Vessel Plus

Review

The role of uric acid in renal damage - a history of inflammatory pathways and vascular remodeling

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Abstract

The association of hyperuricemia with cardiovascular risk, hypertension, atherosclerosis, metabolic syndrome, mortality, and chronic kidney disease has been largely described in clinical studies. Several pathogenetic mechanisms explaining uric acid mediated renal damage have been hypothesized, including crystal deposition, oxidative stress, arteriolosclerosis, and glomerular hypertension. Currently, two explanations for hyperuricemia-induced renal injury are the most widely accepted. Firstly, the fact that uric acid is recognized by receptors involved in the innate immune response as a dangerous molecule appears to be a powerful trigger for the inflammatory cascade, which ultimately lead to renal fibrosis. Secondly, serum uric acid has been demonstrated to be implicated in the renin-angiotensin system activation and nitric oxide synthesis inhibition, which promote endothelial dysfunction and proliferation of vascular smooth muscle cells, resulting in glomerulosclerosis and interstitial fibrosis. In this review, we focus on experimental data demonstrating pathophysiological mechanisms linking uric acid to inflammation and oxidative stress, which contribute to the development and progression of renal injury. In addition, we describe endothelial and vascular dysfunction crucial playmakers in kidney impairment induced by uric acid.

Keywords: Uric acid, inflammation, atherosclerosis, kidney disease

INTRODUCTION

Uric acid (UA) is the end product of nucleic acid metabolism and it is synthesized mostly in the liver, intestines, muscles, kidneys and the vascular endothelium. Due to UA renal excretion, hyperuricemia has always been closely related to chronic kidney disease (CKD). Glomerular filtration rate is undoubtedly one of the main determinants of serum UA levels and the impairment of renal function is the first target organ damage determined by UA[1]. Kidney function impairment and hyperuricemia are well known to increase cardiovascular risk[1-4], even though the independent pathogenic contribution of each one of these variables on increasing cardiovascular risk and mortality is a current research topic[5]. Furthermore, hyperuricemia is closely related to metabolic syndrome, obesity and diabetes which are the natural consequence of insulin resistance and common risk factors for CKD. Due to the fact that insulin reduce urinary urate excretion by tubular reabsorption, CKD and insulin resistance are both bi-directionally related to serum uric acid, with one variable possibly contributing to influence the other one[6].

These complex interactions motivated the search for the possible pathogenetic mechanism by which increased UA levels could cause tissue damage and therefore contribute to the development of hypertension, insulin resistance, CKD, end-stage renal disease, cardiovascular events, and mortality. To the present day, a lot of epidemiological data have associated hyperuricemia with closely related vascular diseases such as kidney disease[2], showing a strong association between higher circulating UA levels and CKD occurrence and progression[7-9]. The current debate on the unclear benefit of treating hyperuricemia to slow CKD increasingly inspires the study of the molecular mechanisms underlying hyperuricemia-mediated organ damage.

Several experimental studies defined potential pathways linking UA to CKD lesions. Mechanisms include, inflammation, oxidative stress, activation of the renin-angiotensin aldosterone system (RAAS), endothelial dysfunction, proliferation of vascular smooth muscle cells (VSMCs), resulting in glomerulosclerosis and interstitial fibrosis[10]. Interestingly, it has been found that UA is recognized by receptors involved in the innate immune response as a dangerous molecule, which acts as a trigger for the inflammatory cascade and therefore has been classified as one of the damage associated molecular patterns (DAMPs). Moreover, oxidative stress increases cytokine release and adipokine synthesis as well as inflammation, all elements that have been suggested as important factors in mediating kidney damage[10]. In the kidney indeed, cytokines induce expression of reactive oxygen/nitrogen species[11], bioactive lipids[12] and adhesion molecules[13] promoting aberrant matrix metabolism[14] and proliferation of resident cells[15].

Furthermore, there is much evidence of the role of UA in aberrant changes in vascular properties. These include endothelial dysfunction[16], promotion of VSMCs proliferation[17], and induction of vasoconstrictive mediators such as endothelin-1 (ET-1) and angiotensin II (Ang II)[18,19]. Experimental evidences suggest a complex but potentially direct, causal role of UA in the pathogenesis of atherosclerosis[7].

In this review, we focus on experimental data demonstrating pathophysiological mechanisms linking UA to the processes leading to vascular and systemic inflammatory response, thus contributing to the development and progression of renal injury. Cellular and hemodynamics effects of UA explaining the experimental, clinical, and epidemiologic relationship that has been described with CKD[6,20,21] are depicted in Figure 1.

HYPERURICEMIA-INDUCED RENAL INJURY

In the first part of this review, we briefly describe the detrimental effects of UA acting as a DAMP and increasing oxidative stress and promoting inflammation. In the second part, we focus on signaling pathways by which UA could cause the renal vascular remodeling.
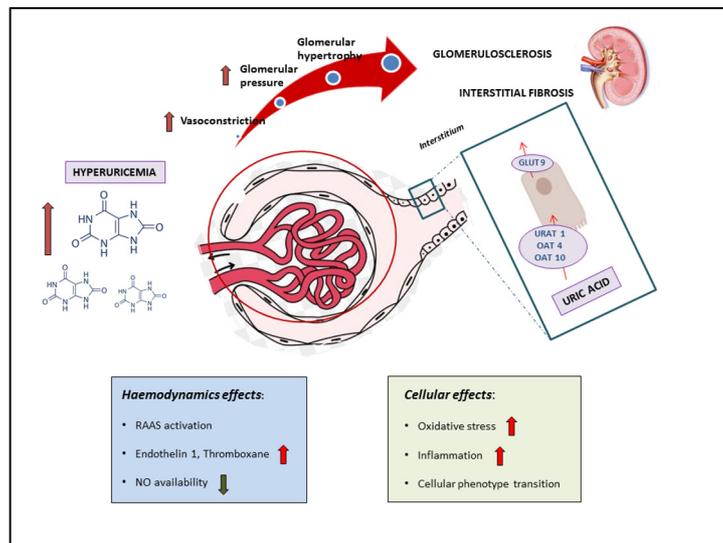
Figure 1. Pathological effects of hyperuricemia on kidney structure and function. Pathophysiology of uric acid - mediated kidney damage. Uric acid enters renal tubular cells through a specific transporter, urate transporter 1 (URAT-1), and two generic transporters, organic anion transporter 4 (OAT-4) and OAT-10. On the basolateral site, the glucose transporter 9 (GLUT-9) is the principal transporter responsible for extrusion of uric acid into circulation. Hyperuricemia might cause hemodynamic effects including increased activity of the RAAS, increased production and activity of vasoconstrictors, such as ET-1, Ang II and thromboxane, and impairment in nitric oxide (NO) availability. These changes lead to impaired endothelium-dependent relaxation and endothelial dysfunction, with negative consequence on kidney structure and function. Moreover, uric acid has been demonstrated to have cellular effects inducing oxidative stress, inflammation and cellular phenotype transition, contributing to glomerulosclerosis and interstitial fibrosis. RAAS: renin angiotensin aldosterone system; NO: nitric oxide; GLUT: glucose transporter; URAT: urate transporter; OAT: organic anion transporter.

The innate immune response

Besides the glomerular and tubular changes directly induced by UA, immune cells populating the kidney recognizes it as a dangerous molecule, and in turn have a direct detrimental effect on renal cells[22]. The main known players of UA immune-recognition are the Nod-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome and toll-like receptors (TLRs), both expressed by renal proximal tubular cells[23].

Inflammasome

Hyperuricemia leads to the formation and deposition of monosodium urate (MSU) crystals, a remarkable event in the pathology of hyperuricemic-related diseases[24]. Several *in vitro* studies demonstrate an inflammatory-related response triggered by MSU irrespective of the cell type, although not all the intracellular pathways have yet been revealed[23]. MSU crystals are able to turn on human primary macrophages to secrete the lysosomal protease cathepsin, proinflammatory cytokines, such as interleukin (IL)-1β, IL-18 [Figure 2], and interferon through the Src/Pyk2/PI3K signaling pathway[25]. An important member of NLRP3 plays a key role in this pathway.

NLRP3 is currently the most well-recognized Nod-like receptor and the most widely studied inflammasome in the field of kidney diseases[26]. The activation of NLRP3 inflammasome requires a priming signal to induce transcription of both NLRP3 and pro-IL-1β, and a second signal to prompt oligomerization of the inflammasome. Several ligands can induce NLRP3 priming, including the TLR 2 ligand Pam3CSK4 (Pam3) and the TLR4 ligand LPS through activation of nuclear factor (NF)-κB[27]. After endocytosis into macrophages, lysosomes attempt to degrade MSU crystal without success. This leads to the lysosomal membrane rupture and lysosomal cathepsins release into the cytoplasm, leading to the activation of the inflammasome[28]. The report by Braga *et al.*[22] demonstrate that soluble UA activates NLRP3 inflammasome
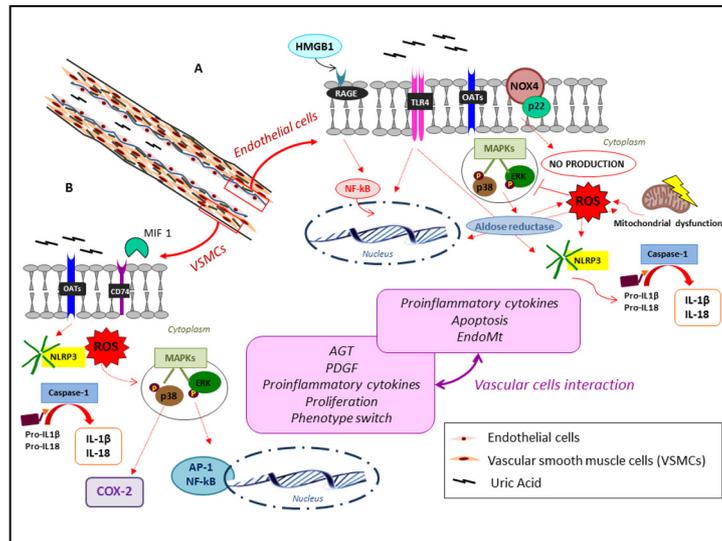
Figure 2. Pathways for uric acid-mediated endothelial (A) and vascular smooth muscle cell (B) damage. In endothelial cells, high uric acid levels stimulate the RAGE signaling pathway and activates NF-κB. This process conduces to the extracellular release of HMGB1 in endothelial cells, and its interaction with RAGE due to its high affinity contributes to the amplification of the inflammatory response, finally inducing endothelial dysfunction. In addition, intracellular uric acid causes P38 and extracellular signal-regulated kinase (ERK) 42/44 MAPK phosphorylation, increasing aldose reductase expression and inducing NOX4 over expression and ROS production. Moreover, uric acid contributes to a pro-inflammatory state, mediated by TLR-4 with NOX4 up-regulation, NLRP3 activation and interleukin production, promoting cellular switching and apoptosis. The ROS produced by xanthine oxidase are an important messenger inducing inflammation and signal transduction, such as mitochondrial dysfunction, leading to apoptosis, increase in proinflammatory cytokines and phenotype transition (A). In VSMCs, uric acid induces proinflammatory cytokine production, apoptosis, and endothelial-mesenchymal transition by several pathways. Uric acid enters the vascular smooth muscle cell where it alters intracellular redox, activates mitogen activated protein kinases (Erk1/2 and p38), COX-2, and nuclear transcription factors (NFκB and AP-1), leading to synthesis of cytokines and PDGF, as well as proliferation and phenotype transition of these cells. The ROS increase and NLP3 activation have similar effects as in endothelial cells. Finally, it has been demonstrated UA-mediated up-regulation of macrophage MIF protein, a cytokine playing inflammatory response induced in VSMCs by oxidized low-density lipoproteins and Ang II during atherogenesis. The two pink boxes summarize the UA-mediated effects on the two cell types, and their interaction brings up an interesting and new aspect of the research, as described in the text (B). AGT: Angiotensinogen; AP-1: activator protein-1; COX-2: inducible cyclo-oxygenase; HMGB1: high-mobility group protein-1; IL: interleukin; MAPK: mitogen-activated protein kinase; MIF: migration inhibitory factor; NLRP3: Nod-like receptor pyrin domain-containing protein 3; NOX4: NADPH oxidase 4; ROS: reactive oxygen species; OAT: organic anion transporter; RAGE: receptor for advanced glycation end products; TLR4: toll-like receptor 4; VSMC: vascular smooth muscle cell.

and induces IL-1β release, cellular redox state changes and mitochondrial changes in macrophages. The subsequent transformation of pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18, respectively, involves the entire cascade determining sterile inflammation[29,30] and amplifying downstream inflammatory signals[31].

Moreover, MSU crystals promote macrophages to secrete transforming growth factor beta-1 (TGF-β1) through mediation of the metastatic tumor antigen 1/transglutaminase 2 (MTA1/TG2) signaling pathway[32]. TGF-β1 is a strong profibrotic cytokine, and aberrant TGF-β1 derived from MSU crystal-induced macrophages, together with above mechanisms, may promote renal fibrosis, as evidenced in *in vitro*[33-35] and *in vivo*[36,37] studies. It has been demonstrated that these extensive biological activities can promote inflammation and dysfunctions in cell metabolism, contributing to the loss of integrity of the glomerular filtration barrier, as outlined in Figure 2. Interestingly, this paves the way for future therapeutic strategies contrasting renal fibrosis based on blocking serum UA internalization or inhibiting its recognition by phagocytes[22,38].

Toll-ike eceptor 4

MSU crystal can be recognized in the extracellular fluid by pattern recognition receptors (e.g., TLRs) expressed on antigen-presenting cells, such as macrophages and tubule epithelial cells as one of the DAMPs which activate immune and inflammatory responses[39,40]. Accumulating evidence demonstrated that TLR4 and other innate immunity-related components (e.g., NLP3, caspase-1, and IL-1β) are essential in the development of UA-mediated inflammation[41], but the mechanisms underlying this pathway still remain largely unclear.

An *in vitro* study by Xiao *et al.*[42] showed that soluble UA enhances *NLP3* expression, caspase-1 activation, IL-1β and intracellular adhesion molecule (ICAM)-1 production in the human primary renal proximal tubular epithelial cells (PTECs) in a TLR4-dependent pathway.

These processes were also proven in mesangial cells[43,44] and confirmed by further studies[43,45]. Milanesi *et al.*[46] documented for the first time the additive effect of UA and Ang II in the stimulation of proinflammatory patterns mediated by TLR4 in PETCs.

The biological activity of TLR4 as a key signal molecule in the immuno-inflammatory network pathway has been proven by *in vivo* studies in mice, suggesting new therapeutic approaches to improve hyperuricemia-mediated immuno-inflammatory renal damage[47,48]. In fact, TLR4 inhibition has proven to reduce soluble UA levels[49,50] and to reduce the severity or slow the progression of the kidney damage[51-53].

Oxidative stress

Oxidative stress is a phenomenon caused by the imbalance between the formation and the removal of free radicals. The most effective free radicals are derived from molecular oxygen, such as superoxide anion (O_2) hydrogen peroxide (H_2O_2), peroxyl radical (ROO) and the very reactive hydroxyl radical (OH) termed ROS, generally considered to be toxic to cells[54]. Many experimental data have suggested a possible role for high hyperuricemia in inducing endothelial dysfunction, and particularly impaired NO bioavailability[55].

Recent studies report that the oxidative stress due to high UA levels directly caused kidney damage and progression of CKD[56]. The mechanisms by which this process takes way continues to be extensively investigated. Hyperuricemia promotes ROS generation and increases oxidative stress inhibiting NO synthesis in several cell types, including mesangial cells, adipocytes, PTECs, and VSMCs[57-59]. This ROS amount determine proliferation, extracellular matrix deposition, and apoptosis[60]. Nevertheless, whether UA contributes to oxidative stress by other specific pathways is still a matter of debate.

The role of urate as a free radical scavenger contributing more than 50% of plasma antioxidant activity has long been recognized by researchers[61]. On the contrary, the description of a redox-dependent effects of UA in PETCs cells primarily produced by NADPH oxidases[62] offers a possible explanation for the paradox by which urate drives oxidative stress when internalized in cells. This last effect may explain the renal protective effects that xantino-oxidase inhibitors showed in some retrospective and randomized studies[63-66]. Moreover, UA proved to contribute to the activation of pro-inflammatory pathways[62,67], as well as the above-mentioned NLP3 inflammasome. This results decrease NO bioavailability, promoting apoptosis of endothelial cells which is part of the so called endothelial dysfunction[68,69].

Mitochondrial damage

Another research hotspot in recent years has become the mitochondrial damage caused by oxidative stress which probably represents the first stimulus leading to the renal tubular epithelial cell apoptosis[70]. One of

the main ROS source is NOX4, a member of NADPH oxidase family. It is highly expressed in the kidney and less in VSMCs and endothelial cells. Some studies have demonstrated the involvement in renal tubular cell apoptosis induced by UA[60,71] and its role in atherosclerosis process is well known[72]. The mitochondria constitute the basis of cell energy metabolism, being the primary place where oxidative phosphorylation occurs. Mitochondrial aerobic activity produces a large amount of adenosine triphosphate (ATP), but also of ROS. When cells are in redox conditions in response to environmental stimuli, such as UA increase, the ROS products exceed the amount that can be cleared. Consequently, the mitochondrial membrane lipid peroxidation occurs, reducing the membrane fluidity and swelling of the mitochondria. This leads to the loss of select permeability and therefore to the mitochondrial dysfunction and cell apoptosis[73].

In this regard, it has been found that UA-induced endothelial dysfunction is associated with mitochondrial dysfunction and reduced ATP generation and, lastly, with apoptosis[73,74]. Molecules such as, alpha lipoic acid, that acts as cofactor in mitochondrial dehydrogenase reactions and with antioxidant properties, improve mitochondrial damage and apoptosis stimulated by UA in endothelial cells, through the activation of protein kinase B (Akt/PKB) signaling[75].

Examining rats with a nephropathy induced by UA, investigators found that the urinary protein, oxidative stress index, and the expression of apoptosis proteins, significantly improved in the group treated with glutathione (serving as an antioxidant)[70,76], creating new insight for treatment development.

Vascular system remodeling

As a matter of fact, microvascular renal lesions are associated with the kidney damage progression by impairing the autoregulatory response to blood pressure and by reducing the glomerular blood flow inducing ischemia. In humans, it has been reported that HU is associated with renal microvascular damage, increased renal resistive index, afferent vasoconstriction in healthy subjects, CKD and hypertensive patients[77-80].

Blood vessels are composed of three concentric layers: the intimal layer, composed of a single concentric coat of endothelial cells; the media, composed of smooth muscle cells; and the adventitia, constituted by a complex of extracellular matrix, fibroblasts, and nerve cells.

The glomerulus is a tuft of capillaries lined by endothelial cells and with smooth muscle cells in their wall. These two primary cell types provide a unique and essential contribution to vessel function. Accumulating evidences suggest that increased serum UA levels are associated with vascular cell dysfunction contributing to the development of vascular stiffness[81] and CKD onset/progression[7]. This may reflect the ability of UA firstly to induce the renin-angiotensin system (RAS) activation contributing to atherosclerosis development, and secondly to cause vascular cell changes resulting in endothelial dysfunction, VSMCs proliferation and phenotype switch. All these processes are pivotal events in vascular system remodeling, and therefore determinants of kidney injury.

RAS activation and atherosclerosis

The central role of the RAS in kidney injury is evidenced by the beneficial effects of the RAS blockade in kidney disease[82]. Ang II is known to be a key player in the pathogenesis of metabolic syndrome, which is known to be related to atherosclerosis, hypertension, insulin resistance and CKD progression[83]. Within the kidney, Ang II induces renal microvascular constriction, especially of the efferent arterioles, playing a major role in the regulation of systemic and glomerular blood pressure and therefore of renal function[84]. The RAS contributes to the pathogenesis of kidney injury also by its direct fibrogenic effect on PTECs and VSMCs[85].

In particular, Ang II induces transforming growth factor beta (TGF-β) production, via p38 MAPK activation and JNK/thrombospondin-1 signaling[85], indirectly upregulates epithelial growth factor receptor in renal proximal tubule further enhancing TGF-β induction[86] and directly upregulates adhesion molecule, mRNA and protein synthesis[87,88] contributing to atherogenesis.

The vascular remodeling is certainly a key player for the development of arteriolosclerosis[89,90]. Recent studies have shown serum UA as an independent risk factor for the presence of arteriolar hyalinosis and intimal thickening of the vessel analyzed in kidney biopsies[91,92]. Moreover, Sánchez-Lozada *et al.*[69] found that raising the serum UA level could induce oxidative stress with endothelial dysfunction, resulting in the development of both systemic and glomerular hypertension, as well as elevated renal vascular resistance and reduced renal blood flow in turn a powerful activator of RAS.

Several experimental observations raise the possibility of a UA-induced nephropathy mediated by the activation of the intrarenal RAS[85,93,94]. Despite the mechanism still unclear, UA is demonstrated to increase inflammatory cytokines and upregulate tissue RAS in rat adipocytes[95]. Sánchez-Lozada *et al.*[69] showed in their preclinical study that UA interferes with the interaction between Ang II and its Ang-type 1 receptor leading to the development of hypertension. The first evidence of the direct effect of UA on the RAS activation in humans was found by Perlstein *et al.*[84] and contributed to a possible explanation of the well documented relationship between hyperuricemia and hypertension[93], glomerular hypertrophy[87], afferent arteriolopathy[85], and interstitial inflammation[93] in *in vivo* studies. Recent studies demonstrating the association between higher serum UA levels and higher ratio of Ang II to angiotensin (1-7) in preterm adolescents support the idea that UA may contribute to increased blood pressure and vascular injury by suppressing angiotensin (1-7)[96].

Despite the well documented efficacy of ACE inhibitors and angiotensin receptor blockers in slowing the kidney disease progression, the nephro-protective treatment needs to be improved and understanding the relationship between UA and RAS might be of utmost importance for this purpose. The recent discovery that RAS and UA play a somewhat additive role both at the renal tubule and at the endothelial level[46,97] confirms that RAAS inhibition alone cannot be the complete solution for the CKD patients and that drugs active on other metabolic pathways could help improve their prognosis.

Endothelial dysfunction
Hyperuricemia is one of the main factors triggering endothelial dysfunction, a primary mechanism for the development of vascular damage[98-101]. The mechanism of endothelial injury involves oxidative stress leading to redox signal pathway activation, the endothelial-to-mesenchymal transition and cytokine and inflammatory factors activation[102].

Experimental studies have shown that human umbilical vein endothelial cells (HUVECs) express 4 different UA transporters: URATv1, ABCG2, MRP4 and MCT9[103,104]. URATv1 plays an important role in the UA internalization[101] then causing oxidative stress through activation of NADPH oxidase, in which the aldose reductase played a central role[99], and reduction of endothelial NO bioavailability [Figure 2]. Moreover, high UA caused P38 and extracellular signal-regulated kinase (ERK) 42/44 MAPK phosphorylation, increasing aldose reductase expression. This process induces NOX4 and ROS over expression with deleterious effects on endothelial cells[99]. In addition, Li *et al.*[99] proposed that UA could induce oxidative stress through the protein kinase C pathway. Consequently, eNOS activity and NO production are reduced, endoplasmic reticulum stress is induced, and endothelial cell die by mitochondrial oxidation and apoptosis[57,103].

Yet, another key effect of UA on endothelial cell lies in its pro-inflammatory nature. UA increases C-reactive protein production through the activation of p38 and ERK 44/42 mitogen-activated protein kinases pathways[68] and, in addition, several studies demonstrate that UA induces cytokine and chemokine expression through NF-κB activation[104,105]. In this regard, Zhen *et al.*[105], demonstrate that NF-κB pathway mediates hyperuricemia-induced endothelium impairment and vascular dysfunction, reducing NO and upregulating IL-6, IL-8 and TNF-α expression. The nuclear protein high-mobility group protein-1 (HMGB1) is a pro-inflammatory cytokine that can interact with the receptor for advanced glycation end products (RAGE). Recently, it has been demonstrated that in endothelial cells, the HMGB1/RAGE signaling pathway contributes to endothelial dysfunction induced by UA[106]. Indeed, when RAGE is blocked with a specific antibody, RAGE, HMGB1, ICAM-1, and VCAM-1 are down regulated as well as the DNA binding activity of NF-κB and the release of IL-6 and TNF-*α*[105]. By proteome analysis of endothelial cells exposed to UA, Oberbach *et al.*[107], reported that UA may promote a variety of signaling pathways involved in metabolic processes which showed to be fundamental for endothelial homeostasis. In particular, UA regulates ubiquitin-proteasome system, the major pathway of protein degradation. The ubiquitin-proteasome system has been indicated to contribute to dysfunction of endothelial cells in vascular complications during uremia[108] and in HUVECs exposed to Ang II[109], modulating eNOS expression and the availability of cofactors and proteins involved in eNOS activation. In UA exposed cells, a significant increase of proteasome activity as well as ubiquitin and ubiquitinated proteins are observed, suggesting a key role of UA in ubiquitin proteasome system regulation in endothelial dysfunction[110].

In vitro and *in vivo* findings suggest that UA contributes to endothelial dysfunction by inducing antiproliferative effects on endothelium and impairing NO production. Several studies reported UA-induced endothelial dysfunction as one of the main mechanisms of kidney disease, as a result of impaired vasodilatation and hemodynamic functions and the potential benefit of urate lowering therapy[110,111].

Phenotype transition of endothelial cells

Phenotype transitions of cells have been regarded as one of the earlier mechanisms of kidney disease. As the induction of epithelial-to-mesenchymal transition on tubular renal cells[112] results in uncontrolled and exaggerated production of collagen and other extracellular matrix proteins leading to renal fibrosis[113,114], the endothelial-to-mesenchymal transition (EndoMT) has been identified as an emerging mechanism of vascular and renal disease, mainly driven by oxidative stress[115,116]. By this process, endothelial cells lose their characteristics (polarity, adhesion, CD31 and eNOS expression) and acquire mesenchymal traits such as a spindle-shape, migratory properties, alpha-smooth muscle actin (α-SMA), fibroblast-specific protein 1 and vimentin expression[117].

Novel findings suggest that UA, increasing NOX activity and, consequently, ROS levels, induces EndoMT in HUVECs and in an animal model of hyperuricemia[118]. Pretreatment with probenecid and antioxidants blunts EndoMT, whereas in hyperuricemic rats, allopurinol partially reverses renal altered expression of vascular endothelial cadherin and α-SMA induced by UA[119].

Ko *et al.*[118] performed *in vitro* and *in vivo* experiment showing as UA induces EndoMT and glycocalyx shedding in cultured vascular endothelial cells. The literature reports as the endothelium is the first line defense against injury[120]. In particular, the loss of the glycocalyx, a gel-like structure formed by proteoglycan core proteins, glycosaminoglycan chains, sialoglycoproteins and adsorbed plasma proteins has been shown to be one of the earliest signs of endothelial injury. Its loss contributes to vascular permeability increase, intravascular thrombosis, loss of NO bioavailability and oxidative stress implicated in several diseases, including kidney involvement[120,121].

As well as an UA-mediated damage mechanism, the detection of glycocalyx shedding is a direct visualization and measurement technique for changes of endothelial cells, being a help for future research on hyperuricemia-induced vascular damage[122].

Specific triggers may induce different levels of EndMT, depending on the underlying cause of renal disease and it could be important to address whether inhibition of EndMT might be a potential therapeutic strategy against renal injury[115].

Vascular smooth muscle cells inflammation, proliferation, and phenotype transition
UA is an independent risk factor for vascular inflammation and remodeling in patients with hypertension or atherosclerosis[123], and this inspired investigators to better understand the role and mechanisms of UA toxicity in VSMCs. One of the first clear demonstration that UA induces arteriolar damage was the finding of expansion of the VSMCs and narrowing of the lumina of the afferent arterioles in hyperuricemic, hypertensive rats[85]. These damages have been shown to be induced by UA *per se* and not by hypertension because they developed in hyperuricaemic rats with blood pressure well controlled by hydrochlorothiazide and were prevented by allopurinol[85].

In vitro studies have elucidated the possible mechanism of UA-mediated arteriolosclerosis firstly through the demonstration of the presence of urate-transport channel URAT1 in human smooth muscle cells[124]. Moreover, there is long-time evidence that soluble UA can induce VSMC inflammatory response increasing cytokine expression in the vessel wall[125], proliferation[13], and VSCM transition from a contractile state to a secretory state[126].

UA has also been shown to possess proliferative and pro-inflammatory abilities, such as to activate intracellular protein kinases (p38 and Erk 1/2) and activator protein-1 (AP-1). Moreover, UA directly affects VSMC by blocking NO release, inhibiting endothelial proliferation and stimulating C-reactive protein production[16,85,125]. Furthermore, UA has proven to induce VSMC proliferation through activation of specific mitogen-activated protein kinases, nuclear transcription factors (e.g., NF-κB). Thus, the VSMCs produce growth factors (e.g., platelet-derived growth factor PDGF), vasoconstrictive substances like Ang II and thromboxane (TXA_2), immune-mediators, and proinflammatory molecules such as C-reactive protein and monocyte chemoattractant protein 1[127].

Kang *et al.*[128] demonstrated a new mechanism involving inducible cyclo-oxygenase (COX-2) by which UA may directly stimulate the VSMC proliferation. The uthors found *de novo* expression of COX-2 mRNA by rat aortic VSMC after incubation with UA, and the prevention of UA mediated proliferation incubating these cells with either a COX-2 inhibitor or with a TXA_2 receptor inhibitor. Moreover, COX-2 was also shown to be expressed *de novo* in the preglomerular rat's vessels, and its expression correlated both with the serum UA and with VSMC proliferation degree[128].

A more recent study from Kırça *et al.*[129] showed that soluble UA induces proliferative pathways in rat VSMCs along with activation of p38 MAPK, p44/42 MAPK, and PDGF receptor in a time and concentration-dependent manner. Interestingly, inhibitory effects of losartan on p38 and p44/42 MAPK activation were also demonstrated in this study, providing a direct proof of p38 and p44/42 MAPK inhibition by reducing UA internalization thorough losartan blockade of URAT1[129].

With the aim to better understand UA-induced vascular injury mechanisms, Fu *et al.*[127] treated mice with UA injection and found up-regulation of macrophage migration inhibitory factor (MIF) protein which is a

cytokine playing inflammatory response induced in VSMCs by oxidized low-density lipoproteins and Ang II during atherogenesis[127]. Moreover, MIF inhibition alleviated UA-induced vascular inflammation and de-differentiation of VSMCs[130] suggesting that this would be a potential therapeutic approach against vascular injury following UA exposition.

The UA-mediated molecular and cellular pathways involved in endothelial and vascular cells dysfunction are summarized in Figure 2.

Endothelial cell/smooth muscle cell interactions
Signaling between endothelial cells and smooth muscle cells is essential for maintaining tone in mature vessels. Their interaction is critical during development, and for repair and remodeling associated with blood vessel growth. Recently it has been depicted the pathways these cells utilize to communicate and how disruptions in these pathways contribute to the organ damage[131].

The individual functions of endothelial cells and VSMCs are dependent on proper crosstalk between these cell types, which begin during embryogenesis[132]. This process is regulated by growth factors and tissue hypoxia, two elements showed to be involved in UA mediated injury.

Technically, these signaling pathways can be divided into two categories: those mediated by soluble or secreted molecule, and those requiring direct physical contact between the two cell types. As regards the diffusible signaling, both *in vitro* and *in vivo* models have demonstrated the role of PDGF signaling in endothelial smooth muscle cell interactions, in particular devoted to smooth muscle recruitment and proliferation, and blood vessel maintenance[133]. Moreover, there is clear evidence that the TGF-β signaling is important for the communication between vascular cells, being receptor-ligand combinations expressed on both endothelial and smooth muscle cells[134].

In several tissues, UA has been shown to stimulate oxidative stress and cellular damage via modulation of TGF-β[135] and the pivotal role of TGF-β-induced tubule-interstitial fibrosis in the progression of CKD is an active topic of research. The attenuation of TGF-β signaling, for instance under angiotensin receptor blockers treatment, has been proven in CKD, by reducing ligands, receptors, and activators[136].

Impaired availability of NO, potentially UA induced, has been found to play a role in signaling via secreted or diffusible factors. In the adult vasculature, it works as hyperpolarizing agents diffuse to VSMCs to cause vascular relaxation[137] that constitutes an efficient mechanism of communication. Likewise, endothelial cell-released contracting agents like ET-1 and Ang II, two known elements strictly involved in UA mediated cellular pathways, are perceived by smooth muscle cells to increase vascular tone[138]. Endothelial cell-dependent regulation of vascular reactivity represents the best-described example of the importance of endothelial-smooth muscle cell interactions, and therefore also in modulation of vascular tone, a key component of the glomerulus.

The second category is represented by contact-dependent signaling, a greater important issue during development then in adults, because of the presence of significant barriers to physical interactions of endothelial-smooth muscle cells in mature blood vessels. However, evidence that cell contact-dependent developmental mediators play a role in adult blood vessel function is an assumption, showing as it might be controlled by Notch signaling[139] and ephrin proteins family, which are noted to modulate blood pressure.

The last key player regarding interaction between cells compounding vascular system is the already in-depth described endothelial dysfunction. A better understanding of how the endothelial dysfunction directly modifies the crosstalk between the two cell types, and the role that UA takes on within this process could be the next advancement in the fight to cure UA-mediated vascular and organ damage.

The magnitude and importance of UA role in the pathogenesis of organ damage might vary and depend on several factors. In the last 20 years, several clinical studies demonstrated as serum UA is a concomitant of high-risk conditions and a predictor of unfavorable outcomes. As a matter of fact, association of hyperuricemia with metabolic syndrome, kidney function impairment, and its impact on cardiovascular disease and mortality is well established[4,6-10,140]. By a pathophysiological point of view, these data are consistent with the hypothesis that UA induces and perpetuates reno-vascular injury, leading to a progressive vicious cycle of further renal damage and thereafter to an increased cardiovascular and all-cause mortality risk. The mechanisms deemed to be implicated in the progression of UA-mediated renal damage are likely multiple and include changes in molecular and cellular systems leading to a wide range of injuries in several tissues, vessels, and organs having a major impact on global health.

CONCLUSION

UA induces immune system activation and alters the characteristics of resident kidney cells, such as tubular epithelial, mesangial, endothelial, and vascular smooth muscle cells, toward a proinflammatory and profibrotic state[141]. Both the hemodynamic and structural changes have been described as key players in UA-induced kidney disease. These findings have led to an increased awareness of UA as a potential and modifiable risk factor in kidney disease, even if the effects of urate lowering therapy are not conclusive.

To the present, UA is considered as a culprit and not only as an innocent bystander in hypertension and progression of renal disease. For this reason, the not univocal demonstration of a benefic effect of urate lowering therapy has to be read as a reflection of incomplete knowledge of UA pathogenetic mechanisms. So, in addition to the need for large clinical trials, more studies are required to better understand the biology of UA.

This review highlights the crucial role of UA in renal cell dysfunction focusing on inflammation and vascular injury, the two main aspects of UA-induced kidney damage. The hope is it will be possible to expand therapeutic strategies by understanding the molecular and cellular processes underlying the occurrence or the progression of CKD induced by hyperuricemia.

DECLARATIONS

Authors' contributions
Design and conduct of the study (ER, DV, GL, RP, GG, FV), data interpretation (FC, PE) and manuscript writing (ER, DV, FV): Russo E, Verzola D, Cappadona F, Leoncini G, Garibotto G, Pontremoli R, Viazzi F

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All authors declared that there are no conflicts of interest.

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Not applicable.

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