Differential Effects of Nonselective Versus Selective β-Blockers on Cardiac Sympathetic Activity and Hemostasis in Patients with Heart Failure

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Carvedilol, a nonselective β-blocker, may be more effective than the selective β-blocker metoprolol in reducing the risk of thromboembolic events in heart failure. The aim of this study was, first, to assess whether there is a differential response in cardiac sympathetic activity by ^{123}I-meta-iodobenzylguanidine (^{123}I-MIBG) imaging when either β-blocker is used. Second, we assessed whether that response correlates with levels of various serum factors that serve as markers for coagulation. **Methods:** In this prospective, randomized, open-label crossover study with masked outcome assessments, stable heart failure patients (left ventricular ejection fraction < 40%) homozygous for the Arg16/Gln27 (n = 13) or Gly16/Glu27 haplotype (n = 8) of the $β_2$-receptor were randomized to equipotent dosages of carvedilol or metoprolol for two 6-wk periods. Primary outcome was sympathetic activity as measured by ^{123}I-MIBG myocardial washout. Secondary outcomes included markers of hemostasis. **Results:** ^{123}I-MIBG cardiac washout was lower during carvedilol than metoprolol treatment (12.9% ± 3.9% vs. 22.1% ± 2.8%, respectively, $P = 0.003$), irrespective of $β_2$-adrenergic receptor haplotype. In addition, treatment with carvedilol resulted in a lower von Willebrand factor than did metoprolol (149% ± 13% vs. 157% ± 13%, respectively, $P = 0.01$), irrespective of $β_2$-adrenergic receptor haplotype. **Conclusion:** Compared with metoprolol, carvedilol resulted in greater reduction of sympathetic activity after 6 wk of treatment and lower von Willebrand factor concentrations in both Arg16/Gln27 and Gly16/Glu27 individuals. Therefore, carvedilol may reduce the risk of thromboembolic events in patients with heart failure, irrespective of $β_2$-receptor haplotype status.

Key Words: heart failure; β-blockers; genetics, coagulation; sympathetic nervous system

Heart failure (HF) is associated with a prothrombotic state, reflected by an increased risk of arterial and venous thromboembolic events (*1–4*). The estimated annual incidence of thromboembolic events is 3%–5% (*2,4*). The increased sympathetic activity present in patients with HF (*5*) can lead to activation of platelets and increases concentrations of coagulation factors, particularly coagulation factor VIII and von Willebrand factor (VWF) (*1,6*). Therefore, the increased risk of thromboembolic events in these patients might be, at least in part, related to a hyperactive sympathetic nervous system (*1,4–6*).

Angiotensin-converting enzyme inhibitors and β-blockers form the cornerstone of treatment in HF. β-blockers inhibit the adverse effects of the increased sympathoadrenergic activity, although all β-blockers may not be equally effective. In a study by Azevedo et al. (*7*), carvedilol, a nonselective $β_1$-, $β_2$-, and $α_1$-receptor blocker, seemed to be more effective than the selective $β_1$-receptor blocker metoprolol in reducing sympathetic hyperactivity in patients with HF. However, this study used metoprolol tartrate in a relatively lower target dosage of 100 mg daily, which has been shown to be inferior to carvedilol in the Carvedilol or Metoprolol European Trial (*8*). In addition, the prothrombotic response on epinephrine or norepinephrine release appears to be $β_2$-receptor–mediated (*6*). Nonselective β-blockers lower the hemostatic response on catecholamine infusions, whereas selective compounds do not (*6*). Hence, treatment with carvedilol could result in a reduction of the prothrombotic state in HF. Indeed, a large observational study showed that the risk of thromboembolic events was lower in HF patients on nonselective β-blockers than in those on selective compounds (*9*).

The efficacy of β-blockers may be mediated through genetic variation in the $β_2$-adrenergic receptor. Two highly prevalent haplotypes (Arg16Gln27 and Gly16Glu27) are associated with altered receptor trafficking and downregulation (*10*). After infusion of isoproterenol, a β-receptor agonist, venodilatation decreased in persons homozygous for Arg16/Gln27, compared with the Gly16/Glu27 haplotype (*11*). The other haplotypes acted intermediately. This difference in functionality might explain in part the increased risk for cardiovascular disease (*12,13*). Patients homozygous for Arg16/Gln27 who had an acute coronary syndrome had a doubled 3-y mortality rate compared with those homozygous for Gly16/Glu27

(14). Noteworthy, it was suggested that the β_2-adrenergic receptor genotype also influences the response in left ventricular ejection fraction improvement by carvedilol in HF patients (15).

This led us to the following hypotheses for the present study: sympathetic and thereby prothrombotic activity in patients with HF is reduced more effectively by the nonselective β-blocker carvedilol than by the selective β-blocker metoprolol. Furthermore, this difference in response to β-blockers is mediated through common β_2-receptor haplotypes.

MATERIALS AND METHODS

Outcomes

The primary outcome of this study was cardiac sympathetic activity as measured by ^{123}I-meta-iodobenzylguanidine (^{123}I-MIBG) myocardial washout. Secondary outcomes regarding sympathetic activity included early and late heart-to-mediastinum ratio in ^{123}I-MIBG scintigraphy. For hemostatic activity, we measured markers for coagulation, fibrinolysis, and platelet activation—that is, factor VIII, VWF, fibrinogen, prothrombin fragment 1 + 2 (F1 + 2), d-dimer, and surface presentation of P-selectin on platelets (P-selectin).

Patient Selection

Patients were recruited from the cardiology outpatient clinics in 6 regional hospitals in The Netherlands. After patients had given written informed consent, they were screened for eligibility, and blood was drawn for DNA collection. Eligible subjects were patients aged 18–80 y, with stable symptoms of chronic HF (New York Heart Association classification I–III) and a left ventricular ejection fraction below 40% as measured within 6 mo before randomization by nuclear scanning, MR imaging, or cardiac ultrasound. Patients were on stable medical therapy for at least 3 mo, including angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (unless contraindicated) and β-blockers on maximal tolerated dosages. Only patients in sinus rhythm were included in the study. Excluded were patients with an acute coronary event or myocardial revascularization within 3 mo before randomization, severe aortic or mitral valve disease, a requirement for intravenous inotropic therapy, severely uncontrolled hypertension (systolic blood pressure $\geq$ 170 mm Hg or diastolic $\geq$ 105 mm Hg), known drug or alcohol abuse, a history of poor treatment compliance, or a systemic disease that might complicate management or reduce life expectancy. Pregnant women or women with childbearing potential on inadequate contraception were also excluded. Since many patients with HF in sinus rhythm used vitamin K antagonists, we also allowed these patients in the study. Anticoagulant treatment is not expected to affect the primary outcome of sympathetic activity or of platelet activity or levels of VWF, factor VIII, and fibrinogen.

Procedures

This study had a prospective, randomized, open-label, masked-endpoint design. All eligible patients with HF were screened for their haplotype of the β_2-receptor. Individuals who were homozygous for Gly16/Glu27 and Arg16/Gln27 and met the study inclusion criteria were randomized to carvedilol or metoprolol (Fig. 1), applying block randomization with block sizes of 4, using the TENALEA randomization service (https://www.tenalea.com). Since included patients were already on β-blocker treatment, they received an equipotent dosage of carvedilol (Eucardic; Roche) or metoprolol succinate (Selokeen ZOC; AstraZeneca). After the first β-blocker treatment period of 6 wk, patients were switched to the other β-blocker, again in an equipotent dosage. Equipotent dosages were considered 25 mg twice daily for carvedilol and 200 mg daily for metoprolol succinate (16). To ensure equipotency, the heart rate of patients was monitored 1 wk after they had been switched to the other β-blocker. If resting heart rate varied by more than 5 beats per minute, the dosage was adjusted accordingly. A comparable heart rate between the 2 β-blockers is important because heart rate reduction is significantly associated with the survival benefit of β-blockers in HF whereas the dose of β-blockers may be less important (17). Compliance with β-blocker treatment was assessed by counting the tablets remaining at the end of the treatment episodes. Sympathetic and hemostatic activity was measured at the end of both treatment periods. As a global estimate of the cardiac condition at the end of each treatment period, N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations were measured (18). A β-blocker washout period was not feasible for ethical reasons. The trial complied with the Declaration of Helsinki and was approved by the Ethics Committee of the Academic Medical Center. The present study was the main outcome of a trial registered on www.trialregister.nl (NTR1067).

β-Receptor Sequencing

Genomic DNA was isolated from 10 mL of peripheral blood using an AutopureLS apparatus according to the manufacturer's protocol (Gentra Systems). β_2-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 was performed with 50 ng of genomic DNA in a 25-μL reaction volume containing 1× Taq DNA polymerase buffer (Qiagen), a 50 μM concentration of each deoxyribonucleoside triphosphate, a 0.4 μM concentration of each primer, and 1 unit of Taq DNA polymerase. The thermal cycling conditions were as follows: 95°C for 2 min, then 20 cycles of 30 s at 95°C (minus 0.5°C during each cycle), 30 s at 65°C, and 30 s at 72°C in a polymerase chain reaction apparatus (T3 Biocycler; Biometra). Then, there were 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C. The program ended with 10 min at 72°C. The sequence reactions were performed using fluorescently labeled dideoxy chain terminations with a Big Dye Terminator ABI Prism kit (Applied Biosystems) according to the manufacturer's protocol and analyses on an Applied Biosystems automated DNA sequencer (model 3730). Sequences were analyzed with the Sequencher package (GeneCodes Co.).

^{123}I-MIBG Scintigraphy

To block thyroid uptake of free ^{123}I, all patients received 100 mg of potassium iodide orally, 1 h before the injection of ^{123}I-MIBG. After a subsequent resting period of at least 30 min, the patients were injected intravenously with approximately 185 MBq of ^{123}I-MIBG (GE Healthcare). Twenty minutes (early imaging) and 4 h (delayed imaging) after ^{123}I-MIBG administration, a 10-min planar anterior image of the chest was acquired. A 20% energy window was centered on the 159-keV photopeak of ^{123}I. Images were acquired using a medium-energy collimator and stored in a 128 × 128 matrix. ^{123}I-MIBG myocardial
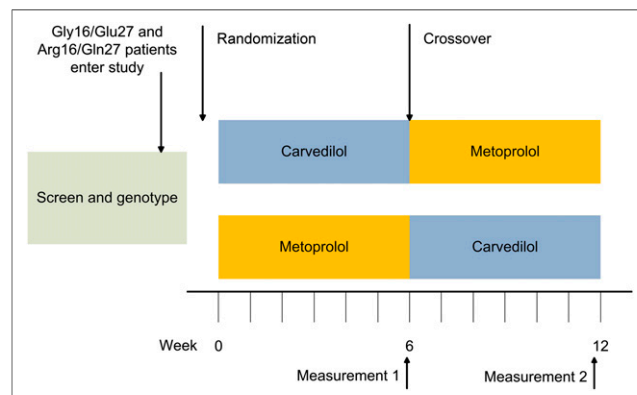
FIGURE 1. Schematic diagram of trial protocol. Measurement = ^{123}I-MIBG scintigraphy and hemostasis.

activity was measured using a manually drawn region of interest around the left ventricle. Mediastinal activity was measured using a fixed 20 × 20 pixel region of interest placed over the upper mediastinum. Post-processing was performed on a HERMES workstation (HERMES Medical Solutions). To evaluate ^{123}I-MIBG myocardial uptake, the heart (H) to mediastinal (M) ratio was calculated from the early and delayed images. ^{123}I-MIBG myocardial washout was defined as the percentage of change in activity from the early and delayed images and is an index of the degree of the sympathetic drive:

$$\left\{ \frac{(\text{early } H/M - \text{late } H/M)}{\text{early } H/M} \right\} \times 100\%.$$

This equation implies that increased adrenergic drive is associated with high ^{123}I-MIBG myocardial washout.

Measurements of Hemodynamics and Blood Collection

Between the early and late ^{123}I-MIBG scintigraphy, measurements of hemodynamics and hemostasis were performed. To avoid any diurnal and dietary influences, all measurements were performed in the morning after an overnight fast. The subjects were supine, and an intravenous cannula was inserted into the antecubital vein of the right arm and filled with diluted saline. After 10 min of rest, blood pressure was measured 3 times at 1-min intervals using a validated oscillometric device (Omron 705IT; Omron Healthcare Europe). The first measurement was discarded, and the mean of the last 2 measurements was used for further analysis. Heart rate was monitored by electrocardiography. After 30 min of rest, blood samples were drawn from the cannula for measurements of hemostasis. The first tube was discarded. Blood for coagulation assays was collected in tubes containing 0.32% trisodium citrate and processed within 15 min. Plasma was prepared by centrifugation at 2,500g twice for 20 min at 16°C, followed by storage at −80°C until assays were performed. Blood for NT-proBNP was collected in heparin-containing tubes and prepared by centrifugation at 1,885g for 5 min.

NT-proBNP was measured by chemiluminescent immunometric assay (Roche Diagnostics). F1 + 2 was measured by enzyme-linked immunosorbent assay (Enzygnost F1 + 2; Siemens Healthcare Diagnostics); factor VIII activity was determined on an automated coagulation system (BCS-XP; Siemens Healthcare Diagnostics) with reagents and protocols from the manufacturer. The fibrinogen concentration was determined from the change in optical signal during prothrombin time determination on the BCS-XP. D-dimer levels were determined with a particle-enhanced immunoturbidimetric assay (Innovance D-dimer; Siemens Healthcare Diagnostics). VWF antigen levels were tested by enzyme-linked immunosorbent assay with antibodies from DAKO. P-selectin was determined by flow cytometry analysis in whole blood as previously described (19).

Statistical Analysis

Sample size calculations to detect a clinical relevance difference of 5.0% in ^{123}I-MIBG myocardial washout between the β-blockers within each haplotype determined that 9 patients with each haplotype were required to provide 90% power with a 2-sided α of 0.05, with the assumption of a 3.0% SD (20) and using a paired Student t test.

In primary analyses, differences between the 2 β-blockers were assessed in patients with both haplotypes grouped together. β-blocker dosages are presented as median and interquartile range. All other outcome data are presented as means ± SEM. Paired Student t tests were used for within-group comparisons when data were distributed normally. The Wilcoxon rank sum test was used when tests of normality failed. In a sensitivity analysis, the primary outcome of ^{123}I-MIBG myocardial washout was assessed in patients without worsening HF symptoms on 1 of the 2 β-blockers. Missing values were imputed with the mean values of the group. Since vitamin K antagonists affect F1 + 2 and d-dimer concentrations, these markers were assessed only in those patients not receiving vitamin K antagonist treatment. Therefore, F1 + 2 and d-dimer levels were also not assessed within each haplotype, since these subgroups were small. Analyses were performed in SPSS, version 16.0 (IBM).

RESULTS

Recruitment of Eligible Patients

Between 2007 and 2009, 86 patients with HF were screened. The Arg16/Gln27 haplotype was present in 21 patients (24%) and Gly16/Glu27 in 13 patients (15%). Of these 34 patients, 13 were not randomized, for several reasons, mainly because the subjects did not meet the study criteria at the time of randomization (Fig. 2). In the Gly16/Glu27 group, 1 patient withdrew consent 2 wk after randomization because of tiredness after the change in β-blockade. Hence, 8 patients with the Gly16/Glu27 haplotype and 13 with the Arg16/Gln27 haplotype entered the study protocol (Table 1). Most patients had ischemia-related HF (67%), and 43% were in New York Heart Association class II. Before randomization, 48% used metoprolol and 33% carvedilol. Of the included patients, 57% were treated with vitamin K antagonists and 62% with antiplatelet agents.

During the trial protocol, 5 patients (4 in the Arg16/Gln27 group and 1 in the Gly16/Glu27 group) experienced an increased shortness of breath after changing from carvedilol to metoprolol,
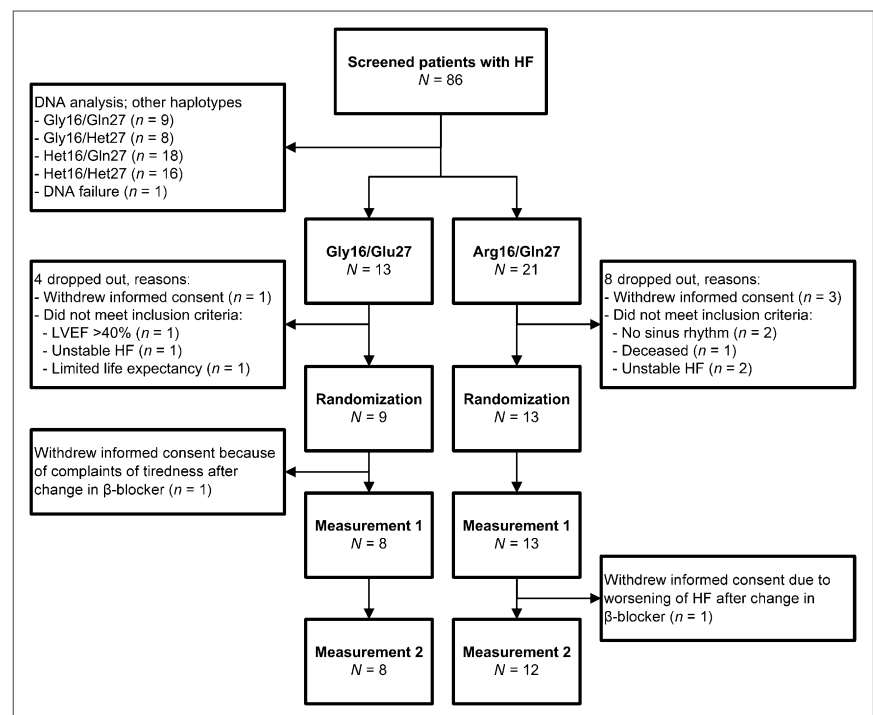

FIGURE 2. Trial profile. LVEF = left ventricular ejection fraction.

TABLE 1
Baseline Characteristics of Participants by Haplotype

Characteristic	All patients (n = 21)	Gly16/Glu27 (n = 8)	Arg16/Gln27 (n = 13)	P
Age (y)	64 ± 2	70 ± 3	60 ± 2	0.02
Male	13 (62)	4 (50)	9 (69)	0.38
Cause of HF				0.20
Ischemic	14 (67)	4 (50)	10 (77)	
Nonischemic				
Hypertensive cardiomyopathy	2 (10)	0 (0)	2 (15)	
Dilated cardiomyopathy	5 (24)	4 (50)	1 (8)	
LVEF (%)	30 ± 2	28 ± 4	31 ± 1	0.47
NYHA classification				0.87
I	6 (29)	2 (25)	4 (31)	
II	9 (43)	4 (50)	5 (39)	
III	6 (29)	2 (25)	4 (31)	
Medical history				
Hypertension	7 (33)	2 (25)	5 (39)	0.53
Diabetes mellitus	8 (38)	2 (25)	6 (46)	0.33
Hypercholesterolemia	7 (33)	3 (38)	4 (31)	0.75
β-blocker before randomization				0.70
Carvedilol	7 (33)	3 (38)	4 (31)	
Metoprolol	10 (48)	4 (50)	6 (46)	
Bisoprolol	2 (10)	1 (13)	1 (8)	
Nebivolol	2 (10)	0 (0)	2 (15)	
Comedication				
RAAS inhibitors	18 (86)	6 (75)	12 (92)	0.27
Loop diuretic	17 (81)	6 (75)	11 (85)	0.59
Vitamin K antagonists	12 (57)	4 (50)	8 (62)	0.60
Antiplatelet agents	13 (62)	5 (63)	8 (62)	0.97
Statin	18 (86)	6 (75)	12 (92)	0.27
Spironolactone	8 (38)	2 (25)	6 (46)	0.33
Digoxin	3 (14)	2 (25)	1 (8)	0.27

LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; RAAS = renin-angiotensin-aldosterone-system.
Qualitative data are expressed as numbers, followed by percentages in parentheses; continuous data are expressed as mean ± SEM.

leading to withdrawal of consent in 1 patient (Arg16/Gln27) 2 wk before the second measurement. In the remaining patients, dosage adjustment of the β-blocker or a (temporary) increment of loop-diuretic dosage reduced the complaints. Other patients did not report changes in complaints between β-blockers. The median administered daily dosages were 12.5 mg (interquartile range, 12.5–37.5) for carvedilol and 75 mg (interquartile range, 50–125) for metoprolol. Compliance with β-blocker treatment was 99%.

β-Blocker Treatment and Sympathetic and Hemostatic Activity

The results of the ^{123}I-MIBG scintigraphy are presented in Table 2. Carvedilol significantly reduced ^{123}I-MIBG myocardial washout, compared with metoprolol (12.9% ± 3.9% vs. 22.1% ± 2.8%, respectively, P = 0.003). The early heart-to-mediastinum ratio was lower and the late heart-to-mediastinum ratio was higher during carvedilol treatment, although the difference was nonsignificant.

Table 2 also provides an overview of the hemodynamic measurements and NT-proBNP concentrations. Heart rate was comparable between the 2 β-blockers. When treated with carvedilol, patients had a lower systolic blood pressure than when they were treated with metoprolol (123 ± 4 mm Hg vs. 129 ± 3 mm Hg, P = 0.01). There was no difference in diastolic blood pressure. NT-proBNP levels did not differ significantly.

When the influence of β-blocker treatment on hemostatic activity was analyzed, carvedilol treatment was associated with lower levels of VWF than was metoprolol treatment (149% ± 13% vs. 157% ± 13%, P = 0.01, Table 3). No differences were observed for factor VIII and fibrinogen concentrations. In patients without vitamin K antagonists, F1 + 2 levels were comparable between the 2 groups, but d-dimer concentrations tended to be higher during carvedilol treatment (0.57 ± 0.25 mg/L vs. 0.40 ± 0.17 mg/L, P = 0.12). There was no difference between the 2 β-blockers regarding the marker for platelet activation (P-selectin).

Sensitivity Analysis

In a sensitivity analysis, we excluded patients with worsening HF on metoprolol treatment (n = 5). Also in this analysis, the primary outcome of ^{123}I-MIBG myocardial washout was significantly lower in patients when on carvedilol than when on metoprolol (9.8% ± 4.3% vs. 18.4% ± 2.7%, respectively, P = 0.01).

β₂-Receptor Haplotypes

Homozygous Arg16/Gln27 patients were approximately 10 y younger, more often had an ischemic cause of HF, more often had hypertension and diabetes mellitus, and used more cardiovascular medication, including statins and spironolactone (Table 1).

Compared with metoprolol, carvedilol significantly lowered ^{123}I-MIBG myocardial washout in patients with both haplotypes (Table 4). In Arg16/Gln27 patients, ^{123}I-MIBG washout was 11.0% ± 5.6% during carvedilol and 21.8% ± 3.6% during metoprolol (P = 0.04). In Gly16/Glu27 patients, these values were 16.1% ± 4.8% vs.

TABLE 2
Carvedilol vs. Metoprolol (6 Weeks of Treatment): ^{123}I-MIBG and Hemodynamic Characteristics

Characteristic	Carvedilol	Metoprolol	P
^{123}I-MIBG scintigraphy			
^{123}I-MIBG myocardial washout (%)	12.9 ± 3.9	22.1 ± 2.8	0.003*
Early heart-to-mediastinum ratio	1.73 ± 0.10	1.85 ± 0.08	0.05
Late heart-to-mediastinum ratio	1.50 ± 0.09	1.43 ± 0.07	0.24
Hemodynamic measurements			
Systolic BP (mm Hg)	123 ± 4	129 ± 3	0.01
Diastolic BP (mm Hg)	74 ± 2	75 ± 2	0.45
Heart rate (beats/min)	66 ± 2	67 ± 2	0.56
NT-proBNP (ng/L)	1,032 ± 297	985 ± 232	0.69

*Primary outcome of this study.
BP = blood pressure; IQR = interquartile range.
Data are presented as mean ± SEM (n = 21), unless otherwise indicated.

22.6% ± 4.6%, respectively (P = 0.02). Also, VWF levels were lower during carvedilol treatment in Arg16/Gln27 patients (143% ± 18% vs. 151% ± 19%, P = 0.03) than in Gly16/Glu27 patients (159% ± 19% vs. 166% ± 18%, P = 0.21), although the difference was nonsignificant. Factor VIII, fibrinogen, and P-selectin did not differ between the 2 β-blockers in either haplotype.

DISCUSSION

In this study, the influence of 6 wk of treatment with carvedilol on cardiac sympathetic and hemostatic activity in stable, chronic HF patients was compared with that of metoprolol succinate, in a crossover design, using equipotent dosages and aiming for comparable heart rates. In addition, we assessed whether the efficacy of β-blockers was mediated through common β$_2$-receptor haplotypes. Carvedilol significantly reduced ^{123}I-MIBG myocardial washout, compared with metoprolol (12.9% ± 3.9% vs. 22.1% ± 2.8%, P = 0.003). This difference in washout was present in both homozygous Arg16/Gln27 and Gly16/Glu27 patients. Finally, during carvedilol treatment, VWF concentrations were lower.

In patients with HF, sympathetic activity is increased to compensate for the loss of cardiac output and circulatory failure. In addition, there is evidence that sympathetic activity induces a procoagulant response (6). Hence, the sympathetic hyperactivity could be an important contributor to the prothrombotic state in HF (1,2). This study showed that, compared with metoprolol, carvedilol resulted in lower cardiac sympathetic activity and lower VWF levels. This finding is in line with a post hoc analysis of the Car-

vedilol or Metoprolol European Trial, in which VWF concentrations showed a decrease during carvedilol treatment, compared with increasing VWF levels in HF patients using metoprolol tartrate (21). Lower levels of VWF are associated with a lower risk of cardiovascular events (22). In combination with the lower cardiac sympathetic activity, this may explain the 16% lower risk of thromboembolic events in patients with HF when treated with nonselective β-blockers than when treated with selective β-blockers (9). In addition, a literature overview suggested a lower thromboembolic event risk in patients with HF when treated with nonselective β-blockers (23). If these findings are corroborated in a larger, randomized, study, a preference for nonselective β-blockers may ensue, especially in those HF patients with a high cardiovascular risk.

Carvedilol induced a relatively lower systolic blood pressure than metoprolol. This difference may be explained at least in part by the fact that carvedilol also has α-receptor–blocking properties. However, whether this difference in systolic blood pressure is related to a lower risk of thromboembolic events is not known. To our knowledge, there are no direct data available on this subject. On the extreme end of the spectrum, it is known that hypertension is associated with a slightly higher risk of venous thromboembolism (24). However, none of the subjects included in our study had hypertension. Dishy et al. have speculated on this subject and stated that increased sympathetic activity with increased catecholamine concentrations may be associated with hypertension, especially in young persons (11,25). Thus, long-term exposure to elevated concentrations of catecholamines in combination with desensitization to the β$_2$-adrenergic,

TABLE 3
Comparison of Markers for Hemostasis Between Carvedilol and Metoprolol After 6 Weeks of Treatment

Parameter	Carvedilol	Metoprolol	P
Hemostatic parameters in all HF patients			
VWF (%)	149 ± 13	157 ± 13	0.01
Factor VIII (%)	155 ± 9	158 ± 9	0.46
Fibrinogen (g/L)	3.7 ± 0.2	3.8 ± 0.2	0.22
Hemostatic parameters in patients without vitamin K antagonists (n = 9)			
F1 + 2 (pmol/L)	228 ± 38	200 ± 31	0.26
D-dimer (mg/L)	0.57 ± 0.25	0.40 ± 0.17	0.12
Platelet activation status in all patients: P-selectin (%)	5.0 ± 0.8	5.0 ± 0.6	0.40

Data are presented as mean ± SEM (n = 21).

TABLE 4
^{123}I-MIBG Myocardial Washout and Hemostatic Outcomes by β-Blocker Treatment and Haplotype

Parameter	Carvedilol	Metoprolol
^{123}I-MIBG myocardial washout (%)		
Arg16/Gln27 ($n = 13$)	11.0 ± 5.6	21.8 ± 3.6*
Gly16/Glu27 ($n = 8$)	16.1 ± 4.8	22.6 ± 4.6*
Hemostasis		
VWF (%)		
Arg16/Gln27 ($n = 13$)	143 ± 18	151 ± 19*
Gly16/Glu27 ($n = 8$)	159 ± 19	166 ± 18
Factor VIII (%)		
Arg16/Gln27 ($n = 13$)	147 ± 11	147 ± 10
Gly16/Glu27 ($n = 8$)	167 ± 17	175 ± 15
Fibrinogen (g/L)		
Arg16/Gln27 ($n = 13$)	3.5 ± 0.2	3.5 ± 0.2
Gly16/Glu27 ($n = 8$)	4.1 ± 0.4	4.2 ± 1.0
P-selectin (%)		
Arg16/Gln27 ($n = 13$)	5.0 ± 1.3	5.1 ± 0.8
Gly16/Glu27 ($n = 8$)	4.9 ± 0.6	4.8 ± 0.7

*$P < 0.05$ for carvedilol versus metoprolol.
Data are presented as mean ± SEM.

vasodilative effects of epinephrine may increase blood pressure, because under these conditions the α-adrenergic pressor effect of epinephrine and norepinephrine would be unopposed by vasodilatation. Increased sympathetic activity and enhanced vasoconstriction have also been implicated in the pathogenesis of preeclampsia (26). Dishy et al. went even further and stated that if the polymorphisms they studied have "similar effects on desensitization in other tissues, including the heart, they may influence the clinical course and survival of patients with congestive heart failure" (11). However, it remains to be established whether the relative small difference in systolic blood pressure between carvedilol and metoprolol may lead to a lower risk of thromboembolic events.

The lower VWF concentrations during carvedilol treatment may be the result of the lower sympathetic activity brought about by carvedilol, which could lead to a reduced prothrombotic state. Factor VIII levels, which are more consistently associated with epinephrine and norepinephrine (6), were, however, unaffected by carvedilol. Carvedilol also acts as a free-radical scavenger, has vasodilator properties due to its $α_1$-receptor blocking capacities, and has antiproliferative properties, all of which may improve endothelial function and thus lower VWF concentrations (27).

We measured cardiac sympathetic activity using ^{123}I-MIBG scintigraphy, a method that is becoming increasingly generally accepted (28). Patients with HF and an increased ^{123}I-MIBG myocardial washout tend to have a poorer prognosis (29). Previous studies found a reduced cardiac norepinephrine spillover by nonselective β-blockers such as carvedilol and propranolol (a nonselective $β_1$- and $β_2$-receptor blocker) but not by metoprolol tartrate (7,30). In addition, the fact that Azevedo et al. (7) observed no difference in muscle sympathetic nerve activity during carvedilol therapy suggests a $β_2$-receptor–enhancing norepinephrine release in the presynaptic cleft. Our study extended these observations by showing a lower ^{123}I-MIBG washout during carvedilol treatment but was the first to compare carvedilol with the currently advised treatment of metoprolol succinate (31).

Finally, we assessed whether sympathetic and hemostatic activity in HF patients is influenced by functional, common $β_2$-receptor

haplotypes. We carefully selected patients who were homozygous for Arg16/Gln27 or Gly16/Glu27. Noteworthy, Arg16/Gln27 patients were on average 10 y younger, more often had an ischemic cause of their cardiomyopathy, and more often had hypertension and diabetes mellitus. Apart from the difference in age, these observations are in line with previous studies (12,32,33). The reason for the difference in age is unclear, since the Arg16/Gln27 haplotype does not influence survival in patients with HF (34) and seems not to be a risk factor for HF (35). A previous retrospective study suggested an improved left ventricular ejection fraction in Gly16/Glu27 individuals when treated with carvedilol (15), but this trial was contradicted by another study (36). Given the differences in baseline in our study, it is difficult to directly compare patients with the 2 haplotypes. Nevertheless, the effect of carvedilol on sympathetic activity was clearly present in both haplotypes. One caveat of the receptor haplotype data is that our study was not designed to compare baseline imaging off medical therapy, for obvious ethical reasons. It may be that there are differential baseline MIBG ratios for the 2 haplotypes and that it is the change from baseline conferred by the β-blockers that reveals the relative differences in haplotype response. More study is needed with respect to understanding the haplotype contributions to pharmacotherapy and signaling.

An important strength of this study was the crossover design for the comparison between β-blockers, which reduces the influence of confounding covariates and limits the number of patients to have sufficient power. Second, by randomizing patients, the possibility of "order" and "carry-over" effects were likely to be equally distributed between the 2 β-blockers.

There were also some limitations that need to be discussed. This was the first study that prospectively assessed differences between 2 β-blockers on hemostatic parameters. We observed significantly lower concentrations of VWF during carvedilol treatment, but other markers were not clearly different. However, this study was not powered to detect significant differences in hemostatic parameters (e.g., d-dimer), and future studies should assess more robustly whether differences in these markers between the 2 β-blockers are important for clinical decisions. A second limitation was that we did not include patients with other variants of the $β_2$-receptor. However, other variants are likely to act intermediately (11,14). Third, for practical reasons, treatment was open-label. To limit the possibility of placebo effects, patients were unaware of which β-blocker was assumed to be more effective, and all investigators were masked to treatment. Fourth, although this study assessed haplotypes of the $β_2$-receptor, these still may be not the causal single-nucleotide polymorphisms but in high linkage disequilibrium with them. Finally, administered dosages of the β-blockers could be considered low, since target doses are 25 mg twice daily for carvedilol and 200 mg daily for metoprolol. However, the dosages in our study were comparable to those observed in the EuroHeart Failure Survey and thus represent daily clinical practice (37).

Based on the assumption that 200 mg of metoprolol equals 25 mg of carvedilol, the average dose given to our subjects did not exactly follow that ratio (factor of 8). Therefore, we might have given a relative underdose of metoprolol or, vice versa, a relative overdose of carvedilol. However, the resting heart rate did not differ between carvedilol and metoprolol, but systolic blood pressure did (123 ± 4 mm Hg vs. 129 ± 3 mm Hg, carvedilol vs. metoprolol, respectively, $P = 0.01$). This difference in systolic blood pressure is probably related to the fact that carvedilol is not a pure β-blocker but also has α-receptor blocking properties.

CONCLUSION

In patients with chronic, stable HF, carvedilol significantly reduced cardiac sympathetic activity as measured by ^{123}I-MIBG scintigraphy after 6 wk of treatment, when compared with metoprolol. In addition, we observed lower VWF levels during carvedilol treatment. These findings could explain the lower risk of thromboembolic events as shown with nonselective β-blocker treatment in HF (9). However, a causal relationship between sympathetic activity and the lower VWF remains unclear. The observed differences between β-blockers were present in both Arg16/Gln27 and Gly16/Glu27 patients, suggesting that these haplotypes are less important in β-blocker–treated, stable HF patients. To establish whether treatment with a nonselective β-blocker such as carvedilol indeed reduces the prothrombotic state in HF and thus the risk of thromboembolic events, a larger prospective study is needed.

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