Abstract

The renin-angiotensin-aldosteron system (RAAS) is a hormonal system that contributes to regulation of blood pressure and volume of extracellular fluids, in both normotensive and hypertensive individuals. Hypertension affects the target organs (kidneys) and leads to a vicious circle that contributes to maintaining a high arterial pressure. Besides the hemodynamic effects of RAAS/Ang II, Ang II has direct impact on structure/function of kidneys, leading to renal injury. Recently, the proinflammatory effect of Ang II has been discovered. Low renal inflammation determined by excessive circulating RAAS, mostly at renal level, promotes and continues the renal disease. This review presents the inflammatory mechanisms initiated by Ang II at renal level, which induce development of renal injury. Identification of such mechanisms might lead to the discovery of new therapeutic approaches for hypertension/target organs damage.

Keywords: RAAS, inflammation, renal injury, hypertension

I. THE RENIN-ANGIOTENSIN-ALDOSTERON SYSTEM

Increased RAAS (renin-angiotensin-aldosteron system) activity, particularly at angiotensin II (Ang II) levels, contributes to target organ damage and increases the risk of cardiovascular events by increasing blood pressure, as well as by its direct effect on the vascular endothelium and cardiac and renal tissues [1], stimulating the inflammatory phenomena and the development of atherosclerosis [2]. Hypertension is closely related to renal disfunctions [3] and represents both the cause and effect of kidney diseases.

In 1871, Traube hypothesized that hypertension may be a homeostatic response threatening the excretory function of the kidney [4]. Excessive secretion of Ang II (antinatriuretic hormone) induces a transient increase in sodium retention. Physiologically, sodium retention becomes normal in a few days, but there are situations when it is maintained at a high level, causing hypertension. Ang II reduces the renal ability to excrete sodium and initiates a series of events that lead to increased blood pressure [5]. Increase in blood pressure, required to return to normal sodium excretion, is achieved by pressure - natriuresis phenomenon. The peripheral vascular resistance often increases in hypertension, although increased blood pressure is initiated by sodium retention and expansion of volume replacement. Increased blood pressure occurs due to direct and indirect effects of peripheral vasoconstrictor Ang II. Chronic administration of Ang II leads to increased sodium retention, developing severe systemic hypertension and pulmonary oedema.

Classically, the renin angiotensin system was considered a circulating hormone structure whose final and bioactive product, Ang II, is produced with the participation of two important enzymes, renin and the angiotensin-converting enzyme [6]. The renin-angiotensin system is a hormonal complex with paracrine, autocrine and intracrine properties. Angiotensine is formed by the action of renin on its specific substrate, angiotensinogen, through a cascade of enzymatic reactions. Renin, a glycosylated carboxypeptidase secreted by the renal juxtaglomerular cells, acts on angiotensinogen (a complex macromolecular protein released by hepatocytes) to form
angiotensin I (an apparently inactive decapeptide). Angiotensin I is converted into a biologically active molecule – angiotensin II (8 aminoacids) by the action of a glycoprotein released by lung tissue, the angiotensin-converting enzyme (ACE). Recently, a homologous angiotensin-converting enzyme called ACE2 was found in heart, kidney, liver, intestine. This new converting enzyme produces the hydrolysis of the last aminoacid of angiotensin I, leading to angiotensin (1-9), a classical inhibitor of ACE1 and a precursor of angiotensin (1-7). Angiotensin-(1-9) may limit the formation and actions of angiotensin II, contributing to local traffic self-regulation [6]. Also, angiotensin II can be generated from angiotensin I by the action of a large number of peptidases called chimase (alternative ways of Ang II formation) [7]. As other peptide hormones, the angiotensins bind to membrane receptors on target cells to exercise their actions (type 1-AT1 and type 2-AT2 angiotensin receptors). Classically, the most important function of RAAS is to maintain blood pressure by Ang II-induced vasoconstriction and sodium retention, mediated by aldosterone in renal collecting tubules [8].

II. INTRARENAL RENIN-ANGIOTENSIN SYSTEM

After more than two decades, a local RAAS that acts independently on the systemic RAAS has been described [9]. An exact delineation of the contribution of local and systemic angiotensin peptides is difficult in terms of recent experiments. Every system in the body contains elements of RAAS. The excretory system (kidneys) is the only one that possesses all RAAS components. In the last decade, Ang II is considered a multifunctional cytokine with multiple non-hemodynamic properties [10]. In addition to electrolytes homeostasis control, Ang II mediates several key events in inflammatory processes [11]. In inflammatory processes, local RAAS activation and synthesis of Ang II leads to increased vascular permeability by VEGF secretion (vascular endothelium-derived growth factor) [12-14] and induces the expression of adhesion molecules (L – selectins, P – selectins and ICAM-1) and their ligands (integrins) [15-17]. Ang II promotes inflammatory cell infiltration of tissues by stimulating the production of cytokines / chemokines.

The renin angiotensin aldosterone system plays an essential role in pathological changes that lead to the progression of kidney disease. Renal injury activates directly and indirectly the local renin angiotensin aldosterone system. For example, hyperglycemia, proteinuria, reduced calcitriol can stimulate local Ang II synthesis [18]. Inflammation, an early stage of kidney damage, is followed by tubulointerstitial fibrosis, tubular atrophy and glomerulosclerosis [19].

III. PROINFLAMMATORY EFFECTS OF RAAS

Mononuclear cell infiltration of the glomeruli and interstitium is present in most renal diseases and plays a crucial role in the development of irreversible structural changes. Immunocompetent cells (T lymphocytes, macrophages and dendritic cells) contain components of the RAAS and contribute to the production of Ang II [20-22]. RAAS plays an essential role in leukocyte infiltration and thus it can influence the evolution of many renal diseases associating immune activation [23].

Ang II is involved in inflammatory processes occurring during kidney damage by regulating the expression of adhesion molecules. Ang II is a chemotactic factor for inflammatory cells, increasing the expression of chemokines and chemokine receptors in both resident cells and infiltrated cells [20]. The inflammatory response can be direct - through the production of MCP-1 and TGF-β1, and indirect - by activating resident cells by macrophages released factors [24].

By AT1 and AT2 receptors pathways, Ang II stimulates transcription of the NF-KB factor. Ang IV has a similar effect. Angiotensin receptor antagonists (sartans) can block only certain pro-inflammatory effects of RAAS. The NF-KB factor activation caused by Ang II occurs via the Rho kinase way.

Ang II stimulates the Ets factor transcription that is the main regulator of vessel infiltration with T lymphocytes and macrophages / monocytes recruited by the vascular wall [25]. Another
mechanism involved in the development of renal inflammation in response to Ang II involves the participation of Toll-like receptor 4 of mesangial cells. An increase in Toll-like receptors 4, mediated by Ang II, results in activation of the NF-KB factor [26].

The essential role in the progression of renal disease is played by recruitment of inflammatory cells in glomeruli and tubular interstitium. Ang II stimulates the growth of VECAM-1 number (vascular cell adhesion molecule-1), ICAM-1 (intercellular adhesion molecule-1), and integrins, and allows the circulating immune cells to adhere to the capillaries.

The NF-KB factor mediates the transcription of chemokine genes responsible for the renal tissue leukocytes infiltration, that include: MCP-1 (monocyte chemotactic protein-1), RANTES.

Ang II can directly stimulate cell proliferation [27]. Leukocytes are an active source of Ang II, thus amplifying the proinflammatory effects [28]. Ang II-mediated proinflammatory effects increase the pathophysiological changes caused by proteinuria [10].

### III.1. THE ROLE OF ANGIOTENSIN AND NF-KB FACTOR IN THE INFLAMMATORY RESPONSE

Ang II plays a special role in the development of inflammation in the kidney. The main vasoactive peptide of RAAS causes migration and adherence of the circulating immune cells in the kidney, at both endothelial and mesangial level. This process involves adhesion of the cytokines and chemokines molecules [29]. On experimental animal models with kidney diseases, administration of angiotensin converting enzyme inhibitors (ACEI) results in decreased cell infiltration and inflammatory markers [29].

By activating the AT1 receptor, Ang II increases the expression of proinflammatory genes for VCAM-1, ICAM-1, IL-6 and MCP-1. The signaling pathways by which these effects are achieved include activation of the NF-KB factor, MAP kinase cascade, Rho protein and redox pathways.

Experimental data show that AT2 receptors are involved in the activation of inflammatory cells in the kidney. The AT2 receptor antagonists, but not the AT1 receptor, decrease the number of inflammatory cells in two animal models of unilateral urethral obstruction receiving systemic Ang II [29-31].

The combined treatment with AT1 and AT2 receptor antagonists inhibits the inflammatory response by two mechanisms: decrease of cell infiltrate and decreased expression of proinflammatory genes.

On unilateral urethral obstruction model, blocking of the AT2 receptor decreases TNF-α and expression of RANTES; stimulation of both types of receptors decreases gene expression, which increases MCP-1 [30]. Involvement of NF-KB is known in renal disease. In vivo activation of the NF-KB factor, produced as a response to Ang II, is partially diminished by AT1 and AT2 receptor antagonists. In contrast, simultaneous administration of both types of receptor antagonists or ECAI totally abolishes this response [29-31]. The NF-KB factor activation in the kidney is differently mediated, according to the place where it occurs. Therefore, in mesangial cells, it is mediated by both types of receptors, in tubulo-interstitial cells only by AT1 receptors, while, in the endothelial cells - via the AT2 receptor.

On the unilateral urethral obstruction model, blocking of the renal NF-KB factor activation by treatment with two different renal inhibitors (pyrrolidin dihydrocarbonate and partenolide) decreases the infiltration with inflammatory cells and the gene expression of some pro-inflammatory factors.

In spontaneously hypertensive rats, inhibition of the NF-KB factor results in the decrease of infiltration with inflammatory cells in renal interstitium and normalization of blood pressure. These data support the hypothesis that, in some experimentally-induced renal disease, blocking of Ang II with ACEI and antagonists of angiotensin receptors (AT1 and AT2), and inhibition of NF-KB pathways are necessary to stop the inflammatory processes.

In conclusion, drugs that modulate the activity of the RAAS, such as ACEI and AT1 receptor antagonists, have a protective effect on the kidney.
III.2. INFLAMMATION AND RENAL INTERSTITIUM

Ang II acts as a cytokine mediating the infiltration and activation of immunocompetent cells in the renal interstitium. Immunocompetent cell infiltration of the renal interstitium is present in non-immune primary injury (the disease evolves with proteinuria, urinary obstruction, polycystic kidney disease, diabetes, hypertension). Mononuclear cell infiltration occurs in the absence of a specific antigen and is the result of increased expression of chemoattractant molecules [22,23].

Under pathological conditions, an increased activation of the RAAS, especially in the tubular cells, may be observed. A decrease in renal interstitium infiltration is achieved by blocking the RAAS (antisense oligonucleotides and drugs). This phenomenon is partially due to decreasing of chemokines (MCP-1 and osteopontin), adhesion molecules and other mediators. Ang II possesses chemotactic properties for mononuclear cells. Tubular RAAS activation leads to an increase in local interstitial infiltration. Mice with a deficit of AT1 receptor and a high level of proteinuria show a marked interstitial infiltration, that is not reduced after ACEI administration. Instead, there is a significant decrease in cellular infiltration after an antagonist of A/B endothelin receptor administration. Ang II stimulates the expression of endothelium. This suggests that other mediators, including vasoactive peptides, endothelin, are involved in interstitial cell recruitment [32].

Johnson et al. hypothesized that sodium-sensitive hypertension appears as the result of tubulo-interstitial damage, interfering with the physiological mechanisms of pressure-natriuresis type. This phenomenon has been studied extensively by the use of Ang II in hypertensive models, due to a massive intake of sodium. The conclusion was that Ang II is the key mediator involved in cell recruitment. Administration of mycophenolate mofenil prevents this type of hypertension and markedly reduces cell infiltration of the interstitium [33].

Chronic administration of L-NAME reduces the synthesis of nitric oxide and the development of hypertension. At interstitial level, an infiltration with macrophages and lymphocytes occurs. When the chronic L-NAME administration is interrupted, normalization of blood pressure is observed, to a lower extent for the animals having received a hypersodated diet during hypertension. Interestingly, both sodium-dependent hypertension models showed a high level of Ang II-producing cells, particularly lymphocytes, accumulated in the renal interstitium. Administration of mycophenolate mofenil led to stabilization of blood pressure and decrease of oxidative stress [34].

In conclusion, the immune cells are capable of producing Ang II in various stages of renal impairment, playing therefore an important role in sodium-sensitive hypertension [35].

The immunocompetent cells recruited by the inflamed interstitium can synthesize Ang II that locally induces differentiation of antigen presenting cells and increases their antigenic activity.

III.3. INFLAMMATION AND RENAL GLOMERULUS

Local RAAS amplifies the antigen-specific immune response in renal glomerular diseases. Ang II is involved in the pathogenesis of the immune complex mediated glomerulonephritis, by activating the NF-KB factor, MCP-1 production and expression of adhesion molecules [36-38]. In glomerulonephritis, the renal RAAS mediates antigen-specific T cell activation. In anti-glomerular basement membrane glomerulonephritis (anti-GMB GN), both humoral and cellular immunity are involved. In acute glomerular injury, the immunoglobulin Fc receptor plays a critical role in PMN recruitment [39]. It was observed that mice deficient in Fc receptor [γ (-/-)] showed protection against primary lethal injury, but still developed glomerular injury characterized by: mesangial proliferation, accumulation of macrophages and formation of deposits in the basement membrane. These changes are prevented by AT1 receptor antagonists administration [35].

Under such conditions, an important role in the formation of glomerular basement membrane deposits is played by Th1 lymphocytes. The antibodies, deposited on the glomerular basal membrane, activate both local and systemic
RASS in a dose-dependent manner. *In vivo* and *in vitro* studies have demonstrated that Ang II plays an important role in lymphocytic proliferation, mostly of Th1 lymphocytes. However, the contribution of systemic RAAS to the development of glomerulonephritis mediated by T cells is not fully understood. There is a large functional diversity between Th1 and Th2 lymphocytes, which is partially explained by the different phenotypes of chemokine receptors [40].

Administration of Ang II in mesangial cells has a potent chemotactic effect for T cells. At this level, Ang II enhances the expression of chemokines IP-10 and MIP1α.

In glomerulonephritis with immune complexes, local RAAS may contribute to the occurrence of antigen-specific T lymphocytes in inflamed glomeruli. The mechanism underlying this phenomenon is achieved, at least partially, through chemokines regulation. The appearance of cellular response to Ang II is achieved through different molecular pathways, which include calcium mobilization, generation of free radicals, activation of protein kinases and nuclear transcription factors [41].

Inflammatory processes caused by Ang II are achieved through the NF-KB factor, which represents the key mediator of these processes. Under its control, Ang II causes an increase in the cytokines and chemokines proinflammatory genes. The NF-KB factor activation pathway involves both types of angiotensin receptors, AT1 and AT2. The pathways by which Ang II activates the Th1 type chemokines are differently regulated. IP-10 is NF-KB dependent, whereas MIP-1α is regulated mainly by NF-AT (nuclear factor calcineurin-dependent on the T activated cells). The NF-AT activity requires a higher concentration of calcium, realized by a massive influx through calcium release-dependent (ICRAC) and calcineurin-dependent (CaN) channels.

The NF-AT pathway activated by Ang II may be involved in pathological changes of vascular smooth muscle cells. CaN / NF-AT are involved in Ang II-induced lymphocytic proliferation, while the Ang II / NF-AT pathway might be involved in tubulo-interstitial damage [42]. Activation of NF-AT was observed in tubulo-interstitial infiltration of chronic glomerulonephritis with anti - basement membrane antibodies and proteinuria in mice γ (−/−).

In conclusion, Ang II acts as a mediator of inflammation, being partially involved in the pathogenesis of kidney disease by infiltration and activation of immunocompetent cells. In some kidney diseases, the immune system can be directly influenced by local RAS activation.

**PERSPECTIVES**

The synthesis of the first oral inhibitor of ACE, captopril, urged the development of new therapeutic hypotheses and opened a new stage in clinical research of RAAS. Blocking of RAAS with IACE has been showed as very efficient in the treatment of hypertension. Subsequently, the hypertensive patients benefit from the discovery of Ang II antagonists discovery, which selectively block the activation of AT1 receptors, without influencing vaso-dilatation kinines. Use of anti-inflammatory medication or blocking of inflammatory cytokines in hypertension may represent a new therapeutic approach in renal disease.

**References**

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