Fixed-dose combination of losartan and hydrochlorothiazide significantly improves endothelial function in uncontrolled hypertension by low-dose amlodipine: A randomized study

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Abstract

Objective: Flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD) in the brachial artery are well-known indices for evaluating endothelial function (ECF). The blood pressure-lowering effects of the combination of losartan (ARB) and low-dose hydrochlorothiazide (H: ARB-H; ARB, 50 mg and H, 12.5 mg) are useful. The aim of the present study was to examine whether the combination of losartan and low-dose hydrochlorothiazide could improve ECF.

Methods: To investigate the effect of ARB-H on ECF in patients with uncontrolled hypertension despite the use of amlodipine (2.5 mg daily), we performed a randomized controlled open-labeled study by using the envelope method and assigned 42 patients to either a control (CTRL) group or an ARB-H combination group, both of which received amlodipine 2.5 mg daily during the treatment period. In addition, both the CTRL (n=21, 69±7 years old) and ARB-H groups (n=21, 69±7 years old) received additional behavioral modification. Before and after 8 weeks of therapy, FMD and NMD were measured in both groups using novel FMD equipment (UNEXEF18G).

Results: Although baseline FMD was not different between the two groups, post-therapy FMD increased in the ARB-H group (2.97±1.56 to 3.95±1.86%, $p<0.05$) but did not change significantly in the CTRL group (2.95±1.43 to 3.11±1.27%, NS). No significant change was seen in NMD when comparing baseline and post-therapy values in either group. No treatment complications were observed.

Conclusion: A fixed-dose combination of losartan and hydrochlorothiazide enhances ECF, suggesting that this combination might have both anti-hypertensive and anti-atherosclerotic effects in patients with hypertension. *(Anadolu Kardiyol Derg 2014; 14: 685-91)*

Key words: flow-mediated vasodilatation, nitroglycerin-induced vasodilatation, angiotensin II receptor blocker, brachial artery, blood pressure

Introduction

The angiotensin II antagonist losartan has excellent blood pressure (BP) control effects in patients with hypertension. Recent studies have demonstrated that the combination of losartan and low-dose hydrochlorothiazide could control BP, even in patients with uncontrolled hypertension. Among the many anti-hypertensive agents, angiotensin II antagonists (angiotensin receptor blockers, ARBs) more effectively prevent target organ damage in hypertensive patients (1), and this effect is independent of its BP-lowering effects (2). In addition, combination therapy with losartan and low-dose hydrochlorothiazide can attenuate the atherosclerotic effect of hypertension in humans (3), because the side effects of hydrochlorothiazide, such as aggravation of both glucose tolerance and uric acid metabolism, could be proatherogenic and cancel the salutary anti-atherosclerotic effects of ARB.

In both experimental (4) and human studies (5-7), combination therapy with losartan and low-dose hydrochlorothiazide significantly alleviated vascular dysfunction, increased nitric oxide (NO), and attenuated intima-media thickness of the common carotid artery in patients with hypertension when compared with losartan therapy alone (4). However, the efficacy of the combination therapy with losartan and low-dose hydrochlorothiazide on endothelial function has not been fully investigated in humans.

Flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD) in the brachial artery are well-known indices for evaluating endothelial function. The purpose of this study was to investigate the effect of the combination of losartan and low-

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dose hydrochlorothiazide on endothelial function, as assessed by brachial artery FMD and NMD, in patients with uncontrolled hypertension despite low-dose amlodipine (2.5 mg) therapy.

Methods

Study population

The study population consisted of 42 hypertensive patients (34 men and 8 women; age, 69.3±6.7 years) who were referred to our hospital as a result of poor BP control, despite treatment with amlodipine (2.5 mg daily, which is widely used as the first-line medication for hypertension in our country) for at least 8 weeks. In addition, secondary hypertension, such as primary aldosteronism, and patients with either renal artery stenosis or chronic renal failure had been worked out and excluded from the study population. Additional exclusion criteria were as follows: (1) the presence of atrial fibrillation; (2) allergic reaction to anti-hypertensive agents; (3) diabetic acidosis; (4) advanced heart block; (5) congestive heart failure; (6) pregnancy; (7) any other acute disorders; and (8) severe hypertensive patients who experienced a hypertensive crisis. Informed consent was obtained from each patient. The study protocol was approved by the institutional review board.

BP measurement

BP measurements were followed by the guidelines of the American Heart Association Scientific Statement (8). The patients were asked to take away all clothing that covered the position of the cuff placement. They were seated comfortably, and the cuff on the upper arm was placed at the level of the right atrium. The patients were instructed to relax, and at least 5 min passed before the BP measurement was carried out. All study patients had systolic BP ≥140 mm Hg and/or diastolic BP ≥90 mm Hg when BP was obtained in the sitting position at the outpatient clinics. BP was measured at least 3 times by the Korotkoff method using a sphygmomanometer, and the measured BP values were averaged.

Study design and study protocol

This was a prospective open-labeled study. Each eligible patient was randomly assigned to one of two 8-week treatment arms. The method of randomization was the envelope method, where each patient was allocated into one of two treatment arms according to patient ID. For example, even-numbered IDs were allocated into one group, and odd-numbered IDs were allocated into the other group, keeping the rule of the consecutive time course visit of each patient's outpatient clinics, or we mixed IDs randomly and chose one of these mixed IDs when the patients met and agreed with enrollment into this study. These arms comprised either a control (CTRL) group or an ARB-H combination group, both of which received amlodipine 2.5 mg daily during the treatment period. In addition to the amlodipine dose maintenance treatment, both the CTRL (n=21, 69.4±6.7 years old) and ARB-H groups (n=21, 69.2±6.7 years old) received additional behavioral modifications, such as diet and/or exercise-therapy. A fixed-dose combination of losartan (50 mg) and hydrochlorothiazide (12.5 mg) was added to the ARB-H group. Patient selection is illustrated in the flow-chart according to the CONSORT statement (9) in Figure 1. In addition, any complications of each treatment were carefully monitored by investigators in each patient visit to the outpatient clinic or during brachial artery ultrasound measurements. Before assigning patients to these treatment arms, BP was measured in a sitting position, venous blood was obtained, and brachial artery endothelial functional testing was performed. Blood samples were tested for routine blood chemistry, including liver and renal function tests, lipid profile, fasting blood sugar, and complete blood counts, according to standard methods. All of these measurements were repeated before and after each treatment.

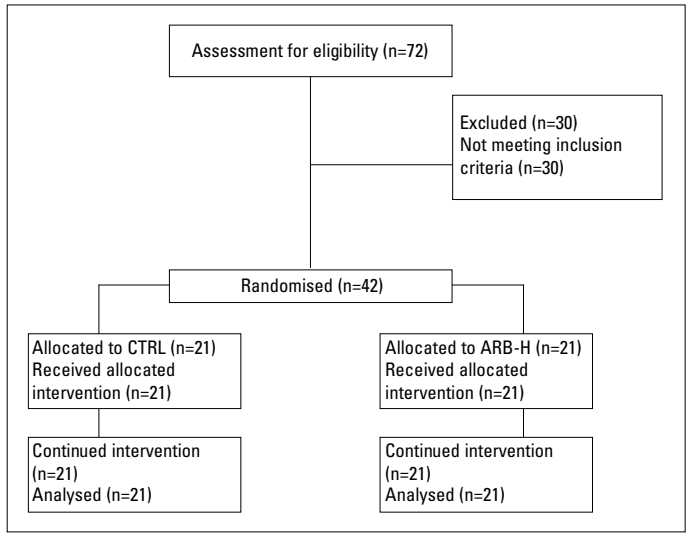

Figure 1. Flow-chart for patient selection

Ultrasound FMD and NMD measurements in the brachial artery

All ultrasound studies were done in a temperature-controlled room [25°C] with the subject in a fasting, resting, and supine state from approximately 14:00 to 17:00. Heavy meals, including a high-fat diet and caffeine-containing beverages, were prohibited beginning on the night before the study. Patients were not allowed to have lunch on the day of the ultrasound study. BP and heart rate were recorded from the left arm every 3 min with an automatic sphygmomanometer (Nihon Korin, BP-203, Tokyo, Japan) during the ultrasound procedure. Vasodilatation responses of the brachial artery were determined by ultrasound technique using a semi-automatic device (EF18G; UNEX, Nagoya, Japan). Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 10-mHz linear array transducer. Then, a BP cuff was inflated to 50 mm Hg above the systolic BP over the proximal portion of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diam-

Table 1. Clinical characteristics of the study population

	ARB-H group (n=21)	CTRL group (n=21)
Age, years	69.2±6.7	69.4±6.7
Male/Female	17/4	17/4
Complications or comorbidities		
Hypercholesterolemia, %	2 (10%)	2 (10%)
Coronary artery disease, %	2 (10%)	2 (10%)
Diabetes mellitus, %	3 (14%)	2 (10%)
Combination treatment, %		
Statin, %	3 (14%)	4 (19%)
β-blocker, %	5 (24%)	6 (29%)
Nitrates, %	1 (5%)	1 (5%)
Anti-platelet agents, %	5 (24%)	5 (24%)
Oral anti-diabetic agents, %	3 (14%)	2 (10%)

ARB-H group: fixed-dose combination of losartan (50 mg) and hydrochlorothiazide (12.5 mg) with additional behavioral modifications, such as diet and/or exercise therapy; CTRL group: amlodipine dose maintenance with additional behavioral modification, as in the ARB-H group; data are expressed as mean±SD or % in parentheses; hypercholesterolemia, total cholesterol >220 mg/dL; coronary artery disease, luminal stenosis >50% in the major branch of the coronary artery by angiogram; diabetes mellitus, fasting blood sugar >126 mg/dL. Unpaired student's t-test was utilized for age comparisons, and Pearson's chi-square was applied for male/female and complication or comorbidity comparisons

eter. The changes in diastolic diameter were continuously recorded. Then, FMD was determined as the maximum change in diameter after cuff release, normalized to the baseline diameter (% of baseline diameter). After a 15-min interval to negate any effect of reactive hyperemia, NMD was assessed. Baseline measurements of brachial artery diameter and flow velocity were obtained again, and 0.3 mg of sublingual nitroglycerin was then administered. Three minutes later, the brachial artery diameter was recorded. The NMD was defined as the percent change of the brachial artery diameter relative to the baseline diameter. These measurements were obtained using the EF18G. The calculations of these values by the EF18G in our laboratory showed that the intra- and inter-observer variability (coefficient of variation) for repeated measures of the diameter before and after reactive hyperemia in the brachial artery was <3% (10, 11).

Statistical analysis

Data are expressed as mean±SD. Categorical variables are summarized as frequency (group percentage) and are compared between the groups using Person's chi-square test. Even if the sample size was small, the histogram of each sample was not skewed (data not shown), so that we presumed each sample in this study was drawn from normally distributed data. Parametric statistical methods were subsequently utilized. Paired student's t-test was used to compare data before and after each treatment unpaired student's t-test was also applied to compare data between the two groups. Pearson product moment correlation was performed between the changes in systolic or diastolic BP and those of FMD or NMD.

In addition, two-way repeated measures ANOVA was utilized to compare the efficacy of the treatment between the CTRL group and ARB-H group. These statistical analyses were performed by SPSS statistical software (Dr. SPSS II for Windows, Nankodo Inc., Tokyo Japan). The sample size was determined by power analysis [G*Power 3.17, (12)], using preliminary data obtained in our laboratory, with the following assumptions: type I error of 0.05 (2-tailed), power of 80%, difference in absolute changes in %FMD between the ARB-H group and CTRL group of 0.76%, and a standard deviation of 1.6%. According to the calculated result, a minimum of 20 patients would yield 80% power to detect a difference in the absolute changes in % FMD. Differences and statistical values were considered significant at $p<0.05$.

Results

Patient profile

The present study consisted of 21 patients in the CTRL group and 21 patients in the ARB-H group, as shown in Table 1. Clinical characteristics were compared between the two groups. Age, sex, and the prevalence of comorbid conditions and complications and combination treatments were similar when comparing the two groups. The mean age in each group was approximately 70 years (elderly populations). The other clinical characteristics are illustrated in Table 1. Approximately 10% of patients in each group had dyslipidemia, 10% had coronary artery disease, and 10% had diabetes mellitus. Consistent with the high prevalence of atherosclerotic risk factors in this study population, the average FMD was approximately 3% in both groups, and the average NMD was approximately 11% in both groups. None of the study patients had FMD ≥6%, which indicates that all patients had impaired endothelial function. In this study, anti-hypertensive medication was not titrated to achieve the level of BP control recommended by hypertension treatment guidelines (13), mainly due to the relatively brief treatment period (8 weeks). In addition, no treatment complications were observed in either treatment group.

Changes in FMD and NMD

The effects of each treatment on hemodynamics, FMD, and NMD are summarized in Table 2. In contrast to the CTRL group, the ARB-H group experienced a significant decrease in systolic and diastolic BP following treatment. As shown in Table 2 and Figures 2 and 3, the ARB-H group experienced a significant increase in FMD after treatment. No significant changes were found in baseline brachial artery diameter or NMD in either group. The FMD values increased in most patients of the ARB-H group, versus 9 patients in the CTRL group (Fig. 2). In addition, according to the results of the two-way repeated measures ANOVA, there were significant differences in the pattern of the effect of each treatment and the increased values of FMD between the CTRL group and ARB-H group (the effect of each treatment, $p<0.001$; the effect of each treatment combined with treatment group differences, $p<0.006$).

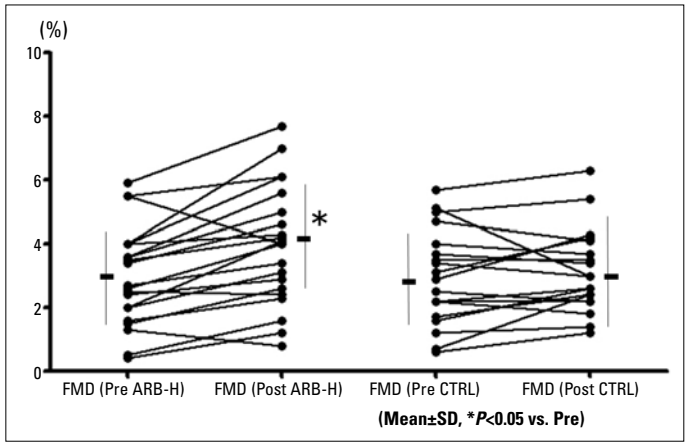

Figure 2. Effect of each treatment on FMD
FMD- flow-mediated dilation; Pre ARB-H: before the fixed-dose combination of losartan (50 mg) and hydrochlorothiazide (12.5 mg), with additional behavioral modification, such as diet and/or exercise therapy; "Post" indicates measurements taken after 8 weeks of therapy; "Pre" indicates measurements taken before initiation of the 8-week treatment regimen. Data presented as mean±SD; *P<0.05 vs. pre ARB-H

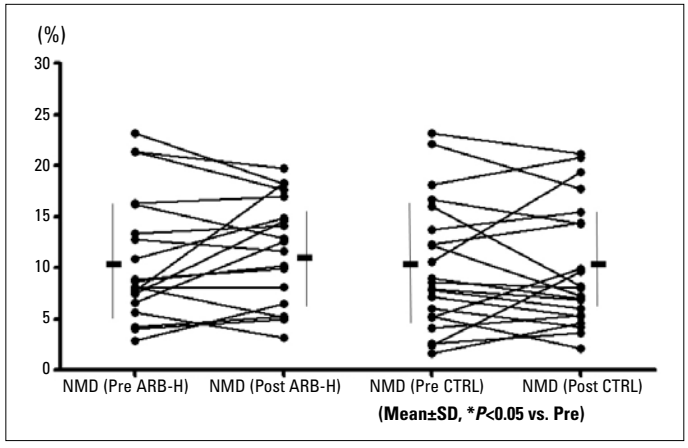

Figure 3. Effect of each treatment on NMD
NMD- nitroglycerin-mediated dilation; NS- no significant differences between Pre and Post. Format is the same as that in Figure 2

In the ARB-H group, the changes in either systolic or diastolic BP in response to treatment did not correlate significantly with changes in FMD (delta systolic BP vs. delta FMD, r=-0.14, n=21, NS; delta diastolic BP vs. delta FMD, r=-0.04, n=21, NS).

Laboratory analysis

No changes were detected in most parameters of blood chemistries in either treatment group, as shown in Table 3.

Discussion

The present study demonstrated that combination therapy with losartan and low-dose hydrochlorothiazide resulted in improvement in endothelial function for 8 weeks, as assessed by brachial artery FMD and NMD, in patients with uncontrolled hypertension and mild endothelial dysfunction, despite low-dose amlodipine therapy. As shown in Figures 2 and 3, although all patients in both treatment groups had brachial artery endothelial dysfunction (FMD <6%) at baseline and although the changes in NMD did not differ when comparing the two groups, 18 of 21 patients (86%) in the ARB-H group had increases in FMD with therapy, whereas only 9 of 21 (43%) patients in the CTRL group had increases in FMD with therapy. These results indicate that endothelial function tended to improve only in the ARB-H group. The clinical characteristics were not significantly differ-

Table 2. Summary of ultrasound measurements of flow-mediated dilation and nitroglycerin-mediated dilation in the brachial artery and the effect of each treatment

	ARB-H group (n=21)		CTRL group (n=21)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Systolic BP, mm Hg	146±5	128±8*	148±7	140±16
Diastolic BP, mm Hg	78±8	70±8*	80±9	78±9
Heart rate, beats/min	65±10	61±9	66±10	64±11
Brachial artery diameter at baseline, mm	4.60±0.52	4.58±0.57	4.32±0.42	4.28±0.39
FMD, %	2.97±1.56	3.95±1.86*	2.95±1.43	3.11±1.27
NMD, %	10.64±5.91	11.63±4.71	10.59±5.50	9.28±5.24

Abbreviations are same as in Table 1; data are expressed as mean±SD-BP, blood pressure; FMD- flow-mediated dilation; NMD- nitroglycerin-mediated dilation; *p<0.05 vs. pretreatment- paired student's t-test was utilized in the intra group comparison, unpaired student's t-test was utilized in the inter group comparison, and two-way repeated measures ANOVA was applied for FMD analysis. In the FMD analysis, the F and P values for the intra group comparison were 16.66 and 0.0001, respectively, and the F and P values for between-group comparisons were 8.54 and 0.006, respectively

Table 3. Effect of each treatment on blood chemistry and blood cells

	ARB-H group (n=21)		CTRL group (n=21)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
LDL-cholesterol, mg/dL	116±27	118±23	110±34	110±36
HDL-cholesterol, mg/dL	49±11	50±13	45±13	45±12
Triglyceride, mg/dL	162±73	186±74	148±57	153±44
s-AST, IU/L	28±7	28±8	30±9	29±9
s-ALT, IU/L	27±12	24±11	30±14	29±17
BUN, mg/dL	16±4	19±5	14±3	15±4
Cr, mg/dL	0.92±0.28	0.97±0.27	0.87±0.22	0.90±0.25
FBS, mg/dL	108±17	108±21	118±27	117±21
WBC, x 10^3/μL	6.00±0.98	6.04±1.13	6.60±1.28	5.72±1.38
RBC, x 10^6/μL	4,38±0.50	4.35±0.48	4.48±0.39	4.42±0.45
Plt, x 10^4/μL	23.5±9.3	21.3±4.4	19.7±5..3	18.4±3.6

ARB-H group: fixed dose combination of losartan (50 mg) and hydrochlorothiazide (12.5 mg), with additional behavioral modifications, such as diet and/or exercise therapy; BUN- blood urea nitrogen; CTRL group: amlodipine dose maintenance with additional behavioral modification, as in the ARB-H group; Cr - serum creatinine; FBS - fasting blood sugar; HDL - cholesterol, high- density lipoprotein cholesterol; LDL - cholesterol, low-density lipoprotein cholesterol; Plt - platelet count; RBC - red blood cell; s-ALT - serum alanine aminotransferase; s-AST - serum aspartate aminotransferase; WBC - white blood cell; Data are expressed as mean±SD. No data showed statistically significant differences. Paired student's t-test was utilized in intragroup comparisons, and unpaired student's t-test was utilized in intergroup comparisons

ent when comparing the two groups. In addition, no significant relationship was observed between the degree of therapeutic improvement in BP and the changes in FMD. Thus, the favorable effect of losartan and low-dose hydrochlorothiazide on endothelial function was not mediated by its effect on BP.

In terms of the effect of losartan only on endothelial function, many previous reports agree with the present study. Losartan has been widely reported to have a salutary effect on endothelial dysfunction induced by elevated BP, which is a hypertensive state in both experimental settings (14, 15) and clinical scenarios (16). In the hypertensive condition, the mechanisms of losartan efficacy were explained by the antioxidant effect (16, 17). The other ARBs have also been reported to have ameliorating effects on endothelial function (18, 19). However, a previous study showed that the powers of this salutary effect on endothelial function varied, depending on the different types of ARB (20), whereas the combined treatment with ARB and hydrochlorothiazide showed an enhancing effect in improving endothelial dysfunction in patients with hypertension (21).

Although the combined treatment with ARB and hydrochlorothiazide on endothelial dysfunction has been reported in hypertensive patients (15), an 8-week improvement in previously impaired endothelial function in response to combination therapy with losartan and hydrochlorothiazide has not been previously reported in humans with uncontrolled hypertension, and this information could be useful in clinical practice. Previous reports (6) have demonstrated that combination therapy with losartan and hydrochlorothiazide could regress the intima-media thickness of the common carotid artery when target BP (systolic BP <140 mm Hg and diastolic BP <90 mm Hg) is achieved in patients with hypertension. Another report (4) showed that chronic administration of losartan and hydrochlorothiazide increased the production of NO in young cardiomyopathic hamsters. These clinical (15, 21) and experimental (22) studies support our findings of improved endothelial function after combination therapy with losartan and hydrochlorothiazide in the present study. However, if either losartan or hydrochlorothiazide was separately prescribed for a 12-week treatment period, no improvement was seen in brachial artery FMD (23). In light of the results from the present study, these data suggest that the combination of losartan and hydrochlorothiazide might be needed to improve brachial artery FMD in patients with hypertension.

The combination of losartan and hydrochlorothiazide actually has the potential to aggravate hypertension or vascular dysfunction through excessive urinary zinc (Zn) loss or through the induction of magnesium (Mg) deficiency (24). However, observations from a previous report (24) and the present study suggest that the combination of losartan and hydrochlorothiazide has other properties that overcome these unfavorable effects, resulting in good blood pressure control and improvements in endothelial function in patients with uncontrolled hypertension, despite amlodipine therapy. This notion is consistent with observations from earlier studies (24, 25), including randomized clinical trials (26-30) showing that anti-hypertensive losartan plus hydrochlorothiazide therapy achieves good blood pressure control and does not produce harmful side effects.

Study limitations

In this study, the CTRL group played the role of the placebo treatment group, since it was not logical to expect a salutary effect of the treatment in the CTRL group on FMD and NMD. Nine patients in the CTRL group showed ameliorated FMD (Fig. 1), and the average systolic BP tended to decrease but not statistically significantly. These findings might be due to a possible placebo effect of the treatment in the CTRL group. Also, a comparison of efficacy between after improving the dose of the medication to 5 mg in patients that use 2.5 mg amlodipine and the treatment arm of the ARB-H group was not conducted. In addition, there are several other limitations to this study. First, the number of the patients was small, and the study was conducted in limited medical centers. However, since most patients who were assigned to combination therapy with losartan and hydrochlorothiazide experienced improvements in FMD, we believe that this result is valid. Second, the mechanisms of the synergistic effect of the combination of losartan and hydrochlorothiazide were not investigated in the present study. However, according to a previous study, this synergistic effect may be mediated, at least in part, by activation of the endothelial nitric oxide synthase (eNOS) pathway and by decreases in oxidative stress (4, 5, 22, 31). In addition, the anti-atherosclerotic properties of losartan may counteract the adverse effects of hydrochlorothiazide on glucose and lipid metabolism to produce an overall favorable effect. Third, even if the combination therapy of losartan and hydrochlorothiazide significantly improved endothelial function, the values of FMD were still abnormally low, belonging to the endothelial dysfunction zone. These findings suggest that an 8-week duration of combination therapy with losartan and hydrochlorothiazide might not be long enough or that the background condition of the study population, such as an elderly population, might be the reason why the 8-week treatment of the combination of losartan and hydrochlorothiazide could not improve FMD into the normal range. This concern should be studied in a future investigation. Fourth, high-sensitivity CRP is now recognized as an important marker for atherosclerosis. However, this parameter was not measured in the present study. This should be also investigated in a duplicate study. Lastly, only the 8-week effect of the combination of losartan and hydrochlorothiazide was investigated in this study. Thus, the long-term effects of combination therapy on endothelial function should be conducted in the future. Regardless, the confirmation of the 8-week efficacy of the combination of losartan and hydrochlorothiazide provides useful information that is relevant to a common clinical scenario-namely, the treatment of vulnerable patients with acute coronary syndrome and hypertension.

Conclusion

The addition of a low-dose, fixed-dose combination of losartan and hydrochlorothiazide resulted in the subacute improvement of endothelial function, suggesting that this combination might have both anti-hypertensive and anti-atherosclerotic effects in hypertensive patients.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - B.T., A.K., M.N.; Design - M.N.; Supervision - M.I.; Resource - M.N.; Material - M.N.; Data collection &/or processing - B.T., M.N.; Analysis &/or interpretation - H.H., Y.T., Literature search - B.T., A.K., M.N.; Writing - B.T., A.K., M.N.; Critical review - B.T., H.H., Y.T., M.N., M.I., A.K.

References

1. Safar ME, Protogerou A, Blacher J. Central blood pressure under angiotensin and calcium channel blockade. Hypertension 2009; 54: 704-6. [CrossRef]
2. Kobayashi N, Ohno T, Yoshida K, Fukushima H, Mamada Y, Nomura M, et al. Cardioprotective mechanism of telmisartan via PPAR-gamma-eNOS pathway in dahl salt-sensitive hypertensive rats. Am J Hypertens 2008; 21: 576-81. [CrossRef]
3. Lacourcière Y, Neutel JM, Schumacher H. Comparison of fixed-dose combinations of telmisartan/hydrochlorothiazide 40/12.5 mg and 80/12.5 mg and a fixed-dose combination of losartan/hydrochlorothiazide 50/12.5 mg in mild to moderate essential hypertension: pooled analysis of two multicenter, prospective, randomized, open-label, blinded-end point (PROBE) trials. Clin Ther 2005; 27: 1795-805. [CrossRef]
4. Crespo MJ, De Mello WC. Chronic administration of losartan plus hydrochlorothiazide improves vascular status in young cardiomyopathic hamsters. Eur J Pharmacol 2001; 420: 133-41. [CrossRef]
5. Cammarata R, Armas-Hernández MJ, Hernández-Hernández R, Armas-Padilla MC, Sosa-Canache B, Pacheco B, et al. Effect of losartan plus hydrochlorothiazide on nitric oxide status in 'nondipper' hypertensive patients. Am J Ther 2007; 14: 161-5. [CrossRef]
6. Ludwig M, Stapff M, Ribeiro A, Fritschka E, Tholl U, Smith RD, et al. Comparison of the effects of losartan and atenolol on common carotid artery intima-media thickness in patients with hypertension: results of a 2-year, double-blind, randomized, controlled study. Clin Ther 2002; 24: 1175-93. [CrossRef]
7. Kinouchi K, Ichihara A, Bokuda K, Kurosawa H, Itoh H. Differential Effects in Cardiovascular Markers between High-Dose Angiotensin II Receptor Blocker Monotherapy and Combination Therapy of ARB with Calcium Channel Blocker in Hypertension (DEAR Trial). Int J Hypertens 2011; 2011: 284823. [CrossRef]
8. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Circulation 2005; 111: 697-716. [CrossRef]
9. Moher D, Schulz KF, Altman D; CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA 2001; 285: 1987-91. [CrossRef]
10. Tomiyama H, Matsumoto C, Yamada J, Teramoto T, Abe K, Ohta H, et al. The relationships of cardiovascular disease risk factors to flow-mediated dilatation in Japanese subjects free of cardiovascular disease. Hypertens Res 2008; 31: 2019-25. [CrossRef]
11. Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, et al. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998; 82: 1535-9. [CrossRef]
12. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007; 39: 175-91. [CrossRef]
13. Mancia G, Grassi G. Impact of new clinical trials on recent guidelines on hypertension management. Ann Med 2011; 43: 124-32. [CrossRef]
14. Koifman B, Topilski I, Megidish R, Zelmanovich L, Chernihovsky T, Bykhovsy E, et al. Effects of losartan + L-arginine on nitric oxide production, endothelial cell function, and hemodynamic variables in patients with heart failure secondary to coronary heart disease. Am J Cardiol 2006; 98: 172-7. [CrossRef]
15. Demirci B, McKeown PP, Bayraktutan U. Blockade of angiotensin II provides additional benefits in hypertension- and ageing-related cardiac and vascular dysfunctions beyond its blood pressure-lowering effects. J Hypertens 2005; 23: 2219-27. [CrossRef]
16. Flammer AJ, Hermann F, Wiesli P, Schwegler B, Chenevard R, Hürlimann D, et al. Effect of losartan, compared with atenolol, on endothelial function and oxidative stress in patients with type 2 diabetes and hypertension. J Hypertens 2007; 25: 785-91. [CrossRef]
17. Yao EH, Fukuda N, Matsumoto T, Kobayashi N, Katakawa M, Yamamoto C, et al. Losartan improves the impaired function of endothelial progenitor cells in hypertension via an antioxidant effect. Hypertens Res 2007; 30: 1119-28. [CrossRef]
18. Mackenzie A, Dunning L, Ferrell WR, Lockhart JC. Angiotensin II Type 1 receptor blockade protects endothelium-derived hyperpolarising factor-mediated relaxation in a rat model of monoarthritis. Life Sci 2013; 92: 1131-7. [CrossRef]
19. Marcus NJ, Philippi NR, Bird CE, Li YL, Schultz HD, Morgan BJ. Effect of AT1 receptor blockade on intermittent hypoxia-induced endothelial dysfunction. Respir Physiol Neurobiol 2012; 183: 67-74. [CrossRef]
20. Mason RP, Jacob RF, Kubant R, Jacoby A, Louka F, Corbalan JJ, et al. Effects of angiotensin receptor blockers on endothelial nitric oxide release: the role of eNOS variants. Br J Clin Pharmacol 2012; 74: 141-6. [CrossRef]
21. Hu ZP, Wang BN, Qian HY, Zhou Q, Wei W, Wang Y. Fixed-dose telmisartan/hydrochlorothiazide in comparison with losartan/hydrochlorothiazide in decreasing serum hepatocyte growth factor and improving endothelial dysfunction in hypertensive patients. Int Heart J 2010; 51: 252-8. [CrossRef]
22. Taguchi K, Matsumoto T, Kamata K, Kobayashi T. Angiotensin II type 2 receptor-dependent increase in nitric oxide synthase activity in the endothelium of db/db mice is mediated via a MEK pathway. Pharmacol Res 2012; 66: 41-50. [CrossRef]
23. Chung NA, Beevers DG, Lip G. Effects of losartan versus hydrochlorothiazide on indices of endothelial damage/dysfunction,

angiogenesis and tissue factor in essential hypertension. Blood Press 2004; 13: 183-9. [CrossRef]
24. Koren-Michowitz M, Dishy V, Zaidenstein R, Yona O, Berman S, Weissgarten J, et al. The effect of losartan and losartan/hydrochlorothiazide fixed-combination on magnesium, zinc, and nitric oxide metabolism in hypertensive patients: a prospective open-label study. Am J Hypertens 2005; 18: 358-63. [CrossRef]
25. Minami J, Furukata S, Ishimitsu T, Matsuoka H. Comparison of therapies between fixed-dose telmisartan/hydrochlorothiazide and losartan/hydrochlorothiazide in patients with mild to moderate hypertension. Int Heart J 2009; 50: 85-93. [CrossRef]
26. Minami J, Abe C, Akashiba A, Takahashi T, Kameda T, Ishimitsu T, et al. Long-term efficacy of combination therapy with losartan and low-dose hydrochlorothiazide in patients with uncontrolled hypertension. Int Heart J 2007; 48: 177-86. [CrossRef]
27. Lindholm LH, Ibsen H, Dahlöf B, Devereux RB, Beevers G, de Faire U, et al; LIFE Study Group. Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention for Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002; 359: 1004-10. [CrossRef]
28. Bakris GL, Weir MR, Shanifar S, Zhang Z, Douglas J, van Dijk DJ, et al; RENAAL Study Group. Effects of blood pressure level on progression of diabetic nephropathy: results from the RENAAL study. Arch Intern Med 2003; 163: 1555-65. [CrossRef]
29. Carr AA, Kowey PR, Devereux RB, Brenner BM, Dahlöf B, Ibsen H, et al. Hospitalizations for new heart failure among subjects with diabetes mellitus in the RENAAL and LIFE studies. Am J Cardiol 2005; 96: 1530-6. [CrossRef]
30. Hosoya T, Kuriyama S, Ohno I, Kawamura T, Ogura M, Ikeda M, et al. Antihypertensive effect of a fixed-dose combination of losartan /hydrochlorothiazide in patients with uncontrolled hypertension: a multicenter study. Clin Exp Nephrol 2012; 16: 269-78. [CrossRef]
31. Yamada Y, Tsuboi K, Hattori T, Murase T, Ohtake M, Furukawa M, et al. Mechanism underlying the efficacy of combination therapy with losartan and hydrochlorothiazide in rats with salt-sensitive hypertension. Hypertens Res 2011; 34: 809-16. [CrossRef]