Withstanding the flow
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Chapter 7

β₂-ADRENERGIC RECEPTOR GENOTYPE INFLUENCES THE EFFECT OF NONSELECTIVE VS. SELECTIVE β-BLOCKADE ON BAROREFLEX FUNCTION IN CHRONIC HEART FAILURE


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Chapter 7

**ABSTRACT**

**Background**

Chronic heart failure (CHF) is characterized by sympathetic overactivity, which is restrained by β-adrenergic receptor (AR) blockade. The efficacy in restoring autonomic balance may however differ between selective and nonselective βAR-blockers. Also genetic polymorphisms in de β2AR, affecting agonist mediated receptor downregulation, may influence the response to βAR-blockade. We hypothesized that nonselective βAR-blockade (carvedilol) restores autonomic balance, as reflected by baroreflex sensitivity (BRS), more effectively than selective β1AR-blockade, affecting agonist mediated receptor downregulation compared to the Arg16/Gln27 haplotype.

**Methods**

In 21 CHF patients homozygous for the Gly16/Glu27 β2AR or Arg16/Gln27 β2AR haplotype baroreflex sensitivity (BRS) was determined after two periods of 6 weeks of carvedilol vs. metoprolol treatment in a cross-over study. BRS was assessed by a sequential cross-correlation between spontaneous systolic blood pressure and interbeat interval oscillations.

**Results**

BRS was higher during carvedilol vs. metoprolol treatment in the Gly16/Glu27 group (10.2±7.1 vs. 5.9±2.3 ms·mmHg⁻¹, p<0.01), but not in the Arg16/Gln27 group (5.5±3.5 vs. 5.5±2.5 ms·mmHg⁻¹).

**Conclusion**

Nonselective βAR-blockade restores autonomic balance as evidenced by baroreflex sensitivity more effectively than selective β1AR-blockade in CHF patients with the Gly16/Glu27 haplotype for the β2AR, but not in patients with the Arg16/Gln27 haplotype.
**INTRODUCTION**

Sympathetic overactivity is a characteristic feature of chronic heart failure (CHF) and a major cause of progression of ventricular dysfunction and cardiovascular complications. This sympathetic dominance is restrained by blockade of β-adrenergic receptors (BAR). The improved outcome in CHF with BAR-blockers forms the basis of current medical management of CHF. The response to BAR-blocker treatment in CHF is however not uniform and may be influenced by BAR-blocker selectivity.

In contrast to selective β-blockade, nonselective β-blockade lowers systemic and cardiac norepinephrine spillover, possibly by blocking prejunctional β2ARs that facilitate the release of norepinephrine at sympathetic nerve terminals. High levels of sympathetic activity attenuate vagal modulation of heart rate (HR) and baroreflex function, whereas BAR-blockade improves vagal HR modulation and baroreflex sensitivity (BRS) in CHF. Both high norepinephrine plasma levels and reduced BRS are independently associated with increased mortality in CHF.

Although part of the variation in responses to β-blocking treatment can be attributed to BAR-blocker selectivity, the effect of a specific BAR-blocker may still differ between patients. Genetic variations in the β2AR likely mediate the diversity in responses to nonselective β-blockade. Two common polymorphisms at codons 16 and 27 of the β2AR, substituting respectively arginine (Arg16) to glycine (Gly16) and glutamine (Gln27) to glutamate (Glu27), have the potential to affect receptor expression and function. The Gly16 and Glu27 polymorphisms generally being linked together form the Gly16/Glu27 haplotype.

In vivo data indicate that the Gly16/Glu27 haplotype is relatively resistant to agonist-mediated desensitization of the β2AR as compared to the Arg16/Gln27 haplotype. Resistance to β2AR desensitization would leave more receptors available for antagonist binding with enhanced responsiveness to nonselective BAR-blockers.

We considered that in CHF, nonselective in contrast to selective BAR-blockade inhibits the β2AR mediated release of norepinephrine, potentially with a greater improvement of autonomic balance. If so, β2AR-blockade would specifically benefit patients with the Gly16/Glu27 haplotype who are relatively resistant to agonist mediated β2AR desensitization. Accordingly we tested the hypothesis that nonselective vs. selective BAR-blockade restores baroreflex function more effectively in CHF patients with the Gly16/Glu27 vs. the Arg16/Gln27 haplotype. To that purpose we determined the effects of carvedilol vs. metoprolol on BRS in CHF patients homozygous for either the Gly16/Glu27 or Arg16/Gln27 haplotype in a cross-over design.

**METHODS**

*Patients*

Patients were recruited from the cardiology outpatient clinics in six regional hospitals in the Netherlands. After patients had given written informed consent, they were screened for eligibility, and blood was drawn for β2-receptor haplotype determination. Eligible subjects were patients aged 18–80 years, with stable symptoms of CHF (New York Heart Association (NYHA) classification I to III), and a left ventricular ejection fraction below 40%, as measured...
within six months prior to randomization by nuclear scan, magnetic resonance imaging or cardiac ultrasound. Patients' medical condition was stable for at least three months. Exclusion criteria were atrial fibrillation, an acute coronary event or myocardial revascularization within three months prior to randomization, severe aortic or mitral valve disease, severely uncontrolled hypertension (blood pressure systolic ≥170 mmHg or diastolic ≥105 mmHg), known drug or alcohol abuse, a history of poor treatment compliance, or a systemic disease that might complicate management or reduce life expectancy. The present study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Academic Medical Centre. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.466

Trial registration number: NTR1067 (http://www.trialregister.nl).

**β₂-adrenergic receptor sequencing**

Genomic DNA was isolated from 10 ml peripheral blood using an AutopureLS apparatus according to the manufacturer’s protocol (Gentra Systems, Minneapolis, MI, USA). β₂-adrenergic genotypes at the alleles encoding for positions 16 and 27 were determined by the big-dye terminator sequencing technique, for which a pair of primers was designed (using Primer3; http://frodo.wi.mit.edu/primer3/). Polymerase chain reaction (PCR) was carried out with 50 ng of genomic DNA in a 25 μl reaction volume containing 1x Taq DNA polymerase buffer (Qiagen, Germany), 50 μmol/l of each dNTP, 0.4 μmol/l of each primer, and 1 U Taq DNA polymerase. The thermal cycling conditions were as follows: 95°C for 2 minutes, then 20 cycles of 30 seconds at 95°C, 30 seconds at 65°C (minus 0.5°C each cycle), and 30 seconds at 72°C in a PCR apparatus (T3 Biocycler, Biometra, Germany). Then 30 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C. The program ended 10 minutes at 72°C. The sequence reactions were performed using fluorescently labeled dideoxy chain terminations with a Big Dye Terminator ABI Prism kit (Applied Biosystems, Foster City, CA, USA) according manufacturer’s protocol and analyses on an Applied Biosystems automated DNA sequencer (model 3730). Sequences were analyzed with the Sequencher package (GeneCodes Co, Ann Arbor, MI, USA).

**Study protocol**

The study used a randomized open label crossover design for comparison of metoprolol vs. carvedilol in CHF patients. Patients homozygous for Gly16/Glu27 and Arg16/Gln27 who met the study criteria were randomized to treatment with carvedilol or metoprolol. Patients on βAR-blocker treatment received an equipotent dosage of carvedilol (Eucardic, Roche, Mijdrecht, the Netherlands) or metoprolol succinate (Seloken ZOC, AstraZeneca, Zoetermeer, the Netherlands). Following the first βAR-blockade 6 week treatment period, patients switched βAR-blocker for another 6 weeks, again in an equipotent dosage (carvedilol 25 mg twice daily and metoprolol succinate 200 mg once daily).454 To assure equipotency resting HR was determined one week following the βAR-blocker switch for each arm of the trial, with dosage adjustment in case of a change in HR >5 beats/min. Compliance to βAR-blocker treatment was assessed by counting the remaining tablets at the end of the treatment episodes. Measurements were performed at the end of both treatment-periods.
Measurements and data analysis

Measurements were performed in the morning after an overnight fast in the laboratory with an ambient temperature of 22° C. After instrumentation in the supine position and following a 10 min period of rest, measurements were performed with the light dimmed and ambient noise minimized for at least 5 min. This procedure was repeated after 2 min in the upright position. Blood pressure (BP) and inter-beat interval were continuously monitored by non-invasive finger photo-plethysmography (Nexfin, BMEye, Amsterdam, The Netherlands) with the finger cuff applied to the mid-phalanx of the left middle finger placed at heart level. The continuous BP signal was A/D converted at 200 Hz and stored on hard disk for off-line analysis. Mean arterial pressure (MAP) was the integral of the arterial pressure wave divided by the corresponding beat interval duration. HR was the inverse of inter-beat interval. Stroke volume (SV) was estimated from the finger pulse pressure using the Nexfin CO-trek® method. Cardiac output (CO) was SV times HR and systemic vascular resistance (SVR) was the ratio of MAP and CO.

BRS was obtained from the 5 min beat-to-beat systolic BP (SBP) and inter-beat interval data by the sequence method in the supine and the upright position. In detail, the cross-correlation between 10 s series of SBP and inter-beat interval samples was computed for delays between changes in SBP and inter-beat interval, called τ, of 0 – 5 s. The τ yielding the highest cross-correlation was selected if significant with α set at 0.05. The regression slope was recorded as one BRS value. Subsequently, the process was repeated for series of SBP and inter-beat interval samples 1 s later. Distributions of individual BRS values are best described as log-normal. Therefore, geometric averages over the 5 min supine and upright periods were used providing one value for each condition.

Statistical analysis

A mixed-effects model was used to determine the effects of the within-subject factors βAR-blocker and posture and the between-subjects factor haplotype. Differences between haplotypes in intra-individual change in BRS with metoprolol vs. carvedilol were evaluated by independent t-test. The level of statistical significance was set at P<0.05. Data are presented as mean±SD.

RESULTS

Patients

Between 2007 and 2009, 86 CHF patients were screened, of whom 8 homozygous for the Gly16/Glu27 haplotype and 13 for the Arg16/Gln27 haplotype entered the study (Figure 7.1, Table 7.1). Median administered daily dosages were 12.5 mg (IQR: 12.5-37.5 mg) for carvedilol and 75 mg (IQR: 50-125 mg) for metoprolol. Compliance to βAR-blocker treatment was 99%.
Table 7.1 Baseline characteristics of CHF patients by β2-receptor haplotype

<table>
<thead>
<tr>
<th>GLY16/Glu27 (n (%))</th>
<th>ARG16/Gln27 (n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>70 ± 8</td>
</tr>
<tr>
<td>Male</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Male</td>
<td>9 (69)</td>
</tr>
</tbody>
</table>

**Cause of heart failure**
- **Ischemic**
  - Gly16/Glu27: 4 (50)
  - Arg16/Gln27: 10 (77)
- **Non-ischemic:**
  - Hypertensive CMP: 0 (0)
  - Dilated CMP: 4 (50)

**NYHA classification**
- I: 2 (25)
- II: 4 (50)
- III: 2 (25)

**LVEF (%)**
- 28 ± 11
- 31 ± 4

**Medical history**
- Hypertension: 2 (25)
- Diabetes Mellitus: 2 (25)
- Hypercholesterolemia: 3 (38)

**β-blocker before randomization**
- Carvedilol: 3 (38)
- Metoprolol: 4 (50)
- Bisoprolol: 1 (13)
- Nebivolol: 0 (0)

**Co-medication**
- RAAS inhibitors: 6 (75)
- Loop diuretic: 6 (75)
- Spironolactone: 2 (25)
- Vitamin K antagonists: 4 (50)
- Antiplatelet agents: 5 (63)
- Statin: 6 (75)
- Statin: 2 (25)

**CMP**: cardiomyopathy, **LVEF**: left ventricular ejection fraction, **NYHA**: New York Heart Association, **RAAS**: renin-angiotensin-aldosterone-system. *p<0.05 vs. Gly16/Glu27.
Baroreflex sensitivity and cardiovascular variables

In the supine position no differences were found in HR, BP, SV, CO, SVR and BRS between β2AR haplotypes or βAR-blockers (Table 7.2). After standing up SV decreased in both groups with both metoprolol and carvedilol. A postural decline in CO was statistically significant in the Gly16/Glu27 patients only. Standing significantly reduced BRS during metoprolol treatment in both groups, whereas this postural decrease in BRS was less evident during carvedilol treatment, particularly in the Gly16/Glu27 patients (Glγ16/Glu27: \( p = 0.76 \), Arg16/Gln27: \( p = 0.06 \) vs. supine). Accordingly, BRS was higher with carvedilol vs. metoprolol in Gly16/Glu27 patients in the standing position (Table 7.2). With carvedilol compared to metoprolol BRS was 4.3±5.4 ms·mmHg\(^{-1}\) higher in patients with the Gly16/Glu27 haplotype whereas this difference was +0.2±2.5 ms·mmHg\(^{-1}\) in the Arg16/Gln27 patients (\( p < 0.05 \) vs. Gly16/Glu27). During carvedilol treatment upright BRS was higher in Gly16/Glu27 compared to Arg16/Gln27 patients, but did not reach statistical significance (10.2±7.1 vs. 5.5±3.5 ms·mmHg\(^{-1}\); \( p = 0.06 \)).
Table 7.2 Cardiovascular variables

<table>
<thead>
<tr>
<th></th>
<th>Gly16/Glu27 Metoprolol</th>
<th>Gly16/Glu27 Carvedilol</th>
<th>Arg16/Gln27 Metoprolol</th>
<th>Arg16/Gln27 Carvedilol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>62 ± 8</td>
<td>62 ± 8</td>
<td>63 ± 8</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>BPsys (mmHg)</td>
<td>135 ± 17</td>
<td>133 ± 23</td>
<td>127 ± 28</td>
<td>123 ± 19</td>
</tr>
<tr>
<td>BPdia (mmHg)</td>
<td>70 ± 10</td>
<td>70 ± 15</td>
<td>70 ± 12</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>92 ± 12</td>
<td>91 ± 17</td>
<td>89 ± 17</td>
<td>87 ± 10</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>78 ± 22</td>
<td>80 ± 22</td>
<td>88 ± 19</td>
<td>83 ± 19</td>
</tr>
<tr>
<td>CO (I·min⁻¹)</td>
<td>4.9 ± 1.6</td>
<td>5.0 ± 1.6</td>
<td>5.6 ± 1.4</td>
<td>5.5 ± 1.4</td>
</tr>
<tr>
<td>SVR (dyn·s·cm⁻⁵)</td>
<td>1877 ± 1074</td>
<td>1769 ± 962</td>
<td>1364 ± 314</td>
<td>1423 ± 408</td>
</tr>
<tr>
<td>BRS (ms·mmHg⁻¹)</td>
<td>10.0 ± 4.7</td>
<td>10.9 ± 7.3</td>
<td>8.7 ± 4.6</td>
<td>9.2 ± 8.0</td>
</tr>
<tr>
<td><strong>Stand</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats·min⁻¹)</td>
<td>74 ± 10</td>
<td>70 ± 9</td>
<td>72 ± 9</td>
<td>74 ± 6</td>
</tr>
<tr>
<td>BPsys (mmHg)</td>
<td>120 ± 20</td>
<td>122 ± 26</td>
<td>124 ± 29</td>
<td>116 ± 25</td>
</tr>
<tr>
<td>BPdia (mmHg)</td>
<td>71 ± 9</td>
<td>72 ± 9</td>
<td>74 ± 12</td>
<td>70 ± 11</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>87 ± 12</td>
<td>88 ± 14</td>
<td>90 ± 17</td>
<td>85 ± 16</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>58 ± 16</td>
<td>63 ± 15</td>
<td>75 ± 20</td>
<td>70 ± 17</td>
</tr>
<tr>
<td>CO (I·min⁻¹)</td>
<td>4.3 ± 1.4</td>
<td>4.4 ± 1.1</td>
<td>5.3 ± 1.3</td>
<td>5.1 ± 1.3</td>
</tr>
<tr>
<td>SVR (dyn·s·cm⁻⁵)</td>
<td>1866 ± 856</td>
<td>1804 ± 678</td>
<td>1439 ± 290</td>
<td>1480 ± 561</td>
</tr>
<tr>
<td>BRS (ms·mmHg⁻¹)</td>
<td>5.9 ± 2.3</td>
<td>10.2 ± 7.1</td>
<td>5.5 ± 2.5</td>
<td>5.5 ± 3.5</td>
</tr>
</tbody>
</table>

HR: heart rate, BPsys: systolic blood pressure, BPdia: diastolic blood pressure, MAP: mean arterial pressure, SV: stroke volume, CO: cardiac output, SVR: systemic vascular resistance, BRS: baroreflex sensitivity. † p<0.01 vs. metoprolol, ‡ p<0.05 vs. supine. Data are means ± SD.

**DISCUSSION**

In the present study we identified an enhanced BRS with carvedilol vs. metoprolol treatment in CHF patients with the Gly16/Glu27, but not with Arg16/Gln27 haplotype for the β2AR. This new finding indicates that β2AR genotype in CHF patients influences the effects of nonselective βAR-blocker treatment on cardiovascular autonomic balance in CHF patients.

Sympathetic dominance in CHF contributes to disease progression and restraining this sympathetic overactivity by βAR-blocker treatment improves outcome.⁴⁵³ Consequently, βAR-blockers together with inhibitors of the renin-angiotensin-aldosterone axis form the mainstay of current medical management of CHF.⁴⁵⁴ Although both metoprolol and carvedilol reduce mortality in CHF⁴⁶⁷,⁴⁶⁸, carvedilol extended median survival of CHF patients by 1.4 years as compared to metoprolol tartrate in the COMET-trial.⁴⁵⁵ It was, however, debated whether the degrees of β₁AR-blockade achieved with the dose regimens used in that study were comparable.⁴⁶⁹ In the present cross-over study patients received both metoprolol succinate and carvedilol and dosages were titrated on HR. The level of HR reduction is significantly associated with the survival benefit of βAR-blockers in CHF whereas the dosage used was shown to be less important.⁴⁷⁰
Genetic polymorphisms of the $\beta_2$AR receptor may account in part for the interindividual variability in responses to nonselective $\beta$AR-blocker treatment. The prevalent $\beta_2$AR haplotypes Gly16/Glu27 and Arg16/Gln27 are associated with altered receptor trafficking and down-regulation. Venodilatation induced by the $\beta$AR agonist isoproterenol diminished in persons homozygous for the Arg16/Gln27 haplotype, whereas it was steady in subjects homozygous for the Gly16/Glu27 haplotype. A diminished $\beta_2$AR-mediated vasodilatation likely explains the higher systolic blood pressure and the increased risk for coronary events in subjects homozygous for the Arg16/Gln27 haplotype. Furthermore, in acute coronary syndrome patients treated with (nonspecified) $\beta$AR-blockers mortality was lower in patients homozygous for the Gly16/Glu27 compared to patients with the Arg16/Gln27 $\beta_2$AR haplotype. In CHF homozygosity for the Gln27 allele in particular was reported to negatively affect outcome, whereas this was not confirmed by others. In these studies patients used various $\beta$AR-blockers and an interaction between $\beta_2$AR genotype and $\beta$AR-blocker treatment may have influenced these findings. Together with previous data on a beneficial response to carvedilol in CHF patients homozygous for the Glu27 allele our findings suggest that nonselective $\beta$AR-blocker treatment efficacy interacts with $\beta_2$AR genotype.

Baroreflex control of HR is mainly effectuated through efferent vagal nerve pathways with low BRS during sympathetic dominance. Reduced vagal activity is associated with increased risk of ventricular arrhythmias and BRS has independent prognostic value for outcome in CHF. Blocking $\beta$ARs increases BRS in healthy subjects in rest and during sympathetic activation by psychological stress. Carvedilol has been shown to enhance BRS in CHF patients, but whether selective $\beta$AR-blockers have the same effect on baroreflex function in these patients is uncertain. A possible mechanism for improved survival with nonselective $\beta$AR-blockade is that blockade of the prejunctional $\beta_2$AR mediated release of norepinephrine likely add to the normalization of autonomic balance with an improvement in baroreflex function. Consequently, nonselective $\beta$AR-blockade may prevent surges in sympathetic activity that potentially induce cardiac arrhythmias. A large part of human activity is characterized by the upright posture, whereas baroreceptor unloading with standing evokes sympathetic activation and parasympathetic withdrawal together with a reduction in BRS. The postural decrease in BRS was absent during treatment with carvedilol, but not with metoprolol, particularly in the Gly16/Glu27 patients. This could be explained by a $\beta_2$AR mediated postural decline in BRS in the Gly16/Glu27 patients, whereas in the Arg16/Gln27 patients an alternative pathway may have been evolved due to desensitization of $\beta_2$ARs.
A potential limitation in this study is the higher age in the Gly16/Glu27 vs. Arg16/Gln27 patients. The inverse relation of BRS with age in both healthy subjects and CHF patients renders an effect of age on the higher BRS in the Gly16/Glu27 CHF patients unlikely. BRS was determined in CHF patients homozygous for the Gly16/Glu27 and Arg16/Gln27 haplotypes and therefore cannot be directly extended to patients heterogeneous for any of these polymorphisms, whereas they may act intermediately. The present findings suggest that nonselective βAR-blockade improves cardiovascular autonomic balance more efficiently than selective β1AR-blockade in CHF patients homozygous for the Gly16/Glu27 β2AR haplotype, but not in patients with the Arg16/Gln27 haplotype. The revealed pharmacogenetic interaction between β2AR genotype and nonselective βAR-blockers may have contributed to the reported improved outcomes with either carvedilol or the Gly16/Glu27 β2AR haplotype in cardiovascular disease. It supports the use of genetic information on drug targets to improve treatment efficacy in CHF.