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Effect of Carvedilol and Nebivolol on Oxidative Stress-related Parameters and Endothelial Function in Patients with Essential Hypertension

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Abstract: Oxidative stress and endothelial dysfunction have been associated with essential hypertension (EH) mechanisms. The purpose of this study was to evaluate the effect of carvedilol and nebivolol on the oxidative stress-related parameters and endothelial function in patients with EH. The studied population included 57 patients, either sex, between 30 and 75 years of age, with mild-to-moderate EH complications. Participants were randomized to receive either carvedilol (12.5 mg) (n = 23) or nebivolol (5 mg) (n = 21) for 12 weeks. Measurements included; 24-hr ambulatory blood pressure (BP), flow-mediated dilatation, levels of nitric oxide estimated as nitrite – a nitric oxide metabolite (NO_2) – in plasma, and oxidative stress-related parameters in plasma and erythrocyte. EH patients who were treated with nebivolol or carvedilol showed systolic BP reductions of 17.4 and 19.9 mmHg, respectively, compared with baseline values ($p < 0.01$). Diastolic BP was reduced by 13.7 and 12.8 mmHg after the treatment with ebivolol and carvedilol, respectively ($p < 0.01$) (fig. 2B). Nebivolol and carvedilol showed 7.3% and 8.1% higher endothelium-dependent dilatation in relation to baseline values ($p < 0.05$). Ferric-reducing ability of plasma (FRAP) and reduced glutathione/oxidized glutathione (GSSH) ratio showed 31.5% and 29.6% higher levels in the carvedilol group compared with basal values; however, nebivolol-treated patients did not show significant differences after treatment. On the other hand, the NO_2 plasma concentration was not modified by the administration of carvedilol. However, nebivolol enhanced these levels in 62.1% after the treatment. In conclusion, this study demonstrated the antihypertensive effect of both beta-blockers. However, carvedilol could mediate these effects by an increase in antioxidant capacity and nebivolol through the raise in NO_2 concentration. Further studies are needed to determine the molecular mechanism of these effects.

Essential hypertension (EH) is a major risk factor for mortality owing to cardiovascular diseases [1]. The multifactorial aetiology of arterial hypertension involves various pathogenic factors determining different therapeutic approaches [2]. In previous years, mechanistic emphasis has been focused on the role of endothelium in the pathophysiology of EH [3]. Endothelial function becomes progressively impaired as blood pressure (BP) increases and the degree of dysfunction is related to the severity of hypertension [4]. Endothelial dysfunction may be determined by genetic factors and partially by the endothelial damage secondary to elevated BP [5,6]. However, several experimental and clinical trials have generated conflicting results concerning the association between risk factors and endothelial function [7,8]. At this point, a better understanding of mechanisms involving inflammation, endothelial dysfunction, sympathetic nerve activation and oxidative stress may allow identification of patients with refractory hypertension [9].

Nitric oxide is a molecule that has been recognized as a crucial modulator of vascular disease. It has a number of intracellular effects that lead to vasorelaxation, endothelial regeneration, inhibition of leucocyte chemotaxis and platelet adhesion [10]. Endothelial dysfunction – which is characterized by impairment of nitric oxide bioavailability – plays an important role in the development of arterial hypertension. Nitric oxide maintains the blood flow, thus relaxing the vascular smooth muscle and regulating oxygen supply to tissues [11,12]. Oxidative stress is involved in the pathophysiology of many cardiovascular conditions, including hypertension. Moreover, oxidative stress may account for endothelial dysfunction, but it is unknown whether this abnormality is a primary event or a consequence of increased BP [13].

Two β-adrenergic blocker agents have been used for the pharmacological treatment of hypertension, nebivolol and carvedilol. Firstly, nebivolol is a third-generation β-adrenergic blocker with pronounced vasodilator properties [14]. Nebivolol has demonstrated antihypertensive efficacy that is comparable with other β-blockers but with more favourable safety and tolerability profiles [15]. Nebivolol received approval for the treatment of hypertension in December 2007 in the United States, and is used in 50 other countries outside North America. A long-term, open-label study was performed outside the United States in patients with stage I to II hypertension. In this trial, nebivolol proved to be safe and effective, with BP-lowering

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effects increasing over time [16,17]. Secondly, carvedilol, unlike nebivolol, is a non-selective α-adrenergic receptor antagonist with β-1 blocking properties. Nevertheless, similar to nebivolol, carvedilol attenuates endothelial dysfunction in experimental models as well as in patients with coronary disease [18,19]. Antioxidant properties of carvedilol include the reduction in reactive oxygen species (ROS) formation *in vitro* by intracellular sources such as mitochondria, even after a short-term treatment with a relatively small dose [20].

Recent evidence suggests that nebivolol has a vasodilator effect not related to alpha receptor antagonism; this property is associated with higher clinical efficacy than that of other beta-adrenoceptor antagonists [21, 22]. However, the association between endothelium-dependent effects and BP reduction is not well understood.

The purpose of this study was to evaluate the effect of carvedilol and nebivolol on the oxidative stress-related parameters and endothelial function in patients with EH.

Materials and Methods

Study design and patient selection. The protocol consisted of a prospective randomized, single blind, clinical trial, conducted in patients with uncomplicated mild-to-moderate EH (defined as diastolic BP < 110 mmHg and systolic < 180 mmHg in absence of therapy). This study was approved by the local Ethics Committee (Clinical Hospital, Universidad de Chile). The study included 57 male and female patients, ages ranging between 30 and 75 years, with normal left ventricular systolic function (defined as an ejection fraction of 50% by echocardiography) and naive to pharmacological hypertensive treatment (fig.1). The enrolment of patients took place at the Cardiovascular Department, Clinical Hospital of the Universidad de Chile, between August 2010 and January 2011. Exclusion criteria included inappropriate echocardiographic window, diastolic BP above 110 mmHg or systolic BP above 180 mm Hg, secondary hypertension, signs or symptoms of heart failure, coronary artery disease, valvular heart disease, atrial fibrillation or any other arrhythmia, atrioventricular conduction abnormality, sinus bradycardia (50 beats/min.), renal failure, systemic connective tissue disease, cancer, acute or chronic inflammatory disease, history of stroke or transient ischaemic attack, known carotid artery stenosis, chronic obstructive pulmonary disease, hepatic insufficiency (serum aminotransferases more than twice the upper limit of normal), history of migraine or frequent headaches, intolerance to β-blocking agents, treatment with vasoactive drugs, and any other reason judged by the investigator to hamper inclusion. Demographic data, medical history and physical examination were obtained at the screening visit (−2 weeks). Treatment was assigned on visit 2 (week 0).

Patients were treated with a daily dose of carvedilol (12.5 mg) (Roche Lab, Santiago, Chile) or nebivolol (5 mg) (Menarini Labs., Florence, Italy). Randomization was carried out centrally and was non-stratified, block based and computer generated. Carvedilol or nebivolol treatment was maintained during 12 weeks. Dietary and physical activity recommendations were not modified during the study.

Ambulatory blood pressure monitoring. Blood pressure was measured by ambulatory BP monitoring on a normal workday (during 24-hr period starting at 08:30 a.m.) using an oscillometric monitor (SpaceLabs 90207, Issaquah, WA, USA) previously checked for accuracy with simultaneous measurements by a mercury sphygmomanometer. This device meets the validation criteria of the British Hypertension Society protocol [23] and fulfils the criteria of the Association for the Advancement of Medical Instrumentation (AAMI) for studies in ambulatory conditions [24]. The oscillometric accuracy showed intra-arterial average differences for systolic BP and diastolic BP (−0.6 ± 5.9 mmHg and 0.9 ± 6.4 mmHg) within the AAMI accuracy standard. The estimated oscillometric reproducibility was −0.3 ± 3.2 mmHg for systolic BP and 0.1 ± 3.5 mmHg for diastolic BP (values are means ± S.D.). An adult cuff and a large adult cuff were used for arm circumferences of 24–31 cm and 32–42 cm, respectively.

Flow-mediated dilatation. Endothelial function was evaluated by measuring the flow-mediated dilatation (FMD) [25]. B-mode scans of the right brachial artery (BA) were obtained in a longitudinal section 5 –10 cm above the elbow, by the same operator, using a 7.5-MHz linear array transducer that was held at the same point throughout the scan by a stereo-tactic clamp. Simultaneous electrocardiographic recordings were obtained and displayed on the ultrasound system video monitor (Philips, Eindhoven, the Netherlands). Imaging studies were performed using lower-arm occlusion technique. After 1 min. of acquisition (basal diameter), a BP cuff was inflated 50 mmHg above the systolic pressure for 5 min. After deflation, the BA diameter was measured (endothelium-dependent dilatation). A repeat baseline scan was obtained after a 15-min. rest period. Endothelium-independent dilatation was achieved by the administration of sublingual glyceryl trinitrate (0.3 mg). FMD and glyceryl trinitrate–induced dilatations were defined as the maximal per cent change in BA diameter compared with baseline. Computer-assisted edge detection brachial analysis software (Medical Imaging Applications, Coralville, IA, USA) was used to calculate BA diameters. The variability of FMD was calculated in healthy individuals 12 weeks after baseline determinations. The variability was 11% with a mean difference of 0.7% between the two measurements. Power analysis indicated that, assuming a two-tailed 5% test, the sample size was sufficient to detect a 1.5% improvement in FMD after therapy with 80% power.

Echocardiographic determinations. Bi-dimensional, M-mode and Doppler echocardiographic tests were performed using standard recording methodology and measurements in all patients, according to recommendations of the American Society of Echocardiography, using a Hewlett-Packard Sonos 2500 echocardiographic device (Andover, MA, USA) and a 2.5- or 2.0-MHz wide-angle phased-array transducer [26]. Data were analysed as the mean of three cardiac cycles. Left ventricular volumes were measured from the apical 4- and 2-chamber views of the 2-dimensional echocardiogram, and LVEF was calculated using the Simpson rule algorithm. Data were analysed as the mean of three cardiac cycles. Measurements were performed by two independent investigators and all the studies were recorded on videotape. Intraobserver and interobserver reproducibility for LVEF in our laboratory were 93.8% and 90.5%, respectively, whereas the agreement between the two observers concerning the classification of diastolic filling pattern was 100%.

Blood samples. Two blood samples were obtained from each patient by antecubital venous extraction. Samples were drawn at the moment of enrolment (day 0) and 12 weeks under nebivolol or carvedilol administration. The samples were collected in chilled vacutainers containing 4 mM disodium EDTA and centrifuged at 3000 × *g* for 10 min. to separate plasma from figurate elements. Red blood cells were washed with saline solution and then subjected to hypotonic haemolysis by dilution with distilled water. Plasma and red blood cell lysates were stored at −70°C, until performing the biochemical analyses.

Estimation of nitric oxide concentration. The level of nitric oxide was estimated as nitrite, a nitric oxide metabolite, measuring transformation of nitrate (NO_3) to nitrite (NO_2) using Griess reaction [27]. Total

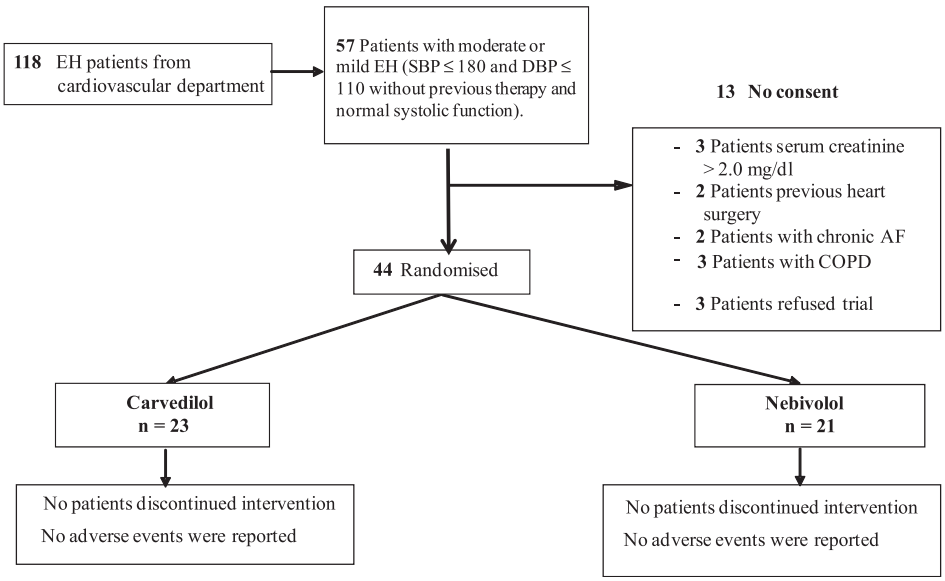

Fig. 1. Flow chart of patients screened and enrolled.

transformed NO_2 was then detected spectrophotometrically. Tolerance limits of interfering species were established at concentrations not causing more than ± 5% error in absorbance values of nitrite (2 μg/mL) and nitrate (2.6 μg/mL) with fixed concentration.

Assessment of oxidative stress-related parameters. Plasma antioxidant status. Plasma antioxidant status was assessed by determination of ferric-reducing ability of plasma (FRAP) with a detection limit of 10 μM [28]. The inter-assay and intra-assay coefficients of variation (CVs) for FRAP were 3.0% and 1.0%, respectively.

Glutathione in erythrocytes. Reduced glutathione (GSH) and oxidized glutathione (GSSG) levels were assayed by fluorometry in erythrocytes and the GSH/GSSG ratio was calculated as a parameter of intracellular redox status [29]. The inter- and intra-assay CVs for GSH and GSSG were 3.1% and 4.2%; and 2.7% and 3.5%, respectively.

Lipid peroxidation. Erythrocyte lipid peroxidation was assessed by thiobarbituric acid reaction at pH 3.5, followed by solvent extraction with a mixture of n-butanol/pyridine (15:1, v/v) [30]. Tetramethoxypropane was used as the external standard, and the levels of lipid peroxides were detected spectrophotometrically at 532 nm and were expressed as mmol malondialdehyde (MDA)/g of Hb. The inter-assay and intra-assay CVs for MDA were 10.5% and 4.8%, respectively.

Plasma 8-isoprostane concentration (pmol/mL), recognized as a reliable biomarker of lipid peroxidation *in vivo* [31], was determined using an ELISA kit (Cayman, Ann Arbor, MI, USA) [30]. The inter- and intra-assay CVs were 9.5% and 10.7%, respectively.

Normotensive group. Thirty normotensive patients who participated in a previous clinical study, and comparable with EH patients in demographic data, were used as a reference group for the determination of normal values of the biochemical parameters [5].

Statistical analysis. Shapiro–Wilk test and distribution plots were used to test distribution normality. For data that did not meet normality criteria, median and interquartile ranges were displayed. Two-sample Wilcoxon rank-sum test was used to evaluate variable medians. Descriptive statistics of continuous variables were presented as means ± standard deviation (S.D.) and compared by repeated measures analysis of variance (RMANOVA). Values of $p < 0.05$ were considered statistically significant. Statistical analysis was performed using Microsoft excel and STATA 10.00 for Windows.

Results

Clinical characteristics.

The 44 patients in the study (31 men and 13 women) [age mean 43.3 years (range, 41–81 years)] were randomized to receive nebivolol (n = 21) or carvedilol (n = 23), fig. 1. Characteristics of the patients prior to the treatment with carvedilol and nevibolol are shown in table 1. No significant clinical differences were found between study groups. Additionally, levels of the BP modulators renin, aldosterone, ET-1, homocysteine, folic acid and vitamin B12 were not significantly different between groups (table 1).

Final evaluation.

Clinical characteristics of EH patients treated with either nebivolol or carvedilol did not show significant differences (table 2). Patients treated with nebivolol or carvedilol showed comparable systolic BP reductions of 17.4 and 19.9 mmHg, respectively. This BP reduction was significant compared with baseline values in both groups ($p < 0.01$) (fig. 2A). Nebivolol and carvedilol induced also a significant diastolic BP reduction in relation to basal values ($p < 0.01$), which values were 13.7 and 12.8 mmHg, respectively (fig. 2B). Treatments with carvedilol and nebivolol did not produce any serious adverse effects during the 3 months of treatment.

Determination of endothelial function.

The endothelial function was measured by echocardiography as described in the Materials and Methods section. There were no

Table 1.
Baseline characteristics of patients before treatment with carvedilol and nebivolol.

	Carvedilol (n = 23)	Nebivolol (n = 21)	*p*-value
Age, years	45.6 ± 2.8	44.9 ± 2.1	0.27
Sex (male/female)	16/7	15/6	0.50
BMI (kg/m^2)	27.6 (6.2)	26.7 (4.7)	0.32
Cardiac frequency (beats/min.)	62 (8)	71 (5)	0.37
Left ventricular ejection fraction, %	56.4 ± 10.8	54.1 ± 7.9	0.61
Left ventricular mass (g/m^2)	83.08 ± 6.4	85.22 ± 88	0.65
Serum glucose (mM)	4.87 ± 0.13	5.18 ± 0.17	0.29
Creatinine (μM)	76.3 ± 2.70	75.9 ± 2.71	0.55
Total cholesterol (mM)	4.78 ± 0.25	4.64 ± 0.31	0.59
HDL-cholesterol (mM)	1.21 ± 0.03	1.28 ± 0.05	0.47
LDL-cholesterol (mM)	2.87 ± 0.24	2.76 ± 0.34	0.68
Serum triacylglycerols (mM)	1.52 ± 0.18	1.35 ± 0.17	0.81
Renin activity(pM/hr)	23.5 ± 1.9	22.9 ± 2.1	0.43
Aldosterone (nM)	0.25 ± 0.06	0.21 ± 0.05	0.82
Homocysteine (μM)	9.07 ± 0.47	9.27 ± 0.41	0.44
Folic acid (nM)	46.7 ± 1.15	45.8 ± 1.19	0.49
Vitamin B_{12} (pM)	248.3 ± 6.3	233.5 ± 7.9	0.58
Daytime			
SBP (mmHg)	139 ± 5.1	141 ± 6.3	<0.01
DBP (mmHg)	97.3 ± 6.6	98.7 ± 5.2	<0.01
MAP (mmHg)	105.3 ± 3.2	103.6 ± 7.1	<0.01

HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, median blood pressure.
Quantitative continuous variables are expressed as median (interquartile range) or mean ± S.D. if presented normal distribution.

differences in basal values of brachial diameter between groups in basal and after treatments (fig. 3A). Nebivolol and carvedilol show 7.3% and 8.1% higher endothelium-dependent dilatation of peripheral arteries produced by reactive hyperaemia ($p < 0.05$), as can be seen in fig. 3B. However, the maximum dilatation obtained with nitroglycerine-mediated dilatation was similar in both groups of patients. These results are shown in fig. 3C.

Antioxidant status, oxidative stress-related parameters and estimation of nitric oxide concentration.

Patients treated with carvedilol show 28.1% and 23.6% lower levels of 8-isoprostane and erythrocyte MDA compared with basal values, respectively ($p < 0.05$). No significant differences were observed in lipid peroxidation levels in patients treated with nebivolol (fig. 4A,B). FRAP and GSH/GSSH ratio show 31.5% and 29.6% higher levels in the carvedilol group compared with basal values; however, nebivolol-treated patients did not show significant differences after treatment ($p < 0.05$) (fig. 4C, D). Additionally, NO_2 concentration was not changed by the administration of carvedilol but nebivolol showed 62.1% higher levels of NO_2 after treatment ($p < 0.05$). These changes are shown in fig. 4E.

The values of oxidative stress parameters between the two groups and normotensive patients are shown in table 3. Hypertensive patients prior to treatment with carvedilol or nebivolol show higher values of lipid peroxidation and lower antioxidant status values (FRAP and ratio GSH/GSSG) compared with normotensive patients with similar clinical characteristics.

Table 2.
Clinical parameters in the carvedilol and nebivolol group after treatment.

	Carvedilol (n = 23)	Nebivolol (n = 21)	*p*-value
Age, years	45.6 ± 2.8	44.9 ± 2.1	0.27
Sex (male/female)	16/7	15/6	0.50
BMI (kg/m^2)	24.6 (4.2)	25.7 (3.9)	0.12
Cardiac frequency (beats/min.)	66 (8)	71 (5)	0.47
Left ventricular ejection fraction, %	58.3 ± 11.7	56.7 ± 9.7	0.71
Left ventricular mass (g/m^2)	93.08 ± 7.4	87.22 ± 10.1	0.55
Serum glucose (mM)	5.17 ± 0.18	5.08 ± 0.21	0.32
Creatinine (μM)	79.2 ± 2.3	80.9 ± 2.71	0.63
Total cholesterol (mM)	4.78 ± 0.25	4.47 ± 0.37	0.59
HDL-cholesterol (mM)	1.17 ± 0.03	1.28 ± 0.05	0.43
LDL-cholesterol (mM)	2.85 ± 0.14	2.66 ± 0.24	0.78
Serum triacylglycerols (mM)	1.54 ± 0.08	1.34 ± 0.07	0.74
Renin activity (pM/hr)	22.7 ± 2.3	20.9 ± 1.8	0.37
Aldosterone (nM)	0.26 ± 0.04	0.24 ± 0.07	0.92
Homocysteine (μM)	9.12 ± 0.51	9.33 ± 0.41	0.74
Folic acid (nM)	45.3 ± 1.12	44.8 ± 1.1	0.39
Vitamin B_{12} (pM)	238.3 ± 7.3	223.5 ± 7.7	0.48

HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, median blood pressure.
Quantitative continuous variables are expressed as median (interquartile range) or mean ± S.D. if presented normal distribution.

Discussion

The main finding of this study confirms that the antihypertensive effect of both drugs is associated with different mechanisms. While nebivolol could induce vasodilatation by nitric oxide-dependent pathway [32, 33], carvedilol shows significant antioxidant properties [34]. In accordance with the pharmacodynamic profiles of carvedilol and nebivolol, we found a comparable reduction in systolic and diastolic BP with the doses used (fig. 2), these findings are consistent with previous studies [35, 36].

Endothelial function represents a valuable surrogate endpoint to assess the impact of therapeutic interventions and at the same time provides an important opportunity to develop new therapeutic approaches [37]. Carvedilol and nebivolol increase the response to endothelial-dependent vasodilatation; after 3 months of treatment, their effect was not modified by nitroglycerine (fig 3A–C). These findings are in agreement with the effect of carvedilol in the reduction in systolic BP and diastolic BP in patients with EH and coronary artery disease [38], and with other reports which found that nebivolol significantly increased forearm vasodilatation in patients with moderate hypertension [39–41].

The result of this study supports the evidence that these beta-blockers have additional properties, exceeding merely beta receptor block. These features may provide additional benefit in the treatment of hypertension. The third-generation drug nebivolol exerts a nitric oxide-mediated vasodilating activity, which has positive effects on intima and media

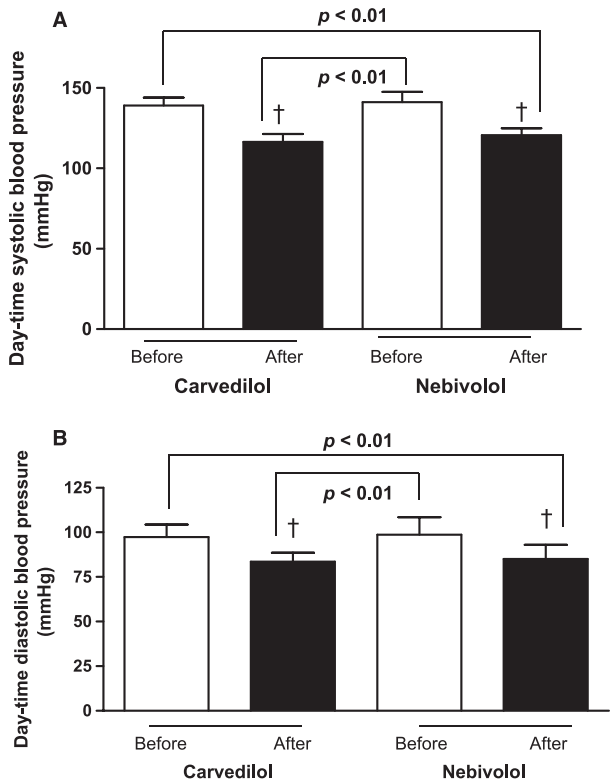

Fig. 2. Graphs representing daytime SBP (A) and DBP (B) in patients before and after being treated with carvedilol (n = 23) and nebivolol (n = 21). Data presented as mean ± S.D. Significant differences ($p < 0.05$): †*versus* before treatment.

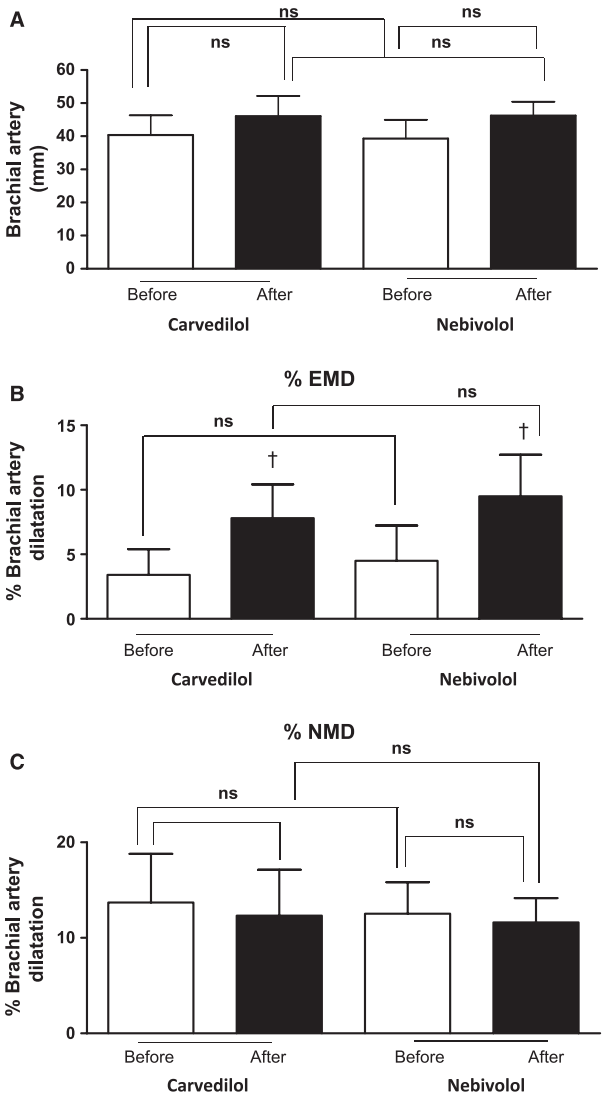

Fig. 3. Graphs representing endothelial function evaluated in dilatation in (A) basal conditions; (B) measurement of flow-mediated dilatation (FMD) and (C) nitroglycerine-mediated dilatation (NMD), in patients before and after treatment with carvedilol (n = 23) and nebivolol (n = 21). Data presented as mean ± S.D. Significant differences ($p < 0.05$): †*versus* before treatment.

thickness and arterial rigidity, a major risk factor in cardiovascular disease. Moreover, anti-proliferative, anti-inflammatory and antioxidative properties have been detected after carvedilol and nebivolol administration, contributing to additional value in the treatment of hypertension [42]. In this study, oxidative stress-related parameters were assessed to evaluate the pleiotropic effect of carvedilol and nebivolol to improve endothelial function. Only after administration of carvedilol, patients showed lower levels with F2-isoprostane and erythrocyte MDA (fig. 4A,B), and higher levels of FRAP and GSH/GSSG ratio (fig. 4C,D) consistent with a minor systemic oxidative stress at the end of treatment, compared with patients who received nebivolol. These findings are in agreement with previous results showing that carvedilol has antioxidant properties, which may provide vascular protection by improving endothelial function, focusing its effect on scavenger properties and decreasing endothelial production of ROS [43]. On the other hand, estimated plasma levels of nitric oxide were not changed by carvedilol and were significantly increased by nebivolol (fig. 4E). The lack of effect of carvedilol is concordant with previous reports [44, 45]. Nevertheless, some preclinical studies have found that carvedilol produced a decrease in BP by increasing plasma nitric oxide levels [46]. On the other hand, nebivolol stimulates endothelial nitric oxide synthesis, thereby increasing the availability of nitric oxide in the endothelium, smooth muscle and platelets, and consequently producing a sustained vasodilation with decreases in peripheral resistance and BP [47]. These described properties are in agreement with the results of the present study.

The increased oxidative stress and decreased antioxidant capacity found in patients with EH at baseline, compared with normotensive patients (table 3) is in agreement with previous studies [48,49]. However, Cracowski and Durand [50] found no significant differences in urinary 8-isoprostane levels between normotensive patients and patients with untreated mild-to-moderate EH, at least in the early stages of the disease. This controversy may be explained by patient inclusion criteria, which did not consider the influence of factors such as physical activity, previous and/or current antioxidant supplementation, high consumption of fruits and vegetables and/or intake of non-antihypertensive (e.g. statins) medication,

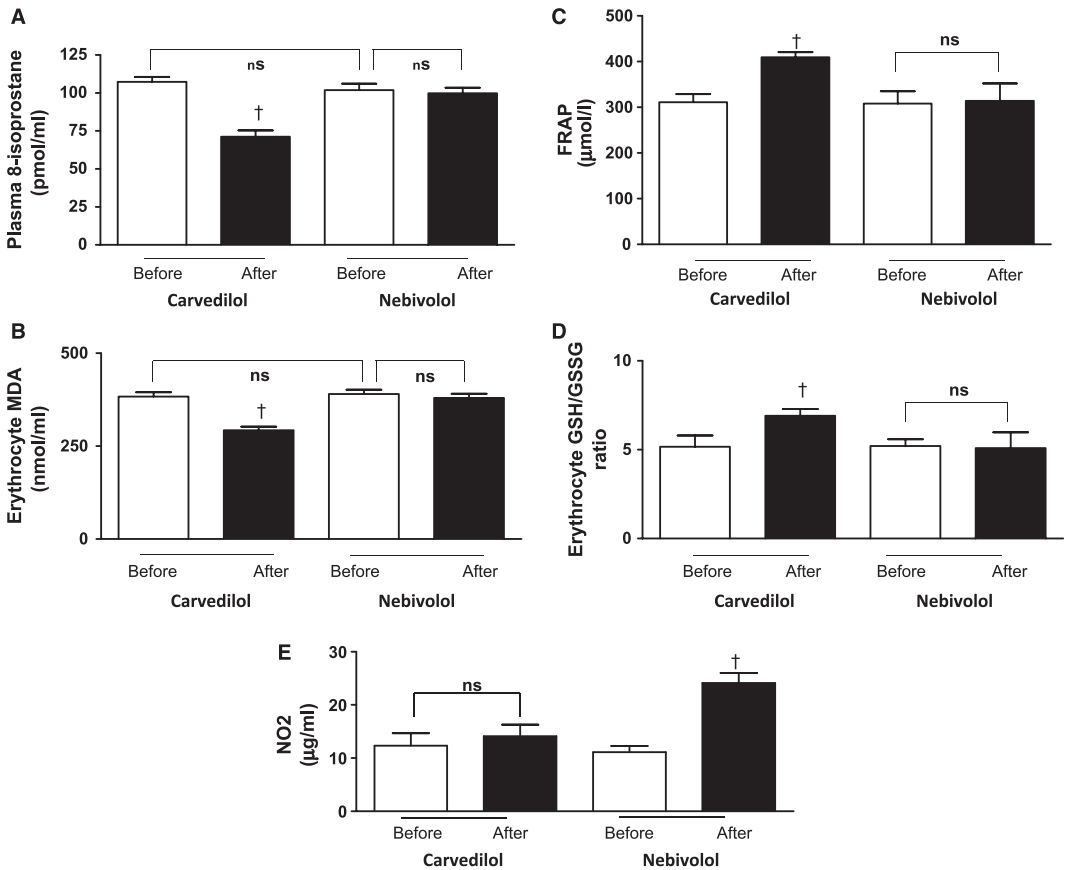

Fig. 4. Graphs representing oxidative stress-related parameters and estimation of nitric oxide levels in blood samples of protocol patients: (A) isorpostane; (B) malondialdehyde (MDA); (C) antioxidant capacity ferric-reducing ability of plasma (FRAP); (D) erythrocyte reduced glutathione (GSH)/oxidized glutathione(GSSG) and (E) and nitrite (NO_2) levels, in patients before and after treatment with carvedilol (n = 23) and nebivolol (n = 21). Data presented as mean ± S.D. Significant differences ($p < 0.05$): † *versus* before treatment.

Table 3.
Plasma and erythrocyte oxidative stress-related parameters in the carvedilol and nebivolol group compared with normotensive patients before treatment (basal samples).

	Normotensive (n = 30)	Carvedilol (n = 23)	Nebivolol (n = 21)	*p*-value
Plasma				
FRAP (µM)	426 ± 17	311 ± 8*	308.7 ± 7*	<0.01
8-Isoprostane (pM)	75.7 ± 4.1	107.3 ± 3.3*	109.3 ± 4.2*	<0.01
Erythrocyte				
GSH/GSSG ratio	5.98 ± 0.15	5.17 ± 0.11*	5.21 ± 0.18*	<0.01
MDA (nmol/g Hb)	311 ± 7	383 ± 12*	390 ± 12*	<0.01

FRAP, ferric-reducing ability of plasma; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde.
Variables are expressed as median ± S.D.
*Significant differences *versus* normotensive group.

all potential confounders of oxidative stress status. The present study considered these factors to obtain a rigorously selected sample of patients to specifically examine the antioxidant effect on BP. The results of the present study have shown a significant decrease in systolic and diastolic BP, after administration of carvedilol or nebivolol in patients with EH compared with their baseline (fig. 2), and these findings are in agreement with other studies [16, 48, 49].

In conclusion, as part of the antihypertensive mechanism of these beta-blockers, nebivolol increases nitric oxide bioavailability while carvedilol seems to reinforce the antioxidant system. However, further studies are needed to determine the precise molecular mechanism of their respective effects.

Acknowledgements

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Conflict of Interest

None of the authors or cooperative members have a proprietary, commercial or any other financial interest in any study procedure or result.

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