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ORIGINAL PAPER

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NF-κB and chemokine-cytokine expression in renal tubulointerstitium in experimental hyperoxaluria. Role of the renin-angiotensin system

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Abstract Recent evidence indicates that the renin-angiotensin system (RAS) seems to play a considerable role in the development of tubulointerstitial (TI) lesions caused by hyperoxaluria (Hox). The purpose of the present study was to evaluate the specific mechanism by which Hox involving RAS induces chemokine and cytokine expression and, therefore, renal TI damage in the ethylene-glycol (ETG) induced hyperoxaluric rat model. Sprague-Dawley rats, separated into five groups, received: G1 regular water, and G2, G3, G4 and G5 1% ETG (a precursor for oxalates) in their drinking water for 4 weeks. An angiotensin converting enzyme inhibitor, benazepril (BZ) 10 mg/kg/day, angiotensin II receptor antagonists, subtype 1 (AT1) losartan (LOS) 40 mg/kg/day and subtype 2 (AT2) PD 123,319 (PD) 10 mg/kg/day, were administered daily to G3, G4 and G5, respectively. At the end of the study, the inflammatory response to Hox was evaluated using anti-NF-κB (p50), anti-IL-6, anti-MCP-1; anti-RANTES and anti-ED1 (monocytes/macrophages) in each group. In spite of the same urine oxalate levels, rats belonging to the hyperoxaluric groups treated with either BZ or LOS showed significantly ($P < 0.01$) less TI lesions together with a lower immunoexpression of inflammatory mediators when compared with untreated hyperoxaluric animals. NF-κB (p50) was increased in tubular cells in the ETG group (43.6 ± 8.7 positive cells/mm^2) and was significantly ($P < 0.01$) reduced by LOS (11.2 ± 4 positive cells/mm^2) and even more by BZ (6.1 ± 2.4 positive cells/mm^2). There was a significant ($P < 0.01$) correlation between NF-κB (p50) positive cells and ED1 cells in the ETG group ($r = 0.88$) and in the ETG + LOS group ($r = 0.92$). LOS showed better control on IL-6 and MCP-1 with respect to untreated rats, while BZ showed the best control on RANTES and ED1 cells in comparison with untreated animals. Renal function was significantly ($P < 0.01$) better preserved in BZ and LOS treated groups compared to both untreated animals and rats with PD, as indicated by creatinine clearance values. These results suggest that Hox stimulates the NF-κB cascade and, therefore, induces the overexpression of inflammatory mediators like IL-6, MCP-1, and RANTES. This pathway seems to be mediated not only by AT1 but also by AT2 receptors of angiotensin II.

Keywords Angiotensin II · Tubulointerstitial lesion · Calcium oxalate crystals

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Introduction

Oxalate, a common constituent of kidney stones, is normally excreted by the kidney. Hyperoxaluric states including primary (hereditary) oxalosis or secondary hyperoxaluria (Hox) can lead to renal tubulointerstitial (TI) damage. In addition, when these pathological circumstances are prolonged, the risk of developing chronic TI disease is increased [1]. Convincing evidence indicates that both oxalate and calcium oxalate (CaOx) crystals are harmful to renal epithelial cells in vivo as well as in cell cultures [2]. Interestingly, recent evidence suggests that local angiotensin II (Ang II) induces a variety of proinflammatory mediators including cytokines and chemokines, and that the nuclear factor κB (NF-κB) activation plays an important role in Ang II-mediated inflammation, independently of the etiology in the majority of glomerular as well as TI diseases [3]. NFκB regulates genes involved in renal disease progression, such as the chemokines monocyte chemoattractant protein-1 (MCP-1) and regulated upon activation of normal T-cells expressed and secreted (RANTES) [4].

Additionally, both MCP-1 and RANTES, which are deeply involved in the inflammatory process, are stimulated by Ang II, and at least the former is overexpressed following contact with CaOx crystals or Hox in tubular epithelial cells [5–7]. CaOx crystal endocytosis and the subsequent tubular epithelial cell responses promote an increase in reactive oxygen species (ROS) production [8]. ROS interacts with interstitial cells leading to an inflammatory reaction and, eventually, tissue injury [9]. Previous studies in our laboratory have shown that interaction against the renin-angiotensin system (RAS) provides substantial benefit involving protection against renal TI lesions [10–17]. In accordance with this information, the purpose of the present study was to evaluate the specific mechanism by which RAS induces chemokine and cytokine expression in CaOx crystal TI injury in a hyperoxaluric rat model.

Methods and materials

Two-month-old male Sprague-Dawley rats initially weighing 250–270 g were housed in metabolic cages, and divided into five groups each of eight animals: a control group (G1), the ethylene glycol (ETG) group (G2), the ETG + benazepril (BZ) group (G3), the ETG + losartan (LOS) group (G4), and the ETG + PD 123,319 (PD) group (G5). All of the animals were allowed to drink tap water, and were fed standard rat chow ad libitum. Over 4 weeks, ETG 1% (as a precursor for oxalates) was continuously administered to G2, G3, G4 and G5 in their drinking water. Every day, BZ 10 mg/kg and LOS 40 mg/kg were administered to G3 and G4, respectively, by gavage. A total of 10 mg/kg/day PD 123,319, a selective Ang II type 2 receptor (AT2) antagonist, was continuously administered to G5 in drinking water.

Biochemical determination of 24 h urine collected at baseline and after 4 weeks was carried out. At these times, blood samples were also obtained for serum determinations. The kidneys were harvested for light microscopy and immunohistochemical studies after 4 weeks. At baseline and at the end of the experiment, systolic blood pressure (SBP) was measured by tail cuff plethysmography, as previously described [12, 16].

Biochemical procedures

Oxalate was determined by enzymatic methods (Sigma Diagnostics, St. Louis, Mo.), while calcium was determined by standard methods using atomic absorption. Creatinine clearances were calculated using the standard formula. Calcium oxalate crystals were identified using brightfield phase contrast and an adapted polarized light microscope, and quantified on ten microscopic fields per sample examined at a magnification of ×400. Crystalluria was graded as: 0 = no crystals per field, 1 = < 10 crystals per field, 2 = 10–25 crystals per field, 3 = 26–50 crystals per field, and 4 = > 50 crystals per field, as previously described [17].

Kidney processing and examination

Kidneys were perfused with saline solution through the abdominal aorta until they were free of blood. Decapsulated kidneys were cut longitudinally and fixed in phosphate-buffered 10% formaldehyde (pH 7.2) and embedded in paraffin. Sections 4 μm thick were cut and stained with hematoxylin-eosin (H-E) and Masson's trichrome.

Immunolabelling and light microscopy

Immunolabelling of specimens was carried out with a modified avidin-biotin-peroxidase complex technique Vectastain ABC kit (Universal Elite, Vector Laboratories, Calif.) and the specimens handled using immunohistochemical standard techniques. With the aim of detecting chemokines, MCP-1 and RANTES, rabbit polyclonal IgG anti-MCP-1 (ab-7202 Abcam, Cambridge, UK), and goat polyclonal IgG anti-RANTES (sc-1410 Santa Cruz Biotechnology, Santa Cruz, Calif.), at a dilution 1:100 were used, respectively. Finally, a goat polyclonal IgG anti-IL-6 (sc-1265 Santa Cruz Biotechnology), and a goat polyclonal IgG anti-NF-κB p50 (sc-114 Santa Cruz Biotechnology), were used.

Morphological analysis

Histological sections were taken from the kidneys of each animal and studied using a light microscope Nikon E400 (Nikon, Melville, N. Y.). For a better identification of calcium oxalate crystals, polarized light microscopy was used. All tissue samples were evaluated independently by two investigators without prior knowledge of the group to which the rats belonged. Interstitium was defined as renal tissue excluding glomeruli, tubules or blood vessels. In order to estimate the TI damage in each group, semi-quantitative scores of crystal deposits and TI lesions were determined on ten microscopic fields per section, examined at a magnification of ×100. The scores were graded each according to the following scale: 0 = absent, 1 = mild (involving ≤ 25% of each microscopic field), 2 = moderate (> 26% and ≤ 50%), 3 = severe (> 51% and ≤ 75%), or 4 = very severe (> 76%), as previously described [17]. When comparing various groups, the same areas of the kidney were analyzed.

Immunohistochemical evaluation was carried out according to the following schedule: transcription factor NF-κB (p50) and ED1(monocyte/macrophage) were expressed as positive cells/mm^2, assessed on 20 consecutive microscopic fields at ×400 magnification; IL-6,

MCP-1 and RANTES were expressed as percentage of positive immunostaining area/mm^2. Data were averaged and the results were expressed as mean ± SD. All measurements were carried out using an image analyzer Image-Pro Plus version 4.5 for Windows (Media Cybernetics, Silver Spring, Md.).

Statistical methods

All of the statistical analyses were performed using absolute values and processed through GraphPad Prism, version 2.0 (GraphPad Software, San Diego, Calif.). The test to determine whether the data fit a normal distribution were performed by the Kolmogorov and Smirnov method. For parameters with a normal distribution, all comparisons among groups were carried out using ANOVA. Values were expressed as mean ± SD. The difference in mean values between groups was assessed by the Tukey-Kramer multiple comparisons test. Statistical analysis for parameters such as histological data with a non-normal distribution was performed by the Kruskal-Wallis test (nonparametric ANOVA) and Dunn's multiple comparison test. Finally, a Spearman rank correlation was performed when appropriate. A value of $P < 0.05$ was considered significant.

Results

At the end of the experiment, there were no significant differences in body weight and SBP among the five groups (Table 1). As expected, a marked and significant increase in urinary excretion of oxalate was observed in rats from groups that received ETG (G2, G3, G4 and G5) (Table 1). Urinary calcium was significantly decreased ($P < 0.01$) in rats from G2 (ETG) and G5 (ETG + PD) in comparison with the other groups. Due to the fact that oxalate was determined in acidified urine samples, which dissolves crystals and consequently indicates the total amount of oxalate, including oxalate precipitated with calcium, and taking into account that calcium was measured in urine that was not acidified, such calcium represents the amount of free calcium ions. The reduction in urinary calcium excretion observed in rats from G2 and G5 may be interpreted as formation of CaOx crystals and its subsequent deposition in the renal interstitium. In accordance with this, animals from G2 and G5 presented a high level of crystalluria and lower creatinine clearance when compared with the other groups ($P < 0.01$). On the other hand, hyperoxaluric rats treated with BZ (G3) and with LOS (G4) had a significantly lower amount of CaOx crystals in their urine, and a higher creatinine clearance relative to untreated hyperoxaluric rats (G2) and hyperoxaluric animals with PD (G5) (Table 1).

Histological and immunohistochemical findings

Polarized light microscopy showed that untreated hyperoxaluric animals (G2) as well as those from G5 (ETG + PD) had diffuse CaOx crystals in the tubular lumens and in the renal interstitium, mainly in the cortex but also in the medulla (Fig. 1). In addition, TI lesions characterized by tubular atrophy and dilatation, inflammatory cell infiltrates mostly involving monocytes and macrophages (ED1 positive cells), in the cortex and in the medulla, were also present in this group (Table 2, Figs. 2A, 3). Interestingly, whereas tubular atrophy and dilatation were significantly diminished in rats from G3 (ETG + BZ) and G4 (ETG + LOS) (Table 2, Figs. 1, 2A, 3), animals from G5 (ETG + PD), despite showing an significant degree of TI lesions, presented a similar reduction in ED1 positive cells in the renal interstitium compared to the control and BZ groups.

A remarkable ($P < 0.01$) increase of NF-κB (p50) immunohistochemical expression in the nucleus of tubular cells was observed in the hyperoxaluric animals which received no treatment (G2) with respect to the other groups (Figs. 2B, 4). However, despite the fact that treated groups (G3, G4 and G5) showed a significantly reduced nuclear expression of NF-κB (p50) in tubular cells, rats with BZ (G3) presented similar values to the control animals (G1), while those with either LOS (G4) or PD (G5) showed a significantly higher expression of this nuclear factor (Figs. 2B, 4). Moreover, when the relationship between the NF-κB (p50) expression in epithelial tubular cells and the magnitude of inflammatory cell infiltration in the renal interstitium (ED1

Table 1 Parameters at 4 weeks. $n = 8$ for all groups. Data are expressed as mean ± SD. NS indicates not significant, ETG ethylene glycol, BZ benazepril, LOS losartan, PD PD 123,319, SBP systolic blood pressure

	G1 Control	G2 ETG	G3 ETG + BZ	G4 ETG + LOS	G5 ETG + PD	*P*
Rat body weight (g)	341 ± 8.9	338.2 ± 7.1	337.5 ± 8.8	329.9 ± 9.3	332.1 ± 7.8	NS
SBP (mmHg)	121.3 ± 1.4	123.6 ± 1.9	119.9 ± 1.7	120.2 ± 2.6	123.8 ± 2.1	NS
Urinary oxalate (μmol/day)	5.9 ± 1.4*	55.3 ± 13.0	53.8 ± 12.1	54.6 ± 8.9	55.1 ± 10.4	
Urinary calcium (μmol/day)	49.1 ± 5.8	17.8 ± 4.5**	47.9 ± 4.9	52.6 ± 6.4	24.5 ± 6.7**	
Crystalluriascore	0.1 ± 0.3*	3.0 ± 0.5**	0.5 ± 0.5	0.5 ± 0.5	2.5 ± 0.5**	
Creatinine clearance (ml/min)	1.41 ± 0.06*	0.91 ± 0.08**	1.20 ± 0.04	1.21 ± 0.05	1.10 ± 0.05**	

* versus all groups $P < 0.01$;
** versus control; ETG + BZ and ETG + LOS $P < 0.01$

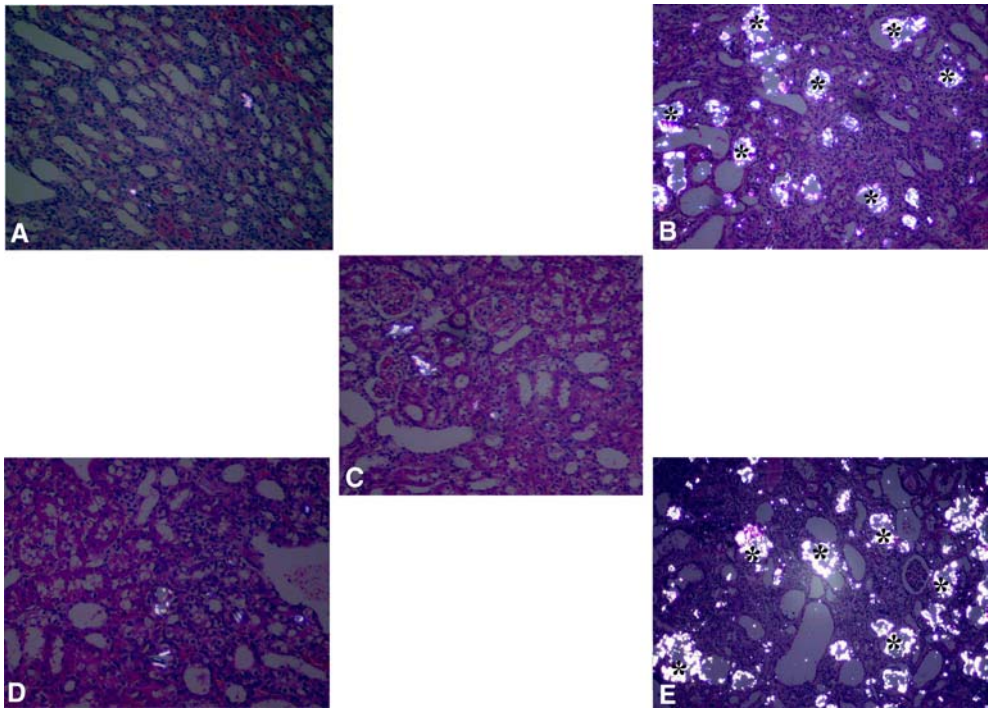

Fig. 1 Sections of renal tissue in all groups using a microscope with polarized light (H-E, ×400). **A** Control group. **B** Group 2 (ETG) and **E** group 5 (ETG + PD), note CaOx crystals in the tubular lumen (*asterisks*), as well as in the interstitium. **C** Group 3 (ETG + BZ), **D** group 4 (ETG + LOS) with minimal deposits of CaOx in the tubular lumen and renal interstitium

positive cells) was observed, there was a positive and significant correlation between these two variables in the hyperoxaluric animals without treatment and also in those belonging to the LOS group (Fig. 5). No correlation was observed in the other groups. There was a notable increase in the immunohistochemical expression of IL-6 in untreated hyperoxaluric rats (G2) and in hyperoxaluric rats with PD (G5). As opposed to this, animals that received either BZ or LOS, but especially the latter, presented the best outcome, which was not different from that of the control group (Table 2, Fig. 6). Similar findings were observed for MCP-1 (Table 2, Fig. 7). Immunohistochemical expression of RANTES in tubular epithelial cells was significantly increased in untreated hyperoxaluric rats when compared with the other groups (Table 2, Fig. 8). In contrast, despite the fact that treatment with BZ, LOS or PD led to a substantial decrease in RANTES immunoexpression, only the animals receiving BZ or PD did not show differences to the control rats, while the LOS group did (Table 2, Fig. 8).

Discussion

In the present study, untreated hyperoxaluric rats exhibited significant CaOx crystalluria together with a significant degree of TI lesions characterized by CaOx crystals in tubular lumens as well as in the renal interstitium along with inflammatory cell infiltration. All of these alterations were associated with a critical increase in the immunohistochemical expression of several inflammatory mediators like NF-κB (p50), IL-6, MCP-1 and RANTES. Furthermore, these animals showed a decline in their renal function as represented by a lower creatinine clearance relative to the other groups. Contrary to this scenario, and as reported in previous experiments from our laboratory [12, 16, 17],

Table 2 Morphological and immunohistochemical evaluation at 4 weeks. $n = 8$ for all groups. Data are expressed as mean ± SD. NS indicates not significant. ETG ethylene glycol, BZ benazepril, LOS losartan, PD PD 123,319, TI tubulointerstial, IL-6 interleukin 6, MCP-1 monocyte chemoattractant protein-1, RANTES regulated upon activation of normal T-cells expressed and secreted

	G1 Control	G2 ETG	G3 ETG + BZ	G4 ETG + LOS	G5 ETG + PD
TI lesion score	0.1 ± 0.3	2.7 ± 0.7†	0.1 ± 0.3	0.2 ± 0.3	1.6 ± 0.5††
Crystaldeposits score	0.0 ± 0.0	1.5 ± 0.7††	0.1 ± 0.1	0.1 ± 0.1	1.0 ± 0.8†††
IL-6 (%)	0.1 ± 0.1*	7.6 ± 1.4††	0.9 ± 0.2**	0.4 ± 0.2	6.9 ± 2.1†††
MCP-1 (%)	0.4 ± 0.3*	16.9 ± 2.2††	6.5 ± 1.5**	0.7 ± 0.2	12.8 ± 4.2†††
RANTES (%)	0.3 ± 0.4***	15.6 ± 2.3††	0.5 ± 0.3**	9.1 ± 2.8	0.2 ± 0.5***

† versus all group $P < 0.01$, †† versus control, ETG + BZ and ETG + LOS, ††† versus ETG + BZ and ETG + LOS, * versus all groups $P < 0.01$, ** versus ETG + LOS$P < 0.01$, *** versus ETG and ETG + LOS $P < 0.01$

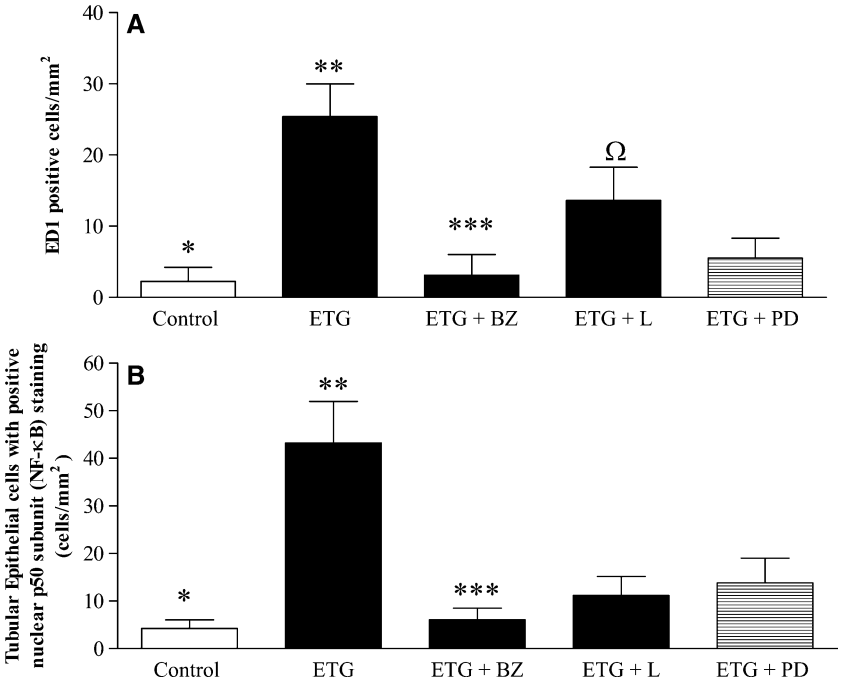

Fig. 2 **A** Quantitative analysis of the ED1 positive cells (monocyte/macrophage) in renal interstitium among the five groups. Values are expressed as mean ± SD. *ETG* ethylene glycol, *BZ* benazepril, *L* losartan. *PD* PD 123,319. (* versus ETG, ETG + LOS and ETG + PD $P < 0.01$. ** versus ETG + BZ, ETG + LOS and ETG + PD $P < 0.01$. *** versus ETG + LOS $P < 0.05$. Ω versus ETG + PD $P < 0.05$). **B** Quantitative analysis of the immunohistochemical nuclear expression of p50 subunit (NF-kB) in tubular epithelial cell (activated cells). (* versus ETG, ETG + LOS and ETG + PD $P < 0.01$. ** versus ETG + BZ, ETG + LOS and ETG + PD $P < 0.01$. *** versus ETG + LOS and ETG + PD $P < 0.05$)

intervention against RAS seems to be favorable in modulating the inflammatory response to Hox and CaOx crystalluria in the renal interstitium, since both angiotensin converting enzyme (ACE) inhibition, by BZ, and Ang II antagonism by blockage of AT1 receptors with LOS led to a remarkable reduction in these lesions with an improvement in renal function.

Urine oxalate overload evokes CaOx crystal formation. Subsequently, the interrelationship between CaOx crystals with tubular epithelial cells generates oxidative stress by stimulating ROS, and promotes local RAS activation, as previously reported]8, 17–20]. Ang II, the main effector peptide of RAS, plays a central role in the pathophysiology of a broad range of renal diseases by inducing inflammatory responses, including ROS overproduction. Currently, it is widely accepted that Ang II is a proinflammatory molecule which participates as a growth factor that regulates cell proliferation, apoptosis

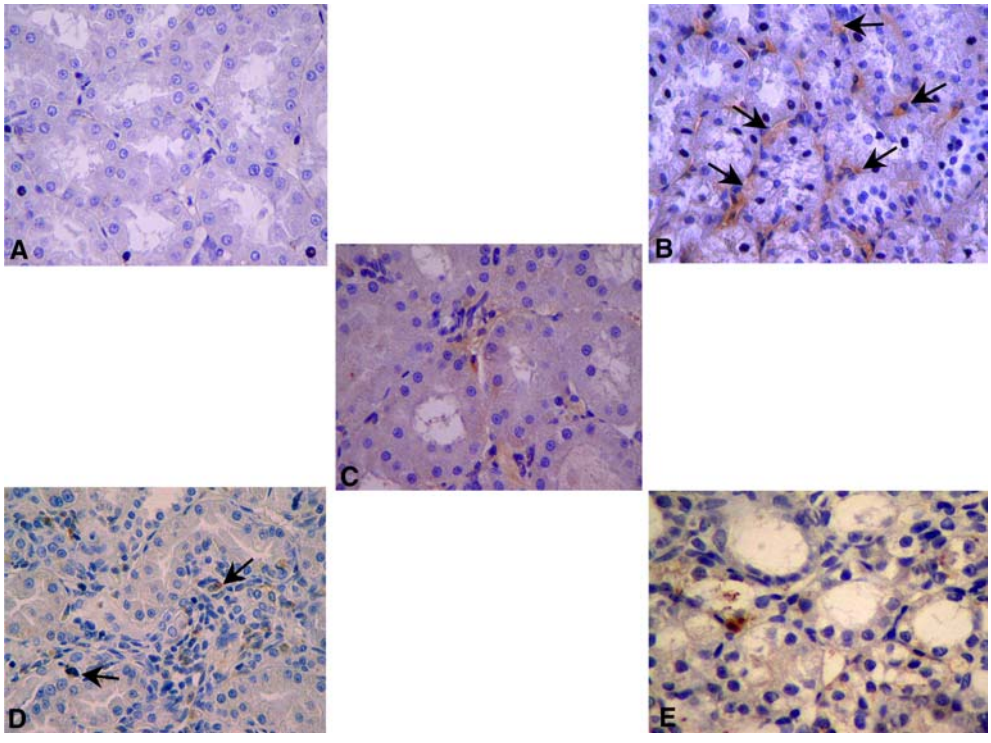

Fig. 3 Renal tissue with ED1 immunostaining (×400). **A** Control group, **B** group 2 (ETG) *arrows* indicate positive cells corresponding to monocytes/macrophages infiltrates, **C** group 3 (ETG + BZ) and **E** (ETG + PD) with an important reduction in ED1 positive cells in both groups. **D** Group 4 (ETG + LOS) partial reduction of inflammatory infiltration (*arrows*)

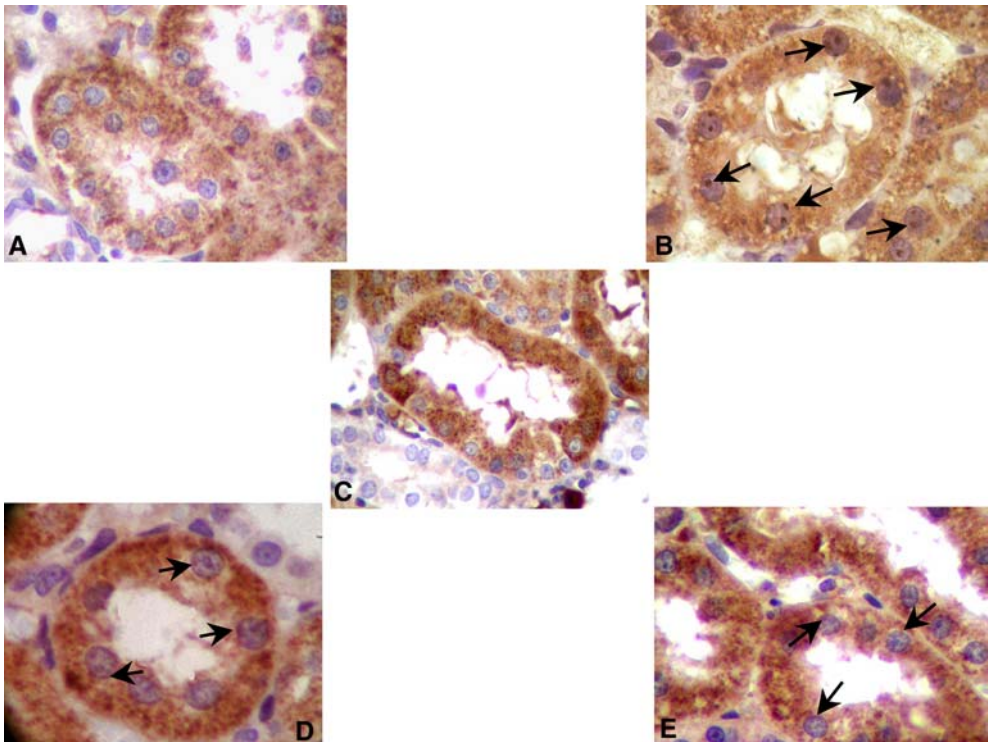

Fig. 4 Representative photomicrographs of renal subunit p50 immunostaining of the complex NF-κB (×1,000). *Arrows* indicate translocation of the activated p50 subunit of NF-κB from cytosol (*brown*) to nucleus in tubular epithelial cells. **A** Control rat (G1), **B** untreated hyperoxaluric rat (G2), **C** hyperoxaluric rat with benazepril (G3), **D** hyperoxaluric rat with losartan (G4), and **E** hyperoxaluric rat with PD 123,319 (G5)

and fibrosis [21–23]. Ruiz-Ortega et al. [24] have reported that systemic infusion of Ang II into normal rats activates NF-κB. Moreover, when these animals were treated with AT1 or AT2 receptor antagonists, different responses were observed. The results from that study indicate that AT1 mainly mediates tubular injury via NF-κB, whereas AT2 receptors participate in inflammatory cell infiltration in the kidney also by NF-κB activation. Since CaOx by itself or by stimulating RAS induces ROS production, and this situation leads to the activation of both NF-κB and RANTES, the control of oxidative stress in Hox, throughout a pharmacological intervention, acquires importance in order to modulate the NF-κB inflammatory cascade and RANTES upregulation.

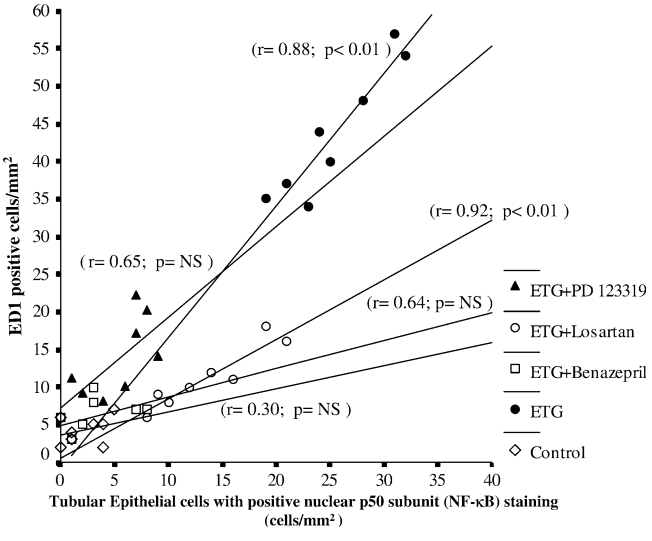

Fig. 5 Relationship between the nuclear expression of the p50 subunit (NF-kB) in tubular epithelial cells (activated cells) and monocyte/macrophage interstitial infiltration (ED1 positive cells) in all groups. *ETG* ethylene glycol, *BZ* benazepril, *L* losartan, *PD* PD 123,319, *NS* not significant

The five members of the NF-κB family are associated in different dimeric forms (p50/p105, p65/ReIA, c-Rel, ReIB and p52/p100). In resting cells, NF-κB dimers remain in the cytoplasm as inactive forms bound to the inhibitory subunit IκB. Upon stimulation (lipopolysaccharide, CaOx cellular overload, ROS), IκB is phosphorylated by the IκB kinase complex and degraded by the proteasome system. Then, free NF-κB dimers expose the nuclear localization sequence and translocate into the nucleus where they activate the transcription of target genes involved in the inflammatory response, and consequently cytokine (IL-6) and chemokine (MCP-1, RANTES) production. In the present study, subunit p50, a dimer part of the complex NF-κB, was increased in renal tissue from untreated hyperoxaluric animals. On the other hand, when ACE inhibition, or blockage of either AT1 or AT2 receptors was carried out, a considerable decrease in the immunohistochemical expression of NF-κB (p50) was found. Nevertheless, the reduction of NF-κB (p50) was not equal in all treatments. Interestingly, BZ presented a higher response in comparison with either LOS or PD. These findings indicate that the stimulation of NF-κB by Ang II in this experimental model of Hox always involves the AT1 receptor, but it also suggests AT2 receptor participation.

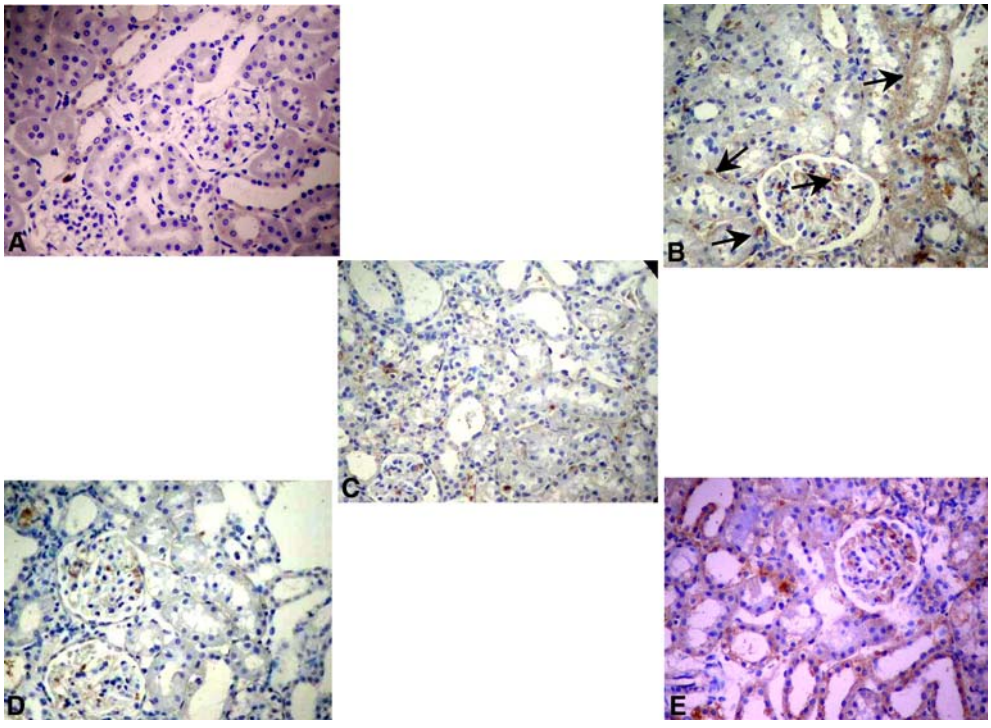

Fig. 6 Representative photomicrographs of renal IL-6 immunostaining (×400). *Arrows* show positive staining in tubular epithelial and interstitial cells, as well as in the glomerular area. Note oxalate crystals (*asterisks*) into the tubular lumens. **A** Control rat (G1), **B** untreated hyperoxaluric rat (G2), **C** hyperoxaluric rat with benazepril (G3), **D** hyperoxaluric rat with losartan (G4), and **E** hyperoxaluric rat with PD 123,319 (G5)

Activation of NF-κB by Ang II stimulates the synthesis of cytokines such as IL-6 and the chemokines MCP-1 and RANTES [21, 25–27]. Because IL-6, MCP-1 and osteopontin (OPN) play a central role in the process of CaOx crystal-induced renal injury [6, 7, 28], a reduction in their corresponding expressions results in a clear benefit with a better renal outcome. OPN has multiple roles for stone formation, such as an inhibitor of crystal growth in the urine and a promoter of crystal aggregation on the cell surface. Despite modulating several steps of CaOx crystallization and seeming to play a significant role in CaOx crystal deposition in the renal interstitium, OPN is a chemokine with similar properties to IL-6, MCP-1 and RANTES. Recently, Umekawa

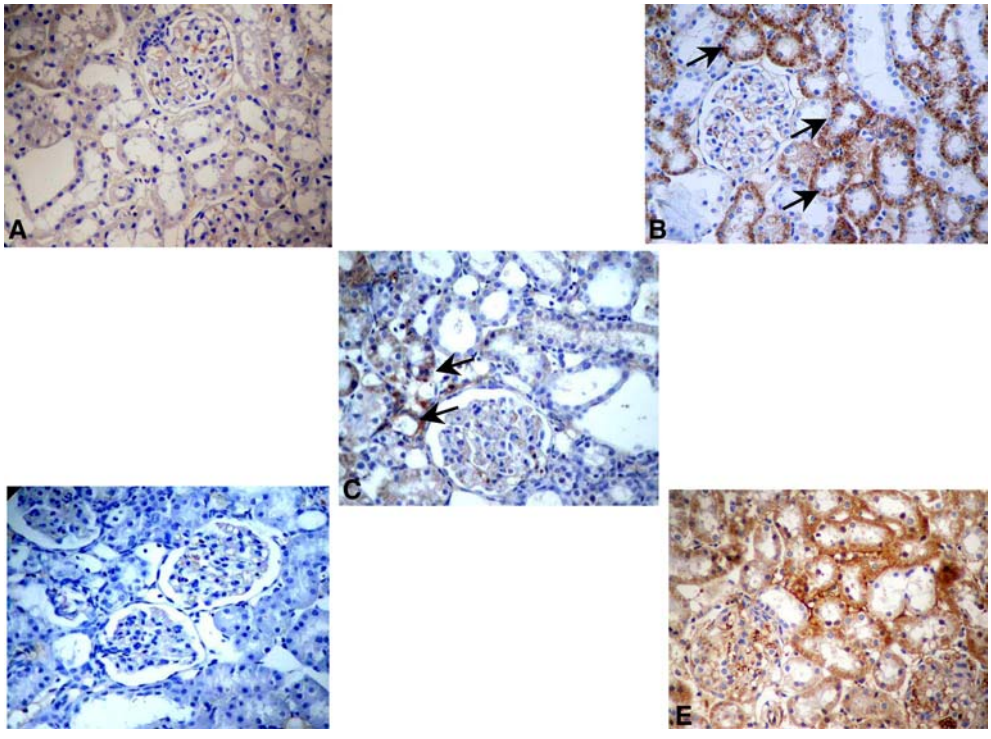

Fig. 7 Representative photomicrographs of renal MCP-1 immunostaining (×400). *Arrows* show positive staining in tubular epithelial cells. **A** Control rat (G1), **B** untreated hyperoxaluric rat (G2), **C** hyperoxaluric rat with benazepril (G3), **D** hyperoxaluric rat with losartan (G4), and **E** hyperoxaluric rat with PD 123,319 (G5)

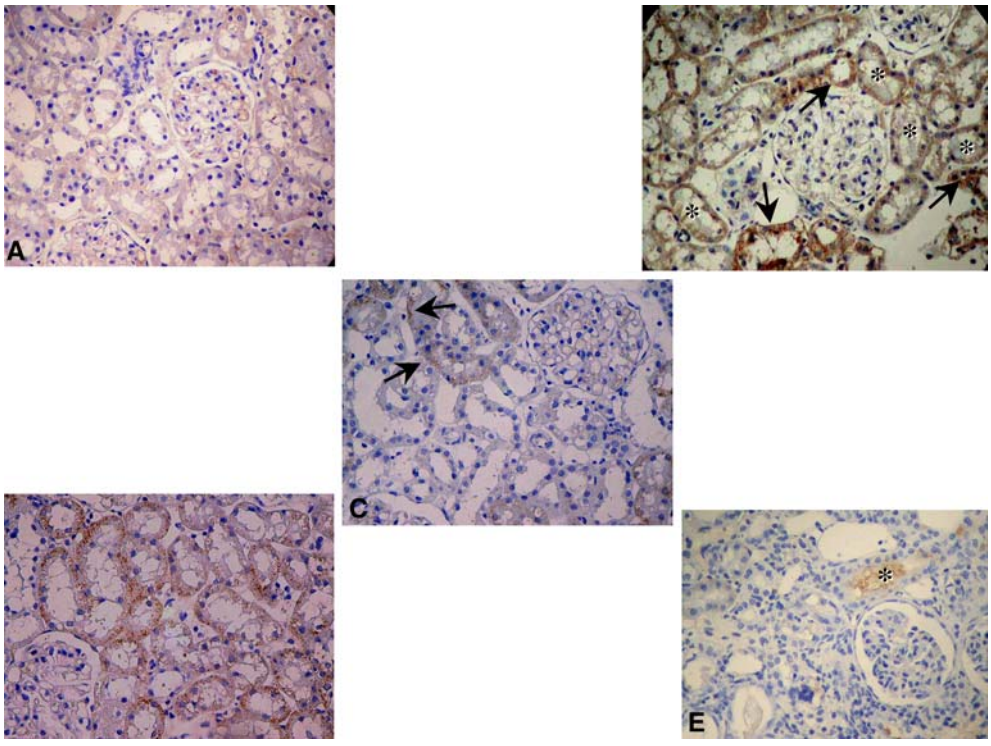

Fig. 8 Representative photomicrographs of renal RANTES immunostaining (×400). *Arrows* show positive staining in tubular epithelial cells. Note oxalate crystals (*asterisks*) into the tubular lumens. **A** Control rat (G1), **B** untreated hyperoxaluric rat (G2), **C** hyperoxaluric rat with benazepril (G3), **D** hyperoxaluric rat with losartan (G4), and **E** hyperoxaluric rat with PD 123,319 (G5)

et al. [20] reported that oxalate-induced upregulation of OPN is in part mediated by RAS throughout AT1 receptor activation. Following these concepts, it is not surprising that in the present study the inflammatory reaction to CaOx crystalluria, characterized by tubular epithelial cell overexpression of IL-6 and MCP-1 in untreated hyperoxaluric rats, was blunted by LOS.

So far, there is no information on RANTES participation in the CaOx crystal-induced TI inflammatory response. Therefore, we consider that the data presented in our study represent the first report on this chemokine linked to Hox and CaOx crystalluria.

Although ROS may stimulate RANTES expression, we consider that the most likely mechanism by which Hox induces RANTES is by the activation of AT2 receptors. This hypothesis is supported by the findings in the present study, in which a significant reduction in RANTES immunostaining was observed in hyperoxaluric animals that received either ACE inhibition or AT2 receptor blockage, but not when LOS was administered. In addition, our findings are in agreement with the convincing experiment of Wolf et al. in which the authors demonstrated, in glomerular endothelial cells, that RANTES is mainly induced via AT2 receptor activation [27].

It is well-known that part of the local Ang II could be generated by non-ACE dependent pathways in various tissues in different species, in particular via chymases when inflammatory cells are present. Despite the fact that some reports indicate that in the rat kidney this mechanism does not seem to be critical in the conversion of Ang I to Ang II [29], we speculate that minimal differences in the results observed in our study involving the expression of IL-6 and MCP-1 between hyperoxaluric rats with BZ and those with LOS may be explained by some limited generation of Ang II by a non-ACE pathway.

In concordance with all of these findings, various studies agree with the hypothesis that Ang II regulates

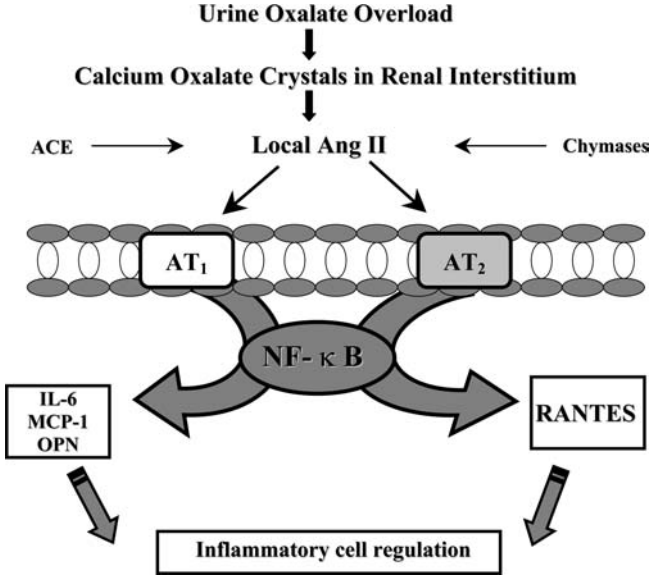

Fig. 9 Renin-angiotensin system participation in the inflammatory response in the kidney in hyperoxaluria. *ACE* angiotensin converting enzyme, *Ang II* angiotensin II, *AT1* angiotensin II type 1 receptor, *AT2* angiotensin II type 2 receptor, *NF-κB* nuclear factor-κB, *IL-6* interleukin 6, *MCP-1* monocyte chemoattractant protein-1, *OPN* osteopontin; *RANTES* regulated upon activation of normal T-cells expressed and secreted

the inflammatory response via activation of NF-κB through AT1 and AT2 receptors [3, 30–32].

Finally, we conclude that Hox stimulates the inflammatory response in renal tissue by the activation of RAS, not only by AT1 but also by AT2 receptors of Ang II. Moreover, this inflammatory reaction seems to be mediated by the activation of the NF-κB and, therefore, the overexpression of IL-6, MCP-1 and RANTES, which are all involved in inflammatory cell regulation, as schematized in Fig. 9. This suggests the possible relevance of obtaining a dual blockage of RAS in order to acquire a better control of the inflammation-repairing process in CaOx crystal injury in renal tissue, as reported in clinical and experimental studies in other kidney diseases with variable degree of TI lesions [33–36].

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