ZOFENOPRIL FUNCTIONS AS ANTIOXIDANT, CORRECTING THE RENAL OXIDATIVE DAMAGES IN A RAT MODEL OF L-NAME INDUCED HYPERTENSION

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Summary

Hypertension (HT) is a major contributor to the development of renal failure, mainly due to increased oxidative stress. The causative relationship between raised blood pressure and overproduction of reactive oxygen species (ROS) is still controversial. Growing evidence attributes to NADP(H) oxidase the main role in ROS production in the renal cortex, while the angiotensin conversion enzyme (ACE) inhibitor, Zofenopril, is known to exert protective effects in kidney ischemia/reperfusion injury. In this study we approached understanding the biochemical background of imbalance between activation of pro-oxidant enzymes and decreased scavenging of ROS in HT, an issue with still many gaps in perception. With this goal we investigated the effects of Zofenopril on kidney cortex in an experimental model of Nω-Nitro-L-Arginine Methyl Ester (L-NAME) induced HT in rats. Animals were divided in 3 groups: HT group (receiving orally 50mg/b.w./day L-NAME, for 6 weeks), HTZ group (HT, receiving orally 15 mg/b.w./day Zofenopril, for 4 weeks), and controls (age-matched C group). Compared to C group, the HT group shows increased blood pressure and serum ACE activity, modification of the kidney cortex normal structure (consisting in tubular atrophy, interstitial fibrosis, obstructive hyalinosis of the renal tubules, effacement of foot processes, mesangial matrix expansion, and reduced number of glomerular filtration slits), biochemical changes indicative for installment of oxidative stress (enhanced NADP(H) oxidase activity, augmented protein expression of p47\textsuperscript{phox}, p67\textsuperscript{phox} subunits of the NADP(H) oxidase, and of p50, and p65 NFκB subunits, and increased protein carbonylation), and reduced antioxidant defense (altered GSH to GSSG ratio and diminished GPx activity). Compared to HT group, the HTZ group shows improvement of structural modifications, along with biochemical changes that demonstrate Zofenopril antioxidant potential. These consist in decreased ROS production and protein expression of NADP(H) oxidase subunits, reduced concentration of protein carbonyls, and activation of the antioxidant defense (improved GSH to GSSG ratio and GPx activity). The original results here reported sustain the renoprotective, antioxidant effect of Zofenopril in the experimental model of L-NAME-induced HT.

Key words: kidney, reactive oxygen species, glutathione, protein carbonyls, NADP(H) oxidase, NFκB

Introduction

Hypertension (HT) and cardiovascular disease (CV) are the most common causes of death in developed countries. In Romania, a survey for CV risk factors (SEPHAR) involving 2017 subjects aged over 18 years showed that more than 40% of these persons have essential hypertension (Dorobantu et al., 2008). The kidneys play a critical role in keeping a person's blood pressure in a healthy range, while high blood pressure may lead to chronic kidney disease.

Nitric oxide synthase (NOS) catalyzes the formation of nitric oxide (NO), a small size gaseous molecule, which plays an important role in the control of vascular and renal function. The endothelial NOS (eNOS) is abundantly expressed in renal vascular endothelium (Touyz, 2011). Uncoupling of eNOS has been evidenced as a contributor of increased oxidative stress in HT rats (Schulz et al., 2008). The oxidative modification of
tetrahydrobiopterin (BH4) to dihydrobiopterin (BH2) by reactive oxygen species (ROS) (Schulz et al., 2008) or by peroxynitrites (·ONOO⁻) (Zou et al., 2002) directs NOS to produce the superoxide anion \( \text{O}_2^- \) rather than NO. Inhibition of NOS activity by \( \text{N}^\omega\text{-Nitro-L-arginine methyl ester} \) (L-NAME) in experimental animals (Sharma et al., 2010) or human subjects has been shown to induce vasoconstriction, arterial HT, tubular, and glomerular lesions, and reduction of renal function (Bevan, et al., 2011) mainly due to the impaired NO signaling (Paulis et al., 2008).

ROS has been proposed as a key mediator of the progression of renal injury associated with essential HT (Shah et al., 2007). The kidney and its vasculature are rich sources of NADP(H) oxidase-derived ROS, contributing to the increased oxidative stress and associated oxidative damages. ROS - induced oxidative modification of proteins contributes to activation of redox-sensitive signaling pathways, such as mitogen-activated protein kinases, tyrosine kinases, and transcription factors (NFkB, AP-1, and HIF-1) (Touyz et al., 2011). Changes in the intracellular redox state through thioredoxin and glutathione systems may also influence signaling events (Chiarugi et al., 2003). Increased production of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) due to an imbalance between prooxidants and antioxidants, and reduced NO synthesis are mainly involved in renovascular injury in HT. Among the defense systems operating against the ROS, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the most important antioxidant enzymes (Shou et al., 1997).

ACE activity regulates renin-angiotensin system. ACE inhibitors block the conversion of angiotensin I to angiotensin II, leading to decreased blood pressure (Dzau et al., 2005). Based on their chemical structure, ACE inhibitors are sulfhydryl-containing agents (such as Captopril and Zofenopril), dicarboxylate-containing agents (such as Enalapril, Ramipril, Quinapril, Perindopril, Lisinopril, Zofenopril, Trandolapril), and phosphonate-containing agents (e.g. Fosinopril). An interesting member is Zofenopril, which was found having a 5-fold more potent anti-hypertensive effect than Captopril (Napoli et al., 2004). Evangelista and Manzini (2005) showed that Zofenopril enhances NO production in endothelial cells, attenuates atherosclerotic lesion development, and inhibits adhesion molecule expression by reducing ROS generation. This study was designed to decipher the molecular events associated with Zofenopril effects in an experimental model L-NAME induced HT.

**Materials and methods**

The enzymatic kits were purchased from Dialab (Austria), non fat dry milk and the antibodies were from Santa Cruz Biotechnology (Germany), and the nitrocellulose membrane was acquired from Bio-Rad Laboratories. All other chemicals were purchased from Sigma-Aldrich (Germany).

**Animal model**

Male Wistar rats (280±30g b.w.) were randomly assigned to one of the three groups: **hypertensive group** (HT, \( n = 7 \), receiving L-NAME 50 mg/ b.w./day in drinking water for 6 weeks), **hypertensive group receiving Zofenopril** (HTZ, \( n = 7 \), receiving L-NAME as above, plus Zofenopril 15mg/ b.w./day in the last 4 weeks of L-NAME administration), and **control group** (C, \( n = 7 \), age-matched rats receiving vehicle only for 6 weeks). Animals were housed at constant room temperature (22°C), on a 12 h light/12 h dark cycle, with free access to standard rodent chow. All the protocols were approved by the ethics committee of the Institute of Cellular Biology and Pathology „Nicolae Simionescu”, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no 85-23, revised 1996).
Also, the experiments were performed according to the procedures defined in the European Council Directive 2010/63/EU. On the day of experiments mean blood pressure, and heart rate were measured invasively with a piezo-resistive transducer (MLT844 Physiological Pressure Transducer, AD Instruments, Germany). The sensor connected to a Bridge Amplifier (ML110/D) was used to detect and convert heart pulses to electric signals. The outputs signals were interfaced to a data acquisition system (PowerLab ML 750/4s analog to digital converter, AD Instruments, Germany). Pressure recordings were analyzed with Chart 5 software for Windows.

**Biochemical parameters of blood serum**

After blood pressure recording, blood was collected, spun down (400xg, 10 min, 4°C), and the supernatant was kept for subsequent assays. The glucose, cholesterol, triglycerides, and creatinine concentrations were assayed with enzymatic kits from Dialab, in accordance with the manufacturer protocol. A fluorimetric method using hippuril-hystidil-leucine as substrate was used to measure tissue angiotensin-converting enzyme (ACE) activity. The fluorescence of the o-phthaldialdehyde-His-Leu adduct was measured (Ex: 365nm, Em: 495nm) (Oliveira et al., 2000).

**Histologic examination of the kidneys**

Paraffin embedded kidneys from each group (fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4), were cut into 7 µm sections and used for histological staining (Masson’s Trichrome).

**Ultrastructural examination of renal cortex**

The rat kidneys from each group were fixed for 90 min. in Karnovski’s fixative, followed by 1.5 hours post-fixation (at 4°C) in 1% osmium tetroxide, dehydrated in serial concentrations of ethanol (70%, 90%, and 100%), and embedded in Epon 812 resin. Ultrathin sections (60 – 90 µm) were stained with uranyl acetate (1 min), and lead citrate (3 min). The ultrastructural alterations of the kidneys were visualized using the TECNAI Spirit Biotwin transmission electron microscope (FEI Company, The Netherlands), with Mega View III CCD Camera (Olympus, Germany).

**Western blotting**

The kidneys were removed, and the cortex was isolated, homogenized in Rippa buffer, spun down (16000 x g, 4°C, 10 min.), and the supernatants saved as renal cortex lysates. The protein concentration was assessed using Amidoblock reagent (Sigma). 60 µg of kidney homogenates were subjected to 10% SDS-PAGE electrophoresis, and the separated proteins were electroblotted onto nitrocellulose membrane. The relevant proteins were evidenced after overnight incubation with primary antibody (rabbit anti-NADPH oxidase subunits, and rabbit anti-NFkB subunits), followed by the secondary antibody (anti-rabbit IgG conjugated with HRP), and detected using ECL kit (Amersham Biosciences). The quantification of resulted bands was performed with the TotalLab Quant software (Nonlinear Dynamics, UK), and reported as arbitrary units.

**Biochemical assay of total, reduced and oxidized form of glutathione**

The antioxidant status in kidney cortex lysates tissue was evaluated based on the reduced glutathione (GSH)/ oxidized glutathione (GSSG) ratio. The kidneys were removed, weighted, homogenized in 10% of 5-sulfosalicylic acid, and spun down at 16000g for 10min. GSH was oxidized by 5,5 dithiobis(2 nitrobenzoic acid) (DTNB) to GSSG, with stoichiometric formation of 5 thio-2-nitrobenzoic acid (TNB). The rate of TNB formation was spectrophotometrically measured at 412nm, being proportional to the amount of GSH+GSSG. Subsequently, GSSG was reduced to GSH by glutathione reductase (GSSG reductase), in the presence of
NADP(H). The results were expressed as GSH equivalents (mU/mg of wet tissue) (Anderson, 1987). For the assay of GSSG content, the renal cortex homogenates were incubated with triethanolamine and 2-vinylpyridine. The reaction mix was treated with glutathione reductase as above, and the NADP(H) was monitored spectrophotometrically at 340nm.

**Biochemical assay of glutathione peroxidase**

The glutathione peroxidase (GPx) activity in renal cortex homogenates was evaluated using the method described by Paglia et al. (1967), and modified by Paget et al. (1998), based on the oxidation of GSH to GSSG, catalyzed by GPx. This reaction is coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADP(H). The spectrophotometric assay monitors the decrease of NADP(H) at 340nm (3 min), and the GPx activity is reported as units per mg protein (1 unit is equivalent to 1 mmol NADP(H)/min.).

**The carbonyl content of proteins**

The protein carbonyls, an index of protein oxidation, was assayed by derivatization with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone (Levine et al., 1990; Nakamura and Goto, 1996). Briefly, equal portions of renal cortex homogenates were precipitated with 10% TCA, and treated either with 10 mM DNPH in 2 N HC1, or with 2 N HC1 only (control), for 1h. The mixture was spun down at 16000 x g, 10 min., and the precipitates were washed three times with ethanol-ethyl acetate (1:1) mixture. Subsequently, the precipitates were dissolved in 6 M guanidine chloride. The carbonyl content was measured at 360 nm, and expressed as nmols per mg protein (considering a molar extinction coefficient of 22,000). The protein content of the sample was assayed with the Amidoblack method.

**Measurement of NAD(P)H oxidase activity**

Lucigenin chemiluminescence assay for measurement of NAD(P)H oxidase activity (Satoh et al., 2006) in renal cortex tissue homogenate was performed using the luminometer Berthold. Briefly, 400 µg protein was diluted in saline phosphate buffer, pH 7.2, and NADP(H) (100 µM) and dark-adapted lucigenin (5µM) were added just before reading. Lucigenin chemiluminescence was recorded for 5 min, and was expressed as units/min./mg of wet tissue (units/min/mg).

**Statistical analysis**

Data were expressed as means ± SD. Statistical evaluation of glucose, cholesterol, triglycerides, and creatinine was performed by t-test with two-tailed distribution. Differences in protein expressions among the three groups (HT, HTZ, and C) were compared by one-way ANOVA. Statistical significance was accepted for p < 0.05.

**Results**

**Characterization of the experimental model: the hemodynamic and biochemical parameters**

The Wistar rats receiving L-NAME in drinking water for 6 weeks develop arterial HT (Table 1). Blood pressure, measured invasively in rat aorta showed an increase with ~ 33.6% of the blood pressure (by comparison with C group). The treatment with the ACE inhibitor, Zofenopril decreases with ~ 14.5% the mean arterial pressure (vs. HT group), and adjust it closer to the level determined in C group (Table 1, fig. 1). L-NAME-treated HT rats showed slight alterations of cholesterol, triglycerides, and creatinine concentrations (vs. C group) (Table 1). Zofenopril administration to HT group improves these parameters close to those in C group (Table 1).

Consistent to the HT condition, ACE activity increases vs. the level in C group, while Zofenopril treatment causes a robust inhibitory effect, reducing it with ~ 82% (as compared to HT group) (Table 1).
Fig. 1 Mean arterial pressure for hypertensive (HT), hypertensive receiving Zofenopril (HTZ) and control (C) groups (expressed as mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive rats</th>
<th>Hypertensive rats treated with Zofenopril</th>
<th>Control rats</th>
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<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>328 ± 16</td>
<td>317 ± 20</td>
<td>366 ± 7</td>
</tr>
<tr>
<td><strong>Mean arterial pressure (mmHg)</strong></td>
<td>131 ± 18</td>
<td>112.2 ± 12</td>
<td>98.5 ± 8</td>
</tr>
<tr>
<td><strong>Systolic pressure (mmHg)</strong></td>
<td>154 ± 11</td>
<td>121 ± 29</td>
<td>118 ± 14</td>
</tr>
<tr>
<td><strong>Dyastolic pressure (mmHg)</strong></td>
<td>110 ± 18</td>
<td>87 ± 12</td>
<td>71 ± 17</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min.)</strong></td>
<td>281 ± 20</td>
<td>297 ± 17</td>
<td>322 ± 15</td>
</tr>
<tr>
<td><strong>Glycemia (mg/dl)</strong></td>
<td>204.4 ± 15.7</td>
<td>100 ± 15.3</td>
<td>151.65 ± 6.3</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/dl)</strong></td>
<td>302.73 ± 40.2</td>
<td>218 ± 15.4</td>
<td>204.66 ± 6.21</td>
</tr>
<tr>
<td><strong>Triglycerides(mg/dl)</strong></td>
<td>107.85 ± 2.45*</td>
<td>70.67 ± 6.8</td>
<td>60.79 ± 5.07</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>0.63 ± 0.063</td>
<td>0.615 ± 0.046</td>
<td>0.53 ± 0.059</td>
</tr>
<tr>
<td><strong>Angiotensin Converting Enzyme (nmole/ml/min.)</strong></td>
<td>49.8 ± 4.8</td>
<td>9.08 ± 1.15*</td>
<td>40.9 ± 6.7</td>
</tr>
</tbody>
</table>

Table 1 Body weight, hemodynamic parameters (mean arterial pressure, systolic, and diastolic pressure, heart rate) and biochemical parameters (circulating concentrations of glucose, cholesterol, triglycerides, creatinine, and the ACE activity) of the HT, HTZ, and C group (mean ± SD; *p<0.05).

**Light microscopy examination of the kidney cortex architecture in experimental model**

Histological analysis of kidneys cortex in HT group shows tubular atrophy (continuous garnet-red staining), interstitial fibrosis consisting in accumulation of collagen around Bowman capsule, and between renal tubules, hypercellularity and hypertrophy of mesangial matrix, obstructive hyalinosis of the renal tubules. All these features demonstrate that long term administration of L-NAME caused renal damage (Fig. 2A).

In HTZ group, the interstitial fibrosis is reduced, hyalinosis and tubular atrophy is diminished, and reduced mesangial expansion / hypercellularity emerged (Fig. 2B). We noted in C group the occurrence of hyaline depots in the renal tubules, along with mesangial expansion (Fig. 2C).

**Electron microscopy of the kidney cortex structure**

Electron microscopic examination of kidney cortex in HT group shows an expanded mesangial matrix, the frequent accumulation of inflammatory cells within the lumen of blood vessels (Fig. 3A), a depletion in number of filtration slits, and the increased effacement of foot processes (pedicels) in glomerular podocytes (Fig. 3B). In HTZ group, the effacement of foot processes is diminished (Fig. 3C) and resembles the situation observed in C group (Fig. 3D).
Fig. 2 Representative images for histological changes in renal cortex (Masson’s Trichrome staining). A) HT group; B) HTZ group; C) C group; the yellow arrows show fibrosis, the green arrows show hyalin deposits; the red arrows show mesangial expansion/hypercellularity.

Fig. 3 Ultrastructural alterations of the kidney glomeruli in HT group (A, B), HTZ group (C), and C group (D). Note in B the loss of slit diaphragm and effacement of pedicels
IC - inflammatory cell; lm-vascular lumen; Pd – podocyte; pd – pedicels; sd – slit diaphragm; GBM- glomerular basement membrane; M –mitochondrion; EC – endothelial cell
**L-NAME-induced HT is associated with oxidative stress in renal cortex, a process modulated by Zofenopril administration:**

(i) **O$_2^-$ concentration as indicator of NADP(H) oxidase activity**

Generation of O$_2^-$ by the NADP(H) oxidase was evaluated by the chemiluminescent reaction with lucigenin. Compared to the C group, in HT group ROS production increases by ~76%. Compared to HT group, ROS production in HTZ group decreases by ~23% (Fig. 4).

(ii) **Protein expression of NADP(H) oxidase subunits**

Immunoblotting was used to assess protein expression of the subunits of NADP(H) oxidase, followed by measurement of bands intensity (Fig. 5). The results show that in HT group there is an increase in protein expression of p22phox, NOX1, p47phox, p67phox by ~21%, ~35%, ~14%, and ~20% respectively (vs. C group); the increase is statistically significant for NOX1, and p67phox (Fig. 5). In HTZ group, the subunits significantly reduced are p22phox, NOX1, and p47phox, by ~45%, ~13%, ~17%, respectively (vs. HT group) (Fig. 5).

(iii) **Protein expression of the redox – regulated transcription factor NFkB subunits**

Immunoblotting experiments showed that in kidney cortex of HT group there is an increased expression of p50 and p65 subunits (by ~37.3%, and ~6.75% respectively, vs. C group) (Fig. 6). In HTZ group, p50 protein expression is reduced by ~43%, and p65 is diminished by ~21% (vs. HT group) (Fig. 6).

(iv) **Protein carbonyls concentration, as marker of oxidative stress in renal cortex**

In this assay, the HT group shows ~81% increase in carbonylated proteins, compared to C group (Fig. 7). In HTZ group, the level of protein carbonyls decreases by ~20%, compared to HT group (Fig. 7).
Glutathione peroxidase (GPx) catalyzes the reduction of organic hydroperoxides (lipid hydroperoxides, DNA hydroperoxides) or H$_2$O$_2$ by GSH. In HT group, GPx activity was ~25% decreased vs. C group. In HTZ group, the GPx activity was ~23% augmented vs. HT group, a process that almost attains GPx activity in C group (Fig. 8).

**Analysis of the antioxidant defense mechanisms in kidney cortex: GSH concentration and GPx activity**

Inhibiton of nitric oxide in HT rats induces in kidney cortex a significant decrease of GSH concentration (by ~21%), and an increase in GSSG concentration (by ~31%) contributing to an imbalance in GSH/GSSG ratio (Table 2). Zofenopril administration augments the GSH concentration in kidney cortex (by ~16%) (vs. HT group) (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>GSH (mU/mg protein)</th>
<th>GSSG (mU/mg protein)</th>
<th>GSH/GSSG ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C group</td>
<td>3.2±0.4</td>
<td>0.74±0.2</td>
<td>4.93±2.7</td>
</tr>
<tr>
<td>HT group</td>
<td>2.5±0.3*</td>
<td>0.98±0.2</td>
<td>2.67±0.7</td>
</tr>
<tr>
<td>HTZ group</td>
<td>3.05±0.5**</td>
<td>0.82±0.1</td>
<td>3.75±0.8**</td>
</tr>
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</table>

Table 2 Reduced and oxidized forms of glutathione in renal cortex from C, HT, and HTZ experimental groups. *p<0.05 for HT vs. C; **p<0.05 for HTZ vs. HT

**Discussions**

Hypertension (HT) is a multifactorial disease involving complex interactions between genetic and environmental factors. Development of experimental models of HT allowed identification of various influences associated with regulation of blood pressure, inheritance of HT traits, and cellular responses to injury. Blood pressure, the most accurate parameter in the diagnosis of HT, is commonly recorded using invasive and noninvasive methods; however, the invasive blood pressure recording is considered the gold standard providing a direct indication of the effect of the investigated factors on the circulatory system (Parasuraman and Raveendran, 2012).

The heart attack, stroke, and kidney failure are among the HT-associated complications. Several pathophysiological
mechanisms contribute to the development of primary HT including: genetic background, high salt intake, low physical activity, obesity, insulin resistance, renin – angiotensin system, excessive production of O$_2^-$, and the sympathetic nervous system. In this experimental study we used the NOS inhibitor L-NAME to induce HT in rats. It was an efficient inductor of HT, as demonstrated by increases in blood pressure and circulating ACE activity (Fig.1, Table 1). Moreover, the structure of the kidney cortex is severely affected (Fig. 2), along with depletion in number of filtration slits, and the increased effacement of foot processes (pedicels) in glomerular podocytes. All these structural changes influence the glomerular filtration properties (Fig. 3). The novelty of the study relies on demonstration of the robust oxidative stress that accompanies the structural alterations in L-NAME-induced HT. We established here the augmentation of several components of oxidative stress (such as O$_2^-$ concentration, protein expression of p22phox, NOX1, p47phox, p67phox NADP(H) oxidase subunits, p40phox, p47phox, p67phox, and Rac (Wiliams et al., 2007). All NADP(H) oxidase subunits are expressed in renal cortex with a prominent representation in glomeruli and podocytes (Gill et al., 2006). Convincing evidence showed an increase in p47phox and p67phox protein in kidney cortex of spontaneous HT rats (Sedeek et al., 2009).

Depending on the level of ROS, different redox-sensitive transcription factors are activated. A significant amount of ROS triggers an inflammatory response through the activation of NF-$\kappa$B and AP-1 (Reuter et al., 2010). NF-$\kappa$B is crucial in inflammation, immunity, cell proliferation and apoptosis. This transcription factor consists in homo- or heterodimers: NF-$\kappa$B1 (p50 and its precursor p105), NF-$\kappa$B2 (p52 and its precursor p100), p65/RelA, and c-Rel and RelB (Hayden et al., 2004).

Another biochemical mechanism generated by ROS is the covalent modification of specific cysteine residues of the redox-sensitive targets leading to carbonylation of lipids, DNA, and proteins (Finkel, 2011) Protein carbonyl content is the most general and well-used biomarker of severe oxidative protein damage, and was found associated with Alzheimer's disease, diabetes mellitus, arthritis, and cancer. Carbonyl groups are formed especially at Pro, Arg, Lys, and Thr amino acid side chains of proteins.

In the defense antioxidant system, the main players are GSH and GPx. The GSH to GSSG ratio is a sensitive indicator of the redox state. The latter intervene in endogenous xenobiotics detoxification, in removal of hydrogen peroxide, of ROS, and in maintenance of free protein sulfhydryl groups. Kidney and liver contain the highest GSH redox activity (Meister, 1988).
Another novel finding reported here is the favorable effect of Zofenopril administration in L-NAME induced HT in rats. Its promising effects are manifest both at systemic level (by reducing blood pressure and serum ACE activity (Table 1, Fig. 1) and locally, in the kidney cortex (correcting the abnormal oxidative stress, and augmenting the antioxidant defense). Additionally, the decrease in protein expression of p50 and p65 subunits of NFkB may suggest an anti-inflammatory action of Zofenopril (Fig. 6). However, further studies are needed to establish whether the anti-inflammatory action of Zofenopril in the HT model here described is due to drug’s capacity to decrease oxidative stress or to L-NAME inhibition of NOS, the enzyme responsible for NFkB activation. Taken together, the results emphasize the renoprotective effects of Zofenopril.

Conclusions
This study supports that HT causes increased oxidative stress and induces severe renal injuries. The anti-hypertensive drug, Zofenopril, known as an ACE inhibitor, exerts renoprotection diminishing the oxidative stress, possibly due to its sulfhydryl moieties.

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References
Dzau, V.: The cardiovascular continuum and rennin - angiotensin - aldosterone system blockade. J. Hypertens., Suppl. 23, 1, S9-17, 2005
Hayden, M.S., Ghosh, S.: Signaling to NF-kappaB. Genes Dev., 18, 18, 2195–2224, 2004
Napoli, C., Sica, V., de Nigris, F., Pignalosa, O., Condorelli, M., Ignarro, L.J., Liguori, A.: Sulphydryl angiotensin - converting enzyme inhibition induces sustained reduction of systemic oxidative stress and improves the...