Research progress in acute hypertensive renal injury by "*in vivo* cryotechnique"

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ABSTRACT

Arterial hypertension has a large prevalence in the general population and as a major hypertensive target organ, the involvement of kidney is usually hard to avoid and gradually develops into chronic kidney disease (CKD). Acute hypertension is defined as a blood pressure greater than 180/120, also known as hypertensive emergency (HE). In acute severe hypertension, the pathophysiology damage to the kidney tends to worsen on the basis of chronic damage, and accounts for more significant mortality. However, the mechanisms of renal injury induced by acute hypertension remain unclear. This review summarizes the clinical and histopathological features of hypertensive renal injury by using "in vivo cyrotechnique" and focusses on the interplay of distinct systemic signaling pathways, which drive glomerular podocyte injury. A thorough understanding of the cellular and molecular mechanisms of kidney damage and repair in hypertension will provide significant insight into the development of new research methods and therapeutic strategies for global CKD progression.

Key words: acute hypertension, hypertensive renal injury, *in vivo* cryotechnique

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INTRODUCTION

It is generally accepted that renal morphology and function are dependent on the maintenance of normal blood pressure. Uncontrolled hypertension can lead to hypertensive renal injury, which is regarded as the second leading cause of chronic kidney disease (CKD), outnumbered only by diabetic nephropathy, and accounts for significant mortality. Arterial hypertension has a large prevalence in the general population, and more than 40% of hypertensive patients have kidney involvement, and 20% of hypertensive patients die of renal failure. [1]

Acute hypertension, defined as a blood pressure greater than 180/120, has been classified as hypertensive emergency (HE) and accounts for approximately 1% of all patients with chronic hypertension.[2] In acute severe hypertension, target organ damage progresses more rapidly. Especially,

the pathophysiology damage to the kidney tends to worsen on the basis of chronic damage.

Given the importance of hypertension for the course of kidney disease, a thorough understanding of the cell and molecular mechanisms of kidney injury and repair, in hypertension, appears fundamental to the development of novel approaches against the progression of CKD worldwide.

HISTOPATHOLOGICAL CHARACTERISTICS OF RENAL INJURY IN HYPERTENSION

Hypertension-induced renal injury, also known as "hypertensive nephrosclerosis", is traditionally characterized by a combination of pathological changes of the pre- and intra-glomerular microvasculature and the tubulointerstitium. And the severity of renal injury is usually related to the

degree and duration of elevated blood pressure. In many cases, hypertensive nephrosclerosis progresses slowly and is classified as "benign nephrosclerosis." In contrast, accelerated nephrosclerosis, characterized by fibrinoid necrosis and/or endomysium cells proliferation, is classified as "malignant nephrosclerosis", and finally, leads to end stage renal disease (ESRD).[3]

Hypertensive renal injury involves different anatomical structures and cell types in the kidney, including the vasculature, glomeruli, tubulointerstitium, capillary endothelial cells, podocytes and immune cells. With the development of hypertension, the muscular arteries and arterioles of the renal parenchyma show progressive intimal thickening subsequent to collagen deposition and spreading of elastic fibers and myofibroblasts, ultimately leads to more vascular resistance, less blood flow in kidney vasculature and renal parenchymal ischemia.

Another histopathological manifestation of hypertensive renal injury is arteriosclerosis of the afferent arterioles, also referred to as "small arterial vitreous hyaline disease." These typical hyaline deposits are the consequence of a pathogenetic cascade of atrophy of vascular smooth muscle cells, increased endothelial leakiness and plasma protein extravasation, leading to subendothelial protein accumulation.

The glomerular involvement due to hypertension is heterogeneous: A variety of pathologies are visible simultaneously, including normal morphology, ischemia, glomerular occlusion, capillary collapse, focal glomerular sclerosis (FSGS) – like lesions, and/or partially hardened glomerular adhesions.

Another hallmark of hypertensive kidney injury is tubular atrophy, accompanied by interstitial fibrosis. During hypertension, the concomitant hemodynamic adaptations results in an oxygen supply-demand mismatch and ultimately tubulointerstitial hypoxia.[4] This relative hypoxia is further aggravated by impaired oxygen delivery due to the increased vasoconstriction hormones, including components of the RAAS, prostaglandins, and endothelin.[5] Hypoxic conditions trigger mitochondrial dysfunction and can activate the transcription factor hypoxia-inducible factor (HIF), which trigger the downstream signal pathway and ultimately contribute to the tubular atrophy and interstitial fibrosis.[6, 7] Following the damage of peritubular capillary bed, the functional nephron is further lost, the tubulointerstitial damage and renal function impairment are further aggravated, which forms a vicious circle.

Besides the characteristic vascular adaptations and glomerular pathology, kidney histology of hypertensive nephrosclerosis may also show trans-differentiation and apoptosis of tubular cells, increased peritubular fibrosis, fibroblasts proliferation, and increased interstitial inflammation.[8]

PODOCYTES PLAY A CRUCIAL ROLE IN THE PATHOGENESIS OF HYPERTENSIVE RENAL INJURY

Glomerular podocytes are highly differentiated cells that cover the outermost layer of the glomerular basement membrane (GBM). Podocytes and their foot processes form tight interdigitating networks that contribute to the hydraulic permeability of the glomeruli and play a crucial role as a filter for macromolecules.

Because of the biological and morphological characteristics of podocytes, podocyte injury is recognized as a central feature in the development of chronic kidney diseases. In fact, podocyte injury and loss has been proved to be causally related to glomerulosclerosis in a variety of kidney diseases such as diabetic nephropathy, focal segmental glomerulosclerosis and minimal change disease.[9–12]

The research on the pathogenesis of podocyte injury mainly focuses on the cytoskeletal abnormality of podocytes, oxidative stress, mitochondrial dysfunction, abnormal autophagy, and apoptosis.[13–15]

The podocyte slit diaphragm (SD) complex is located in the adjacent foot processes, and it is an important structure for sensing extracellular stimulation and introducing extracellular biochemical signals into the cell. Numerous studies have confirmed that the expression abnormality and/or redistribution of SD complex protein leads to SD structure destruction, and can initiate intracellular signal transduction pathway. In a previous study, Takashi Kato, et al. reported that the expression levels of podocyte-specific proteins, nephrin podocin and synaptopodin were decreased in rats' models of malignant hypertensive nephropathy.[16] Consistent results were obtained in the experimental hypertensive nephrosis, such as salt-loaded Dahl rats and malignant stroke-prone spontaneously hypertensive rats, which showed decreased expression of nephrin with podocyte injury.[17] Furthermore, reduced nephrin expression was also found in the renal biopsy specimens from women with pre-eclampsia, a pregnancy-specific disorder characterized by hypertension and proteinuria.[18]

CD2-associated protein (CD2AP) is a multifunctional adaptor protein localized to the cytoplasm and membrane ruffes in glomerular podocytes, and plays a role in cytoskeletal remodeling, cell survival, and endocytosis.[19, 20]

In previous studies, reduced expression of CD2AP has been demonstrated in humans with minimal change nephrotic syndrome and IgA nephropathy.[21] In our previous study, significantly decreased CD2AP expression in glomeruli was also observed in the renal biopsy from the patient with chronic hypertension. Recent studies have suggested that podocytes exposed to high concentration of protein actively endocytose extracellular albumin in a time-dependent manner. When the endocytosed albumin is accumulated and out of podocytes' handing capacity, it causes endoplasmic reticulum (ER) stress and triggers apoptosis of podocytes. Of interest, CD2AP can inhibit the apoptosis of podocytes induced by ER stress.[22] Besides, nephrin and CD2AP also bind to a subunit of phosphoinositide 3-kinase (PI3K), and together they stimulate PI3K-dependent AKT signaling and prevent bad-mediated apoptosis of podocytes in culture.[23]

CALCIUM ION CHANNEL AND DOWNSTREAM SIGNALING PATHWAYS IN HYPERTENSIVE RENAL INJURY

Another research hotspot in podocyte injury is transient receptor pressure channel (TRPC), which is a class of receptor-gated, non-selective cation channel proteins. TRPC6, expressed in the podocyte, has been shown to play a crucial role in the regulation of calcium signaling.[24, 25] TRPC6 is activated and opened when podocytes are stimulated, and calcium ion influx into the cells, which subsequently activates downstream signaling pathways, such as PKA/Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) signaling, Ca^{2+} dependent Rho GTPase signaling, Calcineurin-Nuclear factor of activated T cell (NFAT) transcriptional activation, leading to podocytoskeletal rearrangements and podocyte apoptosis, ultimately leading to proteinuria. When TRPC6 or its upstream signal is blocked, podocyte injury can be alleviated.[26–29]

Connexin43 (Cx43) is a major component of gap junctions (Gjs) and hemichannels, plays a unique role in intracellular communication by directly permitting substances such as calcium, ATP in-and-out of cells. Cx43 also localizes on podocyte SD. It has been shown that up-regulated expression of Cx43 and hemichannel opening in response to kidney stress induced by Ang II, followed by calcium influx and the release of ATP into extracellular fluid.[30, 31] In our previous studies, Cx43 also interacts with SD protein, Nephrin and zonula occludens-1(ZO-1) and contributes to podocyte cytoskeleton rearrangement and apoptosis under hypertension. Hence, we suppose that Cx43 may supplement TRPC6 in the regulation of calcium entry, and there may be

an interaction between Cx43 and TRPC6. Taken all together, progressive observations in animal models and human have shown that podocyte injury is probably a key event in the pathogenesis of hypertensive nephrosclerosis, subsequent to calcium influx, abnormal activity of signal transduction pathways and downstream cytokines resulting from the transient change of hemodynamics.[27] All these findings improve our understanding of the mechanism of podocyte injury during hypertension.

HYPERTENSIVE RENAL INJURY AND HIF

Hypoxia is an important mechanism, contributing to hypertensive nephrosclerosis. Chronic ischemic tubulointerstitial damage, caused by altered hemodynamics, increased oxygen demand, and loss of peritubular capillaries is a hallmark of progressive CKD. Under hypoxic conditions, HIF, and its oxygen-regulated isoforms, HIF1α and HIF2α, are stabilized and promote cellular adaptation to the decreased oxygen supply and influence cell proliferation, survival and metabolism. The increased expression of HIF1α was reported to correlate with glomerular injury and promote hypertensive CKD.[32] HIF1α gene expression in renal endothelia was induced by Ang II in a Nuclear Factor-ϰB (NFϰB)-dependent manner.[32] It has also been shown that reciprocal positive transcriptional regulation leads to persistent activation of HIF1α and NFϰB genes and drives disease progression.[32] The profibrotic action of HIF1α is partly mediated via induction of TGFβ and its proinflammatory downstream target CTGF.[6] Interestingly, HIF2α, which is primarily expressed in non-epithelial cells is associated with ambiguous effects on renal fibrosis. In the early stages of CKD, the activation of HIF2α worsened the renal fibrosis but did not lead to renal functional impairment.[33] In another study, overexpression of HIF2α was sufficient to induce kidney fibrosis.[34] At later stages of CKD, HIF2α activation, in part, activated typical hypoxia-induced target genes of HIF1α such as VEGF, fibronectin, and type 1 collagen but restored the renal vasculature, and thereby ameliorated renal dysfunction and fibrosis.

"IN VIVO CRYOTECHNIQUE" IN BIOMEDICAL RESEARCH AND APPLICATION FOR RENAL INJURY UNDER ACUTE HYPERTENSION

It is well known that hemodynamic factors, such as blood flow and pressure, exert an important influence on kidney structure and function and the effect occurs almost split-second. For the routine immersion or perfusion fixation methods, animal specimens are inevitably exposed

to stresses of ischemia and anoxia, exhibiting only dead morphological states of animal organ tissues without normal blood circulation. Therefore, they always bring about many morphological artifacts, including tissue shrinkage and extraction of components.

There had been a need to develop a new preparation technique for freezing the living animal organs in vivo and then obtaining their acceptable morphology and also immunolocalizations of original soluble components in functioning cells and tissues.

The "in vivo cryotechnique" (IVCT) can realize it by the combination of a precooled cryoknife with isopentane-propane cryogen; all biological processes in the living animal organs were instantly stopped and embedded in the ice microenvironment using the "in vivo cryotechnique". And every component in the cells and tissues was maintained in situ at the time of freezing. Thus, the ischemic or anoxic artificial effects on them could be minimized by the newly developed IVCT. Then the "in vivo cryotechnique" can be also be followed by various preparation steps for morphological analyses.

In 2006, Prof. Li, et al. first visualized the topographical changes of the serum proteins passing through glomerular capillary loops (GCL) clearly by "in vivo cryotechnique" in combination with immunohistochemistry. Under acute hypertensive condition, Albumin and immunoglobulin G (IgG), Ig kappa light chain and IgG1 heavy chain were more clearly immunolocalized along basement membranes and in the Bowman's space,[35] indicating their increased passage through GCL. Moreover, IgG was also more clearly localized in mesangial areas under acute hypertension, compared with that under the normotensive or heart-arrest condition.[35]

In recent years, a variety of podocyte associated proteins, such as podocalyxin, nestin,[36] integrin β1,[37] were quantitatively and qualitatively observed through in vivo cryopreservation. Under acute hypertension condition, these proteins, which are essential for maintaining the normal morphology of podocytes, are down-regulation and disarranged distribution. This is consistent with the results of podocyte injury in hypertensive renal damage. Under the hemodynamic state of acute hypertension, it provides a clearer and more accurate experimental method for the study of the mechanism of genesis and development about renal injury.

OUTLOOK OF NOVEL THERAPEUTIC STRATEGIES IN HYPERTENSIVE RENAL INJURY

Despite recent advances in the understanding of molecular mechanisms, contributing to hypertensive nephropathy, the interplay between the different signaling networks in each cell type is still incompletely deciphered. However, "in vivo cyrptechnique" approach, in conjunction with advanced imaging techniques, comprise excellent opportunities to gain further insight into this complex signaling network.

The bench-to-bedside transition of insight into the molecular mechanisms of renal injury in acute hypertensive patients, and also a profound understanding of the molecular pathogenesis of hypertensive renal injury with other/mixed etiology, may lead to the development novel treatment approaches, such as HIF stabilization, ET1 blockade, anti-inflammatory therapy or immunosuppression. Classifying hypertensive renal injury, based on the individual predominant molecular pathology, rather than traditional clinical etiologies would be an important step in developing focused molecular treatments. There is still a long way ahead, but once achieved, personalized medicine holds promise in acute hypertensive renal injury.

Conflict of Interest

None declared.

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