Gln^{27}→$Glu\beta_2$-Adrenergic Receptor Polymorphism in Heart Failure Patients: Differential Clinical and Oxidative Response to Carvedilol

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Abstract: We investigated the clinical response of chronic heart failure patients with β_2-adrenergic receptor Gln^{27}→Glu polymorphism treated for 6 months with carvedilol, a α/β-antagonist with antioxidant properties. The 6-min. walk test, the left ventricular ejection fraction, heart rate, plasma norepinephrine and malondialdehyde, a stress oxidative marker, concentrations were evaluated at baseline and after treatment for 6 months with carvedilol in 33 stable chronic heart failure patients with the Gln^{27}→$Glu\beta_2$-adrenergic receptor polymorphism. Carvedilol significantly increased the left ventricular ejection fraction, while decreasing the heart rate and malondialdehyde plasma concentrations in chronic heart failure patients with the $Glu^{27}\beta_2$-adrenergic receptor allele. There were however, no significant changes in patients with the $Gln^{27}\beta_2$-adrenergic receptor variant.

This work focuses on β-adrenergic receptors (AR) which are significant modulators of cardiac ino- and chronotropic responses [1]. In the normal myocardium, β_1-adrenergic receptors predominates. In the failing myocardium, however, sympathetic over-activation leads to a selective down-regulation of β_1-adrenergic receptors and a relative increase in β_2-adrenergic receptors of up to 40% above basal [2].

There is appreciable inter-individual variability in the susceptibility to cardiovascular disease and in the response to the associated pharmacological treatments. Genetic polymorphism may be, partly at least, responsible for both susceptibility to disease and inter-individual variability in response to pharmacological treatments [3]. As key regulators of many organ systems, adrenergic receptors are an appropriate target for investigating possible links between receptor polymorphisms, drug responses and susceptibility to, and progression of, disease [4]. The β-adrenergic receptors family members (β_1, β_2 and β_3) are highly polymorphic. The β_2-adrenergic receptors variants are of particular interest because of their pivotal role, both in regulating contractility and heart rate, and as drug targets [5]. There are several polymorphisms in the coding region of the β_2-adrenergic receptors gene which alter receptor functions [6]. In particular, it has been suggested that the Gln^{27}→$Glu\beta_2$AR polymorphism may have an impact on heart failure through altered receptor down-regulation (fig. 1) [6].

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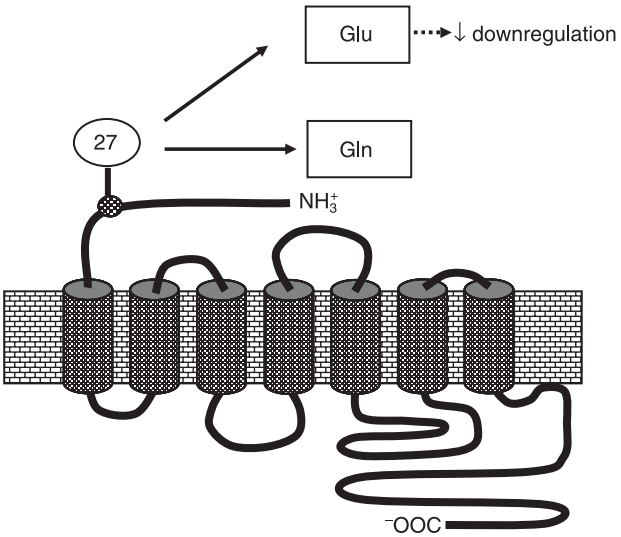

Fig. 1. Gln^{27}→$Glu\beta_2$-adrenergic receptor (AR) polymorphism. The figure shows the *in vitro* effects of the two polymorphic variants. The Glu^{27} is associated with down-regulation of the receptor in its chronic response to agonist.

Carvedilol, an α/β-AR antagonist with antioxidant properties, reduces the risk of morbidity, or death, from cardiovascular causes in patients with chronic heart failure [7–9]. The exact mechanism by which carvedilol improves the prognosis of chronic heart failure is not known, but the possible mechanisms include up-regulation of β-adrenergic receptors in the heart and modulation of post-receptor inhibitory G proteins [10]. In addition, there is evidence that catecholamines induce oxidative stress in terminally differentiated cardiac-muscle cells. This stress can subsequently

trigger apoptosis, a condition possibly prevented by carvedilol [11].

The purpose of the present study was to investigate the influence of $Gln^{27}\rightarrow Glu\beta_2AR$ polymorphism on the variable response to treatment with carvedilol in patients with chronic heart failure. We evaluated clinical parameters and malondialdehyde as a marker of oxidative stress.

Material and Methods

Study design and patient selection. We enrolled 33 patients with chronic heart failure admitted to the Department of Cardiovascular Diseases, P. Catholic University of Chile. All patients were born in Chile and recruited from the Santiago area. Criteria for inclusion were: (i) stable chronic heart failure in NYHA functional classes II–III; (ii) ability to complete a symptom-limited treadmill exercise test; (iii) evidence of left ventricular dilatation and a left ventricular ejection fraction (LVEF) of less than 40%; (iv) treatment with diuretics, digitalis-like drugs and vasodilators such as ACE inhibitors or angiotensin II receptor antagonists.

Criteria for exclusion were: (i) coronary artery bypass surgery, angioplasty or myocardial infarction in the last 6 months; (ii) chronic angina; (iii) uncontrolled hypertension: systolic blood pressure >160 mmHg or diastolic blood pressure >90 mmHg; (iv) hypertensive myocardiopathy; (v) change in maintenance therapy or use of β-adrenoceptor antagonists in the last 2 months; (vi) an implanted pacemaker; (vii) atrial fibrillation; (viii) significant valvular disease; (ix) the presence of other conditions that affect the degree of oxidative stress such as renal insufficiency (plasma creatinine >2 mg/dl), autoimmune diseases, neoplasia, advanced liver or pulmonary disease, and acute or chronic inflammation.

All patients signed an informed consent approved by our Institutional Review Board and Ethics Committee. All patients were treated with carvedilol. The starting dose was 3.12 mg twice daily, which was then increased stepwise at two-week intervals to 6.25, 12.5, 25 and finally, to a target dose of 50 mg per day. The patients were evaluated before and after treatment for 6 months with carvedilol.

Clinical assessment. Clinical evaluations were performed at the Coronary Unit of the Clinical Hospital of the P. Catholic University of Chile. The ischaemic aetiology was determined from the medical history of myocardial infarction, previous bypass surgery or percutaneous coronary intervention, presence of angina or demonstration of ischaemia on exercise testing or scintigraphy. LVEF was determined by radionuclide ventriculography. Performance in a 6-min. corridor walk test was also evaluated

Genotyping. DNA was prepared from peripheral blood samples by hypotonic cell lysis and phenol/chloroform extraction. The region including the $Gln^{27}\rightarrow Glu\beta_2AR$ polymorphism was amplified with a single PCR reaction. The primer sequence for the polymorphic region are (forward) 5′-GCCTTCTTGCTGGCACCCCAT-3′ and (reverse) 5′-CAGACGCTCGAACTTGGCCATG-3′. 5 µl of DNA (approximately 75 ng of DNA) were mixed with 5 µl of solution containing each of the primers (2 µM), 2 µl of $MgCl_2$ (50 mM), 4 µl of buffer (10× PCR). $Gln^{27}\rightarrow Glu\beta_2AR$ polymorphism was identified using BbvI restriction enzyme digestion; fragments were separated by 8%-polyacrylamide gel electrophoresis (fig. 2) [12].

Measurement of plasma norepinephrine concentrations. Plasma norepinephrine samples were collected from an indwelling venous line after patients had been in the supine position in a quiet room for 30 min. Measurement was performed by HPLC, using a commercial kit (Chromsystems Instruments and Chemicals GmbH., Heimburgstrasse 3, D-81243 München, Germany). The inter- and intra-assay precision reported for this method was 6%.

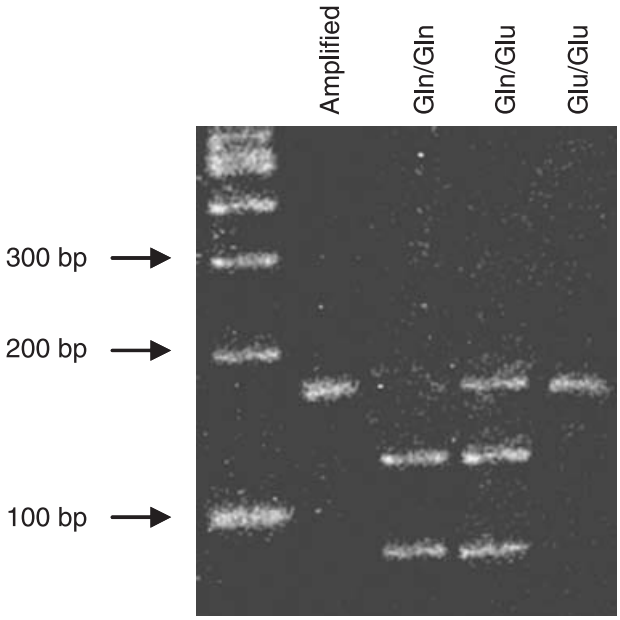

Fig. 2. Electrophoresis of the restriction fragments from the $Gln^{27}\rightarrow Glu\beta_2$-adrenergic receptor (AR) gene. The figure shows the specific cleavage of the restriction enzyme Bbv I on the variant Gln^{27} (CAA) into fragments of 105 and 63 bp.

Measurement of malondialdehyde concentrations. Blood samples (5 ml) were obtained by venipuncture at time zero and during the reperfusion at 0.5 and 24 hr. Each sample was centrifuged at 1250 ×g for 10 min. at 4°. Plasma samples were separated and stored at −20°. Lipid peroxide formation was determined by the presence of thiobarbituric acid-reactive substances (TBARS) as described earlier [13]. Commercially available malondialdehyde was used as standard.

Statistical analysis. Results are mean ± S.D. for continuous variables. The comparisons of the distribution of categorical variables, at baseline, were performed using the Fisher exact test. Differences in LVEF, the 6-min. walk test, heart rate, plasma catecholamine and malondialdehyde plasma concentrations were analysed using paired-sample t-tests. Every group exhibited a normal distribution as analysed by the Kolmogorov-Smirnov test. There were no differences between the standard deviations in each pair of the groups analysed, by Bartlett's test. With several variables, the probability of finding at least one parameter significant by chance is avoided by dividing the statistical probability by the number of variables. A P value ≤0.01 was therefore considered significant.

Results

At position 27 of the $Gln^{27}\rightarrow Glu\beta_2AR$ polymorphism 14 patients were homozygous for the Gln genotype, 12 were homozygous for Glu and 7 were heterozygous. The genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. To analyse the impact of the $Gln^{27}\rightarrow Glu\beta_2AR$ polymorphism, we divided the patients into two groups. The first comprised those homozygous for Gln^{27} (n = 14), the second comprised both those homozygous for Glu and the heterozygous individuals (n = 19).

The distribution of patients by genotype, sex, and age is shown in table 1. There were no differences between genotypes with regard to the diagnosis, risk factors, smoking, medical history and medications at entry.

Table 1.

Characteristics of patients with chronic heart failure.

	β_2AR		
	Gln^{27}	Glu^{27}	P
Characteristic			
N	14	19	
Age (year)	53 ± 16	56 ± 13	NS
Men / women	12/2	13/6	NS
NYHA classes II or III	6/8	11/8	NS
Diagnosis			
Ischaemic (%)	28.6	31.6	NS
Non-ischaemic (%)	71.4	68.4	NS
Risk factors			
Hypertension (%)	57.1	47.4	NS
Diabetes mellitus (%)	14.3	15.8	NS
Hypercholesterolaemia (%)	28.6	26.3	NS
Smoking			
Current (%)	23.1	16.7	NS
Past (%)	38.5	44.4	NS
Past medical history			
Acute myocardial infarction (%)	35.7	31.6	NS
Medications at entry			
Digoxin (%)	50.0	47.4	NS
Diuretics (%)	92.9	73.7	NS
ACE inhibitor (%)	78.6	78.9	NS
Spironolactone (%)	71.4	57.9	NS
Angiotensin II receptor antagonist (%)	7.1	15.8	NS

Values are mean ± S.D. NYHA, New York Heart Association. NS, not significant.

All patients were treated with carvedilol; the maintenance dose was 50 mg during the 6 months. The mean baselines of catecholamine concentrations, plasma MDA concentrations, heart rate, LVEF and the 6-min. walk are shown on table 2. No statistically significant differences were found between the two groups.

Table 2.

Baseline parameters.

	β_2AR		
Parameter	Gln^{27} n = 14	Glu^{27} n = 19	P
LVEF (%)	21.9 ± 9.7	23.6 ± 7.5	NS
Heart rate (beats/min.)	71.7 ± 13	83.5 ± 18	NS
6-min. walk test (m)	475 ± 116	503 ± 70	NS
Norepinephrine (pg/ml)	517 ± 346	498 ± 239	NS
Malondialdehyde (μM)	1.9 ± 1.0	2.0 ± 0.8	NS

Values are mean ± S.D. LVEF, left ventricular ejection fraction. NS, not significant.

After treatment for 6 months with carvedilol, patients carrying the $Glu^{27}\beta_2$AR variant showed an improved clinical response in the LVEF (Glu^{27}: from 23.6 ± 7.5 to 30.6 ± 11.0%, P < 0.01, *versus* Gln^{27}: from 21.9 ± 9.7 to 27.0 ± 12.0%, P = 0.052), heart rate (Glu^{27}: from 83.5 ± 18.0 to 65.6 ± 9.8 beats/min., P < 0.001, *versus* Gln^{27}: from 71.7 ± 13.0 to 62.7 ± 9.2 beats/min., P = 0.092). Results with the 6-min. walk test (Glu^{27}: from 503 ± 70 to 525 ± 83 m, P < 0.035, *versus* Gln^{27}: from 475 ± 116 to 498 ± 115 m, P = 0.394) were not statistically significant (fig. 3C). No differences were observed between the groups in respect of plasma norepinephrine concentrations (Glu^{27} from 498 ± 239 to 456 ± 234 pg/ml, P < 0.478, *versus* Gln^{27} from 517 ± 346 to 455 ± 295 pg/ml, P = 0.416) (fig. 3D). Plasma malondialdehyde concentrations were also significantly decreased in patients with the $Glu^{27}\beta_2$AR variant (Glu^{27} from 2.0 ± 0.8 to 1.0 ± 0.6 μM, P < 0.001, *versus* Gln^{27} from 1.9 ± 1.0 to 1.4 ± 0.5 μM, P = 0.171) (fig. 4).

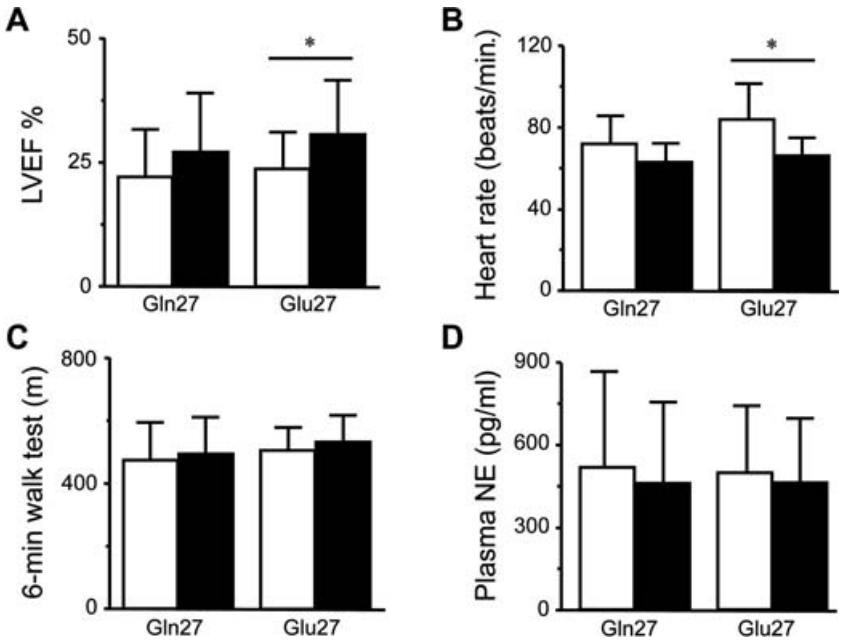

Fig. 3. Effect of the β_2-adrenergic receptor (AR) polymorphism on the clinical response after treatment for 6 months with carvedilol. White bars represent basal states; black, those after 6 months of treatment. Values are mean ± S.D. *P < 0.01 relative to basal state.

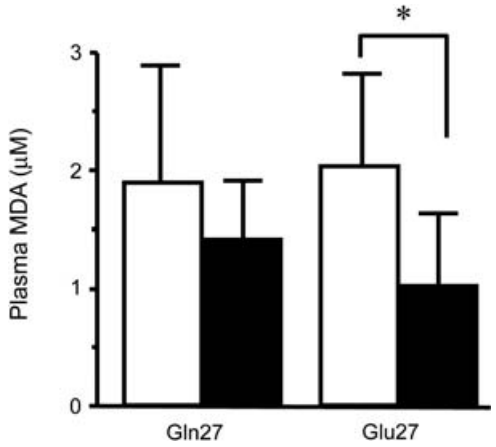

Fig. 4. Effect of the β_2-adrenergic receptor (AR) polymorphism on plasma malondialdehyde (MDA) concentrations after treatment for 6 months with carvedilol. White bars represent basal states; black, those after treatment for six months. Values are mean ± S.D. *P < 0.01 relative to basal state.

Discussion

The over-activation of the catecholaminergic system in the progression of LVEF dysfunction to chronic heart failure has been widely documented [14–16]. The increased catecholamine concentrations observed in patients with ventricular dysfunction initially support, but finally damage, the heart. Catecholaminergic over-activation leads to cardiac desensitization of β-adrenergic receptors, to counteract the catecholamine over-activation. As a result of catecholinergic over-activation, there is an increase in the β_2-adrenergic receptors/β_1-adrenergic receptor ratio in hearts from chronic heart failure patients [14]. One might expect, therefore, that any genetic polymorphisms which increase the up-regulation or activity of β_1-adrenergic receptors and/or down-regulate or decrease the activity of β_2-adrenergic receptors may have protective effects on the myocardium. The functional consequences of β_2-adrenergic receptor polymorphisms have been widely studied in several models *in vitro* and *in vivo*. There are conflicting results concerning the functional significance of specific allelic variants and haplotypes [17–19]. Recent studies *in vivo* have, however, shown that subjects homozygous for the Glu^{27} allele have a greater agonist-mediated responsiveness than do those homozygous for $Gln^{27}\beta_2$AR [20].

We have previously reported that carvedilol improved the prognosis of chronic heart failure patients [21]. We have now investigated the potential effects of the β_2-adrenergic receptor Gln^{27}Glu polymorphism in a 6 month study with carvedilol. Our results show that chronic heart failure patients with the $Glu^{27}\beta_2$-adrenergic receptor allele have a better response to carvedilol treatment (fig. 3). The results also show that patients with Glu^{27} showed a significant improvement in LVEF, whereas patients with Gln^{27} did not. There is good reason for considering heart rate as a high risk factor in cardiovascular disease (fig. 3A) [22].

The predictive utility of heart rate for cardiovascular disease has been documented in patients with myocardial infarction, hypertensive cohorts and patients with stable coronary artery disease from coronary artery surgery study [23]. We have shown here that the effect of carvedilol on heart rate is particularly enhanced in the $Glu^{27}\beta_2$-adrenergic receptor variants (fig. 3B). This fact appears to be important because we also observed elevated and sustained high plasma catecholamine concentrations during the treatment period, as an independent phenomenon on β_2-adrenergic receptor polymorphism (fig. 3D).

No changes were observed in the 6-min. walk test results, independent of the β_2-adrenergic receptor polymorphism (fig. 3C). This clinical parameter appears to be more linked to the endothelial function rather than the cardiac function itself. Carvedilol has previously been shown not to improve endothelial function [24]. Statins improve performance in the 6-min. walk test and endothelial function, but not LVEF or heart rate. This supports the idea that the 6-min. walk test depends more on endothelial than cardiac function [25].

Catecholamine metabolites, formed by oxidation, are initiators of cardiotoxicity [16]. Carvedilol with its antioxidant activity decreased the plasma malondialdehyde concentrations (fig. 4). This decrease in oxidative stress was unrelated to the decrease in plasma norepinephrine concentrations. We have previously shown that carvedilol treatment of patients with stable chronic heart failure for 6 months results in decreased plasma malondialdehyde concentrations [26]. We have now shown that the decrease in malondialdehyde concentrations was also dependent on the $Glu^{27}\beta_2$-adrenergic receptor polymorphism. Another β-adrenoceptor antagonist, metoprolol, without antioxidant properties, also decreased oxidative stress in rats with myocardial infarcts [27]. These observations and our results suggest that the $Glu^{27}\beta_2$-adrenergic receptor polymorphisms may also regulate circulating oxidative stress in chronic heart failure patients in whom plasma catecholamine concentrations are increased [28].

In summary, our results showed that $Glu^{27}\beta_2$-adrenergic receptor polymorphism exerts a differential clinical and oxidative response to treatment with carvedilol for 6 months in patients with chronic heart failure.

Acknowledgements

This work was supported in part by FONDECYT Grant 1010992 (to P.C), FONDAP 15010006 (to S.L), and DID 14-02/01 (to G.D.A). We thank Dr C.I. Pogson for his critical reading of the manuscript.

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