecular mechanisms that may be responsible for the alterations to the vascular system in aging. In the remainder of this review, we will evaluate these experiments and provide evidence that blood vessels play a key role in the progression of renal disease in aging individuals.

Vascular Tone

Aging is associated with changes in blood vessel tone which is determined by the balance between vasoconstrictors and vasodilators. In aging, there is a tendency for the response to vasodilators such as nitric oxide (NO), endothelial-derived hyperpolarizing factor (EDHF) and prostacyclin to be attenuated, while the responsiveness to vasoconstrictors such as angiotensin II (Ang II) is enhanced. This may result in enhanced vasoconstrictive responses in aging which potentially will cause renal damage and ultimately a loss in GFR.

Nitric Oxide

In both humans and animals there is abundant evidence that vasodilation in response to acetylcholine (ACh) is impaired in the majority of vascular beds, including the kidney [9, 10]. In most cases ACh vasodilation is mediated by the local production of NO in the endothelium. NO is formed from the precursor, *L*-arginine through the activity of constitutive and inducible NO synthases (NOS). Total body NO production is reduced in aging rats as measured by the excretion of nitrates and nitrites [11–13] which also coincides with the progression of renal injury and decreases in renal plasma flow [11, 12, 14]. However, these observations have to date not been reproducible in aging man [15]. In addition, elevated systemic levels of the endogenous competitive inhibitor of NOS,

N^G , N^G -asymmetric dimethyl-arginine (ADMA) are observed in both aging rats and humans [13, 14].

Components in the synthetic pathway leading to NO formation have been measured in the kidneys of aging rats. Studies in Sprague-Dawley rats show reductions in eNOS and nNOS protein levels in aging as observed by Western blotting and immunohistochemistry [6, 7, 16]. In contrast, eNOS levels in aging Fischer 344 rats are not altered indicating that there may be differences in gene expression related to the genetic background of the animals [17]. With regard to iNOS, studies by Reckelhoff et al. [18] indicate that iNOS protein expression in the kidney is elevated in aging rats. However, a more appropriate functional measurement than either protein or gene expression of individual NOS enzymes may be total NOS activity. Total NOS activity is diminished in the aging kidney as demonstrated by two independent studies [13, 16].

Plasma and renal cortical levels of the NO precursor *L*-arginine are either maintained [13] or decline in aging animals [12]. Long-term administration of *L*-arginine in 12-month-old rats can improve proteinuria and renal function, although this effect may not be directly through NO, as concurrent experiments with a NO donor, sodium nitrate fail to improve kidney disease [19]. Functionally, aging renal blood vessels tend to be more sensitive to blocking NO. Vasoconstriction induced by administration of the NO inhibitor, *N*-nitro, *L*-arginine methyl ester (*L*-NAME) is enhanced in aging renal blood vessels [10, 11]. This suggests that maintaining NO responses may be more functionally important in aging renal vessels, perhaps by compensating for the enhanced vasoconstriction induced by angiotensin II (Ang II) (see below) to maintain vascular tone.

Endothelial-Derived Hyperpolarizing Factor

It is now well-established that vasodilation of blood vessels by ACh can also be induced by alternative mechanisms other than NO. The currently unidentified EDHF initiates vasodilation by the activation of calcium-induced potassium channels in vascular smooth muscle cells [20]. In aging, impairment of EDHF mediated blood vessel relaxation has been shown in the superior mesenteric artery of rats [21] and human gastroepiploic distal arteries [22]. In addition, studies by Bussemaker et al. [23] show that renal arterial vessels from old spontaneously hypertensive rats, but not Wistar-Kyoto rats exhibit a total loss of EDHF-mediated responses. Preliminary studies also indicate that renal EDHF activity in the ag-

ing rat may be impaired as measured by the relaxation produced with bradykinin and ACh in the presence of NOS inhibitors and prostaglandin synthase inhibitors in the isolated perfused kidney [24].

Angiotensin II

Aging is also associated with enhanced vasoconstrictor responsiveness. One of the main mediators of vasoconstriction is angiotensin II (Ang II) which plays a role in non-vascular effects during the progression of renal disease. Ang II activates renal fibroblasts to become myofibroblasts, stimulates the production of the profibrotic cytokine TGF- β , induces oxidative stress, stimulates chemokines and osteopontin that may cause local inflammation and stimulates vascular and mesangial cell proliferation and hypertrophy [25]. There is currently little information on the expression of Ang II in either aging humans or animals and studies have therefore focused on other components of the renin-angiotensin system (RAS). In both humans and rats, plasma and renal levels of renin are depressed in aging, possibly related to the relative defect in sodium excretion and a tendency towards volume and sodium expansion [26, 27]. In addition, the aging rat shows enhanced renal vasoconstriction in response to either Ang II or Ang I administration [28, 29]. One possibility to explain the increased responsiveness to Ang II in the aging kidney is differential expression of Ang II receptors. There are to date no studies investigating this in the aging kidney, but mRNA levels of both AT₁ and AT₂ receptors are known to be elevated in the aging heart [30]. Interestingly, alterations in Ang II responses in the kidney blood vessels may be one of the early events that occur in aging. For example, Tolbert et al. [31] observed enhanced vascular responses to Ang II in 9-month-old rats. In contrast, differences in NO mediated responses or histological changes within the kidney are commonly not observed until animals are 15–18 months old.

Consistent with a key role for Ang II in aging-related kidney disease are reports that Ang II blockers attenuate the renal effects observed in aging. In the majority of cases, inhibition of the RAS by either ACE inhibition or Ang II receptor blockers has proved effective and improved renal structure and function in aging mice and rats [32]. An elegant study by de Cavanagh et al. [33] demonstrates that either losartan (an Ang II type I receptor blocker) or enalapril (an angiotensin converting enzyme inhibitor) prevents renal damage by preserving mitochondrial function. AT-1 receptor blockers may also act

by enhancing NO activity and thereby improve vasodilatory responses in the aging kidney [34]. However, to date this phenomenon has only been observed in aging spontaneously hypertensive rats.

Angiogenesis and Vascular Growth Factors

There is also evidence that the formation of new blood vessels, i.e. angiogenesis is attenuated in aging. This effect may be more pronounced under conditions in which angiogenesis is normally stimulated such as hypoxia [35]. Consistent with this observation is the finding in the aging rat kidney that there is a focal decrease in both peritubular and glomerular capillary density and proliferation as observed by immunohistochemistry [7]. The loss of capillaries may predispose the tubules to ischaemia and cause a reduction in the glomerular surface area. Indeed, a loss of nephrons is common in aging with glomerular number decreasing from approximately one million to 600,000 by the age of 80 in humans [36]. Nephron loss may eventually lead to glomerulosclerosis due to hyperfiltration, hypertrophy and elevations in glomerular pressure [37].

The control of blood vessel formation involves two families of growth factors that bind to endothelial-specific receptor tyrosine kinases. Vascular endothelial growth factor (VEGF) initiates the development of blood vessels, while the angiopoietins remodel and maintain them. There is some evidence that these factors are altered both systemically and in the kidney during aging. Systemic [38] and renal medullary levels of VEGF [7] have been shown to be reduced in the aging rat. In contrast, a profound upregulation in protein levels of angiopoietin-1 in the kidney cortex has been observed in aging versus young rats [38]. A similar increase was also observed in isolated aging vascular smooth muscle cells [38]. As angiopoietin-1 can stabilize blood vessels, its increase in aging may function to counter the mechanisms leading to impaired angiogenesis and endothelial dysfunction in aging. This data indicates that by protecting the endothelium we may be able to slow the adverse renal effects of aging in the future.

Hypothesis

This evidence allows us to formulate a hypothesis for the progression of renal disease in aging with a central role for the microvasculature as outlined in figure 1. A

Fig. 1. A hypothesis for a role of the microvasculature in progression of aging-associated renal disease. With advancing age, the vascular tone of blood vessels become altered to favour vasoconstriction. In addition, the kidney is more sensitive to additional insults such as high-salt diet, stress and obesity which can cause primary arteriolar disease by inducing further periods of vessel constriction. The primary arteriolar disease sets off a vicious cycle of renal damage eventually leading to nephron loss and ultimately glomerular scarring and elevated blood pressure.

hypothesis along similar lines has been suggested for the development of salt-sensitive hypertension [39]. As we get older the vascular tone of blood vessels becomes altered tending to favour vasoconstriction. Initially, it is likely that different responses of various vasodilators and vasoconstrictors such as NO, EDHF and Ang II are modified to compensate for this change and allow the kidney to function relatively normally. However, when the kidney is subjected to an additional insult such as a high salt load, stress, smoking, alcohol, obesity or even additional oxidative stress these compensatory mechanisms may fail. These insults also activate either the RAS or cause hyperactivation of the sympathetic nervous system and further enhance vasoconstriction. This will lead to a primary arteriolar disease as observed in many aging individuals, particularly those with hypertension. The primary arteriolar disease can then set off a vicious cycle of renal damage with periods of ischaemia, inflammation and peritubular capillary loss (which the kidney is unable to repair due to aberrant angiogenesis). In addition, the development of afferent arteriolar disease may also impair the ability of the kidney to autoregulate. Indeed,

there is evidence that the arteriolar disease and hyalinosis that occur with aging may be associated with impaired autoregulation [32]. These effects will lead to a further alteration on glomerular haemodynamics and may aggravate glomerular and peritubular capillary loss with a subsequent reduction in nephron number. These changes will eventually have functional consequences including a loss in GFR, alterations in sodium handling and ultimately the development of glomerular scarring and elevated blood pressure.

Further Considerations and Conclusions

There are several limitations when evaluating the data from aging animal studies. Firstly, at what age do we consider a subject 'old'? In the studies with rats there is considerable variation with regard to this point, with 12- to 24-month-old animals described as 'aging rats'. Although, this is useful in determining the chronology of events that occur in aging, it causes difficulty when trying to compare data between experiments, particularly when considering genes and proteins as expression patterns may be very different in rats as they get progressively older. Secondly, as already eluded to when describing eNOS expression, the strain of animal used in the experiment is important as there may be genetic variation. Finally, there is a consideration for studies of animal models of renal disease. Currently, the use of animal models is the best way to test future therapies before going to clinical trials. The majority of patients who will be treated clinically with renal disease are elderly. However, most renal disease models in animals are performed in young mice and rats. As gene expression and biological function are dramatically altered in aging it may be more valuable to perform these models in aging mice and rats which may reflect the elderly population more accurately.

In conclusion, we have provided evidence that blood vessels play a key role in the progression of renal disease in aging. Future experiments are required to investigate these mechanisms in more detail and also test whether potential therapies to maintain endothelial integrity and number will slow renal damage in aging individuals.

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