

Distinct Regulation of Natriuretic Peptide Receptors during Renal and Vascular Inflammation in DOCA-Salt and Renovascular Hypertension

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Abstract

This study investigated the differential regulation of atrial and brain natriuretic peptides (ANP and BNP, respectively) and their renal receptors (NPR-A and NPR-C) in two experimental models of hypertension - DOCA-Salt (DS) and Renovascular (RV) - and their association with target organ damage. Male Sprague-Dawley rats were assigned to Control, DS, or RV groups and evaluated at 2, 6, and 12 weeks for systolic blood pressure (SBP), plasma ANP and BNP levels, renal gene and protein expression of NPR-A and NPR-C, and tissue markers of inflammation and fibrosis. Despite comparable increases in SBP, plasma ANP levels rose markedly in DS, while BNP increased only in RV. Renal NPR-A and NPR-C expression showed time-dependent upregulation in DS, but decreased at mid-stage in RV, returning to baseline at 12 weeks. Both hypertensive models exhibited increased expression of proinflammatory (TNF- α , IL-6, NF- κ B) and profibrotic (TGF- β 1) markers in the kidney and thoracic aorta, with DS showing earlier onset of molecular changes. These findings suggest that ANP is more responsive to possible volume overload, as suggested by sodium retention and atrial distension, although direct evidence of fluid expansion was not measured in this study, while BNP reflects pressure-driven stress. The earlier and more pronounced inflammatory and fibrotic responses observed in DS indicate that volume overload may accelerate target organ damage more than pressure overload. This highlights the distinct pathophysiological mechanisms underlying different hypertensive states and supports the relevance of natriuretic peptides as biomarkers and potential modulators of organ injury progression.

Introduction

Natriuretic Peptides (NPs) are a family of peptide hormones including atrial natriuretic peptide (ANP) [1–3], brain natriuretic peptide (BNP) [4] and C-Type natriuretic peptide (CNP) [5]. NPs play essential roles in cardiovascular, renal, endocrine and skeletal homeostasis [6–8] and have been extensively studied during the past few decades, becoming widely used as key clinical biomarkers of cardiovascular diseases with both diagnostic and prognostic significance [8–10].

ANP is synthesized and secreted primarily in the cardiac atria in response to atrial wall stretch due to increased volume or changes in the concentration of water and electrolytes in the body [6, 11]. ANP has natriuretic, diuretic, and vasorelaxant effects, exerting its actions on the blood vessels and kidneys [6]. Moreover, ANP has anti-inflammatory and antimitogenic effects [12]. BNP is mainly synthesized in the heart, where it regulates cardiomyocyte growth and hypertrophy, while CNP localization and vasodilating effects are confined to blood vessels [4, 5].

Three subtypes of renal NP receptors (NPR) have been identified: NPR-A, NPR-B, and NPR-C [13, 14]. NPR-A binds to ANP and BNP leading to increased cyclic GMP (cGMP) as a second messenger. In contrast, NPR-C functions as a clearance receptor for the three peptides [15]. Previous studies of our group were the first to demonstrate that ANP stimulates the phosphatidylinositol 3-kinase pathway, by increasing the hydrolysis of phosphoinositide via NPR-C stimulation [16]. Although three NP receptors exist, we focused on NPR-A and NPR-C because they are the primary mediators of ANP/BNP signalling

in the kidney [13, 14]. NPR-B, which primarily mediates CNP effects in vascular tissues [4, 5], was not examined, and this represents a limitation given its recognized role in vascular homeostasis.

One of the causes of hypertension is the increased renal sodium and water reabsorption, leading to blood volume expansion and resulting in cardiac overload [16–18]. Cardiac muscle stretching induces cardiomyocyte hypertrophy and upregulates the expression of genes related to hypertrophy and cardiac hormones, such as angiotensin II (ANG II) and endothelin, which contribute to systemic hypertension [17]. Renal and vascular tissues are also affected in both essential and secondary hypertension. In previous studies, we described the development of renal inflammation and oxidative stress in early stages in different animal models of hypertension, which further derived in fibrosis and kidney dysfunction [18, 19].

DOCA-Salt (DS) is a well-established experimental model of hypertension based on the administration of deoxycorticosterone acetate (DOCA) and dietary sodium overload [20]. This model is characterized by low plasma renin levels, sodium retention, volume expansion, cardiac hypertrophy and remodelling, endothelial dysfunction, and renal injury with glomerulosclerosis, tubular damage, and proteinuria [21, 22]. In this model, we can also find high levels of circulating catecholamines, ANG II, and endothelin-1 (ET-1) in the heart [23, 24]. Other mechanisms implied in high blood pressure development are central mineralocorticoid receptors activation [25] and augmentation of systemic concentration of ET-1 [26].

Renovascular (RV) hypertension develops in response to unilateral nephrectomy and renal arterial stenosis, an experimental model known as 1 kidney-1 clip (1K-1C). In this model, pressure overload increases as a consequence of increased peripheral resistance. Renin-angiotensin-aldosterone system (RAAS) activation is rapidly followed by water and sodium retention, and activation of the sympathetic nervous system [27, 28]. Although these processes allow for maintaining contractile ventricular function in the early stages, the increased vascular resistance promotes arrhythmias and ventricular remodelling in chronic periods [29]. The 1K-1C experimental model has plasma renin and Ang II levels within the normal range [22]. The human correlation of this model is RV hypertension and unilateral renal artery stenosis.

In the present study, we hypothesized that the development of hypertension and secretion of ANP and BNP are related with simultaneous overexpression of renal NP receptors and inflammatory and fibrotic biomarkers in kidney and vascular tissues, such as thoracic aorta. Altogether, these changes contribute to exacerbate the hypertensive process, leading to renal dysfunction and vascular inflammation and damage.

Materials and Methods

Animals

Male Sprague-Dawley rats provided by the Animal Care Facility of the School of Pharmacy and Biochemistry of the University of Buenos Aires, weighing 180–220 g, were housed in an environment

with controlled humidity and temperature ($23 \pm 2^{\circ}\text{C}$) with a daily 12 h light-dark cycle (light on from 7.00 am to 7:00 pm) with *ad libitum* access to drinking water and standard rodent Purina Chow (Cooperación SRL, Buenos Aires, Argentina). The experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals provided by the NIH, USA (NIH Publications No. 8023, Eight Edition, 2011) and approved by the Ethics Committee for the Care and Use of Laboratory Animals of the School of Pharmacy and Biochemistry of the University of Buenos Aires (Approval REDEC-2023-3497-E-UBA-DCT).

Experimental protocol and experimental models of hypertension

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The rats were randomly divided into three groups: Control (C), DS, and RV. DS hypertension was induced by weekly subcutaneous injections of DOCA (Sigma, St Louis, Missouri, USA) suspended in sesame oil at a dose of 30 mg DOCA/kg of body weight. Throughout the treatment, NaCl 1% w/v was given in the drinking water *ad libitum*. RV hypertensive model 1K-1C was induced by insertion of a silver clip of 0.28 mm lumen in the right renal artery under anaesthesia, accompanied by contralateral uninephrectomy, as previously described [30]. Separate control groups were used: one for the DOCA-salt protocol (vehicle injection + tap water) and one for the 1K-1C protocol (sham surgery). Each hypertensive model was compared with its corresponding control. Each experimental group was divided into three subgroups, corresponding to different durations of the treatment (2, 6, and 12 weeks). A total of 54 rats were used for this study ($n = 6$ rats/subgroup).

Systolic Blood Pressure (SBP) Measurement

At week 0 and after 2, 6, and 12 weeks of treatment, SBP was measured in conscious animals by the tail-cuff method using a sphygmomanometer coupled to a Grass 7C polygraph (Grass Instrument Co., Quincy, MA, USA) between 9 a.m. and 1 p.m. Before the SBP measurement, rats were trained for 3 days and conditioned in a thermostatic ($28 \pm 1^{\circ}\text{C}$) and quiet room for 60 min. Animals were placed in a heated acrylic restrainer at $30 \pm 1^{\circ}\text{C}$ during each measurement. Six measurements were obtained for each rat by the same single-blinded investigator, and the SBP was calculated as the average of the total measurements.

Plasma and Tissue Processing

At the end of each experimental period, the body weight was recorded, and the animals were anesthetized with ketamine (35 mg/kg)/xylazine (5 mg/kg). Blood samples were collected from the abdominal cava vein into ice-cold tubes containing EDTA 15%. To induce diastolic arrest, a solution of KCl 1M was administered. Half of the kidney and a 2 cm-segment of the descending thoracic aorta were immediately excised, washed in cold isotonic saline phosphate buffer, and fixed in formaldehyde 10% v/v

(pH = 7.20) for histological processing. The remaining kidney tissue was snap-frozen in liquid nitrogen and stored at -70°C for gene expression analysis.

Plasmatic ANP and BNP levels

Plasmatic ANP and BNP levels were measured using a commercially available radioimmunoassay kit (Phoenix Pharmaceuticals, Burlingame, CA, USA). The determinations were performed following the manufacturers' protocols.

Immunohistochemistry of Inflammatory and Fibrotic Biomarkers

After removing the capsule, kidneys were incised in a coronal plane, giving mirror image halves. Kidney cortex and aorta samples were embedded in paraffin and processed for immunohistochemistry according to previous studies of our group [53, 54]. Primary specific antibodies for immunostaining were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and used in a 1:100 dilution in PBS. In kidney, the protein expression levels of NPR-A (sc-137041), NPR-C (sc-515449), transforming growth factor beta1 (TGF- β 1) (sc-130348), tumour necrosis factor alpha (TNF- α) (sc-52746), interleukin 6 (IL-6) (sc-130326), and nuclear factor kappa beta (NF- κ B) (p50 subunit) (sc-166588) were measured, while TGF- β 1, TNF- α , IL-6, and nitrotyrosines (NT) (sc-101358) were measured in the thoracic aorta. The immunostaining was performed using a commercial modified avidin-biotin-peroxidase complex kit (Vectastain ABC kit, Universal Elite, Vector Laboratories, Burlingame, CA, USA) to form the avidin-biotin-peroxidase complex. 10 consecutive microscopic fields were analysed per sample with a 400X augmentation. Data was analysed with Image-Pro Plus ver. 4.5 for Windows (Media Cybernetics, Rockville, MD, USA) software. The results were expressed as intensity of optical density (IOD) $\pm$ standard error of the mean (SEM).

mRNA expression levels

Total RNA was extracted from total kidney with Trizol. First-strand cDNA was synthesized from 1 μ g of total RNA using Superscript III Reverse Transcriptase (Invitrogen, Waltham, MA, USA). Real time polymerase chain reaction (RT-PCR) analysis was performed in a SYBR Green mixture containing cDNA and 200 nmol/L of specific primers for 35 cycles, with 30 s each step of 94°C, 55°C, and 72 °C using an Opticon2 thermocycler (MJ Research Inc., MA, USA). Experiments were run in duplicate and expression levels of each mRNA were analysed using Opticon Monitor Analysis Software (MJ Research Inc., MA, USA) and normalized against the β -actin content. Representative quantification graphs were plotted using the relative fluorescence unit versus the PCR amplification cycle. The primer sequences used were:

NPR-A: forward: 5'-TTCAGGCTGCCAAAATTATTACTT-3'

reverse: 5'-GGAAAAGTGGTCTTGGTTGCAGGCT-3'

NPR-C: forward: 5'-TGACACCATTCCGAGAATCA-3'

reverse: 5'-ACCTCCATGGAAAACCTTCTC-3'

β -actin: forward: 5'-CCAACCGTGAAAAGATGACC-3'

reverse: 5'-TCTTCATGAGGTAGTCTGTC-3'

Statistical analysis

All values were expressed as mean $\pm$ SEM. Data was processed using InfoStat/L Software (Córdoba, Argentina). Gaussian distribution was evaluated by the Kolmogorov and Smirnov method and comparisons between groups were performed using two-way ANOVA followed by a Tukey-Kramer test. A value of $p < 0.05$ was considered statistically significant.

Results

Evolution of chronic hypertension

After 2, 6, and 12 weeks of treatment, both DS and RV groups showed significantly higher SBP levels with respect to the C group. At weeks 6 and 12, SBP values were significantly higher compared to week 2 for each experimental group. There were no differences between the DS and RV groups at the three time points evaluated. The C group maintained normal SBP values throughout the study (Table 1).

Table 1
Variation of plasma ANP and BNP levels

SBP (mmHg)	2 wk	6 wk	12 wk
C	128 ± 3	118 ± 2	121 ± 1
DS	153 ± 4*	194 ± 2*&	193 ± 4*&
RV	164 ± 5*	184 ± 2*&	203 ± 4*&
ANP (pg/mL)			
C	72 ± 14	170 ± 68	87 ± 39
DS	174 ± 27*	689 ± 143***	609 ± 38***
RV	169 ± 30*	387 ± 90*#	294 ± 57*#
BNP (pg/mL)			
C	43.3 ± 7.9	38.9 ± 6.2	41.9 ± 7.2
DS	57.9 ± 7.9	55.3 ± 9.2	53.9 ± 5.9
RV	54.4 ± 6.4	99.2 ± 8.0**##	94.4 ± 9.6**##
ANP: Atrial Natriuretic Peptide; BNP: B-type Natriuretic Peptide. C: Control; DS: DOCA-Salt; RV: Renovascular; SBP: systolic blood pressure; wk: week. *p < 0.05; **p < 0.01; p < 0.001 versus control group; #p < 0.05 versus DS group at the same time point; &p < 0.05 versus DS or RV at 2 weeks of treatment, respectively; n = 6.			

Variation of plasma levels of ANP and BNP

We measured circulating levels of ANP and BNP as indicators of cardiac hormone secretion. ANP plasma levels significantly increased in both DS and RV groups compared to the C group at weeks 2, 6, and 12, with a higher magnitude in the DS group (~ 3-fold) than in the RV group with respect to the C group at weeks 6 and 12. In fact, ANP plasma levels in the DS group were significantly higher than in the RV group at these two time points.

In contrast, there were no significant differences in BNP plasma levels among the three experimental groups in week 2. At weeks 6 and 12, BNP levels were significantly higher in RV group compared to both the DS and C groups (Table 1).

Natriuretic peptide receptors NPR-A and NPR-C in renal tissues

We evaluated the tissue distribution and the gene and protein expression of NP receptors in the kidney, a major organ involved in NP function and clearance. We did not observe changes in the expression pattern of NPR-A between groups at the earliest timepoint (Week 2); however, we observed markedly increased immunostaining for NPR-A in the proximal tubules at 6 weeks and 12 weeks in DS treatment, while NPR-A in the RV groups remained similar to the control, and even decreased at 6 weeks (Fig. 1A). These changes can be explained at least in part by increased *Npra* mRNA expression elicited by DS, except at week 2 where the mRNA upregulation does not seem to correlate with the protein expression in the tissue (Fig. 1C, **left panel**)

There were no significant changes in the expression pattern of the clearance receptor NPR-C at week 2 among the experimental groups. However, after 6 and 12 weeks the DS group showed significantly increased levels of renal NPR-C respect to the C and RV groups. (Fig. 1B). Furthermore, the DS group showed renal *Nprc* mRNA expression levels significantly higher compared to the RV and C groups at weeks 2, 6, and 12 (Fig. 1C, **right panel**).

Kidney inflammation and fibrosis

We addressed the expression of the proinflammatory cytokine IL-6, the transcription factor NF- κ B and TNF- α as indicators of the hypertension-associated renal inflammatory process. At the three time points evaluated, NF- κ B was significantly higher in both the DS and RV groups with respect to the C, with the DS group showing significantly higher NF- κ B immunoexpression levels compared to the RV group (Fig. 2, **panel A**). IL-6 immunoexpression was similar at week 2 among the three experimental groups, while at weeks 6 and 12, both the DS and RV groups presented significantly higher IL-6 levels compared to the C group, with higher levels in RV than in DS group. (Fig. 2, **panel B**).

At week 2, no significant changes were found in TNF- α immunoexpression levels among the experimental groups. At week 6, DS showed a significant increase in TNF- α expression level compared to both RV and C groups. By week 12, both RV and DS showed a ~ 20-fold increase in TNF- α expression respect to the C group (Fig. 3, **panel A**).

Finally, we addressed the profibrotic marker TGF- β 1 as an indicator of target organ damage. Interstitial TGF- β 1 expression was prominent in the DS group throughout the course of the treatment compared to C, while it was only significantly increased in RV after 12 weeks (Fig. 3, **panel B**).

Vascular inflammation and fibrosis

Next, we addressed inflammation and fibrosis in the vasculatures. The expression pattern of IL-6 in thoracic aorta showed no changes at week 2 among the experimental groups, a higher immunoexpression level in the DS group with respect to the C and RV groups at week 6, and significantly higher levels of IL-6 at week 12 in both the DS and RV groups compared to the C group (Fig. 4, **panel A**). Meanwhile, NT immunoexpression levels were significantly higher in the DS and RV groups with respect to the C group at week 12, with higher expression in the DS group compared to RV group (Fig. 4, **panel B**). Finally, TNF- α notably increased about 30-fold after 6 weeks of DS. At week 12, immunoexpression

levels of TNF- α in thoracic aorta was significantly higher in DS and RV groups compared to the C group (Figs. 5, **panel A**).

The profibrotic factor TGF- β 1 significantly increased in the vascular wall after 6 and 12 weeks of DS compared to C, while in RV treatment TGF- β 1 was only evident following 12 weeks of treatment. (Fig. 5, **panel B**).

Discussion

Hypertension is a major risk factor for cardiovascular mortality and morbidity through its effects on target organs like the heart, kidney, brain and blood vessels. Remodelling of the heart in response to persistent hemodynamic overload can lead to a spectrum of geometric, functional and hormonal patterns [30]. In the present study, we studied chronic DS and RV hypertension models caused primarily by volume and pressure overload respectively. Our results indicate that, in association with similarly elevated blood pressure, volume overload was linked to earlier target organ damage. These findings are associative and hypothesis-generating rather than mechanistic.

DOCA is an aldosterone analogue that promotes water and sodium reabsorption in the kidney, resembling primary aldosteronism in humans [31, 32]. In contrast, RV hypertension (1K-1C) is characterized by pressure overload predominancy comparable to renal arterial stenosis in humans. In both DS and RV models, we found a time-dependent blood pressure increase, with differences between week 2 versus weeks 6 and 12 of treatment.

We found a significant increase of ANP plasmatic levels in early stages in the DS model ($\approx$ 3 times more than the control group at weeks 6 and 12). This increase reflects the atrial distension induced by the expansion of extracellular volume [33] and acts as a compensatory attempt with natriuretic, diuretic, vasodilatory and anti-inflammatory effects [34].

Renal overexpression of NPR-A and NPR-C receptors was also observed, both at the mRNA and protein levels, especially at 6 and 12 weeks, suggesting activation of the ANP/NPR system as an adaptive mechanism against sodium and water overload. ANP binding to NPR-A, induces conformational change in the receptor triggers signalling pathways that stimulate diuretic, natriuretic, antimitogenic, and anti-inflammatory effects [34–36]. The higher increase of renal NPR-A protein expression levels in shorter periods of time (6 weeks rather than 12 weeks), could be attributed to a protective mechanism against the renal inflammatory state existing in this model [37]. However, this activation did not prevent the progressive development of renal and vascular inflammation and fibrosis, indicating that the ANP system was insufficient to counteract the damage induced by salt and mineralocorticoid overload.

No significant changes were found in this model with respect to BNP plasma levels over time, confirming that BNP does not respond primarily to volume, but rather to pressure overload [38].

In the RV model, ANP plasma levels increased moderately at 6 and 12 weeks, but to a lesser extent than in the DOCA-Salt model. This increase reflects a secondary contribution of ANP, linked more to ventricular hypertrophy and stress than to volume expansion [43]. BNP plasma levels, however, increased significantly from week 6 (≈ 2.5 times higher than control and DS). This elevation is typical of situations of pressure overload and cardiac mechanical stress, acting as a sensitive marker of ventricular dysfunction [43].

No significant overexpression of renal NPR-A or NPR-C receptors was observed (even a transient decrease in NPR-A at 6 weeks), suggesting less direct renal involvement of the natriuretic peptide system in this model. We also observed some discrepancies between mRNA and protein expression of NPR receptors, particularly at early time points. These differences may reflect post-transcriptional regulation, protein turnover, or methodological sensitivity, and should be considered when interpreting receptor dynamics.

Initially regarded solely as a natriuretic peptide (NP) clearance receptor, NPR-C is now recognized to exert additional biological functions [37, 49]. In the DS model, changes in *Nprc* mRNA expression exhibited a pattern similar to that observed for NPR-A. Previous studies suggested that atrial natriuretic peptide (ANP) may exert either pro-oxidant or antioxidant effects depending on the cellular context [35]. In this regard, Costa et al. proposed that ANP stimulates nitric oxide synthase (NOS) activity via NPR-C in the kidney. It has been demonstrated that the concomitant presence of reactive oxygen species (ROS) and increased NOS activity promotes the formation of cytotoxic peroxynitrites, which not only reduce nitric oxide (NO) bioavailability but also exacerbate oxidative stress [36]. A plausible explanation for the NPR-C expression levels observed in the DS model is that, in the kidney, NPR-C may enhance NO production as a protective response against vasoconstriction and renal inflammation.

Regarding the RV model, Tan et al. reported that, in this hypertensive setting, atrial ANP secretion was reduced, whereas ANP mRNA expression was increased in the left ventricle. They further proposed that the elevation in plasma ANP concentrations resulted from enhanced release at ventricular and extra-atrial sites [40]. These findings provide a plausible explanation for the increased plasma ANP levels observed at both weeks 6 and 12 compared with the C group. In addition, an inverse relationship between ANP mRNA levels and NPR-C expression was described: higher ANP mRNA expression was associated with reduced NPR-C expression and, consequently, diminished ANP clearance. As these alterations were accompanied by upregulation of the AT1 receptor and downregulation of the AT2 receptor, the intracardiac effects of ANG II were further amplified. Notably, administration of losartan, an AT1 receptor antagonist, increased atrial ANP release and reversed the effects on NPR-C expression [40].

Complementarily, Jansen et al. investigated NPR-C knockout mice to elucidate the role of this receptor in susceptibility to atrial fibrillation under conditions of elevated ANG II [39]. They demonstrated that ANG II increased atrial fibrillation susceptibility, an effect that was further exacerbated in ANG II-treated NPR-C^{-/-} mice. Similar findings were observed for ANG II-induced atrial fibrosis and the expression of

profibrotic genes, including TGF- β 1 and tissue inhibitor of metalloproteinase-1. Importantly, both atrial fibrillation inducibility and fibrosis were prevented in wild-type mice co-treated with ANG II and a dose-dependent NPR-C agonist (cANF), a selective NPR-C ligand. Collectively, these results underscore a critical protective role of NPR-C in attenuating the pathological actions of ANG II under physiological conditions [39].

In line with these observations, several studies support an antioxidant role for ANP. McLay et al. demonstrated that ANP inhibits inducible nitric oxide synthase (iNOS) activity in proximal tubular cell cultures through NPR-C activation and cGMP-dependent signalling mechanisms [41, 42]. Similarly, Chatterjee et al. showed that, in human proximal tubular cells, ANP attenuates cytokine-induced nitric oxide synthesis via a cGMP-independent pathway mediated by the NPR-C receptor subtype [44]. NPR-C has also been reported to mediate ANP-induced nitric oxide synthase activation in the kidney, underscoring the complexity and context-dependent nature of NPR-C-mediated ANP signalling [36]. We have previously demonstrated that both acute sodium overload and chronic high-salt intake can induce hypertension by enhancing proximal tubular sodium reabsorption, thereby generating relative renal hypoxia through overexpression of renal hypoxia-inducible factor (HIF)- α and ANP as adaptive responses. In this context, the hypertensive arm of the local renin-angiotensin-aldosterone system (RAAS) was activated, as evidenced by increased ANG II levels, AT1 receptor expression [18], and NF- κ B activation [44]. Conversely, vasodepressor and anti-inflammatory pathways were suppressed, including the ACE2-Ang-(1-7)-Mas receptor axis and AT2 receptor expression [18], together with reduced renal endothelial nitric oxide synthase expression [44]. These alterations were associated with increased release of proinflammatory cytokines and chemokines, such as RANTES and TGF- β 1, promotion of fibrotic processes (α -smooth muscle actin expression), and enhanced oxidative stress through NADPH oxidase activation [45, 46].

Consistent with previous reports, aldosterone stimulates sodium and water reabsorption, potassium secretion into the tubular lumen, and proton excretion, processes that are accompanied by increased activity of the Na⁺/K⁺-ATPase pump [47, 48]. Given the high energetic demand of these transport mechanisms and the sustained nature of the stimulus in the DOCA-salt (DS) model—characterized by weekly DOCA administration and high NaCl intake—renal ATP synthesis is markedly increased, resulting in elevated oxygen consumption. Over time, this imbalance leads to relative hypoxia and increased generation of reactive oxygen species (ROS), which in turn promotes NF- κ B activation [49]. Upon nuclear translocation, NF- κ B enhances the transcription of proinflammatory genes encoding TNF- α , IL-6, IFN- γ , IL-2, and IL-8, thereby establishing a self-amplifying inflammatory loop. In addition, ROS can activate mitochondrial uncoupling protein 2, further impairing renal oxygen efficiency and exacerbating tissue hypoxia [50].

Accumulating experimental evidence indicates that hypoxia itself acts as an independent regulator of natriuretic peptide synthesis and release. In this regard, Della Penna et al. proposed an adaptive response to oxidative stress in the kidneys of rats chronically exposed to a high-sodium diet, linking increased HIF-1 α expression with elevated intrarenal ANP levels, a mechanism that may ultimately

aggravate the cellular milieu [44]. Moreover, under conditions of saline overload, exacerbation of RAAS activity has been closely associated with renal hypoxia, largely driven by increased tubular sodium transport [34, 44, 48]. These observations are consistent with the elevated expression of inflammatory markers (TNF- α , IL-6, and NF- κ B) and the profibrotic marker TGF- β 1 in both renal tissue and vasculature observed in the DS model at weeks 6 and 12. Importantly, these biomarkers are known to be dysregulated in a wide range of pathologies, even at early stages [51].

In parallel, vascular nitrotyrosine, an oxidative stress biomarker dependent on nitric oxide availability as a product of tyrosine nitration, has been reported to increase in various tissues under pathological conditions because of excessive ROS production [52]. In both experimental models, vascular nitrotyrosine expression was significantly increased at week 12 compared with the control group, indicating a pronounced pro-oxidative vascular state. In the RV model, the reduction in renal plasma flow resulting from inadequate oxygen and nutrient delivery to the renal parenchyma further promotes renal hypoxia and ROS generation, leading to increased NF- κ B expression and activity and subsequent amplification of inflammatory and fibrotic responses [34]. Accordingly, enhanced renal expression of IL-6 and NF- κ B from week 6 onward, as well as increased renal and vascular TNF- α and TGF- β 1 and vascular IL-6 expression, were observed in the RV model relative to control animals.

In summary, ANP acts as the main marker and modulator in volume-induced hypertension, with early and sustained compensatory activation that attempts to limit renal and vascular damage, although it ultimately proves insufficient. On the other hand, BNP, more than ANP, reflects the systemic pressure stress inherent in the renovascular model where it acts as a marker of pressure overload and progressive cardiac damage. Its elevation could be related to the onset of cardiac and vascular damage, rather than to a renal attempt at compensation.

Transcription of both natriuretic peptide receptors was increased exclusively in the DS model and showed a positive correlation with plasma ANP levels. In addition, several inflammatory and oxidative biomarkers exhibited an earlier increase in the kidney and thoracic aorta in the DS model compared with the RV model, suggesting that volume overload triggers inflammatory responses earlier than pressure overload. Collectively, the findings of the present study indicate that, beyond elevated blood pressure per se, the specific pathophysiological mechanisms underlying arterial hypertension play a critical role in the development of target organ damage. Accordingly, a more detailed understanding of the temporal sequence of events leading to irreversible renal and vascular injury may provide a rational framework for the identification of novel therapeutic targets and the development of more effective intervention strategies. Limitations of this study include the descriptive and correlational design, the use of tail-cuff measurements for systolic blood pressure, and the absence of direct functional assays to confirm mechanistic roles of ANP/BNP and NPR receptors. Therefore, the conclusions should be interpreted as hypothesis-generating.

Abbreviations

ANG II
angiotensin II
ANP
atrial natriuretic peptide
BNP
brain natriuretic peptide
cGMP
cyclic GMP
CNP
C-type natriuretic peptide
DOCA
deoxycorticosterone acetate
DS
DOCA-Salt
ET-1
endothelin-1
IL-6
interleukin 6
IOD
intensity of optical density
1K-1C
1 kidney – 1 clip
NF-kB
nuclear factor kappa beta
NP
natriuretic peptide
natriuretic peptide receptor
NPR
NT
nitrotyrosines
RAAS
renin-angiotensin-aldosterone system
ROS
reactive oxygen species
RT-PCR
real time polymerase chain reaction
RV
renovascular
SBP
systolic blood pressure

SEM
standard error of the media
TGF- β 1
transforming growth factor beta1
TNF- α
tumor necrosis factor alpha.

Declarations

Founding

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Conflict of Interest

The authors declare no conflict of interest exists in connection with this article.

Author Contribution

N.M.K. performed the experiments, analysed the results and wrote the manuscript. M.I.R. performed the immunohistochemistry and analysed the representative images. P.D.P., B.E.F. and S.C. analysed the results and collaborated with the manuscript writing and revision. M.R.C. planned and directed the project, obtained the financial funds, and revised manuscript writing.

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Figures

Figure 1

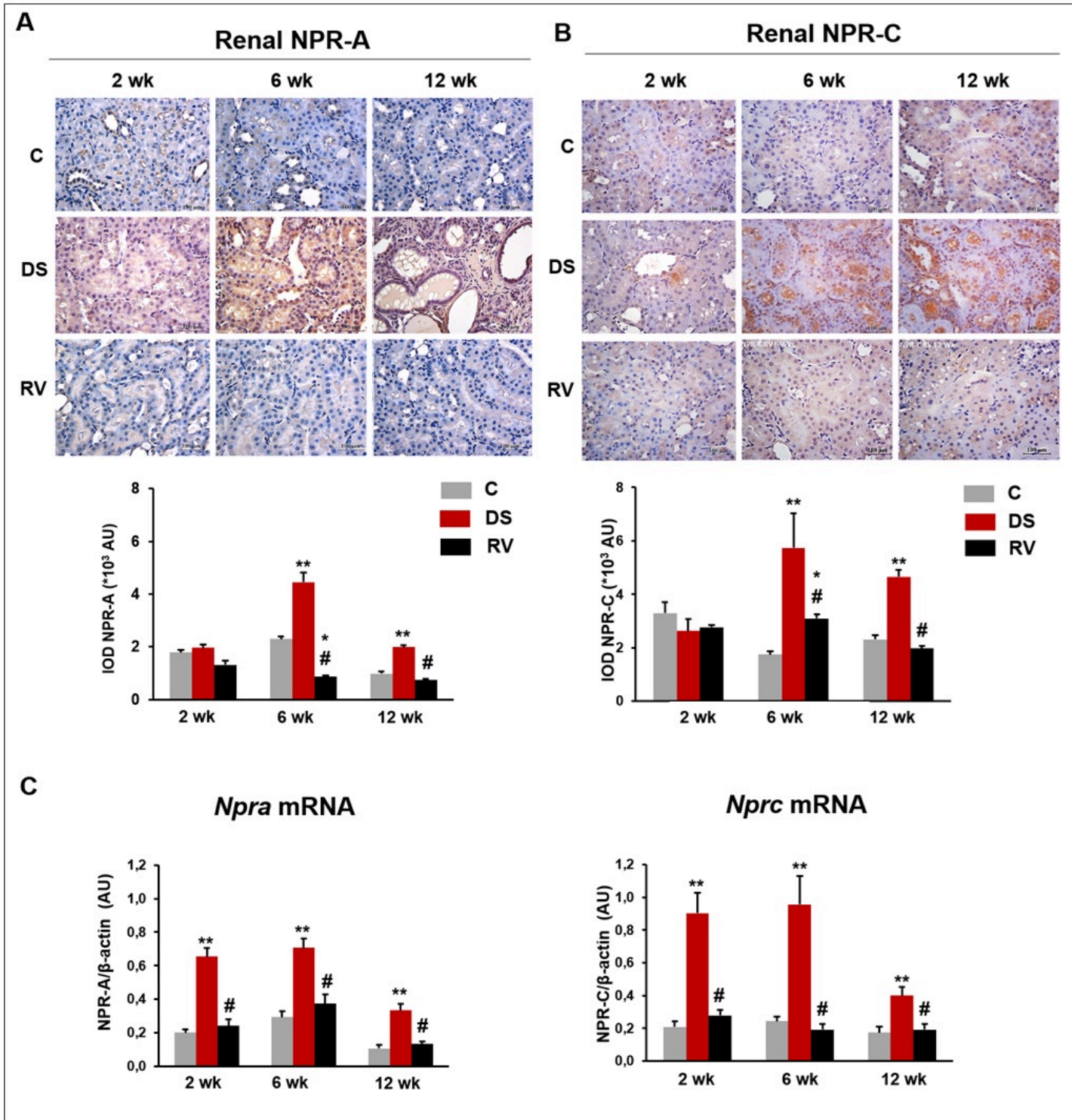


Figure 1

Renal expression of natriuretic peptide receptors. (A) Representative images of immunostaining of NPR-A in kidney sections after 2, 6 and 12 weeks of treatment. (B) Representative images of NPR-C. Images were obtained at 200X magnification. Quantitative results for each marker are shown in the lower panels. (C) mRNA expression (qPCR) of *Npra* and *Nprc* in the kidney. * $p < 0.05$; ** $p < 0.01$ versus control group; # $p < 0.05$ versus DS group at the same time-point; $n = 6$. AU, arbitrary units; C, control

group; DS: DOCA-Salt group; IOD, intensity of optical density; NPR-A, natriuretic peptide receptor A; NPR-C, natriuretic peptide receptor C; RV, renovascular group; wk, week.

Figure 2

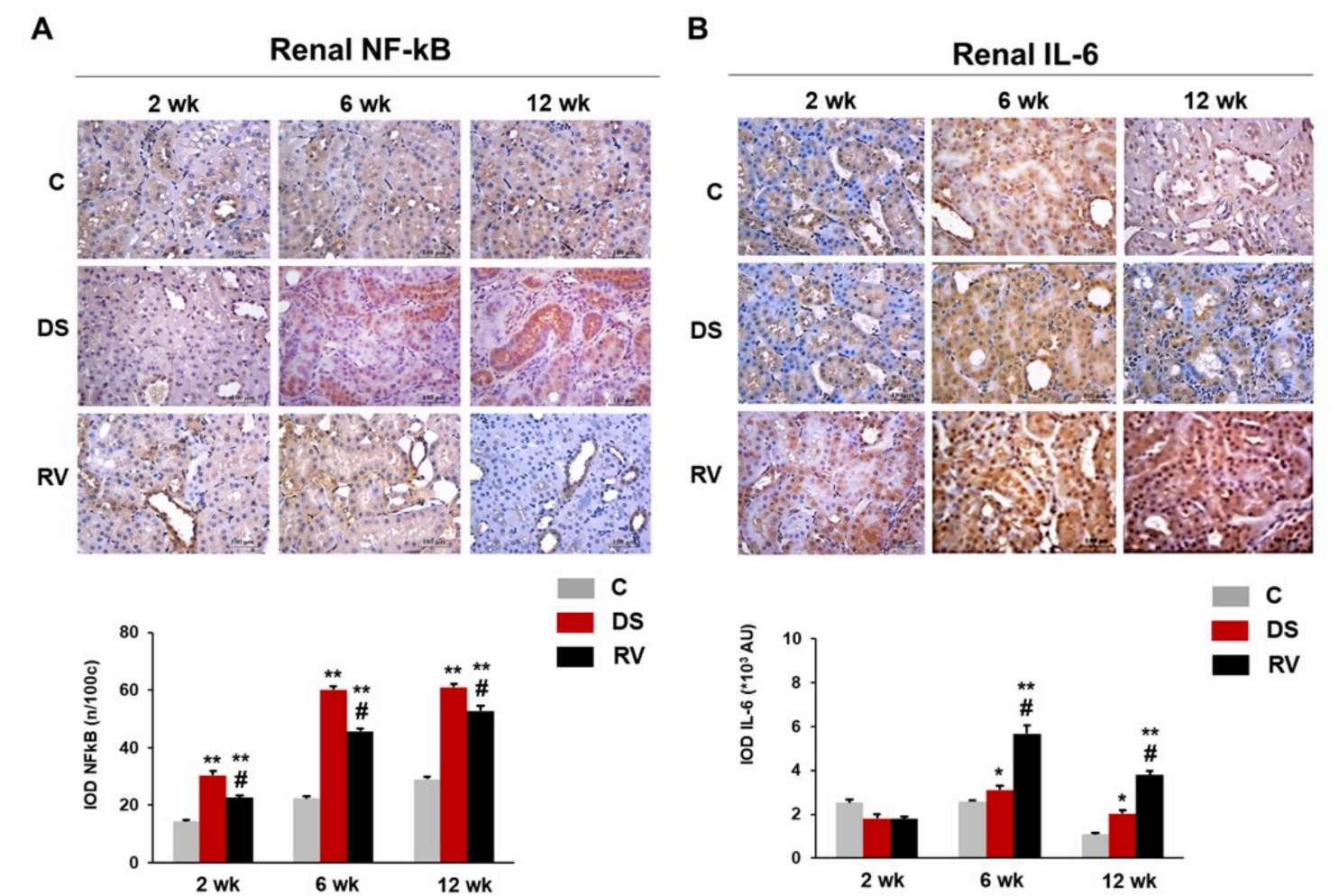


Figure 2

Renal inflammatory markers. (A) Representative images of immunostaining of IL-6 in kidney sections after 2, 6 and 12 weeks of treatment. **(B)** Representative images of NF-κβ. Images were obtained at 200X magnification. Quantitative results for each marker are shown in the lower panels. *p < 0.05; **p < 0.01 versus control group; #p < 0.05 versus DS group at the same time-point; n = 6. C, control group; DS: DOCA-Salt group; IL-6, interleukin 6; IOD, intensity of optical density; NF-κβ, nuclear factor kappa beta; RV, renovascular group; wk, week.

Figure 3

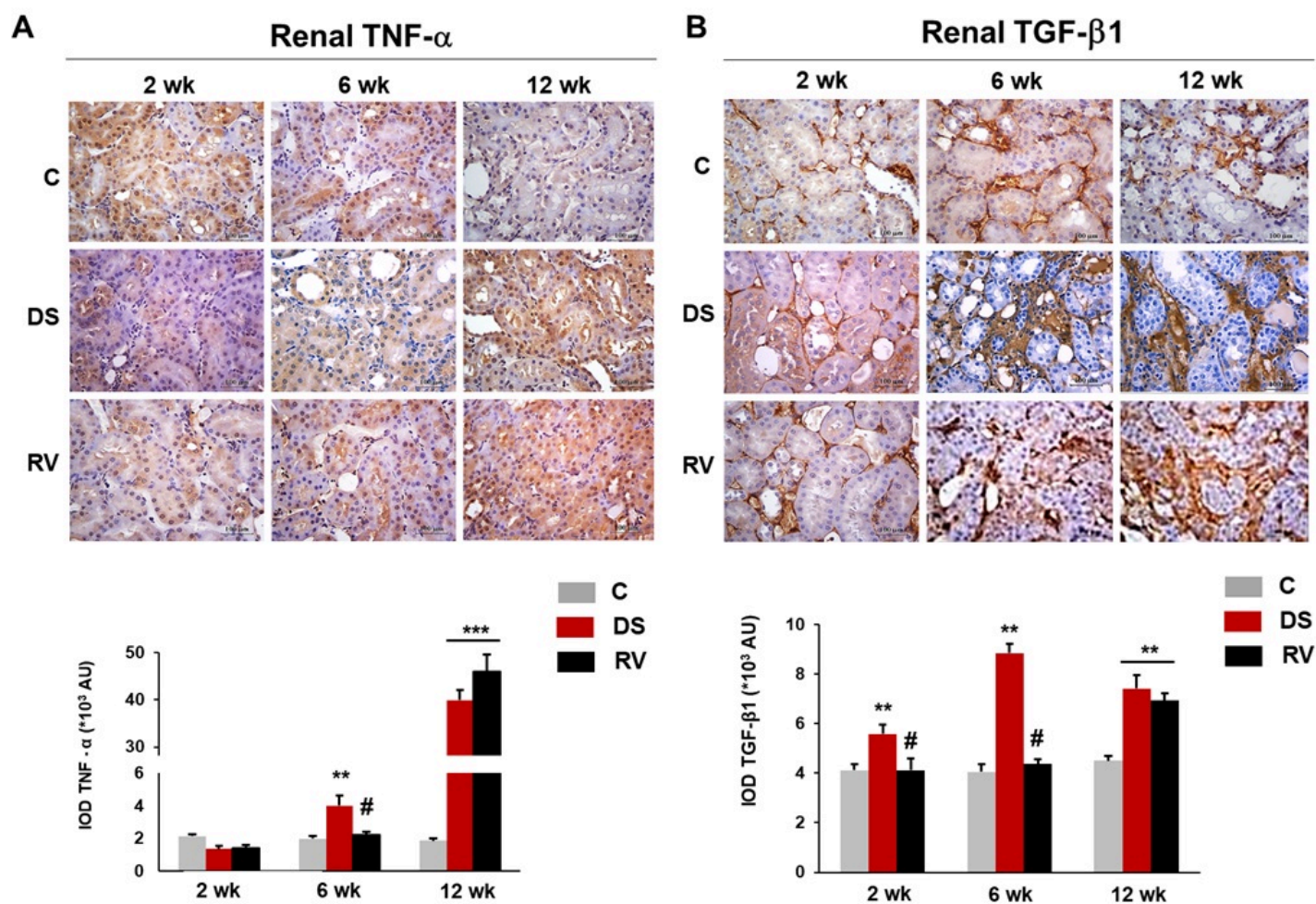


Figure 3

Renal fibrosis markers. (A) Representative images of immunostaining of TNF- α in kidney sections after 2, 6 and 12 weeks of treatment. **(B)** Representative images of TGF- β 1. Images were obtained at 200X magnification. Quantitative results for each marker are shown in the lower panels. **p < 0.01; ***p < 0.001 versus control group; #p < 0.05 versus DS group at the same time-point; n = 6. AU, arbitrary units; C, control group; DS: DOCA-Salt group; IOD, intensity of optical density; RV, renovascular group; TGF- β 1, transforming growth factor beta 1; TNF- α , tumour necrosis factor alpha; wk, week.

Figure 4

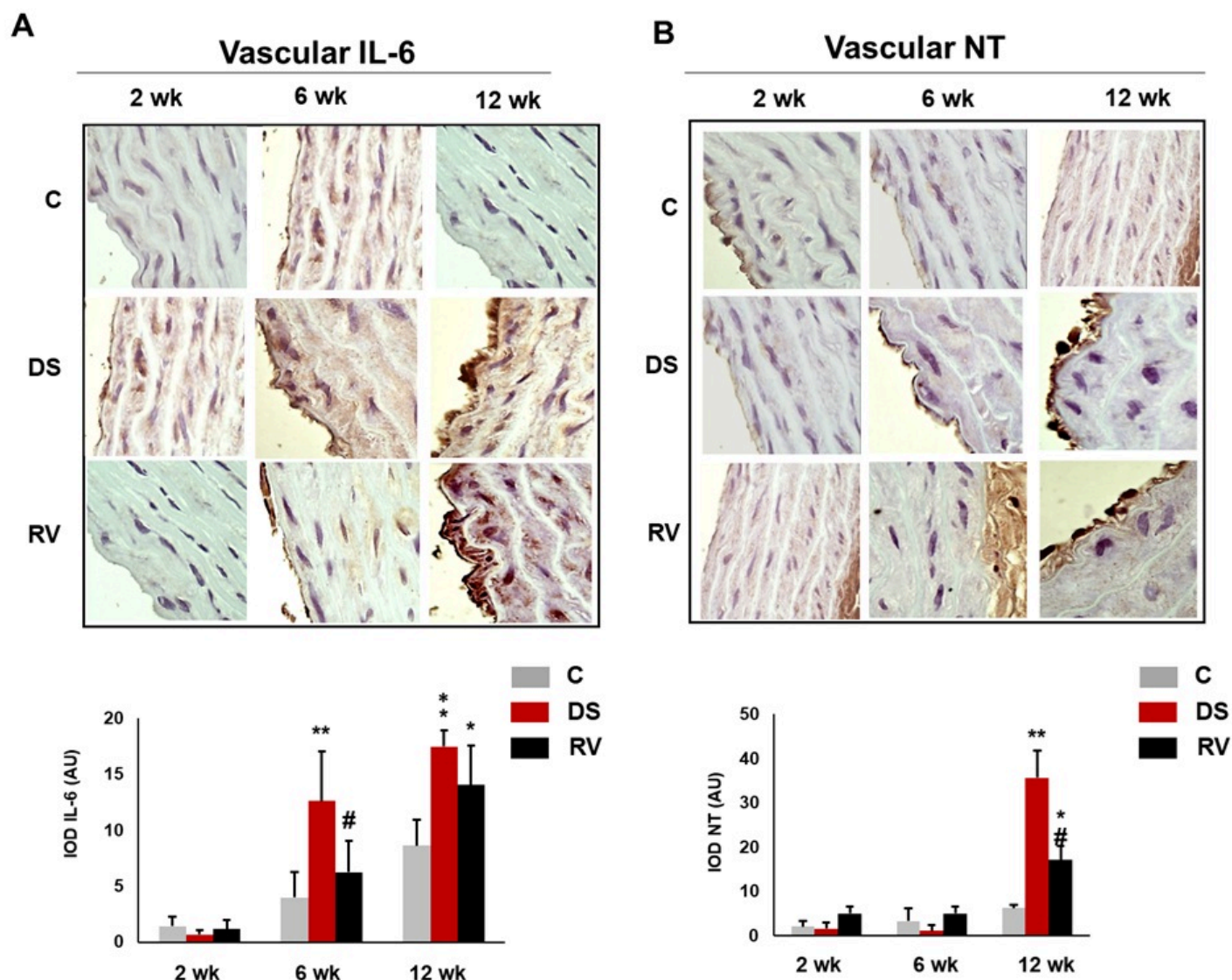


Figure 4

Vascular inflammatory markers. (A) Representative images of immunostaining of IL-6 in kidney sections after 2, 6 and 12 weeks of treatment. (B) Representative images of nitrotyrosines. Images were obtained at 200X magnification. Quantitative results for each marker are shown in the lower panels. * $p < 0.05$; ** $p < 0.01$ versus control group; # $p < 0.05$ versus DS group at the same time-point; $n = 6$. AU, arbitrary units; C, control group; DS: DOCA-Salt group; IOD, intensity of optical density; NT, nitrotyrosines; RV, renovascular group; wk, week.

Figure 5

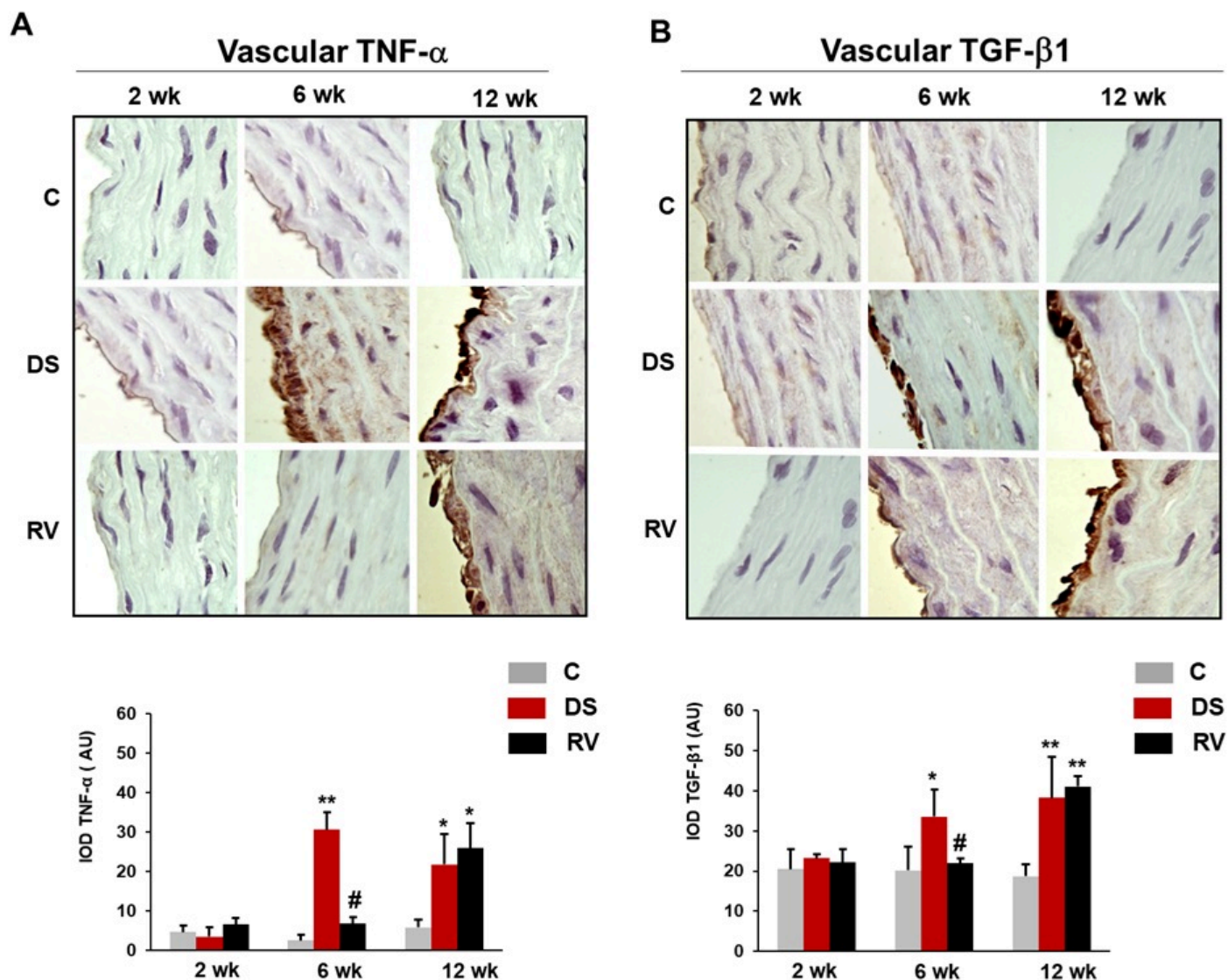


Figure 5

Vascular fibrosis markers. (A) Representative images of immunostaining of TNF- α in transversal sections of the abdominal aorta after 2, 6 and 12 weeks of treatment. (B) Representative images of TGF- β 1. Images were obtained at 200X magnification. Quantitative results for each marker are shown in the lower panels. * $p < 0.05$; ** $p < 0.01$ versus respective control group; # $p < 0.05$ versus DS group at the same time-point; $n = 6$. AU, arbitrary units; C, control group; DS, DOCA-Salt group; IOD, intensity of optical density; RV, renovascular group; TGF- β 1, transforming growth factor beta 1; wk, week.