Therapeutic Efficacy of a Combination of a β 1-Adrenoreceptor (AR) Blocker and β 2-AR Agonist in a Rat Model of Postmyocardial Infarction Dilated Heart Failure Exceeds That of a β 1-AR Blocker plus Angiotensin-Converting Enzyme Inhibitor^S

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ABSTRACT

We had proposed previously a novel combination of β 2-adrenoreceptor (AR) agonist and β 1-AR blocker that in the rat model of postmyocardial infarction (MI) dilated cardiomyopathy exceeds the therapeutic effectiveness of either monotherapy. In the present study, we compared that treatment with a combination of β 1-AR blocker and angiotensin-converting enzyme inhibitor (ACEi), a current standard chronic heart failure (CHF) therapy. Two weeks after coronary artery ligation, rats were divided into groups of similar average MI size, measured by echocardiography, and the following 12-month treatments were initiated: fenoterol (250 μ g/kg/day), a β 2-AR agonist, plus metoprolol (100 mg/kg/day), a β 1-AR blocker (β 1- β 2+); metoprolol plus enalapril (20 mg/kg/day), an ACEi (β 1-ACEi); and a combination of all three drugs (β 1- β 2+ACEi). These treatment groups were compared with each other and with nontreated

(nT) and sham groups. The 12-month mortality was significantly reduced in all treatment groups (44% in β 1- β 2+, 56% in β 1- β 2+ACEi, 59% in β 1-ACEi versus 81% in nT). Bimonthly echocardiography revealed significant attenuation of the left ventricular (LV) chamber remodeling, LV functional deterioration, and MI expansion in all three treatment groups, but effects were significantly more pronounced when treatment included a β 2-AR agonist. The results indicated that a combination of β 1-AR blocker and β 2-AR agonist is equipotent to a combination of β 1-AR blocker and ACEi in the treatment of CHF in rats, with the respect to mortality, and exceeds the latter with respect to cardiac remodeling and MI expansion. Thus, this novel therapeutic regimen for CHF warrants detailed clinical investigation.

Convincing experimental data in rodent models (Pfeffer et al., 1985, 1987) has helped to establish the beneficial clinical effects of renin-angiotensin system blockade with ACE inhibitors (ACEi) in patients with chronic heart failure (CHF) and to include this therapy into clinical practice in 1980s (CONSENSUS Trial Study Group, 1987; The SOLVD Investigators, 1991; Pfeffer et al., 1992; The AIRE Study Investigators, 1993; Køber et al., 1995). Experimental evidence of deleteri-

ous effects of β 1-adrenergic receptor (AR) stimulation on rat cardiac myocytes put forth in 1990s (Mann et al., 1992; Communal et al., 1999) was swiftly translated in successful clinical trials, reporting a 35% reduction in mortality and significant reversal of cardiac remodeling in patients with CHF treated with β 1-AR blockers (Hall et al., 1995; The CIBIS II Investigators, 1999; The MERIT-HF Investigators, 1999). A combination of β 1-AR blockers with ACEi (or angiotensin receptor blockers) was subsequently recommended by American Heart Association, and this combination is presently considered a standard therapy for CHF (HFSA, 2006). Nevertheless, despite spectacular success in treatment during the past decade with this treatment regimen, CHF still remains a major cause of morbidity and mortality, particu-

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ABBREVIATIONS: ACE, angiotensin-converting enzyme; ACEi, angiotensin-converting enzyme inhibitor; CHF, chronic heart failure; AR, adrenoreceptor(s); MI, myocardial infarction; SH, sham operated; LV, left ventricle; EF, ejection fraction; Echo, echocardiography; nT, nontreated; 2D, two-dimensional; EDV, end-diastolic volume; ESV, end-systolic volume; LVM, left ventricular mass; Ees, end-systolic elastance; PRSW, preload recruitable stroke work; Eed, end-diastolic stiffness; Ea, arterial elastance; DCM, dilated cardiomyopathy.

larly in the elderly, and a growing problem in most industrialized countries (Mazza et al., 2005). Thus, the search for novel pharmacological approaches to treat CHF continues.

Evolving experimental evidence in the rat model points to antiapoptotic, and thus cardioprotective, properties of β 2-AR agonists (Communal et al., 1999; Chesley et al., 2000; Zhu et al., 2001; Shizukuda and Buttrick, 2002; Ahmet et al., 2004; Xiao et al., 2004). Furthermore, preclinical translational studies have clearly demonstrated the therapeutic effectiveness of combined β 1-AR blockers and β 2-AR agonist therapy in animal models of CHF (Ahmet et al., 2005, 2008). In contrast to successful translation of experimental work on β 1-AR blockers and ACE inhibitors in animal models to clinical practice, however, the combination of β 1-AR blockers and β 2-AR agonists has not been recognized in the clinical arena, due to largely hypothetical objections: β 2-AR stimulation accelerates the heart rate and, therefore, could be proarrhythmic (Pearce et al., 1989; Martin et al., 1998); chronic β -AR stimulation leads to down-regulation and desensitization of β 2-AR (for review, see Brodde et al., 1995) and, therefore, cannot be effective. An additional barrier to the entree of the β 1-AR blocker and β 2-AR agonist-combined therapy into consideration for a clinical trial is that this combination has not been compared with the standard CHF therapeutic regimen, i.e., an ACE inhibitor combined with a β 1-AR blocker.

Therefore, the present study compared the effectiveness of 12-month treatment regimen of the combination of a β 1-AR blocker and β 2-AR agonist with the standard combination of β 1-AR blocker and ACE inhibitor in a well characterized rat experimental model of post-MI dilated cardiomyopathy.

Materials and Methods

Experimental Design. Male Wistar rats (Charles River Laboratories, Inc., Wilmington, MA), weighing 225 to 280 g, were housed and studied in conformance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Manual 3040-2, 1999), with approval from the Institutional Animal Care and Use Committee. The left descending coronary artery was ligated in 224 rats. An additional 10 rats underwent a sham operation (SH) without actual coronary ligation. Two weeks after surgery, LV dimensions, ejection fraction (EF), and MI size were measured by echocardiography (Echo). Animals with an MI size greater than 20% but less than 50% LV were divided into four groups of similar MI size (average and variability). In three groups of rats, treatment was initiated either with a combination of metoprolol, a selective β 1-AR blocker plus enalapril, an ACE inhibitor (β 1-ACEi), with a combination of metoprolol, and fenoterol, a selective β 2-AR agonist (β 1- β 2+), or with a combination of metoprolol, fenoterol, and enalapril (β 1- β 2+ACEi). Fenoterol, metoprolol, and enalapril (Sigma-Aldrich, St. Louis, MO) were dissolved in the drinking water; the daily dose was adjusted to 250 μ g/kg for fenoterol, 100 mg/kg for metoprolol, and 20 mg/kg for enalapril. The fourth group of animals received no treatment (nT), and fifth group was sham-operated. Treatment was started 2 weeks after coronary ligation and continued for 12 months (4 weeks were counted as 1 month). Animals were inspected daily for signs of moribundity by a person blinded to treatment modalities. Moribund animals were euthanized, and their hearts were harvested for MI measurements. Daily records of dead or euthanized animals were used to calculate continuous mortality curves. Echocardiography was repeated bimonthly after the initiation of treatment. After the final Echo, subsets of rats representing average Echo indices within each group were selected for invasive hemodynamic study, and their hearts were harvested for histological evaluation.

Coronary Artery Ligation. Rats were anesthetized with isoflurane (2% in oxygen). The surgical procedure was performed as described previously (Hochman and Bulkey, 1982).

Echocardiography. Echocardiography (Sonos 5500, a 12-MHz transducer; Hewlett Packard, Andover, MA) was conducted under light anesthesia by sodium pentobarbital (30 mg/kg i.p.) as described previously (Ahmet et al., 2005). In brief, parasternal long axis views were obtained and recorded to ensure that the mitral and aortic valves and the apex were visualized. Short axis views were recorded at the midpapillary muscle level. Endocardial area tracings, using the leading edge method, were performed in a 2D-dimensional mode (short and long axis views) from digital images captured on cine-loop to calculate end diastolic and end systolic LV areas. End-diastolic volume (EDV) and end-systolic volume (ESV) were calculated by a modified Simpson's method. EF was then derived as $EF = (EDV - ESV)/EDV \times 100$. Left ventricular mass (LVM) was calculated from a 2D mode. The MI size at the midpapillary muscles level was estimated from 2D short axis LV images and expressed as a percentage of the LV endocardial circumference. Infarct area was identified as a sharply demarcated section of the LV free wall that failed to thicken during systole. The length of the akinetic part of the LV endocardial circumference was measured from freeze-frame images at end-diastole. Posterior wall thickness was measured from M-mode. All measurements were made by a single observer who was blinded to the identity of the tracings. All measurements were off-line averaged over three to five consecutive cardiac cycles. The reproducibility of measurements was assessed in two sets of baseline measurements in 10 randomly selected rats, and the repeated measure variability did not exceed $\pm 5\%$.

Hemodynamic Measurements. Invasive LV pressure-volume loop analyses were conducted as described previously (Ahmet et al., 2004). Rats were anesthetized with isoflurane (2% in oxygen), intubated, and ventilated. A bilateral thoracotomy was performed in the sixth intercostal space. A 1.4 French-combined pressure-conductance catheter (Millar Instruments Inc., Houston, TX) was inserted into LV through the apex. Traditional load-dependent hemodynamic indices, such as EF, $+dP/dt$, $-dP/dt$, end-diastolic pressure, and isovolumic relaxation time constant (τ), were measured, and load-independent indices, i.e., end-systolic elastance (Ees), preload recruitable stroke work (PRSW), and end-diastolic stiffness (Eed) were determined or calculated. Arterial elastance (Ea) was calculated as index of vascular tension. Arterioventricular coupling, an index of cardiac work efficiency, was calculated as Ea/Ees .

Histological Acquisition. Histological staining and analyses were performed as described previously (Ahmet et al., 2005). In brief, the hearts were isolated and weighed. Myocardial segments from the midpapillary muscle level were imbedded in the paraffin, sectioned (5 μ m), and stained with Masson's trichrome and hematoxylin and eosin staining. MI size was expressed as an average percentage of the LV endocardial and epicardial circumferences that were identified as infarct in the Masson's trichrome-staining sections.

Statistical Analyses. All data are expressed as mean $\pm$ S.E.M. Mortality is reported via Kaplan-Meier survival curves. Differences among survival curves were assessed using logrank statistical analyses (GraphPad Prism 4.02; GraphPad Software Inc., San Diego, CA). For subsequent pairwise comparisons of survival curves the Bonferroni's correction for multiple comparisons was used. Reported Echo indices were analyzed using the repeated measures linear mixed effects model. Each response variable was analyzed for main effects of group and time as well as their interaction. If the group-time interactions were significantly different (statistically) among all groups, further pairwise analyses were conducted and the outcomes were Bonferroni-corrected for multiple comparison. Furthermore, post hoc Bonferroni-corrected comparisons were made for 42 comparisons among the group means at each time point. The model was fit using PROC MIXED in SAS 9.1 (SAS Institute, Cary, NC). Power Analysis (PASS 2008, repeated measures analysis of variance; NCSS, Keyville, UT) indicated that for the main effect the sample

size exceeding 10 animals per group would correspond to a power more than 80%; for the interaction effect, the power for all variables would be more than 70% for samples of 15 or larger. Group differences in hemodynamic or histological data among groups were assessed by Student's *t* test or by one-way analysis of variance with Bonferroni's post hoc corrections as appropriate. Statistical significance was assumed at $p < 0.05$.

Results

Early Mortality after Coronary Ligation and Treatment Assignment. Two hundred and twenty-four rats were subjected to a coronary ligation. Eighty animals died within the first 24 h after surgery, and 14 additional rats died within the first 2 weeks after surgery. There was no mortality among 10 sham-operated rats. Two weeks after surgery, the 130 rats surviving coronary ligation and 10 sham-operated rats underwent echocardiography, at which time their pretreatment (baseline) MI size, LV volumes, and EF were determined. Twenty-two rats, in which MI size was less than 20% or more than 50% LV, were excluded from the therapeutic interventions. One hundred and eight rats with an average MI size of $30 \pm 0.67\%$ LV were assigned to four experimental treatment groups ($n = 27$ in each group) that did not differ with respect to their average pretreatment Echo-derived LV morphometric parameters (EDV and ESV), EF, and MI size. The 10 sham-operated rats served as a control.

Table 1 lists the Echo-derived pretreatment EDV, ESV, and EF for each experimental and sham group 2 weeks after surgery. Early pretreatment LV remodeling was similar in all coronary-ligated groups and consisted of substantial increases in EDV (164–171%), ESV (397–430%), and a 57–62% decline in EF compared with SH. The thickness of posterior wall in coronary ligated rats did not vary significantly from SH at this time.

Mortality. Figure 1 illustrates the Kaplan-Meier survival curves for the four groups of experimental animals and the sham-operated group during 1 year after initiation of treatment. No SH animals died during observation. In the untreated group, mortality reached 50% at 6 months and 81% at 12 months. All treatments had improved survival, by 22 to 37%, compared with the untreated group ($p < 0.01$, logrank test). The treatment effect became statistically significant by 7th month and continued to increase with time. Among different treatment groups, the mortality at the end of 1 year of observation was lowest in $\beta 1$ - $\beta 2+$ group (44%) and highest in $\beta 1$ -ACEi group (59%); the effect of each treatment on mortality statistically differed from the nT group ($p < 0.01$, post hoc test) but did not differ from each other.

Infarct Expansion. Figure 2 illustrates the MI expansion during 12 months of treatment. The top panels present data for all animals. The bottom panels illustrate the results obtained only from the rats that survived until the end of the

Fig. 1. Kaplan-Meier survival curves after induction of MI in a sham-operated group, an untreated group, and three different treatment groups. The number of animals at the beginning of treatment was as follows: SH, 10; and nT, $\beta 1$ -ACEi, $\beta 1$ - $\beta 2+$, and $\beta 1$ - $\beta 2+$ -ACEi, 27 in each.

study. The left panels show the MI expansion among different experimental groups assessed bimonthly from Echo measurements and expressed as the percentage of LV perimeter. The right panels illustrate the MI size, assessed from histological preparations at the termination of the study, or at the time of death. Echo-derived and histological measurements were highly correlated ($R^2 = 0.7$). In nT animals, the average MI expanded from $30.1 \pm 1.3\%$ LV 2 weeks after coronary ligation to $46.2 \pm 1.7\%$ LV at the end of 12 months, i.e., during the 12 months of observation, the MI expanded by 50%. MI expansion was attenuated in all treatment groups ($p < 0.001$ versus nT; see Supplemental Table 1). The least MI expansion, however, occurred in rats in which treatment included a $\beta 2$ -AR agonist, i.e., $\beta 1$ - $\beta 2+$ and $\beta 1$ - $\beta 2+$ -ACEi groups. In fact, the group $\times$ time interaction was significantly different between $\beta 1$ -ACEi group and groups with a $\beta 2$ -AR agonist ($p < 0.001$); the average MI size in $\beta 1$ - $\beta 2+$ group never exceeded the pretreatment level and was significantly smaller than in $\beta 1$ -ACEi group at the 4th, 6th, 10th, and 12th month of treatment (see Supplemental Table 1).

LV Remodeling and Function. Figure 3 illustrates the progression of LV remodeling (EDV and ESV expansion) and functional decline (EF reduction) during the treatment pe-

TABLE 1
Pretreatment LV remodeling and MI size at 2 weeks after coronary ligation

	SH	nT	$\beta 1$ -ACEi	$\beta 1$ - $\beta 2+$	$\beta 1$ - $\beta 2+$ -ACEi
EDV (μ l)	227 ± 6	$616 \pm 14^{***}$	$606 \pm 23^{***}$	$600 \pm 17^{***}$	$614 \pm 22^{***}$
ESV (μ l)	90 ± 5	$466 \pm 17^{***}$	$462 \pm 21^{***}$	$447 \pm 18^{***}$	$477 \pm 23^{***}$
PWth (mm)	1.51 ± 0.04	1.45 ± 0.06	1.40 ± 0.04	1.48 ± 0.03	1.39 ± 0.06
EF%	60.71 ± 0.8	$24.9 \pm 1.4^{***}$	$241 \pm 0.3^{***}$	$26.1 \pm 1.4^{***}$	$23 \pm 1.3^{***}$
MI%	N.A.	30.1 ± 1.3	30.6 ± 1.5	30.3 ± 1.2	31 ± 1.4

N.A., not applicable; PWth, posterior wall thickness.
*** $p < 0.001$ vs. SH.

Fig. 2. Average MI size in untreated and treated rats estimated by monthly Echo (left) or on the basis of histological measurements (right) and presented as percentage of LV. Top, all animals; bottom, animals that survived 12 months after the MI induction. ***, $p < 0.001$ versus nT; †††, $p < 0.001$ versus β1-ACEi (pairwise comparison, group $\times$ time interaction with Bonferroni's correction).

riod. The top panels represent average measurements from all animals at each time point, whereas lower panels represent only animals that survived to the end of the study. Both the top and bottom panels reflect similar patterns. In nT animals, the LV volume monotonically increased by 90 and 120% for EDV and ESV, respectively, whereas EF declined by 50%. At the end of the observation period, EDV in nT animals averaged $1170 \pm 75 \mu\text{l}$, ESV was $1028 \pm 83 \mu\text{l}$, and EF was $12.5 \pm 1.8\%$. For comparison (data are not shown), the LV EDV in SH animals at the end of observation was only $506 \pm 13 \mu\text{l}$, ESV was $242 \pm 10 \mu\text{l}$, and EF was $52 \pm 1.9\%$. The expansion of LV volumes was significantly attenuated in all treatment groups compared with nT ($p < 0.002$; see Supplemental Table 1). However, during the first 6 months of treatment LV expansion in the β1-ACEi group was similar to that in the nT group. Statistical analyses with post hoc comparisons conducted for the first 6 months revealed that both EDV and ESV in β1-ACEi group did not differ from nT, whereas in β1-β2+ and β1-β2+ACEi groups, the beneficial difference from nT was significant for ESV ($p < 0.05$) and approached significance for EDV ($p < 0.07$). During the 12 months of the study, the decline in EF was significantly attenuated, compared with the nT, in group with β2-AR agonist ($p < 0.005$); the preservation of EF in the β1-ACEi

group, however, was significantly less and did not differ statistically from nT.

The Echo-calculated LVM (Fig. 4A) increased in all MI groups compared with SH, and that increase in LVM was reduced by all treatment modalities; however, the differences with nT were statistically significant in β1-ACEi group only ($p < 0.05$; see Supplemental Table 1). Posterior wall thickness (Fig. 4B) became significantly reduced in all MI groups compared with SH. Compared with the nT group, the thinning of posterior wall was attenuated in all treatment groups, but only in the β1-β2+ group was this attenuation statistically significant ($p < 0.02$).

Hemodynamics. Figure 5 presents representative pressure-volume loops recorded from SH, nT, and different treatment groups at the end of the study. Compared with SH, a substantial rightward shift accompanied by reduction of stroke volume and end-systolic pressure, characteristic for CHF, was observed in the nT group. All treatment groups showed significant improvement in cardiac indices, and pressure-volume loop recorded from the rat from β1-β2+ACEi group most closely approached the parameters of SH.

Table 2 lists the results of the pressure-volume loop analyses in randomly selected subsets of rats that survived 12-months observation, i.e., 12.5 months after induction of MI. A

Fig. 3. LV end-diastolic volume (left), end-systolic volume (middle), and ejection fraction (right) in untreated and treated rats estimated by bimonthly Echo during 12 months after induction of MI. Top, all animals; bottom, only animals that survived 12 months after induction of MI. **, $p < 0.002$ versus nT; †, $p < 0.05$ versus β_1 -ACEi (pairwise comparison, group $\times$ time interaction with Bonferroni's correction).

comparison of hemodynamic indices of SH and nT rats clearly indicates an advanced stage of CHF in nT rats: the LV is greatly dilated, cardiac output and stroke volume are reduced by 50%, and EF is reduced 4-fold. Furthermore, a greater than 5-fold reduction in PRSW indicates a pronounced systolic pump dysfunction; and a 3.5-fold elevation in Eed reflects the increased diastolic stiffness of myocardium. All three treatment groups showed a significant improvement of hemodynamic indices compared with the nT group. However, the $\beta_1\beta_2$ and $\beta_1\beta_2$ +ACEi were more effective than β_1 -ACEi in attenuation of LV volume expansion and improvement of EF.

Discussion

The rat model of permanent coronary ligation is the oldest and one of the most established models of chronic heart failure (Selye et al., 1960). Although the ischemic-reperfusion (temporary coronary occlusion) model more relevantly reflects the modern clinical situation of restoration of coronary flow, the permanent occlusion model is better suited to study the time course of infarct expansion, remodeling, performance decline, and morphological changes. It also produces a more uniform pathology; therefore, it is a more suitable for assessment of interventional strategies for DCM. In our experiment, we observed in untreated rats all aspects characteristic of post-MI development of DCM: uniform MI size; progressive MI expansion; LV dilatation; increase of LV

mass; thinning of posterior MI-free wall; progressive functional decline; and, of course, substantial mortality. All of these characteristics have been described and well documented previously (Preffer and Braunwald, 1990; Goldman and Raya, 1995; Krzemiński et al., 2008).

We have been committed to testing different therapeutic regimens in the DCM models (Ahmet et al., 2004, 2005, 2008). Initially, we had tested the effects of selective pharmacological stimulation of β_2 -AR alone, or in combination with β_1 -AR blocker in this in vivo rat model of postmyocardial infarction DCM. The effects of 6 weeks of treatment with a β_2 -AR agonist, fenoterol, alone (Ahmet et al., 2004) or in combination with a β_1 -AR blocker, metoprolol (Ahmet et al., 2005), were compared with metoprolol monotherapy. Treatments were started 2 weeks after coronary ligation. The progression of LV remodeling and MI expansion was monitored by serial echocardiography. At the endpoint of the study, cardiac function was analyzed by pressure-volume loop measurements, and hearts were evaluated histologically. In these short-term, 6-week studies the effectiveness of a β_2 -AR agonist and the combination of a β_2 -AR agonist plus a β_1 -AR blocker was similar, and both significantly exceeded the effectiveness of the β_1 -AR blocker as a monotherapy in attenuation of LV dilatation and functional decline. Both treatments that included β_2 -AR stimulation also reduced myocardial apoptosis and arrested the MI expansion. Moreover, we did not observe any

Fig. 4. LV mass (A) and posterior wall thickness (B) in SH, nT, and three treated groups estimated by bimonthly Echo during 12 months after induction of MI in all animals. *, $p < 0.05$ versus SH; †, $p < 0.05$ versus nT (pairwise comparison, group $\times$ time interaction with Bonferroni's correction).

increase in the number of arrhythmic events during β_2 -AR stimulation.

These studies, however, were of a relatively short duration (6 weeks). In the next experiment in the same model, we compared the effects of long-term, combined therapy with a β_1 -AR blocker, metoprolol, plus a β_2 -AR agonist, fenoterol, and either therapy alone for 12 months with survival as a primary outcome (Ahmet et al., 2008). As in the short-term studies, therapy was started 2 weeks after permanent ligation of the left descending coronary artery. Cardiac remodeling, MI expansion, and LV function were assessed by serial echocardiography and compared with untreated animals. A mortality of 67% observed at the end of 1-year observation in untreated animals was reduced to 33% in the animals treated with the combination of β_1 -AR blocker and β_2 -AR agonist. Progressive cardiac remodeling observed in untreated rats or in rats treated with β_1 -AR blocker alone was

Fig. 5. Representative pressure-volume loops of SH, nT, and three treated groups recorded at the end of the study.

significantly attenuated in animals treated with the combination of both drugs during the first 6 months of treatment. MI expansion was completely prevented only in animals treated with the drug combination; the LV functional decline was significantly attenuated during the entire year. The episodes of arrhythmic events were also lowest in combined treatment group. Furthermore, a reduction of cardiac β_1 -AR density and a reduction in chronotropic and contractile responses to β_2 -AR-specific stimulation in the absence of a reduction of β_2 -AR density that occurred in untreated animals were precluded in rats receiving combined therapy. This explained the additional observation of the study in which the beneficial effect of β_2 -AR agonist as monotherapy lasted only for the first 2 months after initiation of treatment, whereas the combination with a β_1 -AR blocker extended the therapeutic effectiveness throughout all 12 months of observation.

Thus, the effects of β_2 AR agonist treatment in the post-MI model of DCM for only 6 weeks of treatment (8 weeks post-MI) showed its high therapeutic effectiveness. When we extended the treatment period to 12 months, it became apparent that the effect of β_2 AR agonist monotherapy waned after 2 months. The long-term effectiveness of β_2 -AR agonist therapy was possible only in combination with β_1 -AR blocker to prevent β_2 -AR tachyphylaxis and reduce apoptosis. The use of β_2 -AR agonist in combination with β_1 -AR blocker also resulted in preservation of β_1 -AR density and responsiveness of β_2 -AR to stimulation, in which reduction was observed in DCM rats (Ahmet et al., 2008). The fact that the treatment duration in the present study lasted for 12 months is a very important feature of the present study.

The present study extended the series of our previous reports (Ahmet et al., 2004, 2005, 2008) demonstrating therapeutic effectiveness of combined therapy of a β_1 -AR blocker and a β_1 -AR agonist in the rat model of post-MI DCM. In this study, our goal was to specifically compare the beneficial effects of combined β -AR therapy with a combination treatment of a β_1 -AR blocker and an ACE inhibitor, a standard

TABLE 2

Hemodynamic indices in SH rats and treated and untreated rats 12.5 months after MI

	SH (n = 5)	nT (n = 3)	β 1-ACEi (n = 6)	β 1- β 2+ (n = 11)	β 1- β 2+ACEi (n = 8)
HR (beats/min)	263 $\pm$ 13	266 $\pm$ 21	209 $\pm$ 15*	197 $\pm$ 13 ^a	201 $\pm$ 11 ^a
EDV (μ l)	474 $\pm$ 18	1169 $\pm$ 120*	991 $\pm$ 119*	963 $\pm$ 35 ^a	868 $\pm$ 85*
ESV (μ l)	287 $\pm$ 18	1110 $\pm$ 115*	880 $\pm$ 128*	828 $\pm$ 47 ^a	750 $\pm$ 97*
SV (μ l)	242 $\pm$ 9	135 $\pm$ 27*	163 $\pm$ 23*	195 $\pm$ 12 ^a	185 $\pm$ 20*
CO (ml/min)	63 $\pm$ 3	35 $\pm$ 8*	37 $\pm$ 8*	43 $\pm$ 4*	39 $\pm$ 5*
EF (%)	49 $\pm$ 1	12 $\pm$ 3*	18 $\pm$ 5*	20 $\pm$ 1 ^a	28 $\pm$ 3*
ESP (mm Hg)	106 $\pm$ 14	77 $\pm$ 4	90 $\pm$ 4	102 $\pm$ 6 ^a	80 $\pm$ 6 ^b
EDP (mm Hg)	3.0 $\pm$ 0.8	6.1 $\pm$ 2.1	2.8 $\pm$ 1.1	3.2 $\pm$ 0.4 ^a	2.4 $\pm$ 0.3 ^a
Ea (mm Hg/ μ l)	0.43 $\pm$ 0.07	0.70 $\pm$ 0.08*	0.59 $\pm$ 0.07	0.52 $\pm$ 0.05	0.46 $\pm$ 0.07
(+)dP/dt (mm Hg/s)	7079 $\pm$ 1134	2942 $\pm$ 54*	4649 $\pm$ 448 ^a	4943 $\pm$ 337 ^a	4142 $\pm$ 525*
(-)dP/dt (mm Hg/s)	7149 $\pm$ 1288	2362 $\pm$ 161*	4098 $\pm$ 622*	4046 $\pm$ 307 ^a	3766 $\pm$ 472*
τ (ms)	11.5 $\pm$ 0.8	15.0 $\pm$ 1.3*	16.8 $\pm$ 1.6*	16.8 $\pm$ 0.6*	15.0 $\pm$ 0.6*
PRSW (mW/ μ l)	84.6 $\pm$ 6.3	15.2 $\pm$ 1.8*	49.4 $\pm$ 5.1 ^a	48.9 $\pm$ 3.3 ^a	36.6 $\pm$ 3.0 ^{a,b}
Ees (mm Hg/ μ l)	0.39 $\pm$ 0.09	0.48 $\pm$ 0.14	0.50 $\pm$ 0.09	0.52 $\pm$ 0.06	0.44 $\pm$ 0.09
Eed (10 ³ mm Hg/ μ l)	7 $\pm$ 2	25 $\pm$ 4*	6 $\pm$ 1 ^a	9 $\pm$ 2 ^a	7 $\pm$ 1 ^a
dP/dt-EDV (mm Hg/s/ μ l)	24.6 $\pm$ 5.8	6.3 $\pm$ 3.1*	14.9 $\pm$ 3.0	14.6 $\pm$ 2.1*	17.4 $\pm$ 1.4 ^a
Ea/Ees	1.23 $\pm$ 0.14	2.02 $\pm$ 1.01	1.38 $\pm$ 0.29	1.13 $\pm$ 0.15	1.40 $\pm$ 0.36

CO, cardiac output; EDP, end-diastolic pressure; ESP, end-systolic pressure; HR, heart rate; SV, stroke volume.

* $p < 0.05$ vs. SH.^a $p < 0.05$ vs. nT.^b $p < 0.05$ vs. β 1- β 2+.

and widely used regimen in clinical practice. Beneficial effects of β 1-AR blocker-ACE inhibitor combination in experimental model of post-MI CHF had been reported previously (Hügel et al., 1999; Fedorov et al., 2006). In this 1-year-long study, we demonstrated that DCM rats treated with combined β 1-AR blocker- β 2-AR agonist therapy showed 37% increase in survival compared with untreated rats (triple combination of β 1-AR blockade-ACEi- β 2-AR agonist showed 25% improvement)—a survival benefit that did not exceed a standard therapy (an improvement of 22%) but was at least equal to it. However, with respect to cardiac remodeling and MI expansion, the addition of β 2-AR agonist to a therapy (β 1- β 2+ or β 1- β 2+ACEi) clearly exceeded the effectiveness of β 1-AR blocker-ACE inhibitor combination. Specifically, although the β 1-AR blocker-ACE inhibitor combination did attenuate the infarct expansion observed in untreated animals, combined β -AR therapy actually arrested the MI expansion, revealing a statistically significant improvement over the β 1-AR blocker-ACE inhibitor combination. Although standard β 1-AR blocker-ACE inhibitor combination attenuated LV expansion, the addition of a β 2-AR agonist made the treatment significantly more effective during the first 6 months of treatment. Furthermore, the combinations including a β 2-AR agonist were twice as effective as β 1-AR blocker-ACE inhibitor combination in attenuating the functional decline. In addition, although a standard therapy was effective in attenuating the LV mass increase, the β -AR combined treatment was effective in attenuating the thinning of posterior wall, which has been interpreted to reflect myocyte cell death (Anversa et al., 1998). It is important to note that the addition of β 2-AR agonist to the standard therapy of β 1-AR blocker and ACE inhibitor significantly increased the effectiveness of a standard therapy, with respect to LV remodeling and MI expansion; the triple therapy was not more effective than the β 1-AR blocker- β 2-AR agonist combination.

The mechanisms of therapeutic effectiveness of ACE inhibitors in the CHF have been well characterized previously (Wollert and Drexler, 1999; Anand and Florea, 2008; Werner et al., 2008) and encompass numerous signaling pathways from blocking the angiotensin II to suppression of transforming growth factor- β to inhibition of matrix metalloproteinase

activity (Ma et al., 2001; Watanabe et al., 2004; Brower et al., 2007). The mechanisms of cardioprotection by β 2-AR agonist have been investigated in many studies on isolated cardiomyocytes that discovered its marked antiapoptotic effects related to activation of G_i signaling (Communal et al., 1999; Chesley et al., 2000; Zhu et al., 2001; Shizukuda and Buttrick, 2002; Xiao et al., 2004). We believe that this antiapoptotic effect of β 2-AR stimulation was responsible for prevention of cell loss and thus for attenuation of posterior wall thinning in double and triple therapy groups of the present experiment. Because triple therapy (β 1-AR blocker + β 2-AR agonist + ACE inhibitor) was not more effective in our experiment than double therapy with β 1-AR blocker + β 2-AR agonist, it is necessary to conclude that the primary mechanism responsible for the superior attenuation of LV remodeling in the present experiment is a mechanism of double action of β 1-AR blocker and β 2-AR agonist. This mechanism was elucidated in our previous study (Ahmet et al., 2008), i.e., preservation of β 1-AR density and responsiveness to β 2-AR stimulation. Although coupling of β -AR subtypes to specific signaling pathways can be species specific and vary from mouse to rat to humans (Port and Bristow, 2001), the effectiveness of therapies that are now clinically accepted for humans has been demonstrated in animal experimental models. Moreover, successful use of β 2-AR agonist clenbuterol in conjunction with left ventricular assist device to help to attenuate myocardial atrophy before heart transplantation (Hon and Yacoub, 2003) suggests that the translation of current finding to clinical practice seems rational.

In summary, the therapeutic utility of combined treatment with β 1-AR blocker and β 2-AR agonist has been demonstrated in our previous studies in the rat model of post-MI DCM. In the present preclinical study, in the same experimental model, we showed that inclusion of β 2-AR agonist in treatment regimen as double (β 1-AR blocker- β 2-AR agonist) or triple (β 1-AR blocker- β 2-AR agonist-ACEi) therapy exceeds therapeutic effectiveness of combination of β 1-AR blocker and ACE inhibitor. Thus, the β 1-AR blocker- β 2-AR agonist combination alone or in combination with ACEi warrants detailed clinical investigation as a treatment for CHF.

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