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Blockade of the Neprilysin and Angiotensin Ameliorates Oxidative Stress in the Cardiovascular Target Tissues of Dexamethasone-induced Hypertensive Rats

Neprilisin ve Anjiyotensin Blokajı, Deksametazonla Indüklenen Hipertansif Ratların Kardiyovasküler Hedef Dokularındaki Oksidatif Stresi Hafifletmektedir

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Abstract

Aim: The imbalance between production of reactive oxygen species and antioxidant defense determines the degree of oxidative toxicity and severity of subsequent myocardial damage. We aimed to evaluate the anti-oxidative effects of neprilysin and angiotensin inhibition on cardiovascular target tissues in dexhamethasone-induced hypertensive rats.

Methods: Thirty-six Wistar rats were divided into six groups. Three groups received 30 µg/kg/day dexamethasone for 14 days to induce arterial hypertension. Mean arterial blood pressures were verified by carotid artery cannulation. Ramipril (10 mg/kg), and sacubitril/valsartan (80 mg/kg) were administered for 18 days to the hypertensive and normotensive groups. Glutathione peroxidase, superoxide dismutase and malondialdehyde levels were evaluated in cardiovascular target tissues.

Results: Serum and cardiac malondialdehyde levels were lower, while cardiac glutathione peroxidase and superoxide dismutase were higher in treatment groups than in control groups. Aortic malondialdehyde in sacubitril/valsartan group was lower; aortic and renal superoxide dismutase in sacubitril/valsartan and ramipril groups were significantly higher than in control group. Serum glutathione peroxidase was higher in hypertensive sacubitril/valsartan group than in hypertensive control group.

Conclusion: We demonstrated that neprilysin and/or angiotensin inhibition had protective properties against oxidative stress exerted by dexhamethasone-induced hypertension in cardiovascular target organs, which may be mediated by reversal of natriuretic peptides degradation.

Keywords: Neprilysin, renin-angiotensin system, hypertension, oxidative stress, dexamethasone, rat

Öz

Amaç: Reaktif oksijen türlerinin aşırı üretimi ile antioksidan savunma arasındaki dengesizlik, oksidatif toksisite derecesini ve sonraki dönemde miyokart hasarının ciddiyetini belirler. Biz bu çalışmada deksametazonun indüklediği hipertansif ratlarda, neprilisin ve anjiyotensin inhibisyonunun kardiyovasküler hedef dokulardaki anti-oksidatif etkilerini değerlendirmeyi amaçladık.

Yöntemler: Otuz altı Wistar rat altı gruba ayrıldı. Arteriyel hipertansiyonu indüklemek için üç gruba 14 gün boyunca 30 µg/kg/gün deksametazon uygulandı. Ortalama arter kan basıncı, karotis arter kanülasyonu ile ölçüldü. Hipertansif ve normotansif gruplara ramipril (10 mg/kg) ve sakubitril/valsartan (80 mg/kg) 18 gün boyunca uygulandı. Kardiyovasküler hedef dokularda glutatyon peroksidaz, süperoksit dismutaz ve malondialdehit düzeyleri değerlendirildi.

Bulgular: Tedavi gruplarında kontrol grubuna göre serum ve kardiyak malondialdehit düzeyleri düşükken, kardiyak glutatyon peroksidaz ve süperoksit dismutaz düzeyleri daha yüksek bulundu. Sacubitril/valsartan grubunda aortic malondialdehit düzeyi düşük; sacubitril/valsartan ve ramipril gruplarında aortik ve renal süperoksit dismutaz kontrol grubundan anlamlı olarak yüksek saptandı. Serum glutatyon peroksidaz düzeyi, hipertansif sakubitril/valsartan grubunda hipertansif kontrol grubuna göre daha yüksek bulundu.

Sonuç: Neprilisin ve/veya anjiyotensin inhibisyonunun, kardiyovasküler hedef organlarda deksametazonla indüklenen hipertansiyondan kaynaklanan oksidatif strese karşı koruyucu özelliklere sahip olduğunu gösterdik. Bu etkinin, natriüretik peptitlerin degradasyonunun tersine çevrilmesiyle ilişkili olabileceğini düşünmekteyiz.

Anahtar Sözcükler: Neprilisin, renin-anjiyotensin sistemi, hipertansiyon, oksidatif stres, deksametazon, rat

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Introduction

Despite significant advances in medical technology, cardiovascular events still remain one of the major causes of death all over the world (1). Therefore, exposure to cardiovascular toxic substances has important roles in cardiovascular mortality (2,3).

Oxygen is known as one of the most critical elements for life, however, if an electron from molecular oxygen is reduced, superoxide with high-reactive unpaired electron or free radical is formed. Superoxide is involved in cytochrome P450, phagocytosis and detoxifying reactions of prostaglandin biosynthesis. Excessive amounts of superoxide may lead to production of other reactive oxygen species (ROS), which may result in subsequent tissue damage (4).

Neprilysin (NEP) and renin angiotensin aldosterone system (RAAS) blockers are commonly used in the management of cardiovascular disorders. Among them, sacubitril/valsartan, a dual angiotensin II receptor blocker (ARB) and NEP inhibitor, was developed to block the activation of the RAAS and to increase the sensitivity to the natriuretic peptides (5). Favorable effects of NEP inhibition have been attributed to reduced degradation of the natriuretic peptides which cause vasodilation by stimulating particulate guanylate cyclase to produce cGMP (6,7).

Sacubitril/valsartan has demonstrated a greater hypotensive activity than valsartan alone in previous clinical trials (8). Valsartan, a direct angiotensin II receptor antagonist, decreases vasoconstriction and aldosterone production, displaces angiotensin II from the angiotensin II receptor type 1 (AT1 receptor) and reduces blood pressure by antagonizing AT1-induced vasoconstriction, catecholamine release, arginine vasopressin release, water intake, and hypertrophic responses. ARBs also diminish vascular, renal, and cardiac injury by reducing hypertrophy, inflammation, and fibrosis with their inhibitory effect on AT1 receptors (5).

Superoxide anion ($\cdot O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) have been shown to play roles in cardiac diseases (9,10). Imbalance between the production of ROS and the antioxidant defense in the heart determines the intensity of oxidative stress and the grade of consequent myocardial injury. If increased ROS production cannot be controlled by the antioxidant defense, this leads to progression of heart dysfunction and to myocyte hypertrophy, apoptosis and interstitial fibrosis (11).

The first line defense mechanism against ROS-mediated cardiac injury contains several antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). Among these antioxidants, GPx performs several vital functions such as removal of H_2O_2 after the SOD-catalyzed dismutation reaction, and detoxification of the lipid hydroperoxides. Several studies have shown that GPx alone provides higher conservation against oxidative damage than SOD and catalase, which may explain the high efficiency of GPx as an antioxidant (10,12).

The aim of this experimental study was to investigate the effects of NEP/angiotensin inhibition on the oxidative markers of cardiovascular target tissues and serum samples in hypertensive rats.

Methods

Wistar albino rats were housed and maintained at 22 °C, 60±5% humidity and a 12:12 h light/dark cycle with free access to food and water. All experiments were performed in strict accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. Experimental protocols were approved by the Kahramanmaraş Sütçü İmam University Animal Experimentation Ethics Committee (decision no: 2018/08/01, approval date: 06.06.2018).

Drugs

Dexamethasone was purchased from Deva (Dekort, İstanbul, Turkey), ramipril from Sanofi (Delix, İstanbul, Turkey), and sacubitril/valsartan from Novartis Farma S.p.A (Oneptus, Torre Annunziata, Italy). Ramipril and sacubitril/valsartan were freshly prepared at doses of 10 mg/kg per day and 80 mg/kg per day, respectively, homogeneously dissolved in tap water and administered per oral (p.o) to rats with orogastric lavage in 1 mL volume. Dexamethasone (30 µg/kg per day) and sterile saline (sodium chloride) were administered subcutaneously at a volume of 1 mL/kg.

Study Design

Thirty-six Wistar albino rats were given two weeks to acclimatize to the surroundings prior to the experiments. Hypertension was induced by subcutaneous (s.c) injection of dexamethasone at the same hour of the day as previously reported (13,14). On the 4th day of experiments, the rats received dexamethasone (30 µg/kg per day) subcutaneously for 14 days. Dexhametasone-induced hypertension model in rats is usually attributed to the activation of the steroid-induced mineralocorticoid receptor, but studies have shown that glucocorticoids may also increase the blood pressure independent of this receptor. In addition, one of the underlying mechanisms of steroid-induced hypertension is the increase of endogenous vasoconstrictive agents such as norepinephrine and angiotensin II (15). So, in our protocols, antihypertensive medications (ramipril and sacubitril/valsartan) were given between days 1 and

18 for blocking the receptors that are responsible for dexamethasone-induced hypertension.

Mean arterial blood pressure were verified by carotid artery cannulation. The doses in this study (ramipril 10 mg/kg per day, sacubitril/valsartan 80 mg/kg per day) were calculated on the basis of protocols that have showed significant decrease in arterial blood pressure measured by carotid artery cannulation in the rat hypertension model in our previous study (14), and also based upon the fundamental literature (16). The animal groups were as follows (six rats each):

Group 1 (control): received saline p.o from day 4 to 18.

Group 2 (ramipril): received ramipril p.o (10 mg/kg) from day 1 to 18.

Group 3 (sacubitril/valsartan): received sacubitril/valsartan p.o (80 mg/kg) from day 1 to 18.

Group 4 (hypertensive): received s.c dexhamethasone (30 µg/kg) from day 4 to 18 for hypertension induction.

Group 5 (hypertensive-ramipril): received s.c dexhamethasone (30 µg/kg) from day 4 to 18, and ramipril p.o (10 mg/kg) from day 1 to 18.

Group 6 (hypertensive-sacubitril/valsartan): received s.c dexhamethasone (30 µg/kg) from day 4 to 18, and sacubitril/valsartan p.o (80 mg/kg) from day 1 to 18.

After treatments, the heart, thoracic aorta, kidney and blood samples of the rats were collected for the assessment of GPx, SOD and malondialdehyde (MDA).

Assessment of Arterial Blood Pressure

Arterial blood pressure of the rats was measured as previously described (17). Briefly, the rats were anesthetized using an intraperitoneal injection of ketamin (80 mg/kg) and xysilazine (10 mg/kg). The arterial blood pressure was measured from carotid artery through an arterial cannula connected to a pressure transducer coupled with a hemodynamic recorder (BIOPAC MP35 System Inc., Santa Barbara, CA, USA).

Assessment of GPx, SOD and MDA in Tissues and Serum

At the end of the treatments, heart, thoracic aorta, kidney and blood samples of the rats were taken to detect cardiovascular effects of sacubitril/valsartan and ramipril in hypertensive and normotensive rats. GPx, SOD and MDA levels were investigated in serum and tissue homogenates.

The blood of the rats was taken by cardiac puncture into dry tubes. The serum was obtained by centrifugation at 4000x g for 10 minutes. The heart, thoracic aorta and kidney were rapidly removed and washed in cold saline, and homogenized with cold 0.15 Molar (M) KCl (10%, w/v). Tissue homogenates were centrifuged at 600 x g for 10 minutes at 4 °C to remove crude fractions. Subsequently, the supernatants were subjected to centrifugation at 10,000 x g for 20 minutes to obtain the post-mitochondrial fraction. SOD and GPx activities were determined in the post-mitochondrial fraction. Serum and tissue MDA levels were assayed using thiobarbituric acid according to the method of Buege and Aust (18).

SOD activity was measured by the method described by Beyer and Fridovich (19). In this method, xanthine and xanthine oxidase were used to form superoxide radicals which react with 2- (4-iodophenyl) -3- (4-nitro phenol-s phenyltetra-zolium chloride) to form red form azonstain. SOD activity was then measured by the inhibition degree of this reaction. The Beutler method was used for GPx activity measurement (20). The role of GPx was to catalyze the oxidation of reduced glutathione (GSH) to oxidative glutathione (GSHG) via H_2O_2. In the presence of H_2O_2 and T-butyl hydroperoxide, GSSG produced by GPx was reduced to GSH with the help of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH). GPx activity was determined by the spectrophotometric absorbance difference at 340 nm by oxidation reaction of NADPH. Post-mitochondrial fraction protein determinations were made using bicinonic acid.

Statistical Analysis

The continuous variables were defined as mean ± standard deviation. The Kolmogorov-Smirnov test was used to determine whether the distribution of samples was homogenous or not. Analysis of variance with post-hoc Tukey's honestly significant test was used for the analysis of homogeneously distributed data of multiple groups. The Kruskal-Wallis test with post hoc Mann-Whitney U test under Bonferroni correction was used for the analysis of none normally distributed data. The data was evaluated at the 95% confidence interval and a p value of <0.05 was considered statistically significant. The SPSS 17.0 program was used for statistical analysis.

Results

Cardiac Tissue Analysis

As shown in Figure 1, the MDA levels in normotensive ramipril (80.23±25.62 nmoL/g, p=0.018), normotensive sacubitril/valsartan (58.29±18.71 nmoL/g, p=0.006), hypertensive ramipril (60.07±14.40 nmoL/g, p=0.001) and hypertensive sacubitril/valsartan groups (60.00±9.08 nmoL/g, p=0.001) were significantly lower than in normotensive control group (121.48±38.02 nmoL/g). The MDA levels in hypertensive ramipril and hypertensive sacubitril/valsartan groups were significantly lower than in hypertensive control group (p=0.003 and p=0.003, respectively).

As shown in Figure 2, the GPx levels in hypertensive ramipril (601.47±89.65 nmoL/g, p<0.001) and

hypertensive sacubitril/valsartan groups (432.85±69.58 nmoL/g, p=0.001) were significantly higher than in normotensive control group (264.23±102.14 nmoL/g). Although the normotensive ramipril and normotensive sacubitril/valsartan groups had higher GPx levels than normotensive control group, the difference was insignificant. Hypertensive ramipril and hypertensive sacubitril/valsartan groups had significantly higher GPx levels than hypertensive control group (p=0.004 and p=0.004, respectively).

As presented in Figure 3, hypertensive ramipril (44.96±12.69 U/mg, p=0.014) and hypertensive sacubitril/valsartan groups (61.83±27.09 U/mg, p=0.010) had significantly higher SOD levels than normotensive control group (23.58±12.07 U/mg). SOD levels in normotensive control group was significantly higher than in hypertensive control group (p<0.001). Hypertensive ramipril and hypertensive sacubitril/valsartan groups had significantly higher SOD levels than hypertensive control group (p=0.006 and p=0.004, respectively).

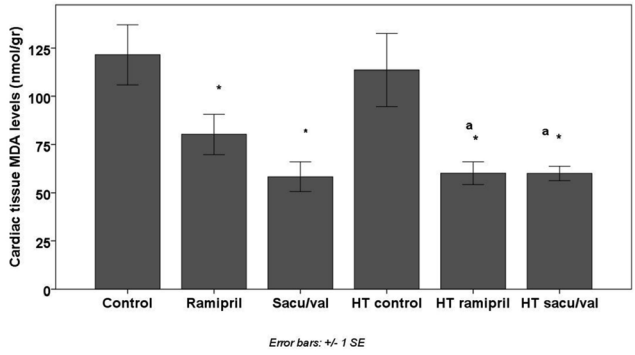

Figure 1. Cardiac tissue MDA levels of the groups.

**MDA levels vs control: ramipril (p=0.018), sacu/val (p=0.006), HT ramipril (p=0.001), HT sacu/val (p=0.001); a MDA levels vs HT control: HT ramipril (p=0.003), HT sacu/val (p=0.003).*

MDA: Malondialdehyde, Sacu/val: Sacubitril/valsartan, HT: Hypertensive

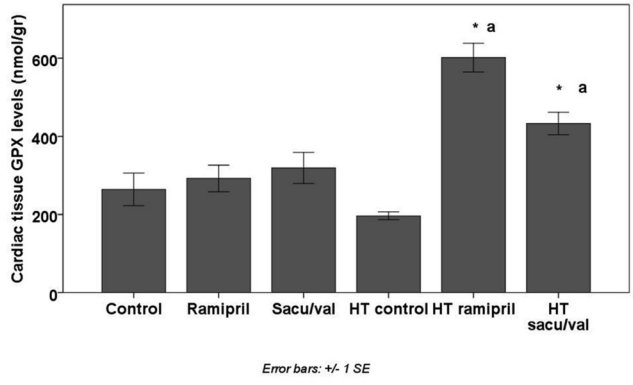

Figure 2. Cardiac tissue GPX levels of the groups.

**GPx levels vs control: HT ramipril (p<0.001), HT sacu/val (p=0.001); a GPx levels vs HT control: HT ramipril (p=0.004), HT sacu/val (p=0.004).*

GPX: Glutathione peroxidase, Sacu/val: Sacubitril/valsartan, HT: Hypertensive

Thoracic Aorta Tissue Analysis

As shown in Table 1, MDA levels in normotensive sacubitril/valsartan group was significantly lower than in normotensive control group (32.59±11.23 nmoL/g vs. 63.48±24.36 nmoL/g, p=0.016). There was no significant difference in GPx levels. SOD levels in normotensive sacubitril/valsartan (32.80±16.83 U/mg, p=0.010), hypertensive control (26.09±5.23 U/mg, p=0.016), hypertensive ramipril (28.98±9.04 U/mg, p=0.016) and hypertensive sacubitril/valsartan groups (31.16±9.77 U/mg, p=0.016) were significantly higher than in normotensive control group (17.11±4.95 U/mg).

Renal Tissue Analysis

As shown in Table 2, there was no significant difference in renal MDA and GPx levels between the groups. However, renal SOD levels in hypertensive control group (19.43±6.47 U/mg, p=0.016) was significantly lower,

Table 1. Levels of oxidant parameters in thoracic aorta tissue

Variable	MDA, nmol/g	GPX, nmol/g	SOD, U/mg
Control	63.48±24.36	312.89±141.73	17.11±4.95
Ramipril	54.06±22.18	371.43±185.51	20.89±6.11
Sacu/val	32.59±11.23[a]	317.83±81.71	32.80±16.93[a]
HT control	61.95±17.37	332.94±92.71	26.09±5.23[a]
HT ramipril	113.68±60.15	412.31±154.10	28.98±9.04[a]
HT sacu/val	56.67±18.67	317.19±133.35	31.16±9.77[a]

MDA: Malondialdehyde, GPX: Glutathione peroxidase, SOD: Superoxide dismutase, Sacu/val: Sacubitril/Valsartan, HT: Hypertensive
[a]MDA levels vs control: sacu/val (p=0.016), SOD levels vs control: sacu/val (p=0.010), HT control (p=0.016), HT ramipril (p=0.016), HT sacu/val group (p=0.016)

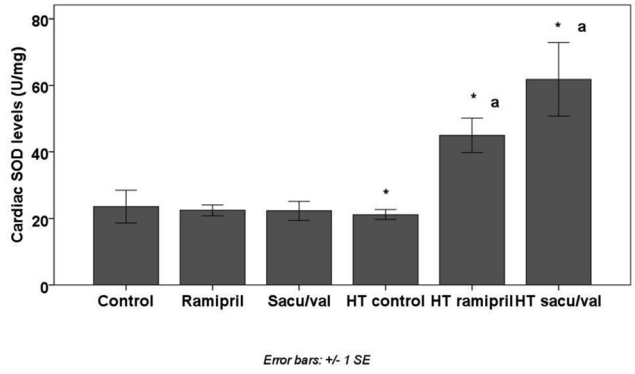

Figure 3. Cardiac tissue SOD levels of the groups.

**SOD levels vs control: HT control (p<0.001), HT ramipril (p=0.014), HT sacu/val group (p=0.010); a SOD levels vs HT control: HT ramipril (p=0.006), HT sacu/val (p=0.004).*

SOD: Superoxide dismutase, Sacu/val: Sacubitril/valsartan, HT: Hypertensive

and SOD levels in hypertensive sacubitril/valsartan group (53.92±20.15 U/mg, p=0.025) was significantly higher than in normotensive control group (30.60±5.17). Renal SOD levels in hypertensive ramipril group (29.36±8.17, p=0.045) and hypertensive sacubitril/valsartan group (53.92±20.15 U/mg, p=0.016) were significantly higher than in hypertensive control group.

Serum Sample Analysis

As shown in Table 3, serum MDA levels in hypertensive ramipril (10.82±3.37 nmoL/mL, p<0.001) and hypertensive sacubitril/valsartan group (9.78±4.94 nmoL/mL, p=0.016) were significantly lower than in normotensive control group (18.24±2.20 nmoL/mL). In addition, serum MDA levels in hypertensive ramipril group and hypertensive sacubitril/valsartan group were lower than in hypertensive control group (p=0.023 and p=0.028, respectively). Serum levels of GPx was significantly higher in hypertensive sacubitril/valsartan group than in hypertensive control group (149.08±31.56 nmoL/mL vs 104.52±35.63 nmoL/mL, p=0.045). Serum SOD levels in hypertensive sacubitril/valsartan group was significantly lower than in normotensive control and hypertensive control groups (p=0.025 and p=0.006, respectively).

Table 2. Levels of oxidant parameters in renal tissue

Variable	MDA, nmol/g	GPx, nmol/g	SOD, U/mg
Control	125.52±12.05	300.99±100.30	30.60±5.17
Ramipril	132.31±8.70	294.24±110.17	29.12±8.25
Sacu/val	153.90±30.63	287.33±86.46	27.26±6.64
HT control	129.35±22.19	276.71±112.14	19.43±6.47[a]
HT ramipril	128.99±16.69	262.98±38.16	29.36±8.17[b]
HT sacu/val	122.46±17.49	182.49±47.90	53.92±20.15[ab]

MDA: Malondialdehyde, GPX: Glutathione peroxidase, SOD: Superoxide dismutase, Sacu/val: sacubitril/valsartan, HT: Hypertensive
[a]SOD levels vs control: HT control (p=0.016), HT sacu/val (p=0.025). [b]SOD levels vs HT control: HT ramipril (p=0.045), HT Sacu/val (p=0.016)

Table 3. Levels of oxidant parameters in serum sample

Variable	MDA, nmol/mL	GPx, nmol/mL	SOD, U/mL
Control	18.24±2.20	123.45±46.74	2.26±0.61
Ramipril	30.10±12.29	114.97±39.79	1.68±0.27
Sacu/val	22.52±6.63	132.83±10.14	2.02±0.57
HT control	18.58±5.96	104.52±35.63	1.96±0.69
HT ramipril	10.82±3.37[ab]	142.38±50.99	1.81±0.71
HT sacu/val	9.78±4.94[ab]	149.08±31.56[b]	1.17±0.35[ab]

MDA: Malondialdehyde, GPX: Glutathione peroxidase, SOD: Superoxide dismutase, Sacu/val: Sacubitril/valsartan, HT: Hypertensive
[a]MDA levels vs control: HT ramipril (p<0.001), HT sacu/val (p=0.016); SOD levels vs control: HT sacu/val group (p=0.025). [b]MDA levels vs HT control: HT ramipril (p=0.023), HT sacu/val (p=0.028); GPx levels vs HT control: HT sacu/val (p=0.045); SOD levels vs HT control: HT sacu/val (p=0.006)

Discussion

In this study, we evaluated the effects of NEP and angiotensin inhibition on oxidative stress in cardiovascular target tissues and its correlation with high blood pressure. We found that oxidative markers were significantly improved after administration of ramipril and sacubitril/valsartan in hypertensive rats.

NEP contributes to the degradation of active natriuretic peptides and other vasoactive compounds. Sacubitril, a NEP inhibitor, prevents neurohormonal activation, vascular tone, cardiac fibrosis and sodium retention (21,22). Combined inhibition of NEP and angiotensin II receptors offers clinical cardio protection in cardiovascular diseases, improves survival, increases functional capacity, and reduces hospitalization in patients with cardiac dysfunction (23).

Previous studies have shown that oxidative stress was involved in the pathogenesis of hypertension. These effects were mediated by inactivation of nitric oxide (NO) by $O_2^{\cdot}$ in the vessels and the renal tissue, and by H_2O_2-induced vessel remodeling. Antioxidant defenses are sufficient to protect the vasculature, leading ROS to function as signaling molecules. The enzymes that produce ROS and the pathways on their removal are related to the source of vascular diseases arising from oxidative stress (24).

ROS in cardiovascular organs are mostly produced by NADPH oxidase, xanthine oxidase and NO synthases (25). These enzymes are activated in a variety of diseases which are commonly seen in clinics, such as atherosclerosis, hypertension, diabetes, and renal insufficiency. Angiotensin II activates NADPH oxidase by stimulating the AT1 receptor (26). Excessive production of ROS contributes to the development of high blood pressure, whereas clearance of ROS decreases blood pressure (27). In our study, we showed that SOD levels were significantly decreased in cardiac tissue of hypertensive rats compared to normotensive rats. Hypertension is, in addition, related to the increased ROS formation in the brain, kidney and vasculature of systems, all of which may contribute to arterial blood pressure. We found that both ACE inhibition and NEP/angiotensin inhibition revealed amelioration in MDA, GPx and SOD levels in the heart, compared to hypertensive non-treated rats. We also revealed that MDA in the heart was significantly reduced, while GPx and SOD were significantly induced with ramipril and sacubitril/valsartan, compared to that in normotensive non-treated rats.

The oxidative stress induced by hypertension in renal tissues causes vicious cycle of hypertension Renal vascular and glomerular architecture expresses the components of the NADPH oxidase (28). Afferent arterioles, glomerulus, proximal tubule, and cortical collecting duct are the

targets of oxidative stress. An increase in superoxide levels in the afferent arteriole degrades NO, and thus, enhances afferent arteriolar vasoconstriction and reduces the glomerular filtration rate. High blood pressure increases the expression of the NADPH oxidase subunit p22phox, activates the NADPH oxidase, and causes endothelial dysfunction in afferent arterioles (29). Similarly, we found that hypertension decreased the SOD levels in renal tissue in comparison to the normotensive rats. Superoxide potentiates intracellular calcium in afferent arterioles (30). The decrease in renal vasoconstriction and the ameliorated renal perfusion through superoxide scavenging lead to improved blood pressure response in hypertensive animals (31). After treatment with ramipril and sacubitril/valsartan, we found that RAAS inhibition significantly increased SOD levels in renal tissue. Furthermore, it appears that hypertensive rats respond better to treatment with sacubitril/valsartan by achieving a greater induction in SOD levels than normotensive non-treated rats.

The NADPH oxidase which is stimulated by angiotensin II, is the main source of superoxide in the vessels (32,33). Regardless of the etiology, hypertension increases the vascular production of ROS in the vessels produced from NADPH oxidase and uncoupled NO (34). Increased vascular superoxide production causes reduced endothelium-dependent vasodilatation. Scavenging of superoxide with SOD ameliorates endothelium-dependent vasodilatation in hypertensive vessels, whereas having minimal effects in the intact vessels (35). Additionally, genetic deletions of SOD isoforms reduce endothelium-dependent vasodilatation (36). We found that, in thoracic aorta, sacubitril/valsartan administration to normotensive rats significantly reduced the MDA level compared that in non-treated normotensive rats. We also demonstrated a better response to treatment with RAAS inhibitors in hypertensive rats with a greater induction in SOD levels in their aorta than in normotensive non-treated rats.

Vascular superoxide surge augments the vascular lesion formation, and may be the mechanistic link between atherosclerosis and hypertension. Vascular ROS may cause vascular smooth muscle hypertrophy, and it has been reported that particularly H_2O_2 was involved in the hypertrophic effect of angiotensin II (37). Additionally, vascular smooth muscle hypertrophy has been reported to be increased in mice overexpressing NADPH oxidase in the vascular smooth muscle (38). The hypertrophic response of vascular smooth muscle is a critical component of vascular remodeling in hypertension. Besides, inhibition of the NADPH oxidase was determined to reduce aldosterone-induced vascular collagen deposition in animal studies (39).

In the serum levels of antioxidants, we found that both ACE inhibition and NEP/angiotensin inhibition exerted amelioration in MDA, compared to that in hypertensive non-treated rats and normotensive rats. We also revealed that GPx in serum was significantly induced by sacubitril/valsartan, compared that in hypertensive non-treated rats. The benefit may be in part due to reduced N-terminal pro-brain natriuretic peptide and increased BNP.

Study Limitations

Our study has significant limitations. Our data provided information about the functional mechanisms of antioxidant effects of sacubitril/valsartan and ramipril in rats, and supported NEP and RAAS blockade with more specific therapeutic agents. There are various processes in the formation and gradual progression of hypertension. Our understanding of the molecular mechanisms of hypertension with oxidant parameters in rats has been weakened due to multifactorial etiology and possible comorbidities related to cardiac dysfunction. Although the present results showed a possible pathogenic role of oxidants in the model of hypertension, we could not exclude the contributions of other ROS sources whose roles have been demonstrated in previous experimental studies. It is likely that there may be a possible interaction or mechanistic relationship between different ROS sources.

Conclusion

This study showed that oxidative toxicology is involved in hypertension. Common medications targeting the NEP and RAAS appear to have significant contribution to management of cardiac diseases by cleansing the excessive amount of oxidative stress in cardiovascular target tissues.

Authorship Contributions

Concept: D.A.A. Design: D.A.A. Data Collection or Processing: D.A.A., M.S., N.E., B.T., S.Y. Analysis or Interpretation: D.A.A., M.S., N.E., B.T., S.Y. Literature Search: D.A.A., M.S., N.E., B.T., S.Y. Writing: D.A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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