Adrenergic Receptor Polymorphisms and Prevention of Ventricular Arrhythmias With Bucindolol in Patients With Chronic Heart Failure

Ryan G. Aleong, MD; William H. Sauer, MD; Alastair D. Robertson, PhD; Stephen B. Liggett, PhD; Michael R. Bristow, MD, PhD, FACC, FAHA

Background—β-blockers prevent cardiac arrhythmias in patients with chronic heart failure and reduced left ventricular ejection fraction, including ventricular tachycardia/ventricular fibrillation (VT/VF). We hypothesized that prevention of ventricular arrhythmias by the β-blocker/sympatholytic agent bucindolol is influenced by genetic variation in adrenergic receptors.

Methods and Results—From a substudy of the β-Blocker Evaluation of Survival Trial (n=1040), we identified those with the high functioning 389Arg versus the lower function 389Gly β1 adrenergic receptor variant, and the loss of function α2c, 322-325 adrenergic receptor deletion versus the 322 to 325 wild-type (Wt)/deletion variant. VT/VF was recorded on case report forms as an adverse event. There were 493 Arg 389 β1 receptor homozygotes (β1, 389 Arg/Arg) versus 547 Gly389 carriers and 207 α2c,322-325 deletion carriers versus 833 homozygous Wts (α2c, 322-325 Wt/Wt). In all genotypes bucindolol was associated with a lower incidence of VT/VF (subhazard ratio, 0.42 [0.27–0.64]; P=0.00006). Bucindolol reduced VT/VF in β1, 389 Arg homozygotes (subhazard ratio, 0.26 [0.14–0.50]; P=0.00005) but not in β1, 389 Gly carriers (subhazard ratio, 0.60 [0.34–1.07]; P=0.09). For genotype combinations, the α2c, 322-325 polymorphism altered the VT/VF bucindolol response in β1, 389 Gly carriers, with α2c deletion genotypes associated with complete efficacy loss. A test of interaction was statistically significant (P=0.028) for the treatment group and a β1, 389/α2c, 322-325 three genotype construct, effectively identifying patients who exhibited enhanced response, no substantial response modification and loss of response.

Conclusions—Bucindolol prevents VT/VF in subjects with heart failure and reduced left ventricular ejection fractions, and this effect is modulated by β1, 389 Arg/Gly and α2c, 322-325 Wt/deletion adrenergic receptor polymorphisms. (Circ Arrhythm Electrophysiol. 2013;6:137-143.)

Key Words: β-blockers ■ genetic polymorphisms ■ pharmacogenetics ■ ventricular arrhythmia

Ventricular arrhythmias are common in patients with chronic heart failure and reduced left ventricular ejection fractions (HFREF). β-blockers decrease the incidence of sudden death (SD)1-3 and ventricular arrhythmias4 in HFREF patients. For heart failure end points, there has been heterogeneity of response to any given β-blocker in multiple large clinical trials that have included >17 000 randomized patients.5 For the β-blocker/sympatholytic agent bucindolol,6 this response variability may be due, in part, to adrenergic receptor (AR) genetic polymorphisms,7-9 and it is possible that such pharmacogenetically based therapeutic heterogeneity is also present for antiarrhythmic effects.

Clinical Perspective on p 143

Two coding AR polymorphisms affect the therapeutic response to bucindolol in patients with HFREF. In the β1-AR primary drug target located on cardiac myocytes a position 1165 single nucleotide polymorphism (C→G) results in an amino acid substitution (Arg→Gly) at position 389.7 Compared with the Gly variant, the 389 Arg variant of the β1-AR has a 3- to 4-fold higher signal transduction capacity,7 higher affinity for agonists including norepinephrine,8,10 and a larger proportion of constitutively active receptors.8,11 In α2c ARs, located on prejunctional nerve terminals and regulating cardiac norepinephrine release through a negative feedback mechanism,12,13 an amino acid position 322 to 325 insertion (wild type)/deletion (Wt/Del) polymorphism results from a 12-nucleotide 964→975 insertion/deletion.14 The Del allele results in a loss-of-function phenotype,14 and an exaggerated sympatholytic response to bucindolol that affects heart failure clinical responses.8

The β-Blocker Evaluation of Survival Trial (BEST) was a placebo-controlled, randomized heart failure clinical trial of bucindolol conducted in HFREF patients with New York Heart Association (NYHA) Class III-IV heart failure and left
ventricular ejection fractions ≤0.35. In a genetic substudy of BEST, bucindolol had a differential effect on mortality and heart failure hospitalizations that was dependent on β₁, Arg389Gly and α₁,322-325 Wt/Del genotypes. We hypothesized that there may also be AR genotype-specific responses to bucindolol for prevention of ventricular arrhythmias, and sought to characterize any potential pharmacogenetic interactions.

**Methods**

**Study Sample**

The BEST protocol, main outcomes, and DNA substudy have been previously described. This study uses the DNA substudy of BEST, a prospectively planned investigation (n=1040) designed to differentiate bucindolol efficacy responses for heart failure end points. In the current study, results for the mild efficacy modulating α₁,322-325 Wt/Del polymorphisms and the impact of their therapeutic interaction with α₁,322-325 Wt/Del polymorphisms for ventricular arrhythmia end points in the BEST DNA substudy. A Cox Proportional Hazards model was used to calculate a hazard ratio (HR) or subhazard ratio (SHR) for time to event for bucindolol versus placebo, with associated probability values. To account for the competing risk of ACM, the model of Fine and Gray was an extension of the Cox model, as implemented in function crr of cran.r-project.org, for each of the end points SD, CA, and VT/VF. Plots were made of cumulative incidence functions versus time, using the SAS macro %CUMINCID available in SAS V9.3 (SAS, Cary, NC). These plots are analogous to plots of survival function (ACM) as the HR. The assumption of proportional hazards in the Fine–Gray model was assessed by visual inspection of plots of log–log(1–cumulative incidence functions), analogous to the suggestion by Cantor of plots of log(−log(survival functions)). Results for competing risk regression were reported as SHRs and for survival function (ACM) as the HR.

**Statistical Analysis**

The current inquiry is a post hoc analysis to investigate the effects of β₁, Arg389Gly polymorphisms and the impact of their therapeutic interaction with α₁,322-325 Wt/Del polymorphisms for ventricular arrhythmia end points in the BEST DNA substudy. A Cox Proportional Hazards model was used to calculate a hazard ratio (HR) or subhazard ratio (SHR) for time to event for bucindolol versus placebo, with associated probability values. To account for the competing risk of ACM, the model of Fine and Gray was an extension of the Cox model, as implemented in function crr of cran.r-project.org, for each of the end points SD, CA, and VT/VF. Plots were made of cumulative incidence functions versus time, using the SAS macro %CUMINCID available in SAS V9.3 (SAS, Cary, NC). These plots are analogous to plots of survival function versus time in the absence of competing risks. The assumption of proportional hazards in the Fine–Gray model was assessed by visual inspection of plots of log–log(1–cumulative incidence functions), analogous to the suggestion by Cantor of plots of log(−log(survival functions)). Results for competing risk regression were reported as SHRs and for survival function (ACM) as the HR.

Randomization in BEST was stratified by 4 binary covariates: sex, race (black, non-black), chronic heart failure cause (ischemic, nonischemic), and baseline left ventricular ejection fractions (≤0.20, >0.20). Analyses of all 4 end points are adjusted via stratification for these same 4 covariates, which is important because there were baseline differences in race and ischemic/nonischemic cause of cardiomyopathy.

**Table 1. Baseline Characteristics, by Genotype Group**

<table>
<thead>
<tr>
<th>DNA Substudy</th>
<th>β₁,389 Arg/Arg (n=1040)</th>
<th>β₁,389 Gly Carrier (n=493)</th>
<th>β₁,389 Gly Carrier+ (n=413)</th>
<th>β₁,389 Gly Carrier+ + Del (n=134)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60.3 (60.0–61.1)</td>
<td>60.1 (59.6–61.2)</td>
<td>60.5 (59.4–61.5)</td>
<td>61.0 (59.8–62.2)</td>
<td>0.083</td>
</tr>
<tr>
<td>Male, %</td>
<td>79</td>
<td>80</td>
<td>79</td>
<td>80</td>
<td>0.16</td>
</tr>
<tr>
<td>Black, %</td>
<td>20</td>
<td>13</td>
<td>26</td>
<td>&lt;0.001</td>
<td>12</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>81.4 (80.6–82.2)</td>
<td>81.2 (80.8–82.4)</td>
<td>81.6 (80.5–82.6)</td>
<td>81.3 (80.0–82.6)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>56</td>
<td>52</td>
<td>59</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>Ischemic etiology, %</td>
<td>35</td>
<td>36</td>
<td>34</td>
<td>0.4</td>
<td>0.0043</td>
</tr>
<tr>
<td>NYHA III, %</td>
<td>92.3</td>
<td>94.3</td>
<td>90.5</td>
<td>29 (9–60)</td>
<td>0.3</td>
</tr>
<tr>
<td>Digoxin, %</td>
<td>90</td>
<td>91</td>
<td>89</td>
<td>80</td>
<td>0.8</td>
</tr>
<tr>
<td>ICD present, %</td>
<td>2.9</td>
<td>3.4</td>
<td>2.4</td>
<td>0.3</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Values are mean (95% confidence interval) except HF duration, given as median (interquartile range). BHR indicates basal heart rate; bpm, beats per minute; Del, deletion; HF, heart failure; HTN, history of hypertension; ICD, internal cardioverter defibrillator; LVEF, left ventricular ejection fractions; NYHA, New York Heart Association; and Wt, wild-type.
in baseline characteristics (Table 1). For VT/VF within pharmacogenetic subgroups, results are also presented as unadjusted analyses. In all time-to-event analyses, subjects were censored at loss of follow-up, at end of study, or at time of cardiac transplantation.

Presentation of Cox/Fine–Gray model regression results included Effect Size, or the effect of bucindolol relative to placebo as a percent measured by (1-hour or SHR)x100, and Relative Effect Size (RES), a measure of pharmacogenetic modulation. RES transforms HR or SHR results into a continuous metric with 1.0 being equivalent to the respective outcome in the parent, all-genotype 1040 patient sample. RES transforms HR or SHR results into a continuous metric with 1.0 being equivalent to the respective outcome in the parent sample. Values >1.0 indicate a greater efficacy than in the parent sample, positive values <1.0 indicate a lesser efficacy than the parent sample but superior to that of placebo, and negative values indicate an efficacy inferior to placebo. RES was calculated as ln (HR or SHR for the specific genotype) divided by ln (HR or SHR for the all-genotype parent population). To identify the degree to which genetic subgrouping resulted in outliers for drug response,^{11} within each polymorphism construct differential efficacy in percent was calculated as the difference between the highest and lowest average RESx100.

Tests of interaction of treatment group and genotype were performed using the Cox/Fine–Gray model, with genotype as an interval-level variable (1 degree of freedom). The tests of interaction used 2 coding schemes for the 3-level genotype. The first coding scheme used 1, 2, and 3, respectively, for the hierarchical 3-group \( \beta_3 \), \( \alpha_2 \), 322-325 pharmacogenetic construct identified as comprising of distinct response groups. The second coding scheme (RES) used RES results observed in the current study and normalized to 1.0. Interaction tests were first performed on the 3-group \( \beta_3 \), \( \alpha_2 \), 322-325 pharmacogenetic construct, and then if results were not \( P<0.05 \) on the 2-group \( \beta_3 \), \( \alpha_3 \) Arg/Gly construct. The estimated statistical power to detect an interaction of genotype and treatment group on VT/VF was approximated by a power calculation assuming no competing risks. Based on an n of 1040 and 96 events, the estimated power was 80% to detect an interaction SHR of 0.32 with a 2-sided \( \alpha \) of 0.05.

Continuous variables are presented as mean (95% confidence interval) and are compared using the \( t \) test; chronic heart failure duration is presented as median (interquartile range) and compared using the Wilcoxon rank-sum test. Categorical variables are compared using the \( \chi^2 \) test. Analysis was performed in SAS (V9.3). Statistical significance was taken as \( P<0.05 \).

Results
Baseline Clinical Characteristics
There were no clinically relevant differences in baseline characteristics between the 1040-subject DNA substudy (Table 1) and the 2708-subject full BEST cohort.\(^6\) There were significant differences in race and history of hypertension between the \( \beta_3 \), \( \alpha_2 \) 322-325 Arg/Arg and Gly carrier (either Gly homozygotes or heterozygotes) groups, as well as between the 2 \( \beta_3 \), \( \alpha_2 \) 322-325 Gly carrier groups. In addition, the percent ischemic/nonischemic cause of cardiomyopathy varied slightly (by 27%) in the \( \beta_3 \), \( \alpha_2 \) 322-325 Gly carrier groups.\(^8\)

Outcomes, Parent Population, and by \( \beta_3 \), \( \alpha_2 \) Arg/Gly Genotypes
In the DNA substudy there were 96 VT/VF first-occurring events. Of these, 78 were VT and 18 were VF. In Table 2, results for the 4 efficacy end points are given as HR or SHR+95% confidence interval for the full DNA substudy, by \( \beta_3 \), \( \alpha_2 \) Arg/Gly genotypes, and within the \( \beta_3 \), \( \alpha_2 \) Gly carrier group by \( \alpha_3 \) 322 to 325 genotype combinations. In the DNA substudy parent population bucindolol treatment was associated with a lower incidence of VT/VF (SHR, 0.42 [0.27–0.64]; \( P=0.00006 \)), but no significant treatment effects on the other end points. In contrast, \( \beta_3 \), \( \alpha_2 \) Arg homozygous (Arg/Arg) subjects had reductions in both ACM (by 38%; \( P=0.042 \)) and VT/VF (by 74%; \( P=0.00005 \)), and had lower SHRs than in the parent population for all 4 end points. Compared with \( \beta_3 \), \( \alpha_2 \) Gly carriers, \( \beta_3 \), \( \alpha_2 \) Arg homozygotes had substantially lower HRs or SHRs, and no end point was statistically significant in \( \beta_3 \), \( \alpha_2 \) Gly carriers. The greater effect sizes in \( \beta_3 \), \( \alpha_2 \) Arg/Arg subjects ranged from 38% (ACM) to 74% (VT/VF), versus a range of 1% (SD) to 40% (VT/VF) in \( \beta_3 \), \( \alpha_2 \) Gly carriers. Results by RES are given in Table 3. RES values were much higher in the \( \beta_3 \), \( \alpha_2 \) Arg/Arg group (average of 1.88 compared with 0.30 in the Gly carrier group), representing respective changes in efficacy of an 88% enhancement versus a 70% decrease compared with the parent population. There was no overlap in the individual RES values of the 4 end points (\( \beta_3 \), \( \alpha_2 \) Arg/Arg range of 1.55–2.19, Gly carrier 0.05–0.59). The differential efficacy between the 2 genotypes was 158%.

Outcomes by \( \beta_3 \), \( \alpha_2 \) Gly Carrier/\( \alpha_3 \) 322-325 Genotype Combinations
HR or SHR results by \( \beta_3 \), \( \alpha_2 \) Gly carrier/\( \alpha_3 \) 322-325 genotype combination for the 4 efficacy end points are given in Table 2. Compared with the \( \beta_3 \), \( \alpha_2 \) Gly carrier/\( \alpha_3 \) 322-325...
Table 3. Relative Effect Sizes, by Individual Genotype

<table>
<thead>
<tr>
<th>End Point</th>
<th>(\beta),389Arg/Arg (P=236, B=257)</th>
<th>(\beta),389Gly Carrier (P=289, B=258)</th>
<th>(\beta),389Gly Carrier+(\alpha),322-325 WT (n=413; P=214, B=199)</th>
<th>(\beta),389Gly Carrier+(\alpha),322-325 Del Carrier (n=134; P=75, B=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACM</td>
<td>1.83</td>
<td>0.32</td>
<td>1.10</td>
<td>−0.15</td>
</tr>
<tr>
<td>SD</td>
<td>2.19</td>
<td>0.05</td>
<td>1.90</td>
<td>−2.95</td>
</tr>
<tr>
<td>CA</td>
<td>1.89</td>
<td>0.26</td>
<td>1.30</td>
<td>−2.94</td>
</tr>
<tr>
<td>VT/VF</td>
<td>1.55</td>
<td>0.59</td>
<td>0.78</td>
<td>0.32</td>
</tr>
<tr>
<td>Average</td>
<td>1.88</td>
<td>0.30</td>
<td>1.33</td>
<td>−1.86</td>
</tr>
<tr>
<td>Range</td>
<td>(1.55–2.19)</td>
<td>(0.05–0.59)</td>
<td>(0.78–1.90)</td>
<td>(−2.95–0.32)</td>
</tr>
<tr>
<td>Differential efficacy (%)</td>
<td>158 vs (\beta),389Gly carrier; 374 vs ((\beta),389Gly carrier+(\alpha),322-325 Del carrier)</td>
<td>NA</td>
<td>319 vs ((\beta),389Gly carrier+(\alpha),322-325Del carrier)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Average, Range, and Differential Efficacy statistics apply to SD, CA, VT/VF only. ACM indicates all-cause mortality; B, bucindolol; CA, cardiac arrest; Del, deletion; NA not applicable; P, placebo; RES, relative effect size; and SHR, subhazard ratio.

Table 4. Effect of Race on the VT/VF End Point Across the 3 Genotype Construct

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Black/Nonblack (%)</th>
<th>VT/VF Blacks No. Events/No. Pts (%)</th>
<th>VT/VF Nonblacks No. Events/No. Pts (%)</th>
<th>All Races No. Events/No. Pts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta), Arg/Arg, n=493</td>
<td>P 24/212 (11/89)</td>
<td>P 3/24 (12.5)</td>
<td>P 31/212 (14.6)</td>
<td>P 34/236 (14.4)</td>
</tr>
<tr>
<td>B 42/215 (16/84)</td>
<td>B 1/24 (2.4)</td>
<td>B 11/215 (5.1)</td>
<td>B 12/257 (4.7)</td>
<td>B 12/257 (4.7)</td>
</tr>
<tr>
<td>(\beta), Gly C+(\alpha), Wt/Wt, n=413</td>
<td>P 26/188 (12/88)</td>
<td>P 0/26 (0)</td>
<td>SHR 0.13 (0.01–2.54)*</td>
<td>SHR 0.28 (0.14–0.50)*</td>
</tr>
<tr>
<td>B 24/175 (12/88)</td>
<td>B 1/24 (4.2)</td>
<td>B 11/175 (6.3)</td>
<td>B 12/199 (6.0)</td>
<td>B 12/199 (6.0)</td>
</tr>
<tr>
<td>(\beta), Gly C + (\alpha), Del C, n=134</td>
<td>P 48/27 (64/36)</td>
<td>P 7/48 (14.6)</td>
<td>P 1/27 (3.7)</td>
<td>P 8/67 (10.7)</td>
</tr>
<tr>
<td>B 43/16 (73/27)</td>
<td>B 3/43 (7.4)</td>
<td>B 3/16 (18.8)</td>
<td>B 6/59 (10.2)</td>
<td>B 6/59 (10.2)</td>
</tr>
<tr>
<td>Interaction tests, P value</td>
<td>0.61 (1.0 interval)</td>
<td>0.18 (1.0 interval)</td>
<td>0.028 (1.0 interval)</td>
<td>0.032 (RES interval)</td>
</tr>
</tbody>
</table>

Interaction Between Genotype and Treatment

As shown for all races in Table 4, for the VT/VF end point there was a statistical interaction between treatment and the 3-group \(\beta\),389/\(\alpha\), construct when the between group intervals were set at 1.0 (\(P=0.028\)) or when the RES values were used to set the intervals (\(P=0.032\)). For the 3 other end points, there were no significant tests for interaction for the 3-group construct, or when tested as a 2-group comparison of \(\beta\),389 Arg/Arg versus Gly carrier.

Based on data in Table 1 results in the 3-group combination genotype construct are potentially confounded by race as the percent blacks is 68% in the \(\beta\),389 Gly carrier+\(\alpha\) group and 13% in the \(\beta\),389 Arg/Arg group. Consequently, we performed an analysis of the VT/VF end point by race in these 3 genotype groups. As seen in Table 4, results in nonblacks were similar to results for all races, and the interaction tests remain statistically significant (\(P=0.018, 0.035\)). Although the interaction \(P\) values in blacks are not significant, there are very few events, and the event rates in the bucindolol group exhibit the same pattern as in nonblacks, that

Wt/Wt combination genotype the \(\beta\) Gly carrier+\(\alpha\) Del carrier group had a smaller effect size for VT/VF, and SHRs >1.0 for ACM, SD, CA, as well as nonoverlap of SHRs between the 2 genetic groups. Although none of the end points in the \(\beta\),389 Gly carrier+\(\alpha\) 322-325 Wt/Wt group were statistically significant, the VT/VF SHR was 0.51(0.26–1.01), \(P=0.054\).

The \(\beta\) Gly carrier+\(\alpha\) Wt/Wt group average RES of 1.33 (Table 3) indicates a mild enhancement of efficacy compared with the parent population. The \(\beta\) Gly carrier+\(\alpha\) Del carrier group had a negative mean RES (−1.86), indicating that the average event rate hazard in the bucindolol group was 186% worse than in the placebo group. There was no overlap in the various end point RES values, which ranged from 0.78 to 1.90 in the \(\beta\) Gly carrier+\(\alpha\) Wt/Wt group to −2.95 to 0.32 in the \(\beta\) Gly carrier+\(\alpha\) Del carrier group. The differential efficacy between the \(\beta\) Gly carrier+\(\alpha\) group and the \(\beta\) Arg/Arg group was, respectively, 374% and 319%, meaning that categorizing patients by combination genotypes further differentiated drug response.

Interaction Between Genotype and Treatment

As shown for all races in Table 4, for the VT/VF end point there was a statistical interaction between treatment and the 3-group \(\beta\),389/\(\alpha\), construct when the between group intervals were set at 1.0 (\(P=0.028\)) or when the RES values were used to set the intervals (\(P=0.032\)). For the 3 other end points, there were no significant tests for interaction for the 3-group construct, or when tested as a 2-group comparison of \(\beta\),389 Arg/Arg versus Gly carrier.

Based on data in Table 1 results in the 3-group combination genotype construct are potentially confounded by race as the percent blacks is 68% in the \(\beta\),389 Gly carrier+\(\alpha\) group and 13% in the \(\beta\),389 Arg/Arg group. Consequently, we performed an analysis of the VT/VF end point by race in these 3 genotype groups. As seen in Table 4, results in nonblacks were similar to results for all races, and the interaction tests remain statistically significant (\(P=0.018, 0.035\)). Although the interaction \(P\) values in blacks are not significant, there are very few events, and the event rates in the bucindolol group exhibit the same pattern as in nonblacks, that
is, increasing from the \([\beta,389 \text{Arg/Arg}]\) group progressing through the \([\beta,389 \text{Gly carrier+}\alpha_2,322-325 \text{Del carrier}]\) group.

**Cumulative Incidence Curves**

For the 3-group combination genotype construct, cumulative incidence curves for time to VT/VF adjusted for ACM competing risk are shown in the Figure. \([\beta, \text{Arg/Arg}]\) subjects had a large relative benefit of bucindolol (Figure A; SHR unadjusted 0.29, \(P=0.0002\)\(^\text{covariate adjusted 0.26, } P=0.00005\)) that was greater than for the \([\beta, 389 \text{Gly carrier+}\alpha_2,322-325 \text{Wt/Wt}]\) group (Figure B; SHR unadjusted or covariate adjusted 0.51, \(P=0.054\)). The \([\beta, \text{Gly carrier+}\alpha_2,322-325 \text{Wt/Wt}]\) subjects exhibited no evidence of therapeutic benefit (Figure C; SHR unadjusted 1.00, \(P=1.00\); covariate adjusted HR 0.76, \(P=0.58\)). The interaction \(P\) value for the unadjusted analyses (interval 1.0) was 0.045.

**Discussion**

This study tested the hypothesis that AR polymorphisms influence the effects of bucindolol on serious ventricular arrhythmia end points in patients with HFREF. The basis for the hypothesis was that for heart failure end points 2 AR polymorphisms do affect the therapeutic response to bucindolol, and the results of the current study provide support that similar pharmacogenetic interactions occur for serious ventricular arrhythmias.

The \(\beta_1,389\) AR polymorphism creates 2 different functional versions of the \(\beta_1\)-AR, with respective allele frequencies of 0.70 (Arg) and 0.30 (Gly) in the general US population. In cardiac-restricted receptor overexpression studies in transgenic mice, compared with the \(\beta_1,389\) AR the \(\beta_1,389\) Arg AR has greater adverse downstream signaling effects, including a more rapid development of a dilated cardiomyopathy,\(^\text{11,12}\) Uniquely, among \(\beta\)-blockers bucindolol acts as an inverse agonist on \(\beta_1,389\) Arg receptors in isolated human heart preparations,\(^\text{7,9}\) and lowers systemic levels of norepinephrine, the cardiac neurotransmitter that selectively signals through \(\beta_1,389\) Arg versus Gly \(\beta_1\)-ARs.\(^\text{9}\) For heart failure end points, bucindolol\(^\text{7}\) but not other \(\beta\)-blockers\(^\text{23,24}\) produces greater therapeutic responses in patients who are \(\beta_1,389\) Arg homozygous, compared with \(\beta_1,389\) Gly carriers. For heart failure end points,\(^\text{7}\) inverse agonism\(^\text{7}\) and norepinephrine affinity\(^\text{9}\) the \(\beta_1,389\) Gly receptor variant is dominant negative.

Bucindolol is a sympatholytic agent in addition to being a \(\beta\)-blocker,\(^\text{6,25}\) and the degree of norepinephrine lowering by bucindolol in HFREF patients is regulated genetically, with the \(\alpha_2\) Del polymorphism associated with large (levels reduced by >200 pg/mL) amounts of sympatholysis and the Wt \(\alpha_2\) AR associated with mild (<100 pg/mL) norepinephrine lowering.\(^\text{8,9}\) For heart failure end points bucindolol is associated with a complete loss of efficacy in \(\beta_1,389\) Gly carriers. This is likely due to exaggerated sympatholysis combined with \(\beta\)-AR blockade adversely affecting signaling through the low norepinephrine affinity, low signal transduction Gly receptor, resulting in inadequate adrenergic support to the failing heart.\(^\text{9}\)

In the current study, for VT/VF and the 3 other ventricular arrhythmia-related end points there was a benefit of bucindolol in \(\beta_1,389\) Arg/Arg subjects not present to the same extent in \(\beta_1,389\) Gly carriers. The VT/VF hazard was substantially (by 74%) reduced (\(P=0.00005\)) in the \(\beta_1,389\) Arg/Arg group, but not in \(\beta_1,389\) Gly carriers. As previously reported,\(^\text{7}\) ACM was also reduced only in the \(\beta_1,389\) Arg/Arg group. Although in the \(\beta_1,389\) Arg/Arg group the lower event rate end points SD and CA did not achieve statistical significance, their respective RES values of 2.19 and 1.89 were higher than the VT/VF RES (1.55). No RES value in the \(\beta_1,389\) Gly carrier group was >0.59, with an average of 0.30 compared with 1.88 in the \(\beta_1,389\) Arg/Arg group. RES is an observational method on which standard

---

**Figure.** Cumulative incidence of ventricular tachycardia or ventricular fibrillation (VT/VF). A, \(\beta_1,389\) Arg homozygotes. B, \(\beta_1,389\) Gly carrier + \(\alpha_2,322-325\) Wt homozygotes. C, \(\beta_1,389\) Gly carrier + \(\alpha_2,322-325\) Del carrier. Analysis is adjusted for competing risk of all-cause mortality. Curves were generated without covariate adjustment for stratification variables; subhazard ratios (SHRs) are given for both covariate adjusted (Cov adj) and unadjusted (Unadj) analyses. B indicates Bucindolol; and P, placebo.
statistics cannot be performed because of uncertain degrees of relation among the multiple end points measured, but there was no overlap in individual RES values between the 2 β,389 genotypic groups. The degree of differential efficacy conferred by subdividing groups by β,389 genotypes was 158%. When β,389 Gly carrier+α,322-325 genotype combinations were analyzed, there was evidence of substantial (by 319%) differential efficacy between the β,389 Gly carrier+α,322-325 Wt/Wt and β,389 Gly carrier+α,322-325 Del carrier groups, which exceeds by >3-fold the differential efficacy observed for heart failure end points in these same genotypes.6 Although no end point HR or SHR in the β,389 Gly carrier+α,322-325 Wt/Wt was statistically significant, there was no overlap in HRs/SHRs or RES values compared with the β,389 Gly carrier+α,322-325 Del carrier group. RES values averaged 1.33 (mild efficacy enhancement) in the β,389 Gly carrier+α,322-325 Wt/Wt group, and −1.86 (event rate hazard in the bucindolol group worse than placebo) for patients with a β,389 Gly carrier+α,322-325 Del carrier genotype. For SD and CA the SHRs converted from <0.70 in the β,389 Gly carrier+α,322-325 Wt/Wt group to, respectively, 1.86 and 2.85 in the β,389 Gly carrier+α,322-325 Del carrier group, suggesting an increased risk for SD on an arrhythmic basis conferred by the addition of α,322-325 Del alleles to β,389 Gly genotypes. The nonoverlap of β,389 Gly carrier+α,322-325 Wt/Wt and β,389 Gly carrier+α,322-325 Del carrier HRs/SHRs and RES values, plus the very pronounced differential efficacy between the 2 groups provided a rational for subdividing β,389 Gly carriers for pharmacogenetic testing against the β,389 Arg/Arg genotype. Furthermore, the significant interaction for VT/VF across the 3-group genetic construct was not confounded by race, because nonblacks (80% of the study population but only 32% of the β,389 Gly carrier+α,322-325 Del carrier) group exhibited the same behavior for bucindolol treatment group event rates and HRs as did all races, and the interaction test remained statistically significant. In addition, in blacks the bucindolol event rates across the 3 groups exhibited the same pattern as in nonblacks. These results for the 3-group construct are similar to those for heart failure end points, and they subdivide patients pharmacogenetically into an enhanced response group [β,389 Arg/Arg], a group with minimal therapeutic modulation [β,389 Gly carrier+α,322-325 Wt/Wt] and a group with no evidence for a favorable therapeutic effect (β,389 Gly carrier+α,322-325 Del carrier). Thus the goal of pharmacogenetics to identify outlier subpopulations for drug response,6,9 measured in this study by differential efficacy, is met by the 3-group construct of β,389 Arg/Gly and α,322-325 Wt/Del genotypes.

β-receptor antagonists, such as metoprolol and bisoprolol, decrease the risk of SD1–3 or developing ventricular arrhythmias4 in patients with HFREF. In the BEST entire cohort from which the population of the current study was derived, SD was nonsignificantly reduced by bucindolol by 12%.6 In the current study, the genotypic group with the largest reduction in ventricular arrhythmia end points by bucindolol was β,389 Arg homozygotes, where event rates were reduced by 38% to 74%. However, the by-genotype analysis also revealed a group with a possible increased risk of SD, the [β1389 Gly carrier+α,322-325 Del carrier] combination genotype. This genotype is prone to large degrees of bucindolol associated norepinephrine lowering, and also has the hypofunctional, low norepinephrine affinity 389 Gly β,-AR.5 This group’s increased SD risk was not directly related to ventricular tachyarrhythmias, inasmuch as the VT/VF HR was 0.76. However, a substantial portion of SD in patients with HFREF is not due to ventricular tachyarrhythmias.26 Increased SD from a sympatholytic agent has been previously observed in patients with HFREF,27 and our data suggest that for bucindolol this effect may be confined to patients who have large degrees of norepinephrine lowering in the presence of β,389 Gly genotypes.

There are several limitations in the present study. First, the reduction in ventricular arrhythmia in the β,389 Arg/Arg group may reflect improved heart failure outcomes. The described pharmacogenetic interactions between bucindolol and β,389α,322-325 polymorphisms are indeed similar to those that occur in heart failure.7,9 However, there were apparent quantitative differences in ventricular arrhythmia and heart failure end points within the 3 β,389α,322-325 genotype construct, related to a more negative average RES for ventricular arrhythmia (−1.86) compared with heart failure end points (−0.14).6 The result was a much greater differential efficacy than for heart failure end points. However, the evidence would support an interpretation that the pharmacogenetic mechanisms that affect serious ventricular arrhythmias are the same as those that affect heart failure outcomes, either in parallel or secondary to changes in heart failure. A second limitation is the small sample size or number of events for some genotypes. Another limitation is the post hoc nature of the arrhythmia analysis, as BEST was primarily designed to investigate total mortality and other heart failure end points. Another limitation is that VT and VF events included both sustained and nonsustained episodes. Therefore, the results of this study are only hypothesis generating, and it should be emphasized that a larger, prospectively designed trial will be necessary to confirm these findings.

Conclusions

The current study demonstrates therapeutic interactions between bucindolol and the β,389 Arg/Gly and α,322-325 Wt/Del AR polymorphisms for treatment effects on ventricular arrhythmias. The data suggest that β,389 Arg/Gly and α,322-325 Wt/Del AR genotypes can be used to better define patient cohorts that would or would not benefit from treatment with bucindolol. The existence of efficacy modifying common genetic polymorphisms in the target(s) of a rhythm modifying agent has not been previously reported, but should be considered in antiarrhythmic drug development.

Disclosures

Dr Aleong has speaking honoraria and consulting from St. Jude Medical. Dr Bristow is an employee, director, and stockholder of ARCA biopharma. Dr Robertson consults with ARCA biopharma. Dr Sauer consults with ARCA biopharma. Dr Liggett is a co-founder of ARCA biopharma and stockholder. Dr Aleong consults with ARCA biopharma.

References

Ventricular Arrhythmias are common in heart failure patients and lead to increased mortality. Although β-blockers have been shown to decrease the incidence of ventricular arrhythmias in heart failure, there is variability in response. Bucindolol is a β-blocker with sympatholytic properties that has been shown to have differential effects on mortality and heart failure admissions via an interaction with 2 adrenergic receptor polymorphism. β and Gly389 polymorphic variants of the β-adrenergic receptor confer impaired coupling to multiple effectors. Circulation. 2002;106:2491–2496.


Adrenergic Receptor Polymorphisms and Prevention of Ventricular Arrhythmias With Bucindolol in Patients With Chronic Heart Failure

Ryan G. Aleong, William H. Sauer, Alastair D. Robertson, Stephen B. Liggett and Michael R. Bristow

*Circ Arrhythm Electrophysiol.* 2013;6:137-143; originally published online December 30, 2012; doi: 10.1161/CIRCEP.111.969618

*Circulation: Arrhythmia and Electrophysiology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/6/1/137

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Arrhythmia and Electrophysiology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation: Arrhythmia and Electrophysiology* is online at: http://circep.ahajournals.org//subscriptions/