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ORIGINAL ARTICLE

Comparison of the effects of barnidipine+losartan compared with telmisartan+hydrochlorothiazide on several parameters of insulin sensitivity in patients with hypertension and type 2 diabetes mellitus

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The aim of this study was to evaluate the effects of barnidipine+losartan compared with telmisartan+hydrochlorothiazide on several parameters of insulin sensitivity in patients with hypertension and type 2 diabetes mellitus. We enrolled 148 normocholesterolemic patients with mild-to-moderate hypertension and type 2 diabetes mellitus. Patients were treated with barnidipine, 20 mg day^{-1}, in combination with losartan, 100 mg day^{-1}, or with telmisartan+hydrochlorothiazide, 80/12.5 mg day^{-1}, for 6 months. We assessed blood pressure (BP) on a monthly basis; additionally, blood samples were collected to assess, at baseline and after 6 months, the following parameters: fasting plasma glucose; glycated hemoglobin; fasting plasma insulin; HOMA index; and some adipocytokines, such as adiponectin (ADN), resistin, leptin, visfatin and vaspin. Patients were also subjected to an euglycemic hyperinsulinemic clamp to assess the M value and glucose infusion rate to ascertain their insulin sensitivity. One hundred and forty-one patients completed the study. The BP was reduced in both groups, although the reduction was greater with barnidipine+losartan ($P<0.001$ *vs.* baseline and $P<0.01$ *vs.* telmisartan+hydrochlorothiazide). Barnidipine+losartan increased the M value and glucose infusion rate during the euglycemic hyperinsulinemic clamp ($P<0.05$ *vs.* baseline and *vs.* telmisartan+hydrochlorothiazide). With respect to the levels of adipocytokines, ADN was increased ($P<0.05$), and resistin and leptin were reduced from baseline with barnidipine +losartan ($P<0.05$ *vs.* baseline), but they were not reduced with telmisartan+hydrochlorothiazide. Visfatin and vaspin were reduced by barnidipine+losartan compared with baseline ($P<0.05$). The adipocytokine levels obtained with barnidipine+losartan were significantly better than those obtained with telmisartan+hydrochlorothiazide ($P<0.05$ for all parameters). In addition to providing a greater BP reduction, barnidipine+losartan improved the insulin sensitivity, as assessed by an euglycemic hyperinsulinemic clamp, and improved some of the adipocytokines related to insulin resistance.
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INTRODUCTION

The global burden of hypertension is substantial and continues to grow. In 2001, an estimated 7.6 million premature deaths worldwide were attributed to high blood pressure (BP), contributing to a relevant proportion of the global disease burden.[1] The prevalence of hypertension among people aged 35–64 years is ~30% in the US population[2] and ~44% in European countries.[3] The current European guidelines recommend a target systolic BP (SBP)/diastolic BP (DBP) of <140/90 mm Hg in the general population.[4] To achieve this target, a single anti-hypertensive agent is often not sufficient, and ~70% of patients are treated with a combination anti-hypertensive therapy.[5] The increased risk of cardiovascular events appears to be related to a large number of factors, including hyperactivation of the renin–angiotensin–aldosterone system,[6] direct vascular damage caused by hyperglycemia in diabetics,[7] systemic subclinical inflammation,[8] aberrant modulation of adipocytokine synthesis[9] and a pathological increase in the rate of vascular remodeling.[10]

According to the European Society of Cardiology guidelines, both angiotensin receptor blockers and calcium channel blockers are recommended for first-line therapy as either monotherapy or in combination.[4] Even if angiotensin converting enzyme (ACE) inhibitors seem to have a lower risk of new-onset atrial fibrillation,[11] the use of

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angiotensin receptor blockers in combination with hydrochlorothiazide is a possible option. Of the angiotensin receptor blockers, telmisartan was superior in the improvement of insulin sensitivity and plasma lipid profile in overweight hypertensive patients compared with eprosartan. This effect is possibly related to the selective stimulating PPAR-ɣ property of telmisartan.[12] In addition, short-term losartan treatment improved several metabolic parameters (*M*-value, adiponectin, retinol binding protein-4, resistin and visfatin) and decreased vascular remodeling biomarkers (metalloproteinases-2 and -9) in hypertensive subjects.[13] However, the metabolic effects of the calcium channel blocker barnidipine are not well defined. Therefore, the aim of this study was to evaluate the effects of barnidipine+losartan compared with telmisartan+hydrochlorothiazide on several parameters of insulin sensitivity in patients with hypertension and type 2 diabetes mellitus.

MATERIALS AND METHODS

Study design

This multicenter, randomized, double-blind, controlled study was conducted at the following centers: Department of Internal Medicine and Therapeutics, University of Pavia, PAVIA, Italy (coordinating site); Ospedale Pesenti Fenaroli, Alzano Lombardo, BERGAMO, Italy; Metabolic Unit, S. Antonio Abate Hospital, Gallarate, VARESE, Italy; and Diabetes Care Unit, S. Carlo Hospital, MILANO, Italy.

The study protocol was conducted in accordance with the Declaration of Helsinki and its amendments as well as the Good Clinical Practice Guidelines. It was approved by each Ethical Committee, and all patients provided written informed consent prior to entering the study.

Patients

We enrolled 148 hypertensive patients with mild-to-moderate hypertension, type 2 diabetes mellitus, who were normocholesterolemic (low-density lipoprotein cholesterol (LDL-C) $<160\ mg\ dl^{-1}$), overweight outpatients, and aged $\geqslant 18$ of either sex (Table 1).

Patients were evaluated for eligibility according to the following inclusion criteria:

- SBP $\geqslant 140$ mm Hg and <180 mm Hg and/or DBP $\geqslant 90$ mm Hg and <105 mm Hg.
- Well-controlled type 2 diabetes mellitus ($HbA_{1c} \leqslant 7.5\%$).

The exclusion criteria were as follows: secondary hypertension; severe hypertension (SBP $\geqslant 180$ mm Hg or DBP $\geqslant 105$ mm Hg); hypertrophic cardiomyopathies due to etiologies other than hypertension; history of heart failure, angina, stroke, transient ischemic cerebral attack, coronary artery bypass surgery or myocardial infarction any time prior to visit 1; concurrent known symptomatic arrhythmia; liver dysfunction (AST or ALT values exceeding the upper limit 2-fold); creatinine $>1.4\ mg\ dl^{-1}$; and known hypersensitivity to the study drugs. Pregnant women, as well as women of childbearing potential, were excluded.

Suitable subjects, identified from the review of case notes and/or computerized clinic registers, were contacted personally or by telephone.

Table 1 baseline characteristics of patients in the two treatment groups

	Barnidipine+ losartan (n = 73)	*Telmisartan+ hydrochlorotiazide (n = 75)*
N	73	75
Age (years)	60.1 ± 8.7	60.2 ± 8.8
Sex (male/female)	36/37	38/37
BMI ($kg\ m^{-2}$)	28.8 ± 1.1	28.5 ± 0.9
SBP (mm Hg)	152.0 ± 10.1	152.8 ± 10.5
DBP (mm Hg)	97.1 ± 6.1	97.8 ± 6.4
HR ($beats\ min^{-1}$)	74.1 ± 8.2	74.6 ± 8.6
Fasting plasma glucose ($mg\ dl^{-1}$)	127.2 ± 8.2	128.4 ± 8.6
HbA_{1c} (%)	7.0 ± 0.4	6.9 ± 0.5
FPI ($\mu U\ ml^{-1}$)	17.6 ± 5.9	17.0 ± 5.4
HOMA-IR	5.5 ± 1.2	5.4 ± 1.1
Duration of diabetes (months)	9.1 ± 6.5	8.8 ± 6.1
Duration of hypertension (months)	3.7 ± 2.2	3.8 ± 2.1
Adiponectin ($\mu g\ ml^{-1}$)	4.6 ± 1.4	4.5 ± 1.3
Resistin ($ng\ ml^{-1}$)	7.5 ± 2.6	7.7 ± 2.6
Visfatin ($ng\ ml^{-1}$)	33.1 ± 19.3	32.8 ± 18.9
Leptin ($ng\ ml^{-1}$)	30.2 ± 15.2	30.6 ± 15.7
Vaspin ($ng\ ml^{-1}$)	1.1 ± 1.1	1.0 ± 0.9
M value ($\mu mol\ min^{-1}\ kg^{-1}$)	5.2 ± 2.9	5.5 ± 3.1
GIR ($mg\ min^{-1}\ kg^{-1}$)	6.2 ± 1.0	6.4 ± 1.2

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FPI, fasting plasma insulin; GIR, glucose infusion rate; HbA1c, glycated hemoglobin; HR, heart rate; SBP, systolic blood pressure.
Data are means ± s.d.

Treatments

The patients fulfilling the inclusion criteria were randomized to receive barnidipine, $20\ mg\ day^{-1}$, in combination with losartan, $100\ mg\ day^{-1}$, or telmisartan+hydrochlorothiazide, $80/12.5\ mg\ day^{-1}$, for 6 months. All drugs were supplied as identical, opaque, white capsules in coded bottles to ensure the study maintained its blinded status. Randomization was performed using a drawing of envelopes containing randomization codes that a statistician prepared. A copy of the code was only provided to the responsible person who was in charge of performing the statistical analysis. The code was only broken after a database lock, but it could have been broken for individual subjects in case of an emergency. Medication compliance was assessed by counting the number of pills returned at the time of specified clinic visits. At baseline, we weighed participants and gave them a bottle containing a >100-day supply of the study medication. Throughout the study, we instructed patients to take their first dose of new medication on the day after they were given the study medication. At the same time, all unused medication was retrieved for inventory. All medications were provided for free.

Diet and exercise

Patients were already following a controlled-energy diet (near 600 kcal daily deficit) based on the American Heart Association recommendations,[14] which included 50% of calories from carbohydrates, 30% from fat (6% saturated) and 20% from proteins. In addition, the maximum cholesterol content was $300\ mg\ day^{-1}$ as well as $35\ g\ day^{-1}$ fiber content. Patients were not treated with vitamins or mineral preparations during the study.

A dietitian and/or specialist doctor provided standard diet advice. A dietitian and/or specialist doctor periodically provided instructions on the dietary intake recording procedures as part of a behavior modification program and then later used the subject's food diaries for counseling. Individuals were also encouraged to increase their physical activity by walking briskly for 20–30 min, 3–5 times per week, or by cycling. The recommended changes in physical activity throughout the study were not assessed.

Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs and 12-lead electrocardiogram. On a monthly basis, we assessed the BP, and blood samples were collected to assess, at baseline and after 6 months, the following parameters: fasting plasma glucose, glycated hemoglobin (HbA_{1c}), fasting plasma insulin, HOMA index, adiponectin, resistin, leptin, visfatin and vaspin. In addition, patients were treated with an euglycemic hyperinsulinemic clamp to assess the M value and glucose infusion rate to assess the insulin sensitivity.

All plasmatic parameters were determined after a 12-h overnight fast. Venous blood samples were collected for all patients between 0800 and 0900 hours. We used the plasma obtained by addition of Na_2-EDTA, $1\ mg\ ml^{-1}$, and

centrifuged at 3000 *g* for 15 min at 4 °C. Immediately after centrifugation, the plasma samples were frozen and stored at − 80 °C for no more than 3 months. All measurements were performed in a central laboratory.

Physicians who were blinded to treatment obtained BP measurements from each patient (left arm) in the sitting position using a standard mercury sphygmomanometer (Erkameter 3000; ERKA, Bad Tolz, Germany) (Korotkoff I and V) with an appropriately sized cuff. The BP was always measured in the morning before daily drug intake (that is, at trough 22–24 h after dosing) and after the subject had rested for 10 min in a quiet room. Three successive BP readings were obtained at 1-min intervals and then averaged.

The heart rate was measured by 30 s of pulse palpation just before the BP measurements.

Body weight was measured with light clothes and without shoes, and the BMI was calculated as the weight (in kg) divided by the height (in m squared).

The glycated hemoglobin level was measured with a high-performance liquid chromatography method (DIAMAT, Bio-Rad, USA; normal values 4.2–6.2%) and intra- and interassay coefficients of variation (CsV) < 2%.[15]

Plasma glucose was assayed by the glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and interassay CsV < 2%.[16]

Plasma insulin was assayed with Phadiaseph insulin radioimmunoassay (RIA) (Pharmacia, Uppsala, Sweden) using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CsV 4.6% and 7.3%, respectively).[17]

The HOMA-IR was calculated as the product of basal glucose (mmol l^{-1}) and insulin levels (μU ml^{-1}) divided by 22.5.[18,19]

The adiponectin level was determined using enzyme-linked immunosorbent assay (ELISA) kits (B-bridge International, Sunnyvale, CA, USA). The intra-assay CsVs were 3.6% for low- and 3.3% for high-control samples, whereas the interassay CsVs were 3.2% for low- and 7.3% for high-control samples, respectively.[20]

The resistin value was measured with a commercially available ELISA kit (BioVendor Laboratory Medicine, Brno, Czech Republic). The intra-assay CsV was 3.4% and interassay CsV was 6.9%.[21]

The leptin concentrations were assessed in duplicate using commercially available ELISA kits (TiterZyme Enzyme Immunoassay kit; Assay Designs, Ann Arbor, MI, USA) according to the supplier's instructions. The intra-assay and interassay CsVs were 4.5% and 6.5%, respectively.[22]

The visfatin levels were measured by the Enzyme Immunoassay kit obtained from Phoenix Pharmaceuticals. The intra- and interassays CsVs were 10% and < 14%, respectively.[23]

Vaspin was measured using commercially available two-site ELISA kits (Adipogen, Seoul, Korea); the intra- and interassays coefficients of variations were 1.74% and 8.32%, respectively.[24]

Glucose clamp technique

The insulin sensitivity (M value) was assessed with the use of the euglycemic, hyperinsulinemic clamp, according to the technique published by De Fronzo *et al.*[25] At 0900 hours, after the subjects had fasted for 12 h overnight, an i.v. catheter (18-g polyethylene cannula, Venflon, Viggo, Helsingborg, Sweden) was placed in an antecubital vein for the infusion of insulin and 20% glucose. A second catheter was retrogradely inserted into a wrist vein. The hand was heated (~70 °C) in a thermo-regulated box with the aim of arterializing venous blood within 20–40 min.[26] The plasma glucose level was assessed at 5–10 min intervals during the clamp. A 10-min priming infusion of insulin (Humulin R, Lilly Corporate, Indianapolis, IN, USA) was administered at rate of 1 mU min^{-1} kg^{-1}, for 2 h, during which the plasma glucose concentration was kept constant at the basal state (95 mg dl^{-1}) with a variable infusion of exogenous glucose. The level of glucose required to maintain isoglycemia equals the whole body disposal of glucose as long as endogenous glucose production is essentially absent. During insulin infusion, the normal fasting blood glucose levels were maintained by adjusting the infusion of a 20% glucose solution. The M value (amount of glucose infused, that is, whole body glucose disposal, expressed as μmol min^{-1} kg^{-1} of body weight (μmol min^{-1} kg^{-1})) was calculated as the mean value for each 20-min interval during the last 60 min of the clamp.

Statistical analysis

Data are expressed as the mean ± s.d.. The statistical analysis of the data was performed with the statistical analysis software (SAS) system, version 6.12 (SAS Institute, Cary, NC, USA). The differences between the two groups in their baseline characteristics were analyzed by the two-tailed Student's *t*-test. Differences between the baseline and 6 months after treatment in each group in terms of the BP and insulin sensitivity parameters were analyzed with the Wilcoxon signed-rank test. Comparisons of changes in the BP and insulin sensitivity parameters between the two groups were evaluated with the Mann–Whitney U-test.[27] Findings of $P < 0.05$ were considered significant. Considering a difference of at least 10% compared with the baseline and an alpha error of 0.05 as clinically significant, the actual sample size was adequate to obtain a power higher than 0.80 for all measured variables.

RESULTS

Study sample

We enrolled 148 patients; 73 were randomized to barnidipine +losartan and 75 to telmisartan+hydrochlorotiazide. One hundred and forty-one patients completed the study, and the results are presented in Table 2. Seven patients did not complete the study and the reasons for premature withdrawal included the following: lost to follow-up (three patients), cough (two patients) and withdrawal of consent (two patients).

Blood pressure

There was a BP reduction in both groups, although the reduction was greater in the group treated with barnidipine+losartan ($P < 0.001$ *vs.* baseline and $P < 0.01$ *vs.* telmisartan+hydrochlorothiazide).

Insulin sensitivity and adipocytokines

Barnidipine+losartan increased the M value and GIR during the euglycemic hyperinsulinemic clamp ($P < 0.05$ *vs.* baseline and *vs.* telmisartan+hydrochlorothiazide). Regarding the levels of adipocytokines, adiponectin was increased ($P < 0.05$), and resistin and leptin were reduced from baseline with barnidipine+losartan ($P < 0.05$ *vs.*

Table 2 Effects of the two treatments on evaluated parameters

	Barnidipine+ losartan (n = 71)	*Telmisartan+ hydrochlorotiazide (n = 70)*
N	71	70
Sex (male/female)	35/36	35/35
BMI (kg m^{-2})	28.6 ± 1.0	28.7 ± 1.1
SBP (mm Hg)	132.3 ± 8.2***°	141.7 ± 9.2**
DBP (mm Hg)	83.4 ± 4.6***°	89.3 ± 5.5**
HR (beats min^{-1})	73.2 ± 7.8	73.7 ± 7.9
Fasting plasma glucose (mg dl^{-1})	125.3 ± 7.7	132.3 ± 9.1
HbA_{1c} (%)	6.9 ± 0.3	7.0 ± 0.6
FPI (μU ml^{-1})	16.9 ± 5.4	17.3 ± 5.6
HOMA-IR	5.2 ± 1.4	5.6 ± 1.7
Adiponectin (μg ml^{-1})	5.3 ± 1.7*^	4.4 ± 1.2
Resistin (ng ml^{-1})	6.9 ± 2.1*^	7.5 ± 2.5
Visfatin (ng ml^{-1})	25.3 ± 17.3*^	32.2 ± 18.5
Leptin (ng ml^{-1})	24.1 ± 12.1*^	30.9 ± 15.9
Vaspin (ng ml^{-1})	0.7 ± 0.5*^	1.1 ± 1.0
M value (μmol min^{-1} kg^{-1})	6.4 ± 3.6*^	5.8 ± 3.3
GIR (mg min^{-1} kg^{-1})	7.3 ± 1.4*^	6.5 ± 1.3

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FPI, fasting plasma insulin; GIR, glucose infusion rate; HR, heart rate; SBP, systolic blood pressure.
Data are means ± s.d.
*$P < 0.05$ *vs.* baseline; **$P < 0.01$ *vs.* baseline; ***$P < 0.001$ *vs.* baseline; ^$P < 0.05$ *vs.* telmisartan+hydrochlorotiazide; °$P < 0.01$ *vs.* telmisartan+hydrochlorotiazide.

baseline), but they were not decreased with telmisartan+hydrochlorothiazide. Visfatin and vaspin were reduced with barnidipine+losartan compared with baseline ($P<0.05$). The adipocytokine levels obtained with barnidipine+losartan were significantly better than those obtained with telmisartan+hydrochlorothiazide ($P<0.05$ for all parameters).

DISCUSSION

In our study, we found that the combination of barnidipine+losartan was more effective than telmisartan+hydrochlorothiazide in reducing the BP, which is in line with the literature reports. Regarding the effects on adipocytokines, adiponectin stimulates the oxidation of fatty acids, suppresses gluconeogenesis and inhibites monocyte adhesion, macrophage transformation, proliferation and migration of smooth muscle cells in blood vessels.[20,28,29] Vaspin is a member of the serine protease inhibitor family, and it has a regulator role in glucose and lipid metabolism.[23] A higher blood vaspin concentration was shown in obese subjects,[30] and this trend was also demonstrated in both nonobese and obese type 2 diabetic patients.[31] Visfatin, instead, is a protein that is expressed by adipocytes as well as the liver, muscle, bone marrow and lymphocytes, where it was first identified as pre-β-cell colony stimulating factor.[32,33] The expression and secretion of visfatin are increased during the development of obesity; however, in contrast with inflammatory cytokines, the increase in visfatin does not decrease the insulin sensitivity. Visfatin exerts insulin-mimetic effects in cultured adipocytes, hepatocytes and myotubes in addition to lowering the plasma glucose levels in mice.[33] Visfatin binds to the insulin receptor with similar affinity, but its binding is at a site that is distinct from insulin.[33] In contrast with insulin, the visfatin levels do not change with feeding and fasting;[33] however, it remains to be determined whether visfatin acts in concert with insulin to regulate metabolism as well as whether this interaction occurs via endocrine or paracrine mechanisms.[32,33]

Treatment with barnidipine+losartan resulted in a better improvement of these parameters compared with telmisartan+hydrochlorothiazide. Given that an angiotensin receptor blocker was present in both drug regimens, the better effect on insulin sensitivity was likely from barnidipine.

Our study has some limitations, such as the short duration of the study. Moreover, we only assessed a few insulin sensitivity parameters and focused our attention on several select parameters.

CONCLUSIONS

In addition to providing a greater BP reduction, barnidipine+losartan improved the insulin sensitivity, as assessed by an euglycemic hyperinsulinemic clamp, and improved some adipocytokines related to insulin resistance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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