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Forum Editorial

Angiotensin II and Reactive Oxygen Species

MARTA RUIZ-ORTEGA and ALBERTO ORTIZ

ANGIOTENSIN II (AngII), the effector peptide of the renin–angiotensin system (RAS), participates in the pathogenesis of several diseases. AngII is a pleiotropic peptide that binds to two specific receptor subtypes, AT_1 and AT_2, and activates complex intracellular signaling systems. AT_1 predominates in vascular tissues and contributes to chronic diseases, such as hypertension, atherosclerosis, cardiac hypertrophy, and renal injury, by promoting cell growth, inflammation, and fibrosis. AT_2 is expressed during fetal development and re-expressed in pathological settings and is involved in vasodilatation and apoptosis (16). The intracellular signals elicited by each receptor are different. AT_1 is linked to calcium mobilization, activation of several kinases, and nuclear transcription factors. AT_2 stimulates the nitric oxide/cycle GMP system, phosphatases, nuclear transcription factors, and ceramides (15, 16). Both receptors promote the production of reactive oxygen species (ROS). ROS generation is one of the main mechanisms involved in AngII-induced tissue damage. The role of ROS in AngII actions has been extensively characterized. Most studies have been performed in the cardiovascular (10, 22) and renal fields (25). Recently, a role for AngII in liver damage has been described (2).

AngII may regulate cell proliferation and hypertrophy and tissue fibrosis via AT_1 and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase–activator protein-1 (AP-1) pathway. ROS are involved in AngII–induced activation of AP-1 and induction of the c-fos and c-jun family of protooncogenes (16). Touyz reviews the mechanisms involved in ROS production in vascular smooth muscle cells (VSMC). In VSMC, AngII stimulates the NADPH oxidase to produce superoxide and hydrogen peroxide. These oxygen species mediate epidermal growth factor receptor transactivation, p38 MAPK, and Akt, leading to cell hypertrophy and proliferation (22). Eguchi group review the mechanisms involved in AngII activation of downstream ROS-sensitive kinases and the pathological significance of their activation (10). In renal proximal tubular cells, AngII caused hypertrophy and induction of $p27^{Kip1}$, an inhibitor of G_1 phase cyclin-dependent kinase/cyclin complexes by a process mediated by ROS and MAPK activation (25). AngII also contributes to a ROS-dependent excessive accumulation of extracellular matrix proteins that may result in fibrosis (16). Activated hepatic stellate cells secrete AngII, which promotes fibrogenesis through the activation of NADPH oxidase (2). AngII regulates fibrosis through the production of endogenous growth factors, such as transforming growth factor-β (TGFβ) and connective tissue growth factor (CTGF) (16). In VSMC, AngII directly regulates CTGF via ROS and Jak activation, and independently from TGFβ (11, 17).

Inflammation is a key process in vascular injury. AngII regulates the inflammatory response through the production of proinflammatory mediators, including adhesion molecules, cytokines, and chemokines, by a redox-sensitive mechanism. AngII can directly activate immune cells and regulate their functions, inducing chemotaxis, proliferation, differentiation, and phagocytosis (15). Immune cells express all the components of the RAS and can produce AngII. Activation of leukocytes and monocytes was found in both patients with essential hypertension and in experimental models (15). Among the intracellular signals involved in AngII-induced inflammatory response, ROS production and the activation of the nuclear factor NF-κB have a special interest. Systemic infusion of AngII in animals causes renal inflammatory cell infiltration mediated by oxidative stress, activation of transcription factors, and endogenous production of inflammatory mediators (15, 18).

The transcription factor NF-κB is activated by many stimuli, including AngII and ROS, and it controls the transcription of many proinflammatory genes. AngII activates the NF-κB pathway via both AT_1 and AT_2 receptors (14, 15). AT_1/NF-κB/gene regulation is mediated by protein tyrosine kinase and MAPK activation, whereas AT_2/NF-κB is dependent on ceramide production (14). AngII-induced ROS activates NF-κB in many cell types, including VSMC, cardiac fibroblasts, and mesangial and tubular cells. Diverse antioxidants blocked the activation of NF-κB elicited by AngII and AT_2 agonist (8, 14), suggesting that ROS act as intermediates of both AT_1 and AT_2. AT_1 upregulates some NF-κB-related genes, such as interleukin-6, monocytic chemotactic protein-1 (MCP-1), and vascular cell adhesion molecule-1 and mediates monocyte binding to endothelial cells, whereas AT_2 regulates RANTES (normal T-cell

Fundación Jimenez Díaz, Universidad Autónoma Madrid, Madrid, Spain.

expressed/secreted) expression (15). Combined therapy with both AT_1 and AT_2 antagonists diminished inflammatory cell infiltration and blocked renal NF-κB activation in the AngII infusion model (5). Tanifuji *et al.* have shown that intracellular signaling systems involved in AngII-induced MCP-1 regulation are cell type-specific (21). In murine proximal tubular cells, intracellular ROS and subsequent RAS and NF-κB activation regulate MCP-1 expression, whereas in mesangial cells protein kinases and MAPK pathways, but not ROS, are the major pathways. Importantly, in both cell types NF-κB activation is crucial in MCP-1 regulation (21). Studies in experimental models of renal damage show that NF-κB activation and oxidative stress could be important mechanisms regulating the progression of tubulointerstitial injury. In models associated with tissue RAS activation, blockade of NF-κB by different strategies, including antioxidants, prevents tissue damage (15). In salt-sensitive hypertension following AngII infusion, there is kidney T-cell infiltration, oxidative stress, activation of NF-κB, and production of AngII. There was a correlation between the intensity of the tubulointerstitial immune infiltrate, oxidative stress, and the number of cells expressing AngII. Treatment with immunosuppressive agents, reduction of the oxidative stress by melatonin administration, or an antioxidant-rich diet ameliorates renal damage (12). Chronic exposure to low doses of lead also caused renal oxidative stress, interstitial inflammation, local expression of AngII, and activation of NF-κB (13). Antioxidant treatment has demonstrated beneficial effects in other models of AngII-induced end-organ damage (9). These data clearly indicate that local AngII generation contributes to renal damage by redox-mediated mechanisms. The activation of NF-κB pathway and the production of ROS in tubuloepithelial cells by AngII could contribute to the renal inflammatory cell infiltration and therefore to the progression of tubulointerstitial damage.

Recently, the transcription factor hypoxia-inducible factor-1 (HIF-1) has emerged as an AngII target (18, 25). Wolf found that in PC12 cells, AngII stabilizes HIF-1 by an AT_2-mediated down-regulation of SM20 (25). In AngII-infused mice, HIF is activated in the kidney by both AT_1 and AT_2 receptors, in association with renal oxidative stress and vascular endothelial growth factor (VEGF) overexpression. In cultured tubuloepithelial cells, AngII regulates VEGF by HIF-1 activation and redox-sensitive processes (18).

Hypertension and aging are associated with oxidative stress. Unraveling the mechanisms leading to oxidative stress may lead to new therapeutic strategies. The oxidation/inflammation theory of aging is reviewed by De la Fuente *et al.* in this issue (3). A number of immune functions that decrease with age were also altered in hypertensive women (3). Moderate exercise and antioxidant diet supplementation improved these immune functions in the elderly and hypertensive patients (3). In circulating cells from hypertensive patients, increased levels of prooxidants, such as ROS, and decreased antioxidant defenses, including superoxide dismutase (SOD), are present (23). In hypertensive patients, Egido's group have found oxidative stress in the kidney, characterized by elevated 4-hydroxynonenal staining, and decreased Cu/Zn SOD. Using an experimental model of hypertension-induced renal injury, which resembles chronic hypertension in the elderly, they have demonstrated that blockade of AngII, by angiotensin-converting enzyme inhibitors and AT_1 antagonists, ameliorates the oxidative stress and evidence of apoptosis (7), suggesting that a potential mechanism of their beneficial effects could be due to the blockade of prooxidant actions of AngII. There is evidence that oxidative stress may be under genetic control. The $-930^{A/G}$ polymorphism of the *CYBA* gene that encodes the essential subunit of the NADPH oxidase $p22^{phox}$, determines the genetic susceptibility of hypertensive patients to oxidative stress (26).

Besides AngII, other components of the RAS could contribute to tissue damage. Recent studies have shown that aldosterone participates in cardiovascular alterations. The aldosterone receptor antagonist, spironolactone, reduced vascular hypertrophy and oxidative stress caused by AngII (24). In experimental hypertension, the blockade of aldosterone receptors ameliorates endothelial dysfunction and reduces systemic oxidation and aortic $p22^{phox}$ mRNA overexpression (20). In this model, AT_1 blockade had similar effects (20), suggesting that production of ROS is a common mechanism of aldosterone and AngII. Finally, Ang degradation peptides also possess biological activities and could participate in tissue damage progression. AT_1 blockade results in increased AngII levels and the formation of Ang peptides (AngIII, AngIV, and Ang1–7) (1). These peptides bind to their specific receptors and might be involved in the systemic effects of AT_1 blockade. In this sense, AngIII through AT_2 and activation of the NF-κB pathway and ROS production could be involved in the inflammatory response (8). Ang1–7, via *mas* receptor, might cause vasodilatation (19), and AngIV, via AT_4/insulin-regulated aminopeptidase, regulate plasminogen activator inhibitor-1 expression and NF-κB/proinflammatory genes (6). Future studies are needed to investigate whether redox mechanisms are involved in these responses of Ang peptides.

Taken together, data presented in this special *Forum* issue illustrate the increasing evidence supporting the critical role of ROS as mediators of tissue injury induced by different components of the RAS system (AngII, angiotensin-related peptides, and aldosterone) in cardiovascular, kidney, and liver diseases.

ABBREVIATIONS

AngII, angiotensin II; AP-1, activator protein-1; AT_1, angiotensin II receptor type 1; AT_2, angiotensin II receptor type 2; CTGF, connective tissue growth factor; HIF-1, hypoxia-inducible factor-1; MAPK, mitogen-activated protein kinase; MCP-1, monocytic chemotactic protein-1; NF-κB, nuclear factor-κB; RAS, renin–angiotensin system; ROS, reactive oxygen species; SOD, superoxide dismutase; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells.

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Address reprint requests to:
Marta Ruiz-Ortega, Ph.D.
Laboratory of Renal and Vascular Pathology
Fundación Jiménez Díaz
Avda Reyes Católicos, 2
28040 Madrid, Spain

E-mail: mruizo@fjd.es

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