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ORIGINAL RESEARCH

Antihypertensive Treatment Approaches and Oxidant-Antioxidant System Relationship in Hypertensive Type 2 Diabetic Patients*

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ABSTRACT

A relationship between endothelial dysfunction and oxidative stress has been shown in the pathogenesis of diabetes and/or hypertension. Antihypertensive drugs (angiotensin-converting enzyme inhibitors [ACEi], angiotensin II type 1 receptor blockers [ARB], and third-generation beta-blockers) can improve oxidative stress. This study evaluated the effects of losartan (ARB) treatment alone and combined with cilazapril (ACEi) or carvedilol on oxidative stress and antioxidants in hypertensive type 2 diabetic patients. Thirty of 56 patients completed the study. All patients received 50 mg losartan daily for 6 weeks, then were randomised into three groups for 6 weeks. In the first group, losartan was increased to 100 mg/day, and in the second and third groups, carvedilol or cilazapril was added to losartan 50 mg/day treatment, respectively. Apolipoprotein B malondialdehyde (basal and Δ-MDA), serum paraoxonase (PON) and arylesterase (AE), erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (Gpx) parameters were studied. The characteristics of the three groups were comparable ($p>0.05$). Blood pressure (BP) decreased significantly in all three groups before and after randomisation in the six-week periods. The largest BP decrease was seen in the ARB+ACEi group (systolic BP: 16.5±7.4 mmHg, diastolic BP: 10.5±2.8 mmHg), and the smallest decrease was seen in the high-dose ARB group (systolic BP: 9±6.1 mmHg, diastolic BP: 3±4.8 mmHg). Throughout the study, no significant changes were detected in basal and Δ-MDA levels and SOD, Gpx, PON and AE activities in inter- and intra-group comparisons. Our observations showed that different antihypertensive therapy approaches effectively lowered BP in hypertensive type 2 diabetics but did not affect oxidant and antioxidant systems in the short term.

Keywords: Type 2 diabetes mellitus. Hypertension. Antihypertensive agents. Oxidative stress. Antioxidants. Losartan. Cilazapril. Carvedilol.

Hipertansif Tip 2 Diyabetik Hastalarda Antihipertansif Tedavi Yaklaşımları ve Oksidan-Antioksidan Sistem İlişkisi

ÖZET

Diyabet ve/veya hipertansiyon patogenezinde endotel disfonksiyonu ile oksidatif stres arasında ilişki olduğu bildirilmiştir. Antihipertansif ilaçlar (anjiyotensin dönüştürücü enzim inhibitörleri [ACEi], anjiyotensin II tip 1 reseptör blokerleri [ARB] ve üçüncü nesil beta blokerler) oksidatif stresi iyileştirebilirler. Bu çalışmada losartan (ARB) tedavisinin tek başına ve silazapril (ACEi) veya karvedilol ile birlikte kullanımının, hipertansif tip 2 diyabetik hastalarda oksidatif stres ve antioksidanlar üzerindeki etkilerini değerlendirildi. Elli altı hastadan 30'u çalışmayı tamamladı. Tüm hastalar 6 hafta boyunca günlük 50 mg losartan aldı, sonra altı hafta için 3 gruba randomize edildiler. İlk grupta losartan 100 mg/gün'e çıkıldı, ikinci ve üçüncü gruplarda losartan 50 mg/gün tedavisine sırasıyla karvedilol veya silazapril eklendi. Apo B-içeren lipoproteinlerin malondialdehit (bazal ve Δ-MDA), paraoksonaz (PON), arilesteraz (AE), eritrosit süperoksit dismutaz (SOD) ve glutatyon peroksidaz (Gpx) parametreleri çalışıldı. Üç grubun karakteristikleri karşılaştırılabilirdi ($p>0.05$). Her üç grupta da randomizasyon öncesi ve sonrası altışar haftalık dönemlerde kan basınçları (KB) anlamlı şekilde azaldı ($p<0.05$). En fazla KB düşüşü ARB+ACEi grubunda (sistolik KB: 16.5±7.4 mmHg, diyastolik KB: 10.5±2.8 mmHg) ve en küçük düşüş yüksek doz ARB grubunda (sistolik KB: 9±6.1 mmHg, diyastolik KB: 3±4.8 mmHg) görüldü. Çalışma boyunca gruplar arası ve grup içi karşılaştırmalarda bazal ve Δ-MDA, SOD, Gpx, PON ve AE aktivitelerinde anlamlı bir değişiklik saptanmadı. Gözlemlerimiz hipertansif tip 2 diyabetiklerde farklı antihipertansif terapi yaklaşımlarının KB'nı düşürmede etkili olduklarını fakat kısa dönemde oksidan ve antioksidan sistemleri etkilemediğini göstermiştir.

Anahtar Kelimeler: Tip 2 diabetes mellitus. Hipertansiyon. Antihipertansif ilaçlar. Oksidatif stres. Antioksidanlar. Losartan. Silazapril. Karvedilol.

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Oxidative stress may have a critical role in endothelial dysfunction in diabetic and/or hypertensive patients[1,2]. Chronic hyperglycemia and dyslipidemia in diabetic patients lead to oxidative stress and persistent inflammation through the production of reactive oxygen species (ROS) and accelerate the loss of renal function[3]. In addition to hemodynamic factors such as hypertension and hyperfiltration, chronic inflammation, insulin resistance, and adipokine dysregulation also play a role in renal damage associated with metabolic syndrome. Furthermore, persistent chronic inflammation is associated with endothelial dysfunction and increased sympathetic activity[4]. Although hypertension is common in patients with type 2 diabetes mellitus (T2DM), most of them do not have clinical signs of kidney damage in the early stages[5]. In addition to glycemic control in patients with T2DM, reasonable blood pressure (BP) control also reduces the risk of developing cardiovascular disease and diabetic microvascular complications, which are the leading causes of death[6].

Treatment of hypertension in patients with T2DM includes lifestyle changes and pharmacological treatment. The renin-angiotensin-aldosterone system (RAAS) antagonists, calcium channel blockers (CCB), thiazide and thiazide-like diuretics, and beta-blockers can be used alone or in appropriate combinations to control BP. However, the presence of renal dysfunction and proteinuria in the patient makes RAAS antagonists the first-line treatment due to their renal protection and cardiovascular advantages. RAAS antagonists can be combined with CCB or diuretics[7]. However, combination therapy with angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II type 1 receptor blockers (ARB) may result in a higher rate of adverse renal outcomes than monotherapy alone[8]. Despite some metabolic adverse effects of other beta-blockers (propranolol, metoprolol, atenolol, etc.)[9,10], carvedilol, a nonselective beta- and alpha1-blocker, may not have adverse effects on glucose control[11]. The relationship between oxidative stress and vascular damage in hypertension may be significant even in the selection of antihypertensive drugs[12,13]. ACEi, ARB, and third-generation beta-blockers, including carvedilol, used alone or in combination, may have beneficial effects on oxidative stress beyond their antihypertensive and antiproteinuric properties, but the literature data on this subject are limited. This study evaluated the short-term impact of losartan alone and high-dose losartan and losartan-cilazapril (ACEi) or carvedilol combination on oxidative stress and antioxidant markers in hypertensive type 2 diabetic patients.

Material and Method

Patient selection

In this study, adult patients diagnosed with (T2DM) who applied for hypertension treatment were evaluated. Eighty-eight patients were enrolled in the study. Exclusion criteria were history of hypersensitivity and allergy to ACEi, ARB or beta-blockers, heart failure, arrhythmias, acute coronary syndrome, bradycardia (pulse rate 50 beats/min), history of thromboembolic event, chronic obstructive pulmonary disease or bronchial asthma, pregnancy, lactation, acute infection or inflammation, dehydration, renal artery stenosis, hyperkalemia (>5.5 mmol/L), use of immunosuppressive drugs, overt proteinuria (>300 mg/d), Stage 3 hypertension (≥180 and/or ≥110 mmHg) and Stage 4 chronic kidney disease (creatinine clearance <30 mL/min/1.73 m^2). The local ethics committee approved the study protocol. Informed consent was obtained from all patients before the study. Of the 32 patients, 25 did not meet the acceptance criteria, three did not accept to be included in the study, and four were excluded for other reasons. Fifty-six patients who met the study criteria were included in the study.

Study design

After a 2-week follow-up period, all patients received losartan 50 mg/day as a single dose for 6 weeks. Then, patients were randomized sequentially into three groups at the end of the 6th week. Losartan dose was increased to 100 mg/day in the first group (ARB100 group, n: 17), and carvedilol (6.25 to 25 mg/day) was added in the second group (ARB50+C group, n: 21) and cilazapril (1 to 5 mg/day) in the third group (ARB50+ACEi group, n: 18) to losartan 50 mg/day treatment for another 6 weeks. BPs of thirty patients were monitored for 2 weeks. Systolic and diastolic BPs were measured on the right arm using a suitable cuff size after the patient rested for 10 minutes in a sitting position. Three measurements were taken at 2-minute intervals, and their averages were taken. In addition, the patients' heights and weights were measured, and their body mass indexes (BMI=weight/height² formula) were calculated.

If potassium levels were below 5.5 mmol/L during follow-up and creatinine levels did not increase by more than 30% from baseline, drug doses were increased for BP control. After randomization, 17 patients were included in the 1st group (losartan 100 mg). Three patients dropped out of the study; one had inadequate BP control. Three patients did not continue in the study for other reasons. Twenty-one patients were included in the 2nd group (losartan 50 mg + carvedilol 6.25-25 mg). 4 patients dropped out of the study. 2 patients had drug intolerance. Four patients did not continue in the study for other reasons.

Eighteen patients were included in the 3rd group (losartan 50 mg + cilazapril 1-5 mg). 2 patients dropped out of the study. 2 patients had drug intolerance. Four patients did not continue in the study for other reasons.

Ten patients in each of the three groups completed the study. The findings of 30 patients who completed the 3-month treatment period were examined. Thirty patients (15 males, 15 females, mean age 58.9±8.73 years, range 45-76) who were hypertensive (systolic and diastolic BP ranges: 140-170 and 85-99 mmHg, respectively) and who had not used any antihypertensive and/or beta-blocker drugs in the last 3 months were included in the study. The known mean duration of diabetes and hypertension were 10.2±5.1 and 8.2±6.8 years, respectively. Patients received oral antidiabetic (n: 21) or insulin (n: 9) treatment and diet for diabetes mellitus.

Laboratory assessments

Fasting blood samples were collected from all patients 2 weeks before treatment (week –2), immediately before treatment (week 0), at week 6 of treatment (week 6), and 6 weeks after randomization to drug groups (week 12). Routine biochemical tests were measured using an autoanalyzer (Aeroset System Abbott, Abbott Laboratories, Diagnostic Department, Illinois, USA) and complete blood count using an autoanalyzer (Abbott Cell-Dyn 3700SL, Abbott Laboratories, Diagnostic Department, Illinois, USA).

Blood was withdrawn from the antecubital vein in the fasting state in EDTA-containing and non-additive tubes and was processed in the laboratory immediately after collection. Sera and plasma were separated by centrifugation at 1,500 g for 10 min. Serum aliquots for paraoxonase/arylesterase measurements were kept at –80 ºC until the analyses were performed. A part of the whole blood was frozen for Gpx determination. Red blood cells for SOD determination were washed with saline and frozen after hemolysis. Oxidative status (basal) and oxidizability of apolipoprotein B-containing lipoproteins were studied in plasma within 24 hours.

Paraoxonase activity was determined as described by Eckerson et al.[14]. The rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm at 25 ºC. The amount of p-nitrophenol generated was calculated from the molar absorptivity coefficient at pH 10.5, which was 18,290 M-1 cm-1. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated using its molar absorptivity coefficient, 1,310 M-1 cm-1[15]. Red blood cell SOD and whole blood Gpx activities were determined using Randox kits (Antrim, UK). Briefly, the determination of SOD activity was based on the production of O2- anions by the xanthine/xanthine oxidase system. Gpx was catalyzed by the oxidation of reduced glutathione in the presence of cumene hydroperoxide. Depletion of nicotinamide adenine dinucleotide phosphate was measured spectrophotometrically at 340 nm. To examine the oxidative status (basal) and oxidizability of apolipoprotein B-containing lipoproteins, this fraction was precipitated with dextran sulfate–magnesium chloride, and then EDTA was removed as described by Zhang et al.[16]. The cholesterol concentration of apolipoprotein B-containing lipoprotein fraction was adjusted to 200 mg/mL with phosphate-buffered saline. The MDA level of apolipoprotein B-containing lipoprotein fraction was measured before (basal) and after 3-h incubation with copper sulfate (final concentration 50 nmol/L) at 37.8 ºC. The basal value was subtracted from the 3 h value to obtain ΔMDA. Basal MDA represented the basal oxidative status of the apolipoprotein B-containing lipoprotein fraction, whereas ΔMDA represented the degree of oxidative modification (peroxidation capacity)[17]. MDA levels in this fraction were determined by the thiobarbituric acid reactive substances (TBARS) assay. MDA produced by the hydrolysis of lipid hydroperoxides heated under acid conditions reacts with TBA to form a complex that absorbs maximally at 532 nm. The complex was measured after extraction into butanol and quantified against MDA standards generated from 1,1V,3,3V tetraethoxypropane, which gave equimolar amounts of MDA under the same reaction conditions[18] The final results were given as nmol MDA/mg cholesterol[19].

Statistical analysis

Data were analyzed using SPSS for Windows (version 29.0.0, IBM SPSS Statistics) licensed package program. Numerical variables were given as arithmetic mean and standard deviation. Fisher-Freeman-Halton exact test was used to compare categorical variables between groups. A non-parametric Wilcoxon signed-rank test was used to compare numerical variables within groups. In comparing differences between groups, pre-treatment and post-treatment values were divided by pre-treatment values, and percentage changes were calculated. The Kruskal-Wallis test was used to compare groups. In the case of significance, the Mann-Whitney U test was used to compare groups. $P<0.05$ was accepted for statistical significance.

Results

Age, gender, diabetes and hypertension durations, oral antidiabetic drug, and insulin usage among the three groups were comparable ($p>0.05$). Other studied basal laboratory values were given in Table I.

No significant change was found in the BP values of the groups in the drug-free period (week –2 vs 0). In

the comparison between the groups, systolic (p=0.015) and diastolic (p=0.033) BP values of the ARB50+C group, and only diastolic BP values of the ARB50+ACEi group (p=0.025) were significantly lower compared to the ARB100 group at week 12. In all three groups, systolic and diastolic BPs decreased significantly (p<0.05) during the losartan 50 mg daily treatment period (week 0 vs 6) and in the post-randomization period (week 6 vs 12). Only in the ARB100 group was the decrease in diastolic BP not significant in week 12 compared to week 6 (p=0.083) (Table II). At the end of the post-randomization treatment period, the most significant decrease in BPs was seen in the ARB50+ACEi group (systolic BP: 16.5±7.4 mmHg, diastolic BP: 10.5±2.8 mmHg), and the smallest decreases in the ARB100 group (systolic BP: 9.0±6.1 mmHg, diastolic BP: 3.0±4.8 mmHg). In the ARB50+C group, systolic and diastolic BP decreased by 13.0±7.4 mmHg and 8.5±7.1 mmHg, respectively.

Table I. The baseline characteristics of patients (week –2)

Variables	ARB100 group (n: 10)	ARB50+C group (n: 10)	ARB50+AC Ei group (n: 10)	P-value
Age (years)	56.4±7.3	56.8±8.3	63.7±9.1	0.145
Gender (M/F)	3/7	5/5	7/3	0.262
DM duration (years)	9.9±4.1	10.8±5.5	9.9±5.9	0.777
HT duration (years)	9.6±6.3	6.5±4.5	8.7±9.2	0.503
OAD/insulin usage (n)	7/3	6/4	8/2	0.879
Weight (kg)	85.6±13.6	74.0±9.9	81.4±12.3	0.163
BMI (kg/m²)	32.0±3.3	29.0±3.7	30.6±5.9	0.465
Systolic BP (mmHg)	149.5±4.9	148.5±9.4	152.2±5.4	0.319
Diastolic BP (mmHg)	91.9±4.0	93.3±3.7	93.1±4.2	0.756
Hemoglobin (g/dL)	13.3±2.1	13.7±1.2	13.8±1.24	0.890
SCr (mg/dL)	0.97±0.27	1.10±0.34	1.14±0.23	0.238
Na^- (mmol/L)	139.1±2.5	141.7±4.6	141.0±1.6	0.194
K^+ (mmol/L)	4.55±0.37	4.71±0.49	4.57±0.43	0.875
FPG (mg/dL)	186.0±54.7	161.8±62.1	138.8±15.2	0.116
2h PG (mg/dL)	268.4±64.1	219.3±110.4	228.7±54.1	0.155
HbA1c (%)	9.02±1.28	8.26±1.91	7.83±1.55	0.215
T-chol (mg/dL)	213±53	199±54	179±36	0.255
LDL-chol (mg/dL)	138±39	135±48	108±22	0.163
TG (mg/dL)	142±60	144±78	130±77	0.717

ARB: angiotensin II type 1 receptor blocker; ACEi: angiotensin-converting enzyme inhibitör; C: carvedilol; M: male; F: female; DM: diabetes mellitus; HT: hypertension; OAD: oral antidiabetic agent; BMI: body mass index; BP: blood pressure; SCr: serum creatinine; Na^-: sodium; K^+: potassium; FPG: fasting plasma glucose; 2h PG: 2-hour plasma glucose; HbA1c: hemoglobin A1c; T-chol: total cholesterol; LDL-chol: low-density lipoprotein cholesterol; TG: triglyceride.

The three groups' weight and BMI values, fasting plasma glucose, serum creatinine, potassium, and sodium levels were comparable. In intragroup comparisons, mean weight (p=0.013) and BMI (p=0.011) values decreased significantly in the ARB100 group at week 12 compared to week 6. In the ARB50+ACEi group, these values decreased significantly in week 6 compared to week 0 (p=0.005) but did not change in week 12. Fasting plasma glucose, serum creatinine, potassium, and sodium levels did not change significantly during the treatment periods in all three groups (Table II). After 12 weeks, 2-hour plasma glucose and HbA1c levels in the ARB100, ARB50+C and ARB50+ACEi groups were 193±26, 177±44 and 219±70 mg/dL and 7.56±0.91, 7.48±1.39 and 6.91±0.79%, respectively. There was no significant difference in these values between the groups. When baseline (week –2) values were compared, 2-hour plasma glucose (p=0.005) and HbA1c (p=0.017) levels decreased significantly in the ARB100 group. The changes in the ARB50+C group were insignificant. Only the HbA1c level decreased significantly in the ARB50+ACEi group (p=0.028).

Table II. Changes in laboratory parameters of the three groups throughout the study

Variables	Period	ARB100 group (n: 10)	ARB50+C group (n: 10)	ARB50+AC Ei group (n: 10)	P-value
Weight (kg)	Week 0	83.5±13.5	73.3±9.5	81.3±12.7	0.180
	Week 6	82.1±13.2	72.8±9.5	79.2±12.0	0.242
	Week 12	80.7±13.4	71.8±10.1	78.8±11.9	0.246
BMI (kg/m²)	Week 0	31.2±3.15	29.2±3.82	30.7±6.27	0.620
	Week 6	30.8±2.97	29.0±3.49	28.8±6.59	0.670
	Week 12	30.0±3.16	28.5±3.89	29.8±5.99	0.910
Systolic BP (mmHg)	Week 0	147±7.1	145±7.0	150±5.9	0.169
	Week 6	136±6.5	133±6.7	138±5.7	0.195
	Week 12	127±4.8	120±8.1	122±9.1	0.045
Diastolic BP (mmHg)	Week 0	89±5.6	89±5.7	93±4.1	0.132
	Week 6	81±3.1	81±6.2	83±4.2	0.431
	Week 12	78±4.2	73±9.1	72±5.4	0.044
FPG (mg/dL)	Week 0	147±22	167±61	153±23	0.703
	Week 6	154±46	163±42	139±17	0.562
	Week 12	133±26	149±43	140±28	0.884
SCr (mg/dL)	Week 0	0.93±0.28	1.17±0.40	1.11±0.21	0.140
	Week 6	0.96±0.30	1.10±0.33	1.13±0.20	0.243
	Week 12	0.99±0.29	1.13±0.34	1.13±0.26	0.278
K^+ (mmol/L)	Week 0	4.78±0.42	4.82±0.38	4.57±0.36	0.356
	Week 6	4.78±0.36	4.72±0.48	4.50±0.23	0.205
	Week 12	4.61±0.31	4.61±0.36	4.76±0.41	0.457
Na^- (mmol/L)	Week 0	140.9±3.54	141.9±2.46	142.1±3.31	0.599
	Week 6	141.5±3.71	140.9±3.63	143.1±3.54	0.507
	Week 12	141.1±4.40	140.9±3.51	143.2±2.34	0.233

ARB: angiotensin II type 1 receptor blocker; ACEi: angiotensin-converting enzyme inhibitör; C: carvedilol; BMI: body mass index; BP: blood pressure; FPG: fasting plasma glucose; SCr: serum creatinine; K^+: potassium; Na^-: sodium.

Basal-MDA and Δ-MDA levels, SOD, Gpx, PON, and AE activities of 30 patients did not change significantly both during the 2-week drug-free period and the 6-week 50 mg losartan treatment period. The baseline values of the three groups at week 6 were similar. When the baseline values before randomization (week 6) were compared in the three groups, no significant difference was found in week 12. There was no difference between the changes in the ARB100, ARB50+C, and ARB50+ACEi groups after six weeks of treatment. No statistical significance was observed when the changes in the ARB50 (n: 30) and treatment periods of the three groups were compared (Table III).

The 30 patients who could continue the study tolerated the treatment regimens well. No significant increase in serum potassium and creatinine levels or hypotension was observed during the study in these patients.

Discussion and Conclusion

Our study showed that losartan (ARB) treatment alone/increased doses and combined with cilazapril (ACEi) or carvedilol (third-generation beta-blocker) significantly reduced BP in patients with type 2 diabetes and hypertension without any significant side effects. The most significant decrease in BPs was observed in the ARB50+ACEi group. However, these antihypertensive treatment protocols did not affect antioxidant and oxidant systems for 3 months.

The choice of antihypertensive drugs in diabetic patients should be individualized according to proteinuria, renal function, and other factors. Although all major classes of antihypertensive medications provide cardiovascular benefits in type 2 diabetes, RAAS antagonists are preferred because of their renoprotection, improvement in insulin resistance, and other favourable metabolic effects[20,21]. The 2023 European Society of Cardiology/European Society of Hypertension (ESC/ESH) guideline emphasizes the importance of initiating dual combination therapy, one of which is a RAS blocker, in diabetic hypertensives to achieve BP targets. SGLT2 inhibitors and GLP-1 receptor analogs may help improve BP control along with glucose control, even in patients receiving multiple antihypertensive therapies. The newer nonsteroidal mineralocorticoid receptor antagonist (MRA) finerenone may be considered[20].

In dual RAAS blockade, ACE inhibition reduces circulating and tissue AII and increases bradykinin, which promotes nitric oxide production, and ARBs completely block AT1 receptors and may also activate AT2 receptors[22]. The combination of ACEi and ARBs is not recommended, mainly because of the risk of hyperkalaemia. Prolonged hypotensive episodes may adversely affect the kidneys, possibly in patients with reduced renal autoregulatory capacity[8]. Dual RAAS blockade may be an option in patients with advanced proteinuric nephropathy[23,24]. However, its effect on CKD outcome is unclear[23]. Another study also found that combining ACEi and ARBs reduced albuminuria in patients with proteinuric diabetic kidney disease but did not slow disease progression in the long term[25]. Dual RAAS blockade may slow or prevent renal

Table III. The changes in oxidant and anti-oxidant systems of patients throughout the study

Drug	Period	PON (U/L)	AE (kU/L)	SOD (U/g Hb)	Gpx (U/g Hb)	Basal MDA (nmol MDA/mg chol)	ΔMDA (nmol MDA/mg chol)
ARB50 group (n: 30)	Week –2	181.3±105.1	104.1±61.1	1042.0±552.7	13.2±8.9	45.212.4	44.9±26.4
	Week 0	205.6±108.9	91.3±30.9	1231.2±791.5	20.3±54.0	44.1±19.2	40.1±30.7
	Week 6	200.9±102.9	95.8±24.4	1263.9±654.2	15.3±11.5	46.2±19.3	62.1±29.5
	0-6 weeks (%)	13.6±86.3	12.7±41.4	23.0±67.9	192.9±725.1	14.3±49.3	185.8±364.4
ARB100 group (n: 10)	Week 6	247.6±119.7	101.6±27.0	1315.9±658.2	15.8±15.1	52.7±26.0	71.2±22.6
	Week 12	241.2±125.4	103.5±27.6	1102.8±414.4	16.2±5.5	43.9±12.4	78.2±21.3
	6-12 weeks (%)	–1.80±18.75	5.11±32.11	–6.54±31.25	259.43±493.3	–2.30±53.40	13.1±20.6
ARB50+C group (n: 10)	Week 6	195.3±110.5	93.0±25.0	1322.9±887.6	13.2±10.4	42.5±18.0	46.0±34.3
	Week 12	226.0±138.3	95.1±22.7	1344.2±688.0	13.8±12.0	48.7±15.5	55.7±37.8
	6-12 weeks (%)	24.39±41.50	4.22±16.74	20.56±70.21	24.11±70.87	40.57±112.92	20.7±24.5
ARB50+ACEi group (n: 10)	Week 6	159.8±56.9	92.7±22.6	1153.0±370.7	17.0±9.0	43.5±11.4	69.3±26.3
	Week 12	151.8±57.2	90.8±25.5	1084.1±270.3	12.6±7.7	46.0±11.4	77.2±23.9
	6-12 weeks (%)	–3.58±20.26	–2.92±7.74	–3.91±9.19	–18.31±42.12	9.50±29.62	15.3±23.9

PON: paraoxonase activity; AE: arylesterase activity; SOD: erythrocyte superoxide dismutase; Gpx: glutathione peroxidase; MDA: malondialdehyde; Hb: hemoglobin; chol: cholesterol; ARB: angiotensin II type 1 receptor blocker; ACEi: angiotensin-converting enzyme inhibitor; C: carvedilol.

function loss, particularly in diabetic CKD patients at high cardiovascular risk, but adverse events should be closely monitored[26,27]. Our study focused on the effects of different regimens on antioxidant and oxidant systems, and no significant renal events were observed in our cohort at 3-month follow-up. Nevertheless, our analysis of renal outcomes of these different protocols up to 1 year is ongoing in this context.

Dysregulated ROS production (oxidative stress) in humans and experimental models influences cellular processes in systems that contribute to the increase in BP in the development of hypertension (such as the cardiovascular, renal, immune, and central nervous systems or RAAS)[28,29]. Regulation of RAAS, which controls BP, activation of the immune system that triggers inflammation, and production of ROS leading to oxidative stress and redox-sensitive signalling play a role in the pathogenesis of hypertension[30]. A study on redox imbalance in hypertensive patients found decreased levels of catalase, glutathione, and MDA and increased levels of Gpx and ceruloplasmin in hypertensives compared with controls[31]. Oxidative stress may be a result of vascular damage observed in hypertension. In a study in hypertensive individuals, when 50 mg atenolol was given with 12.5 mg hydrochlorothiazide without any antioxidant for three months, the increase in serum MDA levels was reduced with the decrease in BP, and the reduction in SOD, Gpx, vitamins E and C was increased[32]. On the contrary, another study showed that oxidative stress did not increase in treated and untreated hypertensive patients compared to normotensive patients[33]. Since our study did not include a healthy control and non-diabetic hypertensive group, comparing the oxidant and antioxidant status of hypertensive patients with or without T2DM with normotensive individuals was impossible.

RAAS antagonists improve endothelial function and prevent vascular remodelling, reducing cardiovascular risk. ACEi and ARBs improve endothelium-dependent vasodilation by stimulating antioxidant effects and NO release[34,35]. The renin blocker aliskiren exhibits antioxidant properties by reducing the increase in ROS produced by RAAS[36]. Nebivolol is a third-generation beta-blocker and is highly selective for the beta1-adrenoceptor[37]. CCB and nebivolol may also improve endothelial function, but classical beta-blockers are ineffective. Carvedilol may also improve endothelial function as a scavenger of oxygen-free radicals[35,38].

There are numerous studies supporting the antioxidant properties of antihypertensive drugs. These drug groups include some beta-blockers such as propranolol, nebivolol, carvedilol and celiprolol (but not atenolol), a CCB amlodipine, enalapril and other ACEi (SH-containing captopril, lisinopril and zofenopril)[39]. Positive changes in oxidative stress by-products and/or antioxidative enzymatic mechanisms may also occur independently of the choice of treatment with beta-blockers such as carvedilol and nebivolol, CCBs (lacidipine), RAAS antagonists (thiol-containing ACEi captopril, ARBs candesartan and olmesartan) or non-pharmacological treatment interventions[40-42]. In a clinical study, adding candesartan to an ACE inhibitor and beta-blocker in patients with heart failure reduced Nt-proBNP and hsCRP but did not alter other markers of inflammation or oxidative stress[43].

The results mentioned above were mainly obtained from in vivo and in vitro hypertensive experimental animal models. Administration of 5 mg amlodipine and 1,000 mg vitamin C daily for three months to hypertensive patients reduced oxidative stress and increased antioxidant status to prevent further vascular damage due to oxidative stress[44]. Additionally, even in non-diabetic patients undergoing chronic hemodialysis, 50-100 mg losartan treatment for three months showed positive effects on oxidative stress[45]. After 3 months of nonpharmacological treatment (n: 20, moderate salt restriction and a low-calorie diet if overweight), beta-blocker (n: 36, atenolol, bisoprolol) or ARB (n: 33, telmisartan) treatments (if necessary, addition of hydrochlorothiazide to reach target BP), a decrease in oxidative damage (MDA etc.) and an increase in antioxidant activity (SOD, catalase, Gpx) were observed in 89 hypertensive individuals[41]. This effect became more pronounced in 42 individuals after 12 months.

In our study, antihypertensive treatment intervention was performed in two six-week periods. Target BP was achieved in all patients (≤130/80 mmHg). Only one patient in the ARB50+C group had a BP of 140/95 mmHg at the end of the 12th week. Different combined antihypertensive regimens or adequate BP control did not cause any changes in oxidant and antioxidant system parameters at the end of the 12th week. This negative result may be related to the small number of patients in our study and the relatively short duration of treatment. In addition, a sample of people with diabetes with partially similar characteristics, without significant proteinuria and significant comorbidities, was enrolled, and there was no healthy control group. Contrary to observations in animal models[46], the inconsistencies seen in the results of different clinical studies may be because many factors such as smoking, diet, lifestyle, genetics, family history and concomitant diseases play a role in the etiology of hypertension in humans[39]. We did not find any study on the effects of combined treatment approaches on oxidant and antioxidant systems in hypertensive patients with T2DM. In hypertensive type 2 diabetic patients, one year of valsartan (80-160 mg/day) treatment improved proinflammatory

mediators that were increased independently of BP and glycemic control, supporting the cardiovascular protective effect of ARBs[47]. We previously reported that these protocols did not affect glycemic control, HOMA insulin resistance and lipid profile in 21 patients from this cohort who were using oral antidiabetic drugs alone[48]. Furthermore, the significant decrease in weight and BMI values in the ARB100 group at week 12 compared with week 6 in our study may also be related to the dietary compliance of the patients in this group. Because 2-h plasma glucose and HbA1c levels also decreased significantly in the ARB100 group compared with the baseline (week −2) values. The changes in the ARB50+C group were not significant. Only HbA1c levels decreased significantly in the ARB50+ACEi group. As a result, the improvement in glycemic control had not yet reached target levels in most patients at the total 14-week follow-up. Considering the relationship between diabetes and oxidative stress, BP control alone may not have been able to correct oxidant and antioxidant systems in patients with T2DM without adequate glycemic control. It may take a longer time for this effect to appear. Serum creatinine, potassium and sodium levels did not change significantly during the treatment periods in the three groups.

In conclusion, our observations show that combination regimens based on RAAS inhibition and new-generation beta-blockers in hypertensive type 2 diabetics reduce blood pressure safely. However, their effects on oxidant and antioxidant systems are similar in short-term use.

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