Urinary cytokine profiles according to the site of blockade of the renin-angiotensin system in nephrectomized rats

Perfil de citocinas urinárias de acordo com o local de bloqueio do Sistema Renina Angiotensina em ratos nefrectomizados

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Abstract

Introduction: It is still unknown how the pharmacological inhibition of the Renin Angiotensin System (RAS) impacts the levels of inflammation and fibrosis biomarkers. **Objective:** This study sought to evaluate the effect of enalapril, candesartan and aliskiren on urinary levels of cytokines in a model of chronic kidney disease (CKD). **Methods:** Male Wistar rats were submitted to surgical removal of ¾ of renal parenchyma to induce CKD (¾ nephrectomy), or subjected to sham surgery (control). Animals were then randomized into five groups: Sham surgery receiving vehicle; ¾ Nephrectomy receiving vehicle; ¾ Nephrectomy receiving enalapril (10 mg/kg); ¾ Nephrectomy receiving candesartan (10 mg/kg) and ¾ Nephrectomy receiving aliskiren (10 mg/kg). Urine output, water intake, mean arterial pressure (MAP) and urinary concentrations of creatinine, urea, albuminuria, Na+, K+, interleukin (IL) -1β, IL-6, IL-10 and transforming growth factor beta (TGF-β) were measured. **Results:** Nephrectomy significantly impaired renal function, increased MAP and altered the levels of all evaluated cytokines in urine. Enalapril, candesartan and aliskiren improved renal function and decreased MAP and IL-6 when compared to vehicle-treated nephrectomized group. Candesartan and aliskiren decreased IL-1β, while only candesartan reduced TGF-β and only aliskiren increased IL-10. **Conclusion:** Enalapril, candesartan and aliskiren presented similar effects on improving renal function and reducing MAP and urinary levels of IL-6 in rats with CKD. On the other hand, cytokine profile differed according to the treatment, suggesting that differential mechanisms were triggered in response to the site of RAS blockade.

Keywords: angiotensins; angiotensin-converting enzyme inhibitors; angiotensin receptor antagonists; kidney failure, chronic; nephrectomy.

Resumo

Introdução: Ainda não se sabe como a inibição farmacológica do Sistema Renina Angiotensina (SRA) afeta os níveis de biomarcadores de inflamação e fibrose. **Objetivo:** Este estudo pretendeu avaliar o efeito de enalapril, candesartan e alisquireno sobre os níveis urinários de citocinas em um modelo de doença renal crônica (DRC). **Métodos:** Ratos Wistar machos foram submetidos à remoção cirúrgica de ¾ do parênquima renal para induzir DRC (nefrectomia), ou submetidos à cirurgia fictícia (controle). Animais foram então randomizados em cinco grupos: Cirurgia fictícia recebendo veículo; Nefrectomia recebendo veículo; Nefrectomia recebendo enalapril (10 mg/kg); Nefrectomia recebendo candesartan (10 mg/kg) e Nefrectomia recebendo alisquireno (10 mg/kg). Débito urinário, ingesta hídrica, pressão arterial media (PAM) e concentrações urinárias de creatinina, ureia, albumina, Na+, K+, interleucina (IL) -1β, IL-6, IL-10 e fator de transformação e crescimento beta (TGF-β) foram medidas. **Resultados:** A nefrectomia comprometeu significativamente a função renal, aumentou a PAM e alterou os níveis de todas as citocinas avaliadas na urina. Enalapril, candesartan e alisquireno melhoraram a função renal e diminuíram a PAM e a IL-6 quando comparado aos grupo de animais nefrectomizados tratados com veículo. Candesartan e alisquireno reduziram IL-1β, enquanto somente candesartan diminuiu o TGF-β e somente alisquireno aumentou a IL-10. **Conclusão:** Enalapril, candesartan e alisquireno apresentaram efeitos similares em relação à melhora da função renal e redução da PAM e dos níveis urinários de IL-6 em ratos com DRC. Por outro lado, o perfil de citocinas diferiu de acordo com o tratamento, sugerindo que diferentes mecanismos sejam desencadeados em resposta ao local de bloqueio do SRA.

Palavras-chave: angiotensinas; antagonistas de receptores de angiotensina; falência renal crônica; inibidores da enzima conversora de angiotensina; nefrectomia.

Introduction

The renoprotective effects of the Renin Angiotensin System (RAS) inhibition in chronic kidney disease (CKD) seems to extend far beyond the control of arterial hypertension.[1-3] Angiotensin converting enzyme inhibitors (ACEi) and angiotensin type 1 (AT1) receptor antagonists (ARA) slow the progression of CKD not only by reducing blood pressure, but also because of their antioxidant, anti-proliferative, anti-inflammatory and anti-fibrogenic effects.[1-3] More recently, renin inhibitors started being used as a novel modality of RAS blockade.[4] Aliskiren, the prototype of renin inhibitors, also presented renoprotective effects in non-diabetic CKD.[5]

Inflammation is known to play an important role in CKD pathogenesis. The levels of inflammation-related molecules, including like C-reactive protein, chemokines and cytokines are increased in patients with CKD.[6-8] CKD patients exhibit a pro-inflammatory profile since initial stages, which, in turn, might contribute to disease progression.[9-11] In addition to inflammation, the RAS plays a pivotal role in the pathophysiology of CKD.[3,12,13]

An exacerbated stimulation of the RAS promotes intrarenal production of Angiotensin (Ang) II that binds to AT_1 receptor producing glomerular and systemic hypertension, endothelial injury, and proteinúria.[12,14,15] Clinical and experimental studies have also drawn attention to the interactions between Ang II and cytokines, chemokines and growth factors, contributing to the progression of CKD.[3,12,16,17] Ang II recruits inflammatory cells, induced the release of cytokines and directly stimulates intracellular signaling mechanisms related to kidney inflammation and fibrosis.[3,12,18,19]

Previous studies have shown that interleukin (IL)-1β and IL-6 are major inflammatory mediators in renal diseases,[6,8,11] whereas transforming growth factor beta (TGF-β) is an important mediator of kidney tissue fibrosis.[18-20] In addition, the interaction of Ang II with these molecules has been previously reported in experimental models and in human renal diseases.[12,13,16,18,19] In addition, some studies have shown the anti-inflammatory actions of IL-10[21-23] in renal diseases. Herein, we aimed to evaluate the urinary cytokine profile in response to three different sites of pharmacological blockade of the RAS in an experimental model of CKD stage III.

Materials and methods

Animals

This study included 60 adult (aged 60 - 90 days), male Wistar rats, weighing 200-250g, Animals were maintained under temperature-controlled conditions with an artificial 12-h light/dark cycle and receiving standard chow and water *ad libitum*. *Rats* were bred at the animal facility of the Faculty of Medicine of Itajubá (MG, Brazil). The study was approved by the Ethics Committee on Animal Welfare of Federal University of Minas Gerais (UFMG, Brazil), according to the protocol CETEA 03/09.

Experimental design and surgical procedures

For surgical procedures, animals received intraperitoneal injection of ketamine (50 mg/kg) and xylazine (25 mg/kg). In order to induce moderate CKD, animals underwent surgical removal of ¾ of the renal parenchyma, according to the guidelines of Ormrod & Miller.[24] As a control group, animals underwent sham surgery.

Sham surgery - control group

Anesthesia, shaving, asepsis and left flank incision were performed to expose the left kidney, followed by dissection of adhering fat and renal capsule, preserving the adrenal gland. After that, a very small slice of renal cortical tissue was removed. Seven days later, right kidney was exposed and manipulated.

Moderate CKD - ¾ nephrectomy

Anesthesia, shaving, asepsis and flank incision were performed with exposure of the left kidney followed by dissection of adherent fat and renal capsule, preserving the adrenal glands. Similarly to the previous group, the left kidney was exposed to remove upper and lower poles with surgical excision of cortical tissue from the outer side portion of the left kidney. After seven days, right renal pedicle was ligated and the right kidney removed.

After recovery from anesthesia, animals were allocated into five experimental groups (n = 10 per group), housed in metabolic cages (Tecniplast, USA), with free access to water and standard rat chow. After 72 hours of adaptation period, animals were randomized to daily receive the following treatments per gavage for 14 days:

1. Control group- sham surgery: distilled water (vehicle);
2. Nephrectomy ¾: distilled water (vehicle);
3. Nephrectomy ¾: enalapril maleate 10 mg/kg;
4. Nephrectomy ¾: candesartan 10 mg/kg;
5. Nephrectomy ¾: aliskiren 10 mg/kg.

All animals were subjected to mean blood pressure measurement by tail plethysmography once a week. Urine output, water and food intake were daily measured. At the end of the experimental period (14th day), blood samples were withdrawn by cardiac puncture, under ketamine and xylazine anesthesia (50 mg/kg and 25 mg/kg, respectively), and centrifuged at 2,500 rpm for 15 min at 4°C. Samples of serum and 24-hour urine were collected at the last day of the experiment and they were stored at - 20°C to measure creatinine, urea, Na^+, K^+, IL-1β, IL-6, IL-10 and TGF-β. At the end of experimental protocol (14th day), the animals were euthanized and the kidneys were removed and cut into 2 mm slices, which were fixed for 24 hours in 10% buffered formalin.

Treatments and doses

The doses and RAS blockers tested have been quite variable in previous studies. Our protocol was based on previous studies that evaluated daily administration of oral treatments in adult rats. Thus, the ACEi of this study was enalapril maleate at a dose of 10 mg/Kg/day based on previous experimental studies with rats.[25,26]

The ARA was candesartan cilexetil at a dose of 10 mg/kg/day based on the study of Lin and co-workers reporting anti-hypertensive and anti-inflammatory effects with daily oral doses of 10 to 15 mg/Kg/day in Dahl salt-hypertensive rats.[27] The reported doses for Aliskiren in experimental studies have varied from 5 mg/Kg/day[28] to 100 mg/Kg/day.[29] For this reason, we performed a preliminary test with the doses of 10 (n = 5) and 100 mg/Kg/day (n = 5) and both doses elicited similar effects on blood pressure, renal function parameters and urinary cytokine measurements (data not shown). Therefore, we adopted the dose of 10 mg/kg/day for Aliskiren.

General measurements and renal function parameters

To evaluate renal function parameters, serum and urinary creatinine were determined by modified Jaffé colorimetric method (Labtest, Minas Gerais, Brazil). Serum urea was determined by the enzymatic method (Labtest, Minas Gerais, Brazil). Serum and urinary sodium and potassium concentrations were measured by flame photometry (CELM, Minas Gerais, Brazil) and microalbuminuria quantification was performed by turbidimetry (Gold Diagnostic analyzes, Minas Gerais, Brazil).

Urinary cytokines measurements

Urinary levels of IL-1β, IL-6, IL-10 and TGF-β1 were measured at day 14 by enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN, USA), following the manufacturer's instructions. All samples were analyzed in duplicate. Urinary concentrations of cytokines were expressed as values relative to urinary creatinine measured simultaneously in the same urine sample (pg/mg Cr). The limits of detection for each cytokine were: 0.1 mg/ml for IL-1β, 0.039 pg/ml for IL-6 and 6 pg/ml for IL-10 and TGF-β1. Measurements of IL-1β, IL-6, IL-10 and TGF-β1 were performed in a single assay to avoid inter-assay variations. The intra-assay variation was less than 3%.

Kidney histopathology

After fixation, the sections were embedded in paraffin, sliced into 5 micra slides and stained with Hematoxylin & Eosin (HE). Afterwards, the histological slides were examined in a blinded manner to experimental protocol under an optical microscope (Nikon Eclipse, Japan). Images of illustrative fields were obtained.

Statistical analysis

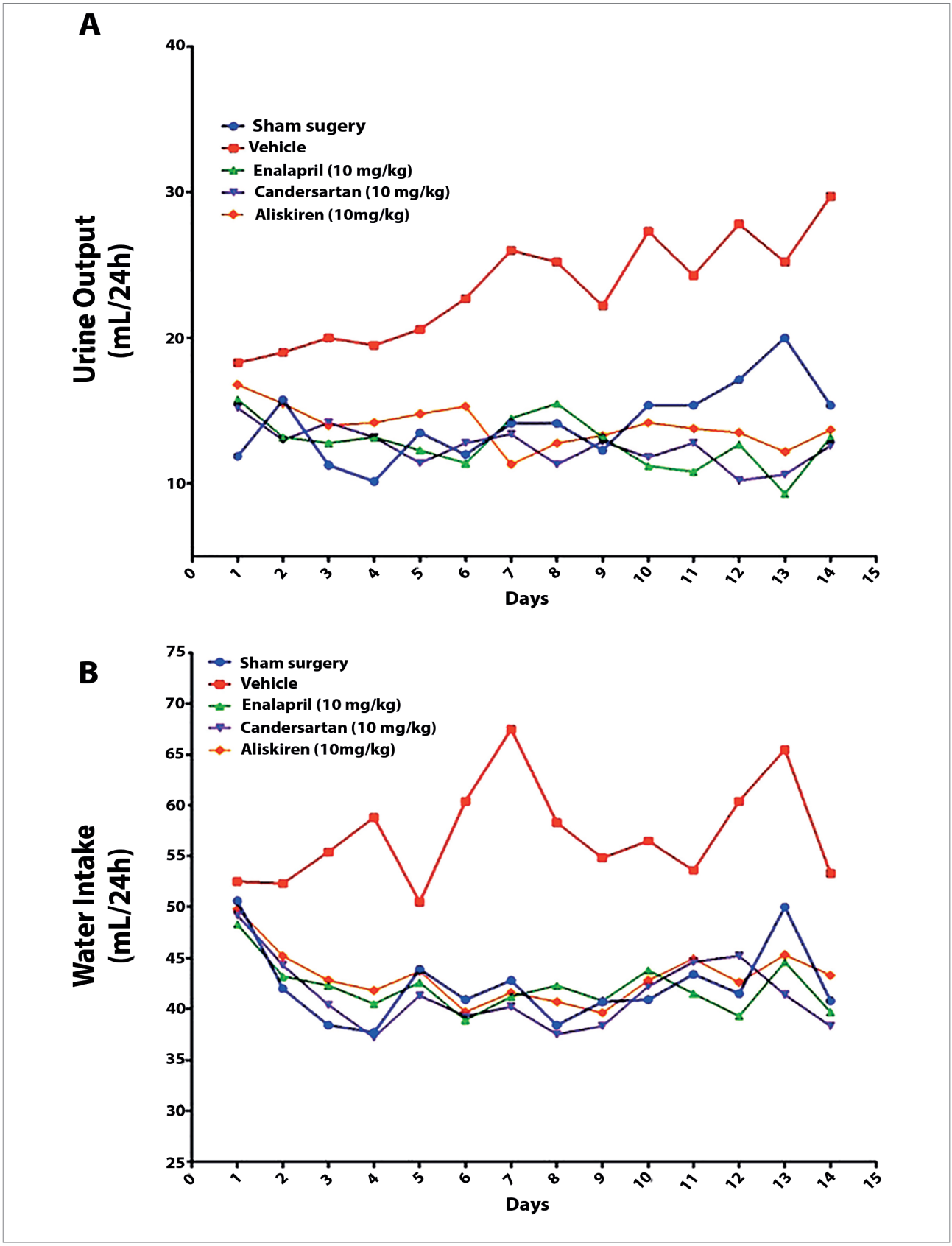
Results were expressed as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) was used to compare means between multiple groups, followed by Student-Newman-Keuls post hoc test. The level of significance was set at $p < 0.05$.

Results

General measurements

Urine output of the animals is shown in Figure 1A. Nephrectomy group that received vehicle showed a significant increase in urine output from day 1 until the end of the experiment when compared with control group ($p < 0.05$) and with treated animals ($p < 0.05$). On the other hand, nephrectomized rats treated with RAS blockers (enalapril, candesartan or aliskiren) were able to maintain urine output similar to control group. There was no significant difference in urine output between treated groups.

Figure 1. Urine output and water intake of rats submitted to sham surgery (control group) or ¾ nephrectomy receiving different treatments: enalapril (10 mg/kg), candesartan (10 mg/kg), aliskiren (10 mg/kg), aliskiren (20 mg/kg) or water (vehicle). Urine output (ml/24h) - panel A - and water intake (ml/24h) - panel B - were assessed daily during 14 days. Lines show the mean of results of five rats per group.



Water intake of the animals is shown in Figure 1B. Nephrectomized and vehicle-administered animals significantly increased water intake in comparison with control group ($p < 0.05$) and with treated animals ($p < 0.05$). As observed for urine output, all treatments (enalapril, candesartan or aliskiren) similarly restored water intake to values comparable to control group. The change in water intake occurred from day 2 until the end of the experiment.

Renal function parameters

Renal function parameters are displayed in table 1. All biochemical parameters of renal function were seriously compromised in the group of nephrectomized rats receiving vehicle when compared with control group (sham surgery) ($p < 0.05$ for all comparisons). On the other hand, the treatments with enalapril (10 mg/kg), candesartan (10 mg/kg) and aliskiren (10 mg/kg) produced significant improvements in all renal function parameters in comparison to nephrectomized animals receiving vehicle ($p < 0.05$). In addition, all treatments similarly restored renal function parameters to values detected in control group (Table 1).

Mean arterial pressure (MAP)

As expected, nephrectomized animals receiving vehicle had significantly higher MAP than control group ($p < 0.05$, Table 2). In contrast, all treatments (enalapril, candesartan and aliskiren) similarly reduced MAP ($p < 0.05$, Table 2) to levels comparable to control animals ($p > 0.05$).

Urinary levels of cytokines (IL-1β, IL-6, TGF-β, and IL-10)

As shown in Figure 2A-D, urinary levels of IL-1β, IL-6, TGF-β, and IL-10 significantly changed after ¾ nephrectomy in comparison with sham-surgery (control group).

Treatments with aliskiren and candersartan significantly reduced urinary levels of IL-1β when compared to nephrectomized group receiving vehicle (85.5 ± 67.8 pg/mg cr *vs.* 1.3 ± 1.1 pg/mg cr following aliskiren treatment and undetectable levels after candesartan administration, Figure 2A). On the other hand, enalapril was not able to reduce urinary levels of IL-1β.

Figure 2B showed that all treatments significantly reduced urinary levels of IL-6 (candesartan: 0.30 ± 0.13 pg/mg cr, aliskiren: 1.37 ± 0.53 pg/mg cr and enalapril 2.50 ± 1.25 pg/mg cr) when compared with vehicle administration (8.60 ± 4 .99 pg/mg cr, $p < 0.05$ for all comparisons), reaching values comparable to control group (0.48 ± 0.18 pg/mg cr).

Only candesartan treatment was able to significantly reduce the urinary levels of TGF-β in comparison with vehicle-administered animals (1.17 ± 0.35 *vs.* 3.42 ± 1.09 pg/mg cr, $p < 0.05$, Figure 2C). In addition, whereas candesartan significantly reduced urinary levels of IL-10, aliskiren increased about four times in comparison with vehicle-administered animals (99.50 ± 36.14 pg/mg cr *vs.* 22.50 ± 21.50 pg/mg cr, $p < 0.05$, Figure 2D). Enalapril did not change urinary levels of IL-10 (11.80 ± 10.52 pg/mg cr) when compared to nephrectomized animals receiving vehicle (Figure 2D).

Table 3 displays all values of the measurements of urinary cytokines.

Renal histopathology

Figure 3 shows representative slices of kidney tissue (400 x magnification) stained by HE from sham-operated rats receiving vehicle (control group, panel A), ¾ nephrectomized rats treated with vehicle (panel B), ¾ nephrectomized rats treated with enalapril (panel C), ¾ nephrectomized rats treated with candesartan (panel D) and ¾ nephrectomized rats treated with aliskiren (panel E). The control group showed normal renal histology (A).

In sharp contrast, ¾ nephrectomized rats receiving vehicle exhibited intense interstitial inflammatory infiltrate, glomerular disruption and abnormal tubular hypotrophy or dilatation with hyaline material in tubular lumen (panel B). Nephrectomized rats treated with enalapril (C) or candesartan (D) or aliskiren (E) presented less severe renal tissue alterations than nephrectomized rats receiving vehicle.

All treatments reduced glomerular and tubular damage in comparison with vehicle administration, showing a renoprotective effect. When compared to sham-operated rats, ¾ nephrectomized rats treated with RAS blockers had an augmentation of mesangial cellularity and of interstitial inflammation, discrete glomerular hypertrophy and mild tubular hypotrophy.

Discussion

Our data showed that ACE inhibition, AT_1 receptor antagonism and renin inhibition were similarly

Table 1 Renal function parameters of rats submitted to sham surgery (control group) or ¾ nephrectomy receiving different treatments (vehicle, enalapril, candesartan or aliskiren)

Parameters	Sham surgery	Vehicle	Enalapril 10 mg/kg	Candesartan 10 mg/kg	Aliskiren 10 mg/kg
Serum Na^+ (mEq/l)	141.3 ± 2.3	157.0 ± 2.1#	141.0 ± 2.3*	143.8 ± 3.0*	145.3 ± 3.8*
Serum K^+ (mEq/l)	4.3 ± 0.2	5.8 ± 0.3#	4.5 ± 0.5*	4.4 ± 0.4*	4.8 ± 0.6*
Urinary Na^+ (mEq/l)	286.8 ± 28.2	185.3 ± 20.2#	268.3 ± 21.2*	274.3 ± 18.2*	265.3 ± 22.8*
Urinary K^+ (mEq/l)	212.4 ± 32.3	173.5 ± 12.1#	210.0 ± 17.3*	219.2 ± 17.1*	208.2 ± 12.3*
Serum urea (mg/dL)	33.4 ± 3.5	162.3 ± 7.5#	40.3 ± 7.8*	38.3 ± 6.5*	40.8 ± 7.7*
Serum creatinine serum (mg/dL)	1.1 ± 0.2	5.3 ± 1.0#	1.5 ± 0.5*	1.4 ± 0.6*	1.8 ± 1.0*
Creatinine clearance (mL/min)	0.42 ± 0.04	0.16 ± 0.02#	0.38 ± 0.03*	0.37 ± 0.06*	0.36 ± 0.08*
Microalbuminuria (mg/day)	0.42 ± 0.4	52.3 ± 4.1#	1.0 ± 0.7*	0.8 ± 0.5*	1.0 ± 0.9*

Table 2 Mean blood pressure of rats submitted to sham surgery (control group) or ¾ nephrectomy receiving different treatments (vehicle, enalapril, candesartan or aliskiren).

Mean Blood Pressure (mmHg)	
Groups	Day 14
Control (sham surgery)	108 ± 6
Vehicle (¾ nephrectomized + water)	161 ± 5#
Enalapril 10 mg/kg (¾ nephrectomized)	102 ± 8*
Candesartan 10 mg/kg (¾ nephrectomized)	105 ± 4*
Aliskiren 10 mg/kg (¾ nephrectomized)	122 ± 6*

Mean blood pressure of animals in all experimental groups was measured by tail plethysmography once a week. Results are presented by mean ± SEM of ten mice per group. *$p < 0.05$: for comparisons with vehicle-administered group. #$p < 0.05$: for the comparison between vehicle and control group (sham surgery).

effective in reversing biochemical changes, reducing blood pressure, proteinuria and renal tissue damage produced by ¾ nephrectomy. On the other hand, the treatments with enalapril, candesartan and aliskiren changed urinary cytokines in a different way. In comparison with vehicle administration, AT_1 receptor antagonism with candesartan significantly reduced urinary levels of all cytokines (IL-1β, IL-6, TGF-β and IL-10), whereas ACE inhibition with enalapril only decreased IL-6 levels. In contrast, renin inhibition with aliskiren was the only treatment that increased urinary levels of the anti-inflammatory cytokine IL-10.

The ¾ nephrectomy produced significant changes in renal function parameters, including urine output, plasma and urinary concentration of electrolytes, plasma levels of nitrogen waste products, creatinine clearance and urinary protein excretion. Polyuria and polydipsia can be attributed to alterations in mechanisms of urine concentration, related to the inability of vasopressin to stimulate reabsorption of water in the collecting ducts of these animals.[30,31] The detection of lower urinary concentrations of sodium and potassium was also related to the reduced capacity of urine concentration.

On the other hand, Chamberlain & Shirley[32] obtained different results in relation to sodium and potassium in rats submitted to unilateral and subtotal nephrectomy. The authors detected that overall sodium and potassium excretion changed little in unilateral nephrectomized rats, while in animals submitted to 5/6 nephrectomy, a small reduction in sodium and potassium excretion was observed only during the first week after surgery[32].

These differences may be attributed at least in part to the use of different rat strains, since we used Wistar rats and the study of Chamberlain & Shirley[32] evaluated Sprague-Dawley animals. In addition, Chamberlain & Shirley[32] did not study renal function parameters following ¾ nephrectomy as in our study. It should be also mentioned that rats subjected to subtotal nephrectomy have elevated plasma levels of vasopressin[30] and decreased gene expression of aquaporins 1, 2 and 3.[31]

Figure 2. Effects of the administration of Aliskiren, Candesartan, Enalapril or Vehicle on urinary levels of cytokines (IL-1β, IL-6, IL-10 and TGF-β) in rats submitted to ¾ nephrectomy. Panels A to D display IL-1β (A), IL-6 (B), TGB-β (C) and IL-1 (D).

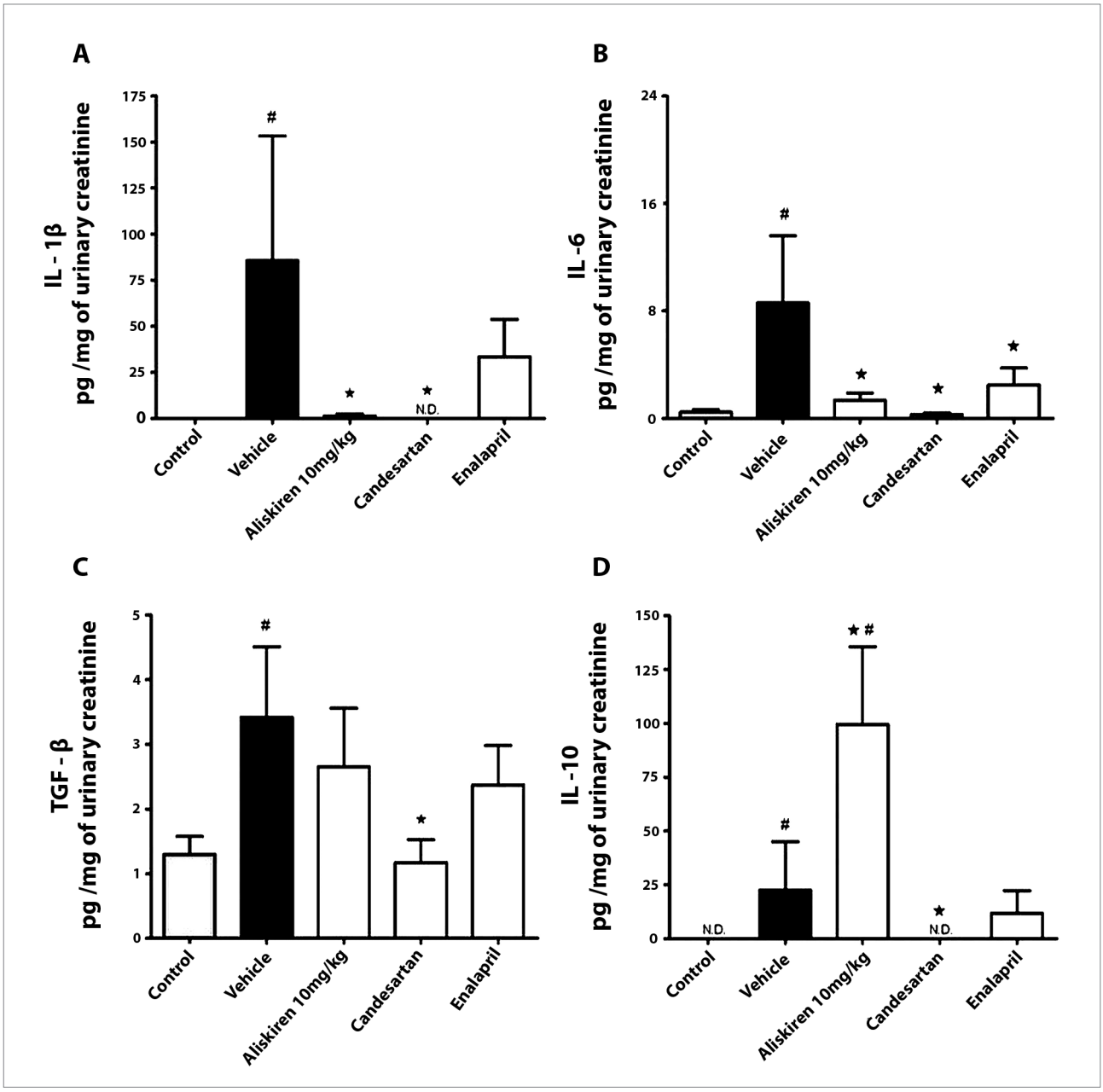
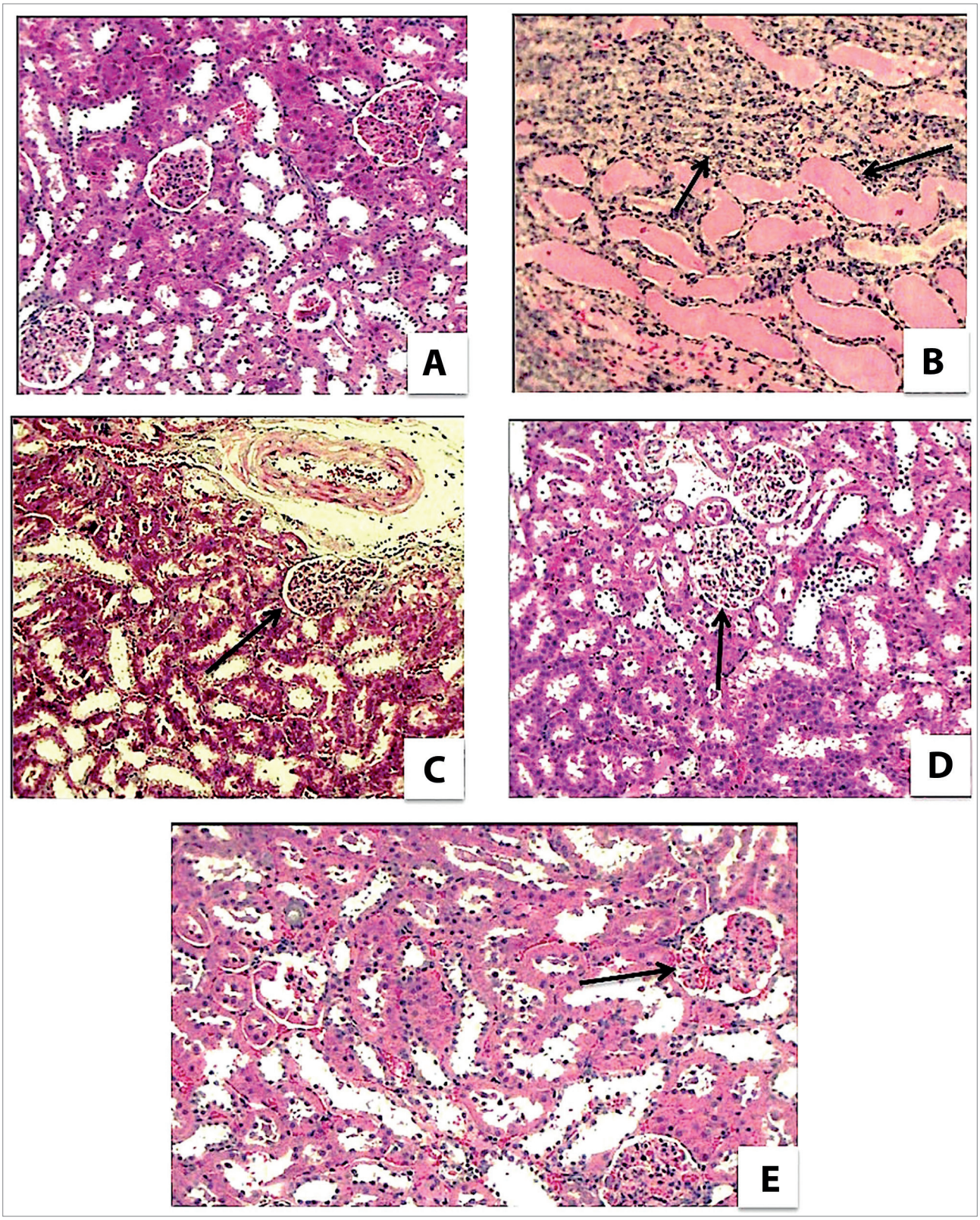


Table 3 Urinary levels of cytokines (pg/mg creatinine) of rats submitted to sham surgery (control group) or ¾ nephrectomy receiving different treatments (vehicle, enalapril, candesartan or aliskiren)

Cytokines (pg/mg cr)	Sham surgery	Vehicle	Enalapril 10 mg/kg	Candesartan 10 mg/kg	Aliskiren 10 mg/kg
IL-1β	N.D.	85.50 ± 67.80#	27.33 ± 23.45	N.D*	1.30 ± 1.10*
IL-6	0.48 ± 0.18	8.60 ± 4.99#	2.50 ± 1.25*	0.30 ± 0.13*	1.37 ± 0.53*
TGF- β	1.34 ± 0.47	3.42 ± 1.09#	2.48 ± 0.96	1.17 ± 0.35*	2.75 ± 0.83
IL-10	N.D.	22.50 ± 21.50#	11.80 ± 10.52	N.D.	99.50 ± 36.14*

N. D. = non-detectable levels *$p < 0.05$: for comparisons with vehicle-administered group. #$p < 0.05$: for the comparison between vehicle and control group (sham surgery).

Figure 3. Representative slices of renal tissue (400x magnification) stained by HE from sham-operated rats receiving vehicle (control group, panel A), ¾ nephrectomized rats treated with vehicle (panel B), ¾ nephrectomized rats treated with enalapril (panel C), ¾ nephrectomized rats treated with candesartan (panel D) and ¾ nephrectomized rats treated with aliskiren (panel E). Panel A represents normal renal histology. Panel B shows intense inflammatory infiltrate and tubular dilatation with hyaline material in the lumen (black arrows). Panel C shows more preserved glomerular structure (black arrow) and less intense tubular damage. Panels D and E show preserved tubular and glomerular structures with mild glomerular hyperthrophy and mesangial hypercelularity (black arrows).



In our study, we observed that all tested treatments reversed polyuria, polydipsia and changes in sodium and potassium concentrations produced by ¾ nephrectomy. It was not possible to determine the mechanisms by which RAS blockers normalized these parameters. However, Ang II is known to increase vasopressin secretion and dipsogenic effects.[33] Thus, the reduction of Ang II synthesis in response to enalapril or aliskiren and the antagonism of AT_1 receptors due to candesartan might reduce the release of vasopressin and water intake with consequent normalization of the volume and concentration of the urine.

All treatments also reduced serum creatinine levels, blood pressure and urinary protein excretion in ¾ nephrectomized animals. The improvement of renal function, blood pressure control and reduction of proteinuria in response to ACE inhibitors and AT_1 blockers have been widely reported in experimental and clinical studies.[1-3,12-14] However, our study is the first that evaluated the effects of aliskiren in rats subjected to ¾ nephrectomy.

Clinical studies have shown that aliskiren potentiates the antihypertensive effects of calcium channel blockers, diuretics, ACE inhibitors or AT_1 receptor antagonists.[34,35] In addition, aliskiren reduced proteinuria and delayed the progression of renal dysfunction if compared with placebo.[4] More recently, the treatment with aliskiren alone or in combination with AT_1 receptor antagonism significantly reduced proteinuria in patients with non-diabetic CKD.[36]

Experimental studies have shown that surgical removal of large amounts of the renal parenchyma increases the expression of several proinflammatory genes and stimulates infiltration of the remaining renal tissue by macrophages.[37,38] According to these findings, the present study found a significant increase of the urinary concentrations of IL-1β, IL-6, IL-10 and TGF-β following ¾ nephrectomy in rats.

However, the profile of cytokines differed according to the treatment. For instance, the AT_1 antagonist candesartan reduced all evaluated molecules (IL-1β, IL-6, TGF-β and IL-10). In experimental models of CKD, the treatment with candesartan significantly decreased the mRNA for TGF-β in renal tissue and by so doing slowed the progression of renal injury.[39-42] Candesartan administration also reduced renal IL-1β and IL-6 levels and improved renal function in Zucker obese rats.[42] In diabetic patients, treatment with candesartan reduced serum levels of IL-6.[41]

Less pronounced changes in cytokine profile were elicited by enalapril administration, since only urinary IL-6 concentrations were significantly reduced. Similarly, Ding *et al.*[43] showed a reduction of renal expression of IL-6 in Wistar rats submitted to unilateral right nephrectomy.

Alternatively, in rats subjected to subtotal nephrectomy, Ghosh *et al.*[44] reported a reduction of IL-1β and TGF-β levels following the treatment with enalapril. The results obtained with aliskiren differed somewhat from the other treatments. The renin inhibitor reduced urinary concentrations of pro-inflammatory cytokines, IL-1β and IL-6, and did not change TGF-β levels.

Moreover, aliskiren was the only treatment that increased urinary levels of IL-10. According to our findings, previous studies also reported the effects of aliskiren in pro-inflammatory cytokines.[4,5,45-48] Matavelli *et al.*[45] showed that the treatment of diabetic rats with aliskiren reduced IL-6 and TNF-α levels in renal interstitium by decreasing renal concentrations of Ang II. Accordingly, Wang *et al.*[46] reported that aliskiren prevented the increase in the levels of renal tissue mRNA for the expression of proinflammatory cytokines (TNFα and IL-1β), attenuated albuminuria and glomerulosclerosis in a mice model of diabetic nephropathy.

In contrast to our data, other investigators found that aliskiren reduced renal TGF-β levels in a mice model of progressive renal fibrosis[48] and decreased urinary concentration of this cytokine in patients with nondiabetic kidney diseases.[47] These divergent findings related to TGF-β might be due to differences in experimental models and/or in the degree of fibrosis.

However, this study is the first showing an elevation of urinary IL-10 following aliskiren administration. It has been previously reported that IL-10 exerts anti-inflammatory effects in other experimental models different from CKD.[21-23] In these studies, anti-inflammatory pharmaceuticals and/or phytotherapy products increased IL-10 levels as part of their mechanism of action.[21-23] We might speculate that the elevation in IL-10 levels could contribute, at least in part, to the beneficial effects of renin inhibitors also at renal tissue. Further studies are necessary to support our hypothesis.

We are aware of the limitations of our study. The main point is that we did not evaluate the mechanisms beyond the interactions between RAS blockers and urinary cytokines. In addition, our study did not allow us to precisely determine the role of urinary cytokines in CKD pathogenesis. On the other hand, some aspects may strength our findings, including the rigorous experimental protocols and the low intra-assay variability for cytokines measurements.

In conclusion, this study showed that ¾ nephrectomy resulted in changes in renal function parameters, elevated blood pressure, produced kidney tissue injury and increased urinary concentrations of pro-inflammatory cytokines (IL-1β and IL-6), a mediator of fibrosis (TGF-β) and an anti-inflammatory cytokine (IL-10). The pharmacological blockade of RAS at different levels improved renal function, reduced blood pressure and altered the profile of urinary cytokines.

All treatments reduced the pro-inflammatory cytokine IL-6. However, only renin inhibition was able to increase the anti-inflammatory mediator, i.e., IL-10. Our results did not allow the identification of specific mechanisms of action of each treatment, but suggest that local release of molecules related to inflammation and fibrosis may vary according to the site of RAS blockade and these differences may contribute to the effects of these medications in CKD.

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