INTERACTION BETWEEN ANGIOTENSIN II, HYPERTENSION AND INFLAMMATION IN RAT KIDNEY

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INTERACTIONS BETWEEN ANGIOTENSIN II, HYPERTENSION AND INFLAMMATION IN RAT KIDNEY (Abstract): The renin-angiotensin system is one of the most important regulatory mechanisms of blood pressure homeostasis. Hypertension, a risk factor for chronic kidney disease, is able to induce renal injury by promoting inflammation. Inflammatory cytokines can induce fibrosis in various organs, including the kidney. Aim: The study verifies if angiotensin II is implicated in kidney T cells infiltration. Material and Methods: The experiment was performed on Wistar male rats, divided in two groups who received either a sham operation (control group, n=9) or continuous angiotensin II (Ang II) infusion (300ng/kgc/min)(Ang II group, n=9) subcutaneously, via minipumps. Blood pressure was measured noninvasively (tail cuff). After 14 days, the animals were sacrificed under anesthesia with xylazine/ketamine. The tissue obtained of each kidney was used for histopathological exam or flow cytometry. Renal inflammation was assessed using a flow cytometric analysis. The Histo Quest program quantified automatically the cellular elements and performed a statistical analysis on the obtained data. Results: Inflammation was observed in Ang II Wistar rats group, accompanied by an increase in renal CD45RC+, CD45RA+, CD19+. Conclusions: Our research underlines an undefined role for T cells in the genesis of high blood pressure up to that time and sustains the involvement of inflammation in the pathogenesis of this prevalent disease. Thus, T cells might correspond to a novel therapeutic target for the management of hypertension. Keywords: ANGIOTENSIN II, HYPERTENSION, KIDNEY, INFLAMMATION

Renin-angiotensin-aldosteron system (RAAS) is a hormonal system that contributes on regulation of arterial pressure (1, 2). The increase of RAAS, especially by Ang II levels, affects target organs and increases the risk of cardio-vascular disease by raising blood pressure and also by a direct effect that Ang II has on vascular endothelium of renal and cardiac tissue (3, 4), promoting inflammatory phenomena and arteriosclerosis. Ang II reduces the renal capacity of sodium excretion and initiates a range of events that leads to increased blood pressure (5, 6). Ang II also mediates key events related to inflammatory processes (7). Ang II leads to the accumulation of inflammatory cells in the tissues by stimulating the production of cytokines / chemokines. RAAS plays an essential role in morphopathologic changes that
produce progression of renal diseases. Renal inflammation represents the early stage of renal injury and is followed by tubulointerstitial fibrosis, tubular atrophy and glomerulosclerosis (8).

**MATERIAL AND METHODS**

Male Wistar rats weighing about 250g, were divided into two groups: the first group (control) made of animals who received continuously isosmotic solution using osmotic minipumps Alzet, model 2001-1μl / h (9 rats) and the second group (9 rats) who received continuously Ang II infusion (300ng/kgc/min) (Bachem - 1008593) subcutaneously, via minipumps (Alzet), for 14 days. Systolic blood pressure was measured noninvasively using tail-cuff plethysmography (Biopac), every 3 days. After 14 days, the animals were sacrificed under anesthesia with xylazine/ketamine. Each kidney was divided into 2 equal pieces: a piece was immersed in 4% paraformaldehyde for tissue fixation in order to perform a histopathological exam (Hematoxylin-Eosin or Szekely trichromic methods) and the other was used to perform immunophenotyping (flow cytometry). The Histo Quest analysis program allowed the study of renal periglomerular and tubulointerstitial inflammatory reaction. Renal inflammation was assessed by quantifying CD45RC, CD45RA expression, using a flow cytometric analysis (6). Results were expressed as mean ± SEM. All protocols were approved by the Animal Care and Use Institutional Committee of the University of Medicine and Pharmacy "Gr. T. Popa".

**RESULTS**

**Systolic blood pressure**

The values of systolic blood pressure (BP) were similar among the two groups before the treatments. However, systolic BP progressively and significantly increased to 200±7 for Ang II vs. 120±5 mmHg for Sham at day 14 (fig.1).

![Fig. 1](image)

**Kidney inflammatory infiltration area**

Histo Quest program allowed the mapping and the separation and quantification of all nuclei related to the periglomerular and tubulointerstitial infiltrate (fig. 3-14). We used as analysis criteria the following parameters: nuclear size and intensity of the nuclei in Hematoxylin-Eosin stain.

**Flow cytometric analysis**

CD45RA. As demonstrated in figure 16A, B, chronic Ang II infusion significantly (p<0.005) increased CD45RA (5.5±0.5% for Ang II vs. 2.7±0.8% for sham). Absolute numbers of T cells (CD45RA) in kidneys
from sham and angiotensin II infused rats were expressed as the means ± the SEM.

CD45RC. As shown in figure 17A, B, chronic Ang II infusion significantly decreased CD45RC (p<0.05). Data are expressed as the means ± the SEM.

Fig. 3. Periglomerular inflammatory analysis in control group

Fig. 4. Scattergram: graphical representation of periglomerular inflammatory infiltrate (2.26%) (control group)

Fig. 5. Histogram: graphical representation of periglomerular inflammatory infiltrate (2.26%) (control group)

Fig. 6. Periglomerular inflammatory analysis in Ang II group
Fig. 7. Scattergram: graphical representation of periglomerular inflammatory infiltrate (20.37%) (Ang II group)

Fig. 8. Histogram: graphical representation of periglomerular inflammatory infiltrate (20.37%) (Ang II group)

Fig. 9. Tubulointerstitial inflammatory infiltrate analysis in control group

Fig. 10. Scattergram: graphical representation of tubulointerstitial inflammatory infiltrate (1.65%) (control group)

Fig. 11. Histogram: graphical representation of tubulointerstitial inflammatory infiltrate (1.65%) (control group)
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Fig. 12. Tubulointerstitial inflammatory infiltrate analysis in (Ang II group)

Fig. 13. Scattergram: graphical representation of tubulointerstitial inflammatory infiltrate (45.86%) (Ang II group)

Fig. 14. Histogram: graphical representation of tubulointerstitial inflammatory infiltrate (45.86%) (Ang II group)

Fig. 16. Flow cytometric analysis in kidneys cells -CD45RA (A: Sham; B: Ang II)
DISCUSSION

The chronic kidney disease might be determined by varied conditions as hypertension, diabetes, nephritis, inflammatory and infiltrative diseases, etc. Into a varying number of cases, renal injury becomes chronic, progressive and irreversible and often requires substitution therapy (dialysis or renal transplantation) (9). Although renal impairment begins at the glomerular, tubular or renovascular level, the progression to chronic kidney disease is accompanied by common histological and functional changes, going to progressive glomerulo-sclerosis. Hypertensive nephropathy is initiated by an increased intraglomerular pressure that activates and destroys glomerular cells, including mesangial and epithelial cells, and also the podocytes. These cells produce proinflammatory and vasoactive mediators that contribute to the cell damage, the decrease of renal plasma flow and of the glomerular filtration rate (10, 11).

In a study carried out by Lombardi et al. (12), they showed that after chronic administration of 435ng/Kg/min Ang II for 2 weeks, to male Sprague-Dawley rats, renal histological changes including focal vascular damage (damage of peritubular capillaries) and tubulointerstitial injury occured (tubular atrophy and mononuclear cell infiltration). These changes are found both in the juxtaglomerular area and in the superficial cortex (13).

In the present study we intended to demonstrate that Ang II-induced hypertension leads to lymphocytic infiltrate in the kidney. In our experiment, we observed that in the control group, consisting of animals that received isosmotic solution through minipumps and were subjected to the same laboratory conditions, histological examination revealed a normal appearance of the juxtaglomerular apparatus and a normal appearance of the renal glomerulus, afferent and efferent arteriole and renal tubules. The histological examination of kidney sections from rats chronically treated with Ang II for 14 days revealed presence of inflammatory infiltrate with lymphocytes.

CONCLUSIONS

In our study angiotensin II increased the activation of T cell markers in kidney tissue. T cells have an important role in the genesis of high blood pressure, pointing the inflammatory infiltrate involvement in the pathogenesis of this disease. Thereby, T cells might represent a novel therapeutic
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