Gender-Specific Association between Preproendothelin-1 Genotype and Reduction of Systolic Blood Pressure during Antihypertensive Treatment— Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA)

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Summary

Background: Studies suggest that endothelin-1 contributes to the pathogenesis of hypertension. A G5665T gene polymorphism of preproendothelin-1 has been shown to be associated with higher blood pressure in overweight patients. No study has yet determined the effect of this polymorphism on the change in blood pressure during antihypertensive treatment.

Hypothesis: This study aimed to determine this effect in hypertensive patients with left ventricular (LV) hypertrophy during antihypertensive treatment with either irbesartan or atenolol.

Methods: We determined the preproendothelin-1 genotype using minisequencing in 102 patients with essential hypertension and LV hypertrophy verified by echocardiography, randomized in a double-blind fashion to treatment with either the AT_1-receptor antagonist irbesartan or the $beta_1$-adrenoceptor antagonist atenolol.

Results: The change in systolic blood pressure (SBP) after 12 weeks of treatment was related to the preproendothelin-1 genotype in men; after adjustment for potential covariates (age, blood pressure, and LV mass index at study entry, dose of irbesartan/atenolol, and type of treatment), those carrying the T-allele responded on average with a more than two-fold greater reduction than those with the G/G genotype (-21.9 mmHg [3.9] vs. -8.9 [2.3], p = 0.007). No significant differences in blood pressure change between G/G and carriers of the T-allele were seen among women.

Conclusions: Our finding suggests a gender-specific relationship between the G5665T preproendothelin-1 polymorphism and change in SBP in response to antihypertensive treatment with irbesartan or atenolol, suggesting the endothelin pathway to be a common mechanism included in the hypertensive action of the drugs.

Key words: endothelin, irbesartan, atenolol, hypertension, polymorphism, gender

Introduction

The endothelins are a family of peptides that are extremely potent vasoconstrictors.[1,2] Endothelin-1 (ET-1), the major isoform in the vascular endothelium, is generated in two steps from a 203-amino acid residue precursor preproET-1. The precursor is converted into the polypeptide bigET-1, from which ET-1 is cleaved by endothelin-converting enzymes (ECE). Endothelin-1 exerts both arterial and venous vasoconstriction, direct positive inotropic and chronotropic effects on the heart,

and hypertrophic effects on vascular smooth muscle cells, fibroblasts, and isolated cardiomyocytes.[3] The effects of ET-1 appear to be mediated through two receptor subtypes, ET_A and ET_B. In the heart, ECE is expressed in the endocardium and myocardium.[4] PreproET-1 mRNA is expressed by human cardiomyocytes and interstitial cells that synthesize and secrete mature ET-1.[5, 6] Both ET_A- and ET_B-receptors are present in the human myocardium.[7]

Hypertension has been associated with increased ET activity. Plasma ET-1 levels are higher in patients with essential hypertension than in normotensive subjects,[8] and parallels the degree of cardiac hypertrophy. The ETA receptor gene is overexpressed in arteries of hypertensive patients,[9, 10] suggesting that ET-1 may contribute to the pathogenesis of hypertension by means of ET dysfunction or proliferation of vascular smooth muscle cells. Chronic treatment with an ET-receptor antagonist in hypertensive patients reduces blood pressure, which provides additional support for the involvement of ET-1 in hypertension.[11]

The G5665T polymorphism of the preproET-1 gene causes a Lys/Asn change at codon 198 of the protein.[12] Two studies have shown that overweight patients carrying the T-allele have higher blood pressure than those with the G/G genotype.[12, 13] Another study demonstrated that systolic blood pressure (SBP) during pregnancy was higher among carriers of the T allele in a group consisting of normal and pre-eclamptic women, and that plasma ET-1 levels were significantly higher among T/T homozygotes.[14]

No study has yet determined the impact of this polymorphism on the response to antihypertensive treatment. We aimed to examine the relation between this polymorphism and the change in blood pressure in hypertensive men and women with left ventricular (LV) hypertrophy during antihypertensive treatment with either the angiotensin II type 1 (AT$_1$) receptor antagonist irbesartan or the beta$_1$-adrenoceptor antagonist atenolol.

Methods

Study Population

The subjects participated in the Swedish Irbesartan Left Ventricular Hypertrophy versus Atenolol (SILVHIA) trial, which has been described previously.[15, 16] Briefly, Caucasian men and women with mild to moderate essential hypertension and echocardiographically verified LV hypertrophy were enrolled, with the primary goal of evaluating the efficacy of irbesartan compared with atenolol on blood pressure reduction and regression of LV hypertrophy. The Penn convention was used for calculation of LV mass, which was corrected for body mass index (LVMI). Left ventricular hypertrophy was considered present if LVMI was > 131 g/m^2 for men and > 100 g/m^2 for women. After subjects rested for at least 10 min in the seated position, blood pressure was measured by trained study-nurses using a mercury sphygmomanometer, and was determined as the average of three measurements taken 1 min apart. Inclusion required a diastolic blood pressure (DBP) of 90–115

mmHg. During treatment, blood pressure was measured at trough (24 ± 3 h after the last dose). Secondary hypertension was excluded by means of a physical examination and routine laboratory tests. All antihypertensive agents were withdrawn before the start of a 4–6 week, single-blind, placebo lead-in period, after which the patients received either irbesartan 150 mg or atenolol 50 mg once daily as monotherapy in a double-blind fashion. The doses were doubled after 6 weeks if DBP was ≥ 90 mmHg. In all, 115 patients were randomized to receive either irbesartan 150 mg or atenolol 50 mg once daily, and the dose was doubled after 6 weeks if a patient's DBP was ≥ 90 mmHg. A total of 102 patients completed the first 12 weeks of monotherapy in the SILVHIA trial, and data from this first period are considered in this study. Of the 102 patients, 49 patients had been treated with irbesartan and 53 with atenolol. The appropriate ethics committees approved the study and the participating patients gave their informed consent. The study was completed in accordance with institutional guidelines.

Genomic DNA was extracted from ethylene diamine tetraacetic acid (EDTA)-blood using spin columns (QIAamp® DNA Blood Mini Kit, Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was conducted using AmpliTaq™ Gold DNA polymerase (Applied Biosystems, Foster City, Calif., USA) with the 5'-biotinylated forward primer 5'-CTT TTG CCA AAG GGT GAT TT-3' and the reverse primer 5'-AGG GTG GAG AGT GCA GAG TC-3' manufactured by Interactiva (The Virtual Laboratory, Ulm, Germany), giving a 240 bp product. Reaction volumes (50 µl) containing PCR buffer II, 3.5 mmol/l MgCl$_2$, 0.25 mmol/l of each dNTP, 0.4 µmol/l of each primer, 1 U of Taq DNA polymerase, and approximately 100 ng of genomic DNA were used. The PCR conditions consisted of an initial activation step at 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 60 s, after which a final elongation step at 72°C for 10 min concluded the reaction (GeneAmp PCR system 9700, Applied Biosystems). Genotyping for the preproET polymorphism was conducted with solid phase minisequencing.[17] The PCR products were captured in streptavidin-coated microtiter plate wells and rendered single stranded. The polymorphic nucleotide was detected by single nucleotide extension with a radioactively labelled nucleotide (^{3}H[dCTP] or ^{3}H[dATP], Amersham, U.K.) of a detection primer (5'-ACA TAA CGC TCT CTG GAG GG-3'), which was designed to anneal immediately adjacent to the polymorphic site. The genotype of the samples was defined by the ratio between the incorporated ^{3}H-labeled nucleotides.

Data are presented as mean values ± standard error (SE). The estimated adjusted mean difference in blood pressure change at 12 weeks between G/G and carriers of the T allele (two categories) was calculated with the general linear models (GLM) procedure of the SAS software (SAS Institute, Cary, N.C., USA) for each treatment group. Due to known gender differences in the ET system,[18–28] data were stratified by gender. Two different models were used: one univariate and one multivariate model, including the potential covariates age, SBP, DBP, and LVMI at study entry, dose of atenolol or irbesartan (all continuous), and type of treatment. Interaction be-

TABLE I   Baseline patient characteristics stratified by treatment, genotype, and gender

	Men		Women	
	G/G (n = 49)	G/T (n = 18)	G/G (n = 22)	G/T (n = 13)
Treatment (% irbesartan/atenolol)	45/55	44/56	64/36	38/61
Age (years)	54.5 (1.1)	51.6 (1.7)	56.0 (2.2)	54.4 (2.5)
BMI (kg/m^2)	28.1 (0.8)	26.5 (0.6)	26.3 (0.6)	27.3 (1.4)
SBP (mmHg)	161.3 (2.5)	167.3 (4.1)	162.2 (4.7)	157.1 (6.0)
DBP (mmHg)	105.0 (1.0)	108.6 (1.3)	100.0 (1.7)	97.4 (1.1)

Mean values (± standard error).
Abbreviations: BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure.

tween gender and ET genotype was tested by inclusion of a product interaction term into the multivariate model. A probability (p) of < 0.05 was considered significant.

Results

Genotype distribution (71 G/G [70%], 29 G/T [29%], and 2 T/T [1%]) was consistent with Hardy-Weinberg equilibrium (p = 0.84). Carriers of the T allele were considered as one group since only two patients with the T/T genotype were found. Fifty-three patients (35 G/G and 18 carriers of the T allele) had been given atenolol and 49 (36 G/G and 13 carriers of the T allele) irbesartan. Among patients given atenolol, 54% received 50 mg, 42% 100 mg, and the rest either 25 mg or 75 mg. Among patients given irbesartan, 67% received 300 mg, 28% 150 mg, and the rest either 75 mg or 225 mg. Baseline characteristics of the patients stratified by gender and genotype are shown in Table I. There were no significant differences between the genotypes in each group.

There was a significant interaction between gender and genotype for change in SBP and DBP at 12 weeks (p 0.001 and 0.04, respectively). Change in blood pressure at 12 weeks, stratified by gender and genotype, is shown in Table II. We found a significant difference between genotypes in the reduction of SBP among men. In the multivariate model, carriers of the T-allele responded on average with a more than two-fold

greater reduction than those with the G/G genotype (−21.9 mmHg [3.9] vs. −8.9 [2.3], p = 0.007). This genetic difference in SBP change was seen both in men treated with irbesartan and in those treated with atenolol. No statistically significant differences in blood pressure change between G/G and carriers of the T-allele were seen among women.

Discussion

Our study suggests a gender-specific relationship between the G5665T preproET-1 polymorphism and the degree of reduction of SBP during antihypertensive treatment with the AT_1-receptor antagonist irbesartan and the beta$_1$-adrenoceptor antagonist atenolol.

Evidence suggests that there is a difference between gender in the ET system. Ovarian hormones have been shown to suppress or modulate ET-1 production and its effects; 17β-estradiol attenuates ET-1-induced coronary artery constriction both in vitro[18] and in vivo,[19] and plasma ET-1 levels are higher in men than in age-matched women.[21] Endothelin-1 decreases during estrogen replacement therapy, during pregnancy (when estrogen levels are high), and during estrogen administration in transsexual male patients,[21, 22] and in the absence of ovarian hormones, preproET-1 mRNA increases, as does ET-1.[23, 24] Furthermore, testosterone administration in transsexual female patients increases ET-1 levels in plasma.[21] In addition,

TABLE II   Blood pressure change at 12 weeks stratified by gender and genotype

	Men			Women		
	G/G (n = 49)	G/T (n = 18)	p Value	G/G (n = 22)	G/T and T/T (n = 13)	p Value
SBP change (mmHg)	−9.0 (2.2)	−23.0 (3.7)	0.002	−18.4 (3.9)	−7.9 (5.0)	0.11
SBP change (mmHg) - adjusted	−8.9 (2.3)	−21.9 (3.9)	0.007	−17.6 (3.0)	−9.4 (4.0)	0.12
DBP change (mmHg)	−9.3 (1.4)	−14.4 (2.4)	0.07	−11.3 (1.4)	−8.2 (1.8)	0.18
DBP change (mmHg) - adjusted	−8.9 (1.4)	−13.2 (2.3)	0.12	−11.0 (1.2)	−8.6 (1.7)	0.28

Adjustment was made for age, SBP, DBP, and LVMI at baseline, and dose of irbesartan/atenolol. Mean values (± standard error).
Abbreviation: LVMI = left ventricular mass index. Other abbreviations as in Table I.

the total number of ET-1-receptors is increased in human saphenous vein from men in comparison with postmenopausal women not receiving estrogen replacement therapy, without gender differences in ET-1 binding K_d. In men, ET_B-receptors have been shown to mediate tonic vasoconstriction, whereas in women ET_B-receptors mediate tonic vasodilation.[27] In the light of these studies, it would seem likely that a functional impact of the G5665T preproET polymorphism would be different between men and women, which was the case in our study.

In the present study, presence of the T-allele predicted the change in SBP in a similar way both in males treated with irbesartan and in those treated with atenolol. This is in contrast with our previous findings that the angiotensin-converting enzyme (ACE) I/D polymorphism predicted the blood pressure response to irbesartan only.[29] Thus, the present data suggest that endothelin is involved in the mechanism whereby both the AT_1-receptor antagonists and the beta$_1$-receptor antagonists reduce blood pressure. The strength of this study is that the subjects represent a clinically well-characterized group, randomized to treatment in a prospective, double-blind trial. The major limitation is the small number of subjects; thus, the findings should be interpreted with caution until confirmed in larger populations.

Conclusions

Our findings suggest a relationship between the G5665T preproET-1 polymorphism and reduction of blood pressure in response to antihypertensive treatment with the AT_1-receptor antagonist irbesartan and the beta$_1$-adrenoceptor antagonist atenolol among men with essential hypertension and LV hypertrophy.

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