Effects of Low-Dose Combination Therapy with an Angiotensin-Converting Enzyme Inhibitor and a Diuretic on Flow-Mediated Vasodilation in Hypertensive Patients: A 6-Month, Single-Center Study

Cevad Sekuri, MD, Ozgur Bayturan, MD, Hakan Gocer, MD, Talat Tavli, MD, and Ugur Kemal Tezcan, MD

Department of Cardiology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey

ABSTRACT

Background: Combination therapy with an angiotensin-converting enzyme (ACE) inhibitor and a diuretic has been shown to be highly effective in hypertension. Clinical trials have demonstrated that ACE inhibitors may improve endothelial cell dysfunction in hypertension. However, the effectiveness of the combination treatment in endothelial cell dysfunction is unknown.

Objective: This study investigated the effects of a new low-dose combination, perindopril 2 mg plus indapamide 0.625 mg, on brachial artery flow-mediated vasodilation (FMD) and left ventricular diastolic function in hypertension.

Methods: Patients aged 18 to 75 with newly diagnosed stage I or II hypertension were eligible. Endothelium-dependent brachial artery FMD and endothelium-independent vasodilation were assessed at baseline. Patients were treated with oral perindopril 2 mg plus indapamide 0.625-mg tablets once daily for 6 months. FMD measurements were then repeated. Percentage changes in FMD from baseline to 6 months, as well as left ventricular diastolic function parameters (isovolumic relaxation time [IVRT] and mitral diastolic E-wave deceleration time [EDT]), indicated the effectiveness of the intervention.

Results: Twenty-nine Turkish patients were enrolled (17 women, 12 men; mean [SD] age, 54.5 [9.5] years [range, 38–75 years]). The mean (SD) baseline FMD was 7.00% (2.39%) (endothelial cell dysfunction) and increased significantly to 8.68% (2.78%) at 6 months ($P = 0.02$); FMD improved in 15 patients (51.7%). At baseline and 6 months of therapy, mean (SD) IVRT was 101.7 (12.4) ms and 95.5 (7.7) ms, respectively ($P < 0.001$), and EDT was 234.7 (33.9) ms and 217.9 (25.6) ms, respectively ($P < 0.001$).

Conclusions: In this small sample of hypertensive patients, a low-dose combination ACE inhibitor and diuretic significantly improved brachial artery FMD

and left ventricular diastolic function. The improvement in FMD values was independent of the stage of hypertension. These findings suggest a relationship between improvement in endothelial cell function and diastolic function. (*Curr Ther Res Clin Exp.* 2003;64:715–724) Copyright © 2003 Excerpta Medica, Inc.

Key words: flow-mediated vasodilation, hypertension, angiotensin-converting enzyme inhibitors, diuretics.

INTRODUCTION

Hypertension is a known risk factor for the progression of atherosclerosis and cardiovascular disease (eg, coronary events, heart failure, arrhythmias, and stroke).^{1,2} Endothelial cell dysfunction is early pathophysiologic evidence of atherosclerosis.³ In vitro studies have shown that endothelial cell function is abnormal in the early stages of atherosclerosis, even before the plaque exists and clinical symptoms develop.⁴ The most important functional consequence of endothelial cell dysfunction is a decrease in the ability to produce and release endothelial-derived release factor (EDRF).⁵ Two studies^{6,7} suggest decreased EDRF level and impaired endothelial cell function in hypertensive patients. Two clinical trials^{8,9} have demonstrated that endothelial cell dysfunction improves with angiotensin-converting enzyme (ACE) inhibitor administration. However, combination regimens with diuretics have become important because the success rate is low with monotherapy in hypertension.¹⁰ Although blood pressure (BP) is controlled more effectively with combination therapy than with monotherapy,^{11,12} the effect of BP on endothelial cell function has so far not been studied.

The objective of this study was to investigate the effects of a new low-dose combination of an ACE inhibitor, perindopril 2 mg, and a low-dose diuretic, indapamide 0.625 mg, on brachial artery flow-mediated vasodilation (FMD) and diastolic parameters in patients with mild or moderate hypertension. The hypothesis of the study was that treatment with the combination of perindopril and indapamide for 6 months would result in improved endothelial cell function compared with baseline. The primary efficacy parameter was the net change in the FMD of the brachial artery between baseline and after 6 months of therapy.

PATIENTS AND METHODS

Patients

The study population included patients recruited from the Department of Cardiology, Celal Bayar University (Manisa, Turkey) between October 2000 and May 2001. Patients were eligible for the study if they were aged 18 to 75 and had newly diagnosed stage I (mild) or II (moderate) hypertension based on Joint National Committee on Prevention, Detection, Evaluation, and Treatment of

High Blood Pressure (JNC) VI guidelines¹³ (stage I: systolic/diastolic BP [SBP/DBP] 140–159/90–99 mm Hg; stage II: SBP/DBP 160–179/100–109 mm Hg). Patients whose SBP/DBP was <140/<90 mm Hg with drug therapy were considered to have normalized BP.

Patients were excluded from the study for the following reasons: type 1 diabetes mellitus (DM); low-density lipoprotein cholesterol >160 mg/dL; SBP/DBP >180/>110 mm Hg; clinically significant hepatic or renal dysfunction; valvular heart disease; second- or third-degree atrioventricular block; and a history of cardiovascular or cerebrovascular events or treatment with lipid-lowering agents in the previous 6 months. Patients also were excluded if they had received ACE inhibitors, diuretics, hormone replacement therapy, or antioxidant therapy in the previous 6 months. Pregnant, possibly pregnant, or breastfeeding women were excluded from the study. Women of childbearing age were required to use an effective method of birth control throughout the study because of possible teratogenic effects of ACE inhibitors.

Study Design

The study protocol was approved by the local ethics committee, and written informed consent was obtained from all patients.

Patients received oral perindopril 2 mg plus indapamide 0.625-mg* tablets once daily for 6 months. Measurements were performed at baseline and at 6 months. After the initial diagnosis of hypertension, endothelial cell dysfunction was determined by measuring the responses of brachial artery flow to reactive hyperemia (an index of endothelium-dependent vasodilation) and sublingually administered glyceryl-trinitrate (endothelium-independent vasodilation) (FMD). Brachial artery endothelial cell function was assessed at baseline and at the end of the study period using a previously validated technique.^{14,15} The diameter of the target artery was measured from 2-dimensional sonograms. A 7.5-MHz linear phased-array ultrasound transducer attached to an ImagePoint ultrasound system (Hewlett-Packard Company, Palo Alto, California) was used; the operating parameters of the equipment were not changed during the study. The study medication was withdrawn 72 hours before brachial measurements, and any vasoactive agents were discontinued ≥ 12 hours before brachial measurements.

Brachial artery analysis was performed by an experienced cardiologist blinded to whether the measurement was a baseline measurement or an intervention measurement. Baseline measurements were carried out after the patients were at rest at room temperature (22°C) for ≥ 10 minutes. Measurements were performed using pulse-wave Doppler sonography in the central area of the artery. The magnified images were obtained at the end-diastolic period in a longitudinal section of the brachial artery 2 to 15 cm superior to the antecubital

*Trademark: Preterax® (Les Laboratoires Servier, Cedex, France).

fossa in the dominant arm. Depth and gain settings were set to optimize the views of the lumen arterial wall interface. Subsequently, 3 measurements were performed within 2 minutes after the deflation of a cuff that had been inflated to a pressure of 300 mm Hg for 5 minutes, and mean values were calculated. After a 15-minute vessel recovery period, glyceryl-trinitrate 400 µg was administered sublingually (glyceryl-trinitrate-induced dilatation [NID]), and the final measurement was performed within 4 minutes. Electrocardiography was performed continuously, and BP was recorded in the left arm at 5-minute intervals. Increased arterial blood flow after removal of the pressure cuff (ie, reactive hyperemia) results in FMD. FMD was calculated as the percent change in brachial artery diameter from baseline to peak following reactive hyperemia (%FMD). Endothelial cell dysfunction was defined as <10% vasodilation. Pretreatment and posttreatment percentage changes in arterial vasodilation were obtained for each study.

We used a pulsed-Doppler technique to measure the parameters of trans-mitral diastolic flow from apical 4-chamber window. The effects of treatment on diastolic parameters (isovolumic relaxation time [IVRT] and mitral diastolic E-wave deceleration time [EDT]) were analyzed statistically.

Statistical Analysis

The paired *t* test, Wilcoxon signed rank test, and Fisher exact test were used in the statistical analyses. *P* < 0.05 was considered significant. The data were processed using the Statistical Package for Social Sciences version 10.0 (SPSS Inc., Chicago, Illinois).

RESULTS

The study population consisted of 29 white, Turkish patients (17 women, 12 men; mean [SD] age, 54.5 [9.5] years [range, 38–75 years]) (Table I). Eighteen patients (62.1%) had stage I hypertension and 11 (37.9%) had stage II hypertension. Fourteen patients (48.3%) were obese (body mass index >30 kg/m²), and 4 patients (13.8%) had type 2 DM. Mean (SD) serum lipid levels at baseline were as follows: total cholesterol, 189.0 (31.2) mg/dL; high-density lipoprotein cholesterol, 46.8 (10.7) mg/dL; low-density lipoprotein cholesterol, 111.9 (27.8) mg/dL; and triglycerides, 152.6 (65.7) mg/dL. All patients tolerated the studies well.

Complete brachial artery sonography data were available for all patients. FMD was inversely related to the baseline diameter of the brachial artery. No significant effect was found on brachial artery diameter after 6 months of therapy. All of the patients in the study had endothelial cell dysfunction (<10% vasodilation) at baseline. However, after the treatment period FMD improved in 15 patients (51.7%) and decreased in 14 patients (48.3%). Mean FMD was 7.00% (2.39%) at baseline and 8.68% (2.78%) after 6 months of combination therapy (change, 1.68% [0.69%]; *P* = 0.02) (Figure and Table II). The ratio of the

Table I. Baseline demographic and clinical characteristics of study patients (N = 29).

Characteristic	Value
Age, y	
Mean (SD)	54.5 (9.5)
Range	38–75
Sex, no. (%)	
Women	17 (58.6)
Men	12 (41.4)
Race	
White	29 (100.0)
Hypertension, no. (%)	
Stage I	18 (62.1)
Stage II	11 (37.9)
Obesity (BMI >30 kg/m ²), no. (%)	14 (48.3)
Type 2 DM, no. (%)	4 (13.8)
Serum lipids, mean (SD), mg/dL	
TC (NV, <200)	189.0 (31.2)
HDL-C (NV, men, 35–65; women, 35–80)	46.8 (10.7)
LDL-C (NV, 60–130)	111.9 (27.8)
TG (NV, 10–90)	152.6 (65.7)

BMI = body mass index; DM = diabetes mellitus; TC = total cholesterol; NV = normal value; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides.

improvement of endothelial cell dysfunction was similar in patients with stage I and II hypertension. No significant change in NID was found with drug therapy.

At 6 months, FMD improved statistically significantly in patients with total cholesterol <200 mg/dL ($n = 19$ [65.5%]; $P = 0.001$) and ≥ 200 mg/dL ($n = 10$ [34.5%]; $P = 0.02$). Mean IVRT improved from 101.7 (12.4) ms at baseline to 95.5 (7.7) ms after treatment ($P < 0.001$). Mean EDT was 234.7 (33.9) ms at baseline and 217.9 (25.6) ms after treatment ($P < 0.001$). In our laboratory, intraobserver variation was <1% and interobserver variation was <2%.

Significant reductions were found in mean SBP and DBP after 6 months of therapy. Mean (SD) SBP decreased from 155.2 (10.3) mm Hg at baseline to 135.0 (12.7) mm Hg at 6 months ($P < 0.001$), and DBP decreased from 96.6 (6.1) mm Hg to 84.1 (8.2) mm Hg ($P < 0.001$). BP normalized in 22 patients (75.9%) after the treatment.

DISCUSSION

Our results demonstrated that 6 months of therapy with low-dose perindopril–indapamide, an ACE inhibitor–diuretic combination, improved endothelium-dependent FMD in the brachial artery of patients with hypertension. FMD was inversely related to the baseline diameter of the brachial artery in our study, as it was in other studies.^{8,16} All of the patients in this study had endothelial

Figure. Change in the mean percentage of flow-mediated vasodilation (FMD) at baseline and after 6 months of therapy with perindopril 2 mg plus indapamide 0.625 mg (N = 29 study patients). * $P = 0.02$ versus baseline.

cell dysfunction at baseline as a result of hypertension, as has been demonstrated in previous reports.^{6,7} However, endothelial cell dysfunction significantly improved in approximately half of the patients after treatment. Moreover, the low-dose perindopril-indapamide combination improved left ventricular diastolic function parameters (ie, IVRT and EDT).

Endothelium plays a key role in vascular homeostasis through the release of autocrine and paracrine mediators.¹⁷ A healthy endothelium has antiatherogenic effects in addition to vasodilation. Endothelial cell dysfunction occurs in the presence of atherosclerosis or its risk factors, particularly hypertension and hypercholesterolemia.^{18,19} Hypertension is a major risk factor in patients with coronary artery disease, and endothelial cell dysfunction usually occurs concomitantly with hypertension in these patients. Animal²⁰ and human²¹ studies have demonstrated that endothelial cell function is impaired, endothelium-dependent relaxation is affected, and vasoconstriction develops with hypertension.

Because healthy endothelium has great importance in vascular hemostasis, endothelial cell dysfunction and its treatment have been widely investigated. Various studies have examined how antihypertensive drugs affect endothelial cell dysfunction. Experimental and clinical studies have suggested that ACE inhibition has a beneficial effect on endothelial cell function.^{8,22}

ACE inhibitors are thought to improve vascular functions by various mechanisms. Increasing angiotensin II and stimulating endothelium-derived hyperpolarizing factor/nitric oxide synthesis through increasing bradykinin levels are among these mechanisms. Moreover, ACE inhibitors have been reported to

Table II. Mean (SD) brachial artery measurements at baseline and after 6 months of treatment with perindopril 2 mg plus indapamide 0.625 mg (N = 29 study patients).

Parameter	Baseline	6 Months
Baseline diameter, mm	4.8 (0.5)	4.9 (0.5)
FMD		
Diameter, mm	5.2 (0.5)	5.4 (0.5)
%	7.00 (2.39)	8.68 (2.78)*
NID		
Diameter, mm	57.86 (6.75)	60.37 (7.44)
%	14.29 (8.95)	14.03 (7.46)
IVRT, ms	101.7 (12.4)	95.5 (7.7) [†]
Age <50 y	97 (13)	93 (7)
Age ≥50 y	104 (11)	97 (8)
EDT, ms	234.7 (33.9)	217.9 (25.6) [†]
Age <50 y	230 (40)	217 (31)
Age ≥50 y	237 (30)	218 (22)
Arterial BP, mm Hg		
Systolic	155.2 (10.3)	135.0 (12.7) [†]
Diastolic	96.6 (6.1)	84.1 (8.2) [†]

FMD = flow-mediated vasodilation; NID = glyceryl-nitrate-induced dilation; IVRT = isovolumic relaxation time; EDT = mitral diastolic E-wave deceleration time; BP = blood pressure.

**P* = 0.02 versus baseline.

[†]*P* < 0.001 versus baseline.

inhibit angiotensin II formation in the endothelial cell, and increased expression of endothelial nitric oxide synthase may be beneficial in the restoration of endothelial cell function.²³ Augmentation of endothelium-dependent vasodilation has been demonstrated with ACE inhibitors in 2 animal studies.^{24,25}

The effects of ACE inhibitor–thiazide combinations, which frequently are used in clinical practice to control BP, on endothelial cell dysfunction are unknown. In this study, we assessed the effects of an ACE inhibitor and a thiazide diuretic combination (low-dose perindopril [2 mg] and indapamide [0.625 mg]) on endothelial cell dysfunction and diastolic parameters in patients with stage I or II hypertension. Because monotherapy is usually not a sufficient first-line therapy in hypertension, the JNC VI report¹³ addresses the administration of fixed low-dose combination therapy as a first-line therapy in hypertension. The effect of ACE inhibitor–thiazide combination therapy on FMD is unknown. In our study, we determined that low-dose indapamide administered in combination with a low-dose ACE inhibitor improved endothelial cell dysfunction and left ventricular diastolic parameters.

The Brachial Artery Normalization of Forearm Function (BANFF) study⁸ (comparative study of ACE inhibition, angiotensin II antagonism, and calcium channel blockade on FMD in patients with coronary disease) and TREND⁹ (Trial on

Reversing ENdothelial Dysfunction) studies indicated that endothelial cell dysfunction improved when quinapril, an ACE inhibitor, was administered. However, in the BANFF study, no significant change was seen in endothelial cell dysfunction at the end of 8 weeks of monotherapy with enalapril (an ACE inhibitor), amlodipine (a dihydropyridine calcium channel blocker), or losartan (an angiotensin II receptor blocker) groups. Only a nonsignificant trend toward a difference in improvement in endothelial cell dysfunction with quinapril compared with enalapril was found in that study. Treatment with perindopril has been reported to improve endothelial cell dysfunction in patients with congestive heart failure.²⁶ However, according to a MEDLINE search (key words: *indapamide*, *endothelial cell dysfunction*, and *flow-mediated vasodilation*; years: 2002 and 2003), the literature includes no studies of the effect of indapamide on endothelial dysfunction.

Because the sample size in the present study was small, the results should be interpreted with caution. Larger studies should be conducted to support the results.

CONCLUSIONS

In this small sample of patients with stage I or II hypertension, a low-dose ACE inhibitor and diuretic combination provided significant improvement in brachial artery FMD, an index of endothelium-dependent vasodilation. The improvement in FMD values was independent of stage of hypertension. This combination therapy also yielded improvements in the parameters of left ventricular diastolic function (ie, IVRT and EDT). These findings suggest a correlation between improvement in endothelial cell function and diastolic function.

ACKNOWLEDGMENT

This study was sponsored by Servier Company (Istanbul, Turkey).

REFERENCES

1. Thomas F, Rudnichi A, Bacri AM, et al. Cardiovascular mortality in hypertensive men according to presence of associated risk factors. *Hypertension*. 2001;37:1256–1261.
2. Psaty BM, Furberg CD, Kuller LH, et al. Association between blood pressure level and the risk of myocardial infarction, stroke, and total mortality: The cardiovascular health study. *Arch Intern Med*. 2001;161:1183–1192.
3. Healy B. Endothelial cell dysfunction: An emerging endocrinopathy linked to coronary disease. *J Am Coll Cardiol*. 1990;16:357–358.
4. Ross R. The pathogenesis of atherosclerosis—an update. *N Engl J Med*. 1986;314:488–500.
5. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980;288:373–376.

6. Furumoto T, Saito N, Dong J, et al. Association of cardiovascular risk factors and endothelial dysfunction in Japanese hypertensive patients: Implications for early atherosclerosis. *Hypertens Res.* 2002;25:475–480.
7. Ouvina SM, La Greca RD, Zanaro NL, et al. Endothelial dysfunction, nitric oxide and platelet activation in hypertensive and diabetic type II patients. *Thromb Res.* 2001; 102:107–114.
8. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). *J Am Coll Cardiol.* 2000;35:60–66.
9. Mancini GB, Henry GC, Macaya C, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study [published correction appears in *Circulation.* 1996;94:1490]. *Circulation.* 1996;94:258–265.
10. Hansson L, Zanchetti A, Carruthers SG, et al, for the HOT Study Group. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: Principal results of the Hypertension Optimal Treatment (HOT) randomised trial. *Lancet.* 1998;351:1755–1762.
11. Cushman WC, Ford CE, Cutler JA, et al, for the ALLHAT Collaborative Group. Success and predictors of blood pressure control in diverse North American settings: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *J Clin Hypertens.* 2002;4:393–404.
12. Black HR, Elliott WJ, Neaton JD, et al. Baseline characteristics and elderly blood pressure control in the CONVINCE trial. *Hypertension.* 2001;37:12–18.
13. The sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure [published correction appears in *Arch Intern Med.* 1998;158:573]. *Arch Intern Med.* 1997;157:2413–2446.
14. Anderson TJ, Uehata A, Gerhard MD, et al. Close relationship of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol.* 1995;26:1235–1241.
15. Sorensen KE, Celermajer DS, Spiegelhalter DJ, et al. Non-invasive measurement of human endothelium dependent arterial responses: Accuracy and reproducibility. *Br Heart J.* 1995;74:247–253.
16. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet.* 1992;340: 1111–1115.
17. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med.* 1993;329: 2002–2012.
18. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med.* 1986;315:1046–1051.
19. Campuzano R, Moya JL, Garcia-Lledo A, et al. Endothelial dysfunction and intima-media thickness in relation to cardiovascular risk factors in patients without clinical manifestations of atherosclerosis [in Spanish]. *Rev Esp Cardiol.* 2003;56:546–554.
20. Zhu Z, Zhu S, Zhu J, et al. Endothelial dysfunction in cold-induced hypertensive rats. *Am J Hypertens.* 2002;15:176–180.
21. Rizzoni D, Agabiti-Rosei E. Endothelial factors and microvascular hypertensive disease. *J Cardiovasc Pharmacol.* 2001;38(Suppl 2):S15–S18.
22. Vanhoutte PM, Boulanger CM, Illiano SC, et al. Endothelium-dependent effects of converting-enzyme inhibitors. *J Cardiovasc Pharmacol.* 1993;22(Suppl 5):S10–S16.

23. Zhuo JL, Mendelsohn FA, Ohishi M. Perindopril alters vascular angiotensin-converting enzyme, AT(1) receptor, and nitric oxide synthase expression in patients with coronary heart disease. *Hypertension*. 2002;39:634–638.
24. Finta KM, Fischer MJ, Lee L, et al. Ramipril prevents impaired endothelium-dependent relaxation in arteries from rabbits fed an atherogenic diet. *Atherosclerosis*. 1993;100:149–156.
25. Nunes VW, Fortes ZB, Nigro D, et al. Influence of enalapril on the endothelial function of DOCA-salt hypertensive rats. *Gen Pharmacol*. 2000;25:117–125.
26. Joannides R, Bizet-Nafeh C, Costentin A, et al. Chronic ACE inhibition enhances the endothelial control of arterial mechanics and flow-dependent vasodilatation in heart failure. *Hypertension*. 2001;38:1446–1450.

Address correspondence to:

Cevad Sekuri, MD
Erzene Mahallesi 113 sok. No. 31
Camyuva-Bornova
Izmir 35100
Turkey
E-mail: csekuri@hotmail.com