https://doi.org/10.31925/farmacia.2022.1.14 **ORIGINAL ARTICLE**

THE PLASMA LEVEL OF SOLUBLE ENDOGLIN IN HYPERTENSIVE PATIENTS UNDERGOING LONG-TERM TREATMENT WITH CANDESARTAN

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Abstract

Endoglin is a transmembrane glycoprotein involved in regulating endothelial function and homeostasis. We investigated the effect of candesartan compared with that of different regimens of blood pressure (BP)-lowering agents on the plasma level of soluble endoglin (sEng), a biomarker involved directly in endothelial homeostasis. A total of 395 patients were enrolled, of which 129 had normal BP (group A, control group), 133 were hypertensive patients treated with candesartan (8, 16, or 32 mg/day) in monotherapy (group C) and 133 were hypertensive patients treated with different types of antihypertensive agents (beta-blockers, calcium-channel blockers, diuretics) as monotherapy (group B). Classical methods of assessing endothelial damage (flow-mediated dilatation, intima-media thickness) as well as other biochemical parameters and investigations were undertaken. Correlations with the plasma level of sEng were performed. A lower plasma level of sEng was found in patients undergoing long-term BP-lowering treatment with candesartan compared with that in hypertensive patients undergoing long-term treatment with beta-blockers, calcium-channel blockers, or diuretics ($p < 0.001$). Diastolic BP and the blood urea nitrogen (BUN) levels were protective factors for sEng. The end-diastolic diameter of the left ventricle and neutrophil count were risk factors for soluble endoglin and thus for endothelial dysfunction. By quantifying a glycoprotein involved in endothelial function/homeostasis regulation, sEng, could be a specific biomarker of vascular and cardiac lesions and predict the efficacy of antihypertensive treatment.

Rezumat

Endoglina solubilă (sEng) este o glicoproteină transmembranară implicată în reglarea funcției și a homeostaziei endoteliale. 395 de pacienți au fost înrolați în studiu, dintre care 129 cu tensiunea arterială (TA) normală (grupul A), 133 pacienți hipertensivi sub tratament cu candesartan (8, 16 sau 32 mg/zi) în monoterapie (grupul C), iar 133 au fost pacienți hipertensivi sub alte regimuri de tratament antihipertensiv în monoterapie (grupul B). S-au evaluat leziunile endoteliale prin metode clasice, și au fost efectuate investigații biochimice, toate acestea fiind corelate cu nivelul plasmatic al sEng. Un nivel mai scăzut al sEng plasmatice a fost găsit la pacienții sub tratament cronic cu candesartan, comparativ cu cel al pacienților sub tratament cronic cu alte clase farmacologice ($p < 0,001$). TA diastolică și nivelul acidului uric au fost identificați ca factori de protecție pentru sEng. Diametrul *end*-diastolic al ventriculului stâng și numărul de neutrofile au fost identificați ca factori de risc pentru sEng și, prin urmare, pentru disfuncția endotelială. În concluzie, endoglina solubilă ar putea fi un biomarker specific al leziunilor vasculare și cardiace, ce ar putea cuantifica eficacitatea tratamentului antihipertensiv.

Keywords: candesartan, ARB, soluble endoglin, endothelial dysfunction, hypertension, myocardial fibrosis

Introduction

Endoglin (also called cluster of differentiation-105) is a transmembrane glycoprotein involved in endothelial function/homeostasis regulation. It is a type-III auxiliary receptor for transforming growth factor (TGF)-β. Endoglin belongs to the zona-pellucida family of proteins and highly expresses vascular endothelial cells. Studies have shown that endoglin expression is increased in patients diagnosed with endothelial dysfunction or atherosclerosis [3, 9, 43].

Endoglin is composed of disulphide-linked subunits and includes three regions: an extracellular region (consisting of 561 amino acids), a single transmembrane domain, and a cytoplasmic region (composed of serine/threonine). Metalloproteinase (MMP)-14 shows high expression on endothelial cells. MMP-14 can cleave the receptor's extracellular domain proteolytically to generate the circulating form of endoglin called "soluble endoglin" (sEng). This form can be found in plasma after different endothelial-damaging stimuli and acts as an antagonist for TGF-β signalling [3, 43]. Another form of endoglin is located in the cell membranes of various tissues.

sEng has been reported to compete over TGF-β1 receptors. This mechanism decreases signalling and endothelial nitric oxide synthase (eNOS) activation to induce: dysregulation of arterial vasodilatation; higher expression of cell-adhesion molecules; increased vascular permeability; endothelial dysfunction; arterial hypertension; anti-angiogenic effects; atherosclerosis [28]. It seems that sEng can interact with the signalling of TGF-β/eNOS and bone morphogenic protein-9 and result in impaired endothelial function [28]. Moreover, increased levels of sEng are associated with hypercholesterolemia [22], atherogenesis [36], acute myocardial infarction and other major cardiovascular events such as stroke or heart failure [33]. High levels of sEng have been found in pregnant women who developed preeclampsia subsequently [23, 45].

Endoglin plays a key part in angiogenesis. Inhibiting endoglin expression attenuates angiogenesis markedly. Studies have shown that mice lacking endoglin die from defective vascular development [8, 29, 30].

Endoglin has been found to be expressed in cardiac fibroblasts and to modulate the profibrogenic actions of angiotensin II (Ang II) [4, 6]. Several studies have reported that the circulating maternal autoantibody agonist of the angiotensin II type-I receptor agonistic autoantibody (AT1-AA) has an important role in pre-eclampsia pathophysiology and that tumour necrosis factor (TNF)-α is an essential mediator for this auto-antibody. Hence, by stimulating AT1 receptors, AT1-AA induces TNF-α expression, increasing the plasma levels of sEng and soluble fms-like tyrosine (sFlt)-1: these two cytokines have important importance roles in preeclampsia development [18, 35, 50].

Several studies have reported that angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II type I receptor blockers (ARBs) such as candesartan attenuate Ang II-induced endoglin expression and that statin treatment reduces the level of sEng and increases its expression in the aorta [9, 21, 24].

The present study was designed to evaluate the effect of candesartan compared with different regimens of blood pressure (BP)-lowering treatment on the plasma level of sEng. This biomarker is directly involved in endothelial homeostasis.

Materials and Methods

Study design

This comparative, cross-sectional, prospective study was conducted in the Cardiology Clinic of Timișoara City Hospital (Timișoara, Romania) from March 2015 to September 2016.

In total, 395 patients were enrolled in the present study. Of these, 129 had normal BP (group A, control group), 133 were patients suffering from hypertension treated with candesartan (8, 16 or 32 mg/day) as monotherapy (group C), and 133 were patients suffering from hypertension treated with different types of antihypertensive agents, such as beta-blockers (metoprolol, 50 or 100 mg/day or nebivolol, 5 mg/day), calcium-channel blockers (amlodipine, 5 or 10 mg/day or lercanidipine, 10 or 20 mg/day) or diuretics (indapamide, 1.5 mg/day or furosemide, 20 mg + spironolactone, 50 mg/day) as monotherapy (group B).

All patients were matched by age and sex and had BP-lowering treatment for > 1 year, administered every morning.

The Ethical Committee of "Victor Babeș" University of Medicine and Pharmacy of Timișoara, Romania, approved the study protocol. All procedures were following the ethical standards of the institutional research committee and with the Declaration of Helsinki and its later amendments or comparable ethical standards.

Patient selection

All recruited patients provided written informed consent and participated voluntarily. Moreover, they underwent screening for primary disease and comorbidity, family medical history, as well as laboratory and paraclinical investigations.

Patients enrolled in the control group were examined first for suspicion of essential arterial hypertension. However, after 24 h, the BP-monitoring device at home did not reveal this diagnosis.

All recruited hypertensive patients were enrolled based on age > 18 years, diagnosis of essential arterial hypertension for ≥ 1 year (> 140/90 mmHg) and monotherapy with one of the drugs/classes mentioned above.

Patients with other diseases (e.g., atherosclerotic disease carotid or peripheral, diabetes mellitus (DM),

coronary artery disease, heart failure, hepatic and/or renal diseases, acute/chronic inflammatory disease) were excluded from the study. Also, patients treated with ACEIs or other ARBs were excluded from this study based on their similar action in reversing endothelial dysfunction.

Patients enrolled in group C received long-term treatment with candesartan (8, 16 or 32 mg/day) as monotherapy. Patients enrolled in group B received long-term treatment with other types of antihypertensive agents (beta-blockers, calcium-channel blockers, diuretics) as monotherapy.

Biochemical assays

Examination of patients was undertaken in the morning in a temperature-controlled room after fasted > 10 h. Samples of venous blood were collected after ≥ 15 min of rest. Standard laboratory methods within the hospital carried out standard biochemical analyses (e.g., serum glucose and total cholesterol). Plasma levels of sEng, pentraxin-3, and high-sensitivity C-reactive protein (hs-CRP) were measured by Bioclinica SA Laboratory (Timișoara, Romania). Plasma levels of sEng and pentraxin-3 were determined with a quantitative sandwich enzyme immunoassay kit (R&D Systems, Minneapolis, MN, USA).

The normal range for sEng given by Bioclinica SA Laboratory was 2.54 - 7.06 ng/mL. The normal range for pentraxin-3 is < 1.18 ng/mL.

Arterial BP

BP was measured at the right brachial artery on the same morning in a temperature-controlled room after ≥ 15 minutes of rest in the supine position. BP was expressed as the mean value of three measurements.

The presence/absence of the endothelial dysfunction was assessed through a functional assessment (measurement of flow-mediated vasodilatation (FMD) of the brachial artery) and structural assessment (intima-media thickness (IMT) of carotid arteries).

FMD assessment

This procedure was undertaken after the patient had stopped taking vasoactive medication for ≥ 8 h, after a fast, without smoking for 8 h, and after 15 min of rest in a quiet room. The patient was positioned supine with the arm in a comfortable position. The brachial artery was imaged above the antecubital fossa in the longitudinal plane, using a 9 MHz linear transducer. The dimension of the brachial artery was measured manually with an electronic calliper. Ischemia was produced by inflating the cuff of a manometer placed at the distal forearm at a pressure of 50 mmHg higher than the systolic BP of the patient. Release of the cuff after 5 min generated an increased diameter of the brachial artery, with the maximal diameter being measured 1 min after cuff release. The brachial artery diameter was measured at the same time as the cardiac cycle.

FMD was calculated as the percentage change in diameter from rest to 1 min after ischemia, and a 20% increase was considered normal.

Measurement of carotid IMT

Measurement of carotid IMT was done in B-mode using a high-resolution ultrasonography system (VIVID S5, General Electric (Boston, MA, USA) equipped with a 9 MHz linear-array transducer. All three measurements were made on the distal 1 cm of the bilateral common carotid artery, and the arithmetic mean of the value was documented. An increased IMT was considered when IMT was > 0.9 mm, and a plaque was considered if there was localized thickening > 1.5 mm.

Echocardiography

Echocardiography was undertaken using a high-resolution ultrasonography system (VIVID S5, GE Healthcare, USA). A cardiologist assessed the consequence of hypertension over the structures and functions of the heart. All patients with a low ejection fraction (EF < 40%) were excluded, and the following parameters were recorded: diameter of the left atrium (DLA); dimension of the interventricular septum (IVS); the posterior wall of the left ventricle (PWLV); EF; end-diastolic diameter of the left ventricle (EDLV). The same certified physician made all measurements.

Statistical analysis

Numerical data are presented as the mean ± standard deviation (SD) and median (25^{th} - 75^{th} percentiles). Categorical data are presented as the frequency (%). We used SPSS v17 (IBM, Armonk, NY, USA) for analyses. $p < 0.05$ was considered significant. Differences between related groups were analysed using the Wilcoxon signed ranks test and independent groups using the Mann–Whitney U-test. The plasma level of sEng and other numerical variables was conducted using the multivariate regression model, and the strength of the correlation was obtained using Spearman's correlation coefficient. A risk analysis was made for sEng > 7.06 ng/mL by calculating the nominal variables' odds ratio (OR).

Results and Discussion

The clinical, haematology and echocardiography results of enrolled patients are presented in Table I. There was no significant difference between the groups regarding sex distribution. Patients from the control group were younger than those suffering from hypertension from groups B and C.

The BP (systolic and diastolic) values in groups B and C were significantly higher than in the control group, with no significant differences between the two hypertension groups.

The plasma level (in ng/mL) of sEng was significantly lower in group C (4.25 ± 1.05) compared with that of group B (5.59 ± 2.39) and group A (4.99 ± 2.81) ($p < 0.001$).

Table I

The clinical, haematology and echocardiography results

Parameters	Group A Control (n = 129)	Group B other anti-HTN (n = 133)	Group C Candesartan (n = 133)	p ANOVA One Way / Chi2	Significant difference-Multiple comparisons
Age (years)	55.74 ± 13.86	62.38 ± 13.54	61.62 ± 10.26	< 0.001	< 0.001 (1) (2)
Men (total)	68 (129)	55 (133)	49 (133)	0.029	-
%	52.7%	41.4%	36.8%		
Women (total)	61(129)	78 (133)	84 (133)		
%	47.3%	58.6%	63.2%		
SBP (mmHg)	121.84 ± 18.03	139.26 ± 18.83	139.44 ± 16.48	< 0.001	< 0.001 (1) (2)
DBP (mmHg)	76.69 ± 10.01	83.36 ± 13.89	83.57 ± 10.4	< 0.001	< 0.001 (1) (2)
HTN duration (months)	-	107.86 ± 85.9	121.5 ± 91.18	0.21 (3)	-
Heart rate (beats/min)	69.95 ± 7.99	75.95 ± 10.9	69.93 ± 8.67	< 0.001	< 0.001 (1) (3)
Endoglin (ng/mL)	4.99 ± 2.81	5.59 ± 2.39	4.25 ± 1.05	< 0.001	0.026 (2), < 0.001 (3)
hs-CRP (mg/dL)	0.3 ± 0.39	0.25 ± 0.25	0.32 ± 0.52	0.394	-
Pentraxin-3 (ng/mL)	1.47 ± 1.27	1.34 ± 1.25	0.9 ± 0.63	< 0.001	< 0.001 (2), 0.005 (3)
FMD (%)	17.17 ± 0.06	10.44 ± 0.06	15.28 ± 0.07	< 0.001	< 0.001 (1) (2) (3)
E.D. (presence)	31 (129)	20 (133)	34 (133)	0.079	-
%	24.0%	15.0%	25.6%		
E.D. (absence)	98 (129)	113 (133)	99 (133)		
%	76.0%	85.0%	74.4%		
CC IMT right (mm)	0.84 ± 0.29	0.93 ± 0.2	0.92 ± 0.21	0.006	0.018 (1), 0.026 (2)
CC IMT left (mm)	0.84 ± 0.22	0.95 ± 0.18	0.92 ± 0.18	< 0.001	< 0.001 (1), 0.008 (2)
Smokers (yes)	51 (129)	98 (133)	84 (133)	< 0.001	-
%	39.5%	73.7%	63.2%		
Smokers (no)	78 (129)	35 (133)	49 (133)		
%	60.5%	26.3%	36.8%		
Glucose (mg/dL)	110.98 ± 49.95	112.63 ± 44.74	112.37 ± 40.66	0.951	-
Cholesterol (mg/dL)	198.65 ± 55.64	202.68 ± 51.58	199.04 ± 52.27	0.793	-
Triglicerides (mg/dL)	122.88 ± 81.35	146.25 ± 184.61	99.72 ± 27.98	0.006	0.006 (3)
Creatinine (mg/dL)	0.86 ± 0.21	0.86 ± 0.22	0.85 ± 0.25	0.788	-
Potassium (mmol/L)	4.35 ± 0.41	4.25 ± 0.45	4.4 ± 0.38	0.015	0.017 (3)
INR	1.16 ± 0.31	1.06 ± 0.24	1.05 ± 0.15	0.001	0.005 (1), 0.002 (2)
ALAT (U/L)	38.83 ± 14.99	40.23 ± 15.28	41.67 ± 17.65	0.359	-
ASAT (U/L)	24.77 ± 8.78	23.99 ± 8.26	24.45 ± 9.81	0.781	-
ESR (mm/h)	9.19 ± 5.35	13.23 ± 8.03	15.54 ± 9.01	< 0.001	< 0.001 (1) (2), 0.049 (3)
BUN (mg/dL)	18.12 ± 6.12	17.52 ± 7.01	18.45 ± 6.05	0.485	-
BMI (kg/m^2)	23.86 ± 3.86	26.48 ± 3.95	28.86 ± 2.06	< 0.001	< 0.001 (1) (2) (3)
Fibrinogen (mg/dL)	315.76 ± 48.61	315.37 ± 48.64	305.77 ± 43.02	0.146	-
DLA (mm)	35.31 ± 6.6	37.71 ± 7.37	37.89 ± 5.77	0.002	0.006 (1), 0.007 (2)
IVS (mm)	10.75 ± 2.31	11.38 ± 2.35	11.36 ± 1.59	0.024	0.044 (1), 0.017 (2)
PWLV (mm)	10.02 ± 1.58	10.54 ± 1.87	10.77 ± 1.5	0.001	0.009 (1), < 0.001 (2)
EDLV (mm)	49.51 ± 6.77	52.65 ± 5.51	50.41 ± 4.76	< 0.001	< 0.001 (1), 0.003 (3)
E.F. (%)	55.88 ± 8.09	52.72 ± 8.8	54.35 ± 7.27	0.007	0.007 (1)

Data are represented as mean ± standard deviation (SD). SBP – systolic blood pressure; DBP – diastolic blood pressure; HTN – essential arterial hypertension; hs-CRP – high-sensitivity C-reactive protein; PTX3 – pentraxin-3; E.D. – endothelial dysfunction; FMD – flow-mediated vasodilatation; CC IMT – common carotid intima-media thickness; INR – international normalized ratio; ALAT – alanine aminotransferase (GTP); ASAT – aspartate aminotransferase (GOT); ESR – erythrocyte sedimentation rate; BUN – blood urea nitrogen; BMI – body mass index; DLA – diameter of the left atrium; IVS – diameter of the interventricular septum; PWLV – the posterior wall of the left ventricle; EDLV – end-diastolic diameter of the left ventricle; EF – ejection fraction. In the mean ± SD comparison cases, we applied the ANOVA test (between the 3 groups), and in the frequency comparison cases, we applied the Chi2 test. Statistical significance was considered at a p-value < 0.05. (1) = p-value obtained for group B *vs.* group A comparisons; (2) = p-value obtained for group C *vs.* group A comparisons; (3) = p-value obtained for group C *vs.* group B comparisons.

The plasma concentration of pentraxin-3 (in ng/mL) was significantly lower in group C (0.9 ± 0.63) compared with that in group A (1.47 ± 1.27) and group B (1.34 ± 1.25) ($p < 0.001$).

Endothelial dysfunction was assessed by two parameters: FMD of the brachial artery and IMT of the carotid arteries. Thirty-one patients (24%) presented with endothelial dysfunction in group A compared with 20 patients (15%) in group B and 34 patients (25.6%) in group C. Group B had the lowest number of patients with endothelial dysfunction. However, the FMD (in %) level was the smallest in this group (10.44 ± 0.06

in group B *vs.* 15.28 ± 0.07 in group C *vs.* 17.17 ± 0.06 in group A, $p < 0.001$). IMT (in mm) was significantly higher in group B (0.95 ± 0.18) compared with that in group A (0.84 ± 0.22) ($p < 0.001$) and group C (0.92 ± 0.18) ($p < 0.01$).

In both groups of hypertensive patients (B and C), there were more smokers than that in the control group (A), and the difference was significant (73.7% *vs.* 63.2% *vs.* 39.5%, $p < 0.001$).

Echocardiography parameters showed some significant differences between groups. The DLA (in mm) was increased significantly in both groups of hypertensive patients (B and C) compared with that in the control group (37.71 ± 7.37 in group B *vs.* 37.89 ± 5.77 in group C *vs.* 35.31 ± 6.6 in group A, $p = 0.002$). The dimension of the IVS and PWLV was increased significantly in hypertensive patients compared with that in the control group (IVS (mm): 10.75 ± 2.31 in group A *vs.* 11.38 ± 2.35 in group B *vs.* 11.36 ± 1.59 in group C, $p = 0.024$; PWLV (mm) 10.02 ± 1.58 in group A *vs.*10.54 ± 1.87 in group B *vs.* 10.77 ± 1.5 in group C, $p < 0.001$). The EDLV (in mm) was lower in the control group (49.51 ± 6.77) than that in group C (50.41 ± 4.76), and the difference was not significant, but the EDLV was significantly higher in group B (52.65 ± 5.51) ($p < 0.001$). The EF of the left ventricle (%) was higher in the control group (55.88 ± 8.09) than that in group C (54.35 ± 7.27), but was significantly lower in group B (52.72 ± 8.8) ($p = 0.007$).

The majority of patients (72.2%) in group C were under long-term treatment with candesartan at 16 mg/day, 23.3% took 8 mg/day and 4.5% took 32 mg/day.

Although patients in group C had the lowest plasma level of sEng, patients under long-term treatment with 32 mg/day of candesartan had an average plasma level of sEng < 3.5 ng/mL (Figure 1).

Figure 2 presents the average plasma level of sEng in group B under different treatment regimens. The highest plasma concentration of sEng (in ng/mL) was in patients under long-term treatment with beta-blockers (5.84 ± 2.61 *vs.* 4.25 ± 1.05, $p < 0.0001$) compared with those under long-term treatment with calcium-channel blockers (5.68 ± 2.17 *vs.* 4.25 ± 1.05, $p < 0.0001$). The lowest average plasma level of sEng was in patients under long-term treatment with diuretics (5.11 ± 2.29 *vs.* 4.25 ± 1.05, $p < 0.0005$) (Figures 3, 4, 5 and 6).

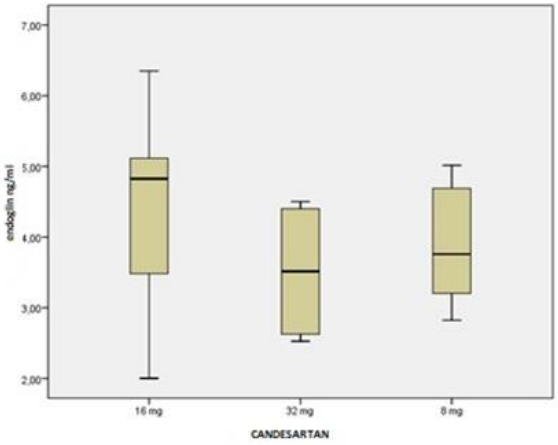

Figure 1.
Group C's boxplot, related to the dose of candesartan

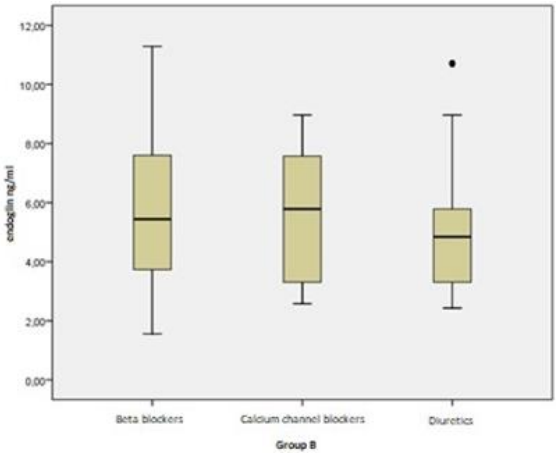

Figure 2.
Group B's boxplot, related to the three types of treatment

Using multivariate regression, the variables associated significantly with the plasma level of sEng as an independent variable were evaluated (Table II).

Table II

Multiple linear regression was performed for Group B (soluble endoglin–dependent variable, R2 = 0.997)

Predictors	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
DBP	0.092	0.007	0.536	13.196	**< 0.001**	0.078	0.106
EDLV	0.289	0.011	0.666	25.307	**< 0.001**	0.266	0.311
HCT	-0.398	0.020	-1.335	-19.490	**< 0.001**	-0.439	-0.357
NEUT	-3.267	0.065	-2.395	-50.280	**< 0.001**	-3.396	-3.138
EO	-14.988	1.213	-0.686	-12.352	**< 0.001**	-17.397	-12.578
RDW-CV	-0.803	0.132	-0.329	-6.069	**< 0.001**	-1.065	-0.540

Statistical significance was considered at a sig. value < 0.05**.** DBP – diastolic blood pressure; EDLV – end diastolic diameter of left ventricle; HCT – haematocrit; NEUTRO – neutrocytes; EO – eosinophils; RDW – Red cell distribution width

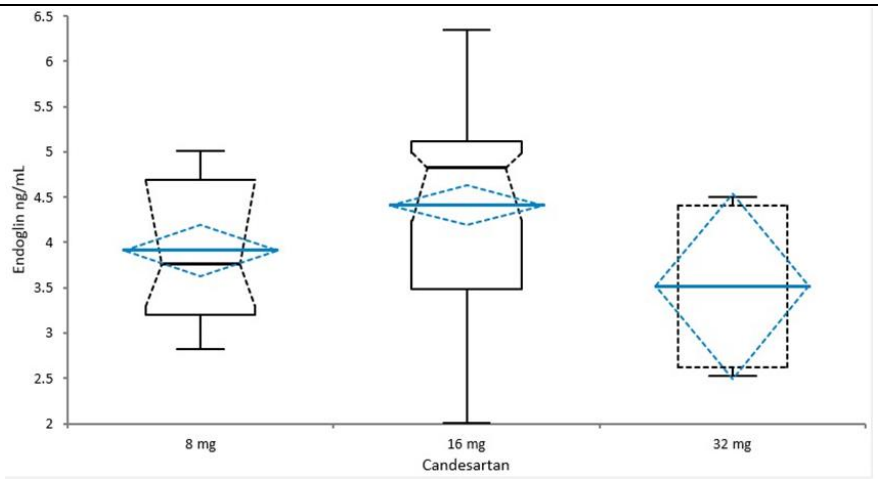

Figure 3.
Mean (in blue) and median (in black) endoglin plasma levels in Group C, related to candesartan's dose

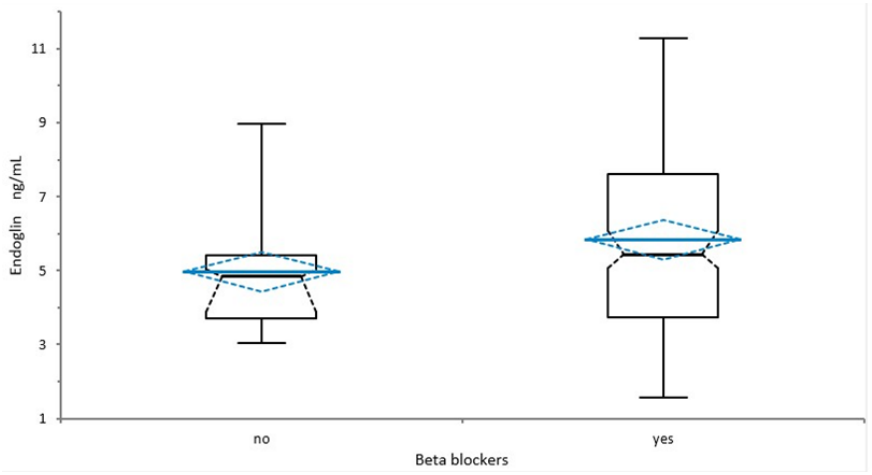

Figure 4.
Mean (in blue) and median (in black) endoglin plasma levels in Group B, in patients under treatment with beta-blockers (yes – under treatment with beta-blockers; no – different antihypertensive treatment)

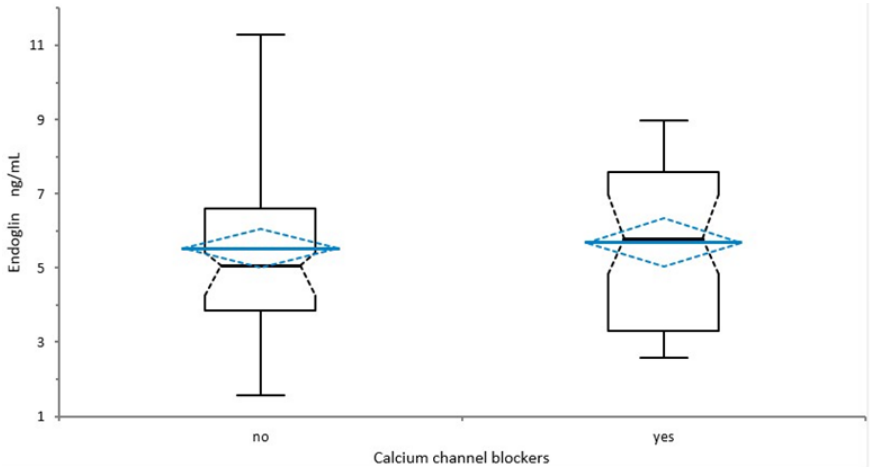

Figure 5.
Mean (in blue) and median (in black) endoglin plasma levels in Group B patients under treatment with calcium channel blockers (yes – under treatment with calcium channel blockers; no – different antihypertensive treatment)

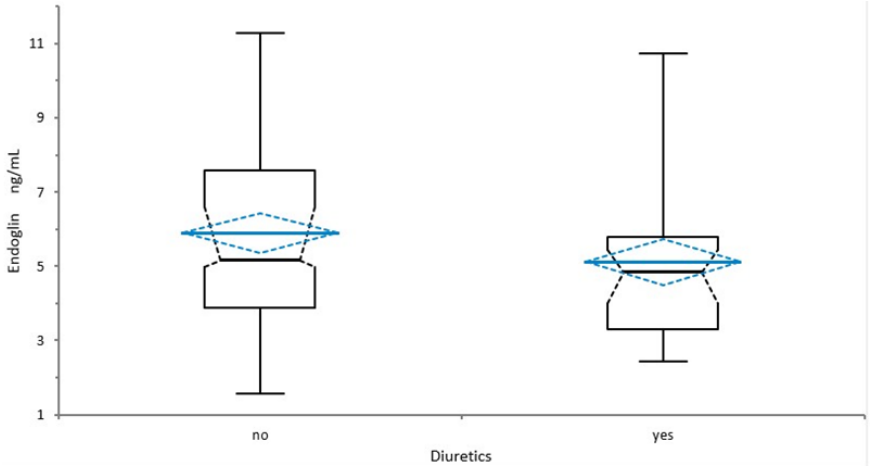

Figure 6.
Mean (in blue) and median (in black) endoglin plasma levels in Group B patients under diuretics treatment (yes – under treatment with diuretics; no – different antihypertensive treatment)

Table III presents the risk analysis undertaken for group B. The blood urea nitrogen (BUN) level and diastolic BP were protective factors for the plasma level of sEng. However, EDLV, RBC width, mean platelet volume, haematocrit, neutrophil count, and eosinophil count were risk factors for endothelial dysfunction. Being older than 56.5 years was not a risk factor for an increased plasma level of sEng. Also, there was no significant association between age and the plasma level of sEng (chi-squared test, p = 0.887, OR = 0.943, 95%CI, 0.42 - 2.14).

Table III
Risk analysis in Group B

Parameters	$p^{sign.}$	OR	95% interval of confidence	
			Lower limit	Upper limit
BUN – protective factor	< 0.001 s	0.01	0.004	0.027
DBP – protective factor	0.025 s	0.32	0.11	0.89
EDLV – risk factor	< 0.001 s	5.77	1.98	16.81
RDW-CV– risk factor	< 0.001 s	4.38	1.83	10.43
MPV – risk factor	< 0.001 s	15.06	1.97	115.15
HCT – risk factor	< 0.001 s	7.68	2.14	27.58
NEUTR – risk factor	0.011 s	2.8	1.25	6.28
EO – risk factor	0.019 s	2.79	1.16	6.73

Statistical significance was considered at a sig. value < 0.05. DBP – diastolic blood pressure; EDLV – end diastolic diameter of left ventricle; HCT – haematocrit; NEUTRO – neutrocytes; EO – eosinophils; RDW – Red cell distribution width

Diastolic BP and EDLV were correlated positively with the plasma level of sEng in group B. In contrast, the haematocrit, neutrophil count, eosinophil count, and red blood cell (RBC) width were correlated negatively (also observed in Table IV).

Risk analysis could not be carried out in Group C because we set the cut-off value for sEng > 7.06 ng/mL, and no patients had a plasma level above this value.

Tables IV and V present the correlations between the plasma level of sEng and the other parameters studied in group B and group C. In group B, we found a weak inverse correlation between the plasma level of sEng and hs-CRP level, creatinine level, IMT, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin level, neutrophil count, eosinophil count, and platelet width. Direct weak correlations were found with IVS diameter, EDLV, and mean platelet volume, and a direct medium-level correlation was found with RBC width.

In group C, we found a weak direct correlation between the plasma level of sEng and fibrinogen level, cholesterol level, platelet count, and eosinophil count (Table V). Interestingly, the plasma level of sEng correlated inversely with the level of hs-CRP, triglycerides, glucose, potassium, as well as DLA, IVS diameter, EDLV, white blood cell (WBC) count, mean corpuscular haemoglobin concentration, lymphocyte level, neutrophil count and basophil count. The plasma level of sEng correlated inversely and at a medium level with the pentraxin-3 level, creatinine level, and DLA (Table V).

We evaluated the effect of candesartan compared with that of different regimens of BP-lowering treatment on the plasma level of sEng. We found a lower plasma level of sEng after long-term treatment with candesartan (a BP-lowering drug with endothelium-protective effects) compared with that using other treatment regimens. Candesartan is an inhibitor of the renin-angiotensin-aldosterone system and part of the ARB family. It is

used as a pro-drug, mainly in treating hypertension and heart failure [7, 13, 14, 31]. Candesartan is employed as monotherapy or in combination with other drugs to reduce cardiovascular morbidity and mortality. It can confer endothelial protection and ameliorate endothelial dysfunction [10, 48]. Candesartan is also prescribed for migraine, left-ventricular hypertrophy, diabetic nephropathy/retinopathy, stroke and cerebrovascular accidents [5, 15, 27].

Table IV

Significant Spearman correlation between soluble endoglin values and some parameters in Group B

Parameters	ρ coefficient	p^{sign}
DBP	0.190	0.031 s
EDLV	0.247	0.004 s
MPV	0.311	< 0.001 s
EO	-0.228	0.008 s
hs-CRP	-0.207	0.017 s
Creatinine	-0.313	< 0.001 s
E. F.	-0.202	0.02 s
MCV	-0.298	0.001 s
MCH	-0.307	< 0.001
PDW	-0.298	< 0.001 s
MPV	0.311	< 0.001 s
IVS	0.252	0.003 s
EDLV	0.247	0.004 s

s – significant correlation; for p > 0.05 correlation was considered insignificant. hs-CRP – high sensitivity C reactive protein; SBP – systolic blood pressure; CC IMT – common carotid intima-media thickness; DLA – diameter of left atrium; IVS – diameter of interventricular septum; PWLV – posterior wall of the left ventricle; EDLV – end diastolic diameter of left ventricle; EF – ejection fraction; WBC –white blood cells; HCT – haematocrit; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration; PLT – platelet count; LYMPH – lymphocytes; NEUTRO – neutrocytes; EO – eosinophils; BASO – basophils; RDW – Red cell distribution width; PDW – Platelet distribution width; MPV – Mean platelet volume

Candesartan antagonizes AT1 receptor of Ang II to inhibit the negative actions of Ang II and, thus: induce vasodilatation and decreased BP in vascular smooth muscle; cause dilatation of efferent arterioles in the kidneys and inhibition of reabsorption of sodium, water and bicarbonate; affect aldosterone secretion and release of antidiuretic hormone. We chose candesartan because: (i) it is the most used ARB in the clinic; (ii) it has a slow dissociation rate from the receptor, with a partial-to-complete decrease of the response and a parallel rightward shift in the Ang II concentration-response curve [10, 13, 14, 48].

Both groups of patients with hypertension (B and C) were similar in age, sex distribution, and BP level (Table I). Group-B patients presented higher heart rates than the other two groups (A and C). This aspect was a particularity of the group because the patients were enrolled randomly (Table I).

A low plasma level of sEng was found in group-C patients under long-term treatment with candesartan compared with that for other therapy regimens (though all values were in the normal range given by Bioclinica SA Laboratory). These values of soluble endoglin are similar with other data reported in the literature where hypertensive patients under chronic antihypertensive treatment had lower soluble endoglin plasma levels (4.39 ± 1.04 ng/mL) compared with the control group (5.21 ± 1.10 ng/mL) and compared with patients who associated diabetes and hypertension (5.02 ± 0.98 ng/mL) [17]. The plasma level of sEng has been reported to be higher in patients with gestational hypertension (the median being 6.2 ng/mL) and much higher in women with preeclampsia (the median being 30.2 ng/mL) [6].

Table V

Significant Spearman correlation between soluble endoglin and other parameters in Group C

Parameters	ρ coefficient	p^{sign}
hs-CRP	-0.201	0.02 s
Pentraxin 3	-0.514	< 0.001 s
Fibrinogen	0.257	0.003 s
Cholesterol	0.175	0.044 s
Triglycerides	-0.195	0.025 s
Creatinine	-0.481	< 0.001 s
Glucose	-0.197	0.023 s
Potassium	-0.269	0.002 s
WBC	-0.317	< 0.001 s
DLA	-0.494	< 0.001 s
IVS	-0.281	0.001 s
PWLV	-0.395	< 0.001 s
EDLV	-0.227	0.009 s
NEUT	-0.247	0.004 s
MCHC	-0.227	0.009 s
PLT	0.197	0.023 s
EO	0.208	0.016 s
LYMPH	-0.203	0.019 s
BASO	-0.221	0.011 s

s – significant correlation; for p > 0.05, the correlation was considered insignificant. hs-CRP – high sensitivity C reactive protein; SBP – systolic blood pressure; CC IMT – typical carotid intima-media thickness; DLA – diameter of the left atrium; IVS – diameter of the interventricular septum; PWLV – the posterior wall of the left ventricle; EDLV – end-diastolic diameter of the left ventricle; EF – ejection fraction; WBC – white blood cells; HCT – haematocrit; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration; PLT – platelet count; LYMPH – lymphocytes; NEUTRO – neutrocytes; EO – eosinophils; BASO – basophils; RDW – Red cell distribution width; PDW – Platelet distribution width; MPV – Mean platelet volume

This finding of a low plasma level of sEng correlates with data from Behl and colleagues. They reported that Ang II modulates endoglin expression and, thus, blockers of AT1 receptors will decrease endoglin expression [5, 19]. Moreover, candesartan was reported recently to decrease the plasma level of sEng in gestational hypertension in animal models [38]. The explanation underlying this effect is based on stimulation of AT1 receptors by circulating levels of maternal TNF-α; the production of two anti-angiogenic factors (sEng and sFlt) is increased in gestational hypertension

and, thus, by blocking AT1 receptors, candesartan decreases sEng production. Moreover, candesartan seems also to block the nuclear factor-kappa B signalling pathway, which is involved in endothelial function and progression of gestational hypertension [18, 35, 37, 38, 50]. In another study that we performed, we found a low plasma level of sEng in patients under long-term treatment with perindopril [9]. Based on this argument and their action on the renin-angiotensin system, we excluded patients undergoing long-term therapy with ACEIs.

In group C, the plasma level of pentraxin-3 was lower than in group B (Table 1) [10]. Pentraxin-3 is an acute-phase protein, part of the same family as CRP and serum amyloid P component. Pentraxin-3 has been recognized as an important determinant and an early marker of endothelial damage [11, 49]. Studies have shown that a low plasma level of pentraxin-3 ameliorates the endothelial response/function due to its interaction with MMP-1 and P-selectin [11, 16]. Endothelial cells can release pentraxin-3 in response to inflammatory stimuli such as interleukin-1β, TNF-α and lipopoly-saccharide. Overexpression of pentraxin-3 decreases the formation of nitric oxide (due to up-regulation of expression of MMP-1 and P-selectin) and, by binding to fibroblast growth factor-2, induces an autocrine anti-angiogenic effect [12, 16]. *In vitro* studies have shown that pentraxin-3 can inhibit the expression of peroxisome proliferator-activated receptor-gamma, liver X receptor-α and adenosine triphosphate-binding membrane cassette transporter A-1 [2].

The level of sEng and pentraxin-3 was lower in patients undergoing long-term treatment with candesartan. However, this group presented the highest percentage of patients with endothelial dysfunction, higher erythrocyte sedimentation rate and highest body mass index than group B. Moreover, the FMD was higher in the group of patients undergoing long-term treatment with candesartan compared with that in group-B patients. Potassium levels were higher in patients under treatment with candesartan (as expected) due to its mechanism of action (Table I). Group-B patients had a higher IMT, triglycerides level and more smokers than group-C (Table I).

The echocardiography parameters studied in the three groups of patients provided information on the effects of long-acting antihypertensive therapy on myocardial performance. There was an increase in EDLV in groups B and C compared with that in group A. Still, and the value increased significantly only in group B. There was also a decrease in the EF in groups B and C. Still, the decrease was significant only in group B. These data suggested that long-acting antihypertensive treatment with candesartan protects against deterioration of cardiac contractile performance due to arterial hypertension. Also, a protective role of candesartan on structural vascular alterations (characterized by IMT of carotid arteries) was observed.

In group B, patients under long-term treatment with beta-blockers had the highest plasma level of sEng (Figure 2). Propranolol has been reported to decrease the level of membrane-bound endoglin (but not sEng) and to have anti-angiogenic properties in patients with hereditary hemorrhagic telangiectasia [1].

We noted correlations between the plasma level of sEng and the remainder of the investigated parameters in groups B and C (Tables II, III, IV and V). They are interesting, but unfortunately, we could not find explanations for the effects observed.

Diastolic BP was correlated directly with the plasma level of sEng (Table II). Diastolic BP was found to be a protective factor for sEng in group B (Table III). sEng has been linked to hypertension and preeclampsia [1, 32, 47]. Hypoxia has been reported to induce the formation of reactive oxygen species, which can convert cholesterol to oxysterol species that bind to liver/retinoid X receptors and, thus, increase the plasma level of MMP-13 and decrease the level of tissue inhibitor of metalloproteinase-3. Then, MMP-14 cleaves membrane-bound endoglin and generates sEng. After-wards, sEng dysregulates the binding of TGF-β1 to its receptors, antagonizing eNOS activation and vaso-dilatation and, thus, inducing (together with sFlt1) hypertension [22, 44, 45]. Also, the maternal level of angiotensin II has been linked to a pro-inflammatory chemokine in the placenta [41]. Interestingly, the BUN level was also a protective factor for sEng in group B (Table II).

The correlations found between sEng and echo-cardiography parameters (EDLV and IVS) in groups B and C can be explained by the: (i) link between sEng and myocardial fibrosis (sEng being a component of TGFβ1 signalling in cardiac fibroblasts); (ii) effect of candesartan on reducing myocardial remodelling and fibrosis by limiting the action of Ang II (a high plasma level of sEng correlated positively with EDLV in group B and inversely in group C) (Tables II, III, IV and V) [34, 42].

Counts of neutrophils and eosinophils were found to be risk factors for sEng (Table II), and they correlated negatively with the plasma level of sEng (Table IV) and WBC count (Table V), further demonstrating that sEng can be a marker of endothelial lesions.

In group C, we found a direct, weak correlation between the plasma level of sEng and cholesterol level. This finding suggested that a reduction in the plasma level of sEng is strongly related to a decrease in total cholesterol level. Kapur *et al*. reported, in an animal model, that sEng and hypercholesterolemia can induce endothelial dysfunction [26]. Moreover, Vitverova and colleagues suggested that measuring the sEng level can reflect treatment efficacy in cardio-vascular diseases [46]. We also found that the fibrinogen level was correlated directly with a reduced plasma level of sEng (Table V).

Another direct correlation found in group C was between the plasma level of sEng and the platelet count, which suggested a possible link between sEng, platelets and endothelium interactions (Table V), as reported previously [39, 40]. An inverse correlation was also found in group B between the plasma level of sEng and platelet width, suggesting that a lower level of sEng correlates with a low platelet count (Table IV).

In groups B and C, the plasma level of sEng correlated negatively with the creatinine level (though this correlation was more robust in group C) (Tables IV and V). Recently, Dogfish and colleagues showed that the plasma level of sEng is increased in patients with diabetic nephropathy as the degree of endothelial dysfunction progresses [21].

Interestingly, the plasma level of sEng was negatively correlated with the WBC count and neutrophil count in group C, both of which are markers of endothelial lesions, thereby suggesting another connection between sEng and endothelial dysfunction. Jerkic and Letarte showed that endothelial endoglin-deficient cells in mice had increased cell permeability [24]. The main limitation of our study was the small study cohort. This limitation hampered our ability to study the molecular mechanisms involved.

Conclusions

A lower plasma level of sEng was found in patients under long-term BP-lowering treatment with candesartan than in hypertensive patients undergoing long-term treatment with beta-blockers, calcium-channel blockers, or diuretics. Diastolic BP and the BUN level were protective factors for sEng. The EDLV and neutrophil count were risk factors for sEng and thus for endothelial dysfunction.

By quantifying a glycoprotein involved in endothelial function/homeostasis regulation, sEng could be a specific biomarker of vascular and cardiac lesions and predict the efficacy of antihypertensive treatment.

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Conflict of interest

The authors declare no conflict of interest.

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