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β_2 AR Agonists in Treatment of Chronic Heart Failure: Long Path to Translation

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Abstract

The main clinical manifestations of advanced chronic heart failure (CHF), e.g. in dilated cardiomyopathy (DCM), are reduced systolic and diastolic functions, increased arterial elastance and arterio-ventricular uncoupling, accompanied and exacerbated by an excessive sympathetic activation and extensive abnormalities in the β AR signaling. Loss of cardiomyocytes due to apoptosis is one mechanism that undoubtedly contributes to cardiac remodeling and functional deterioration associated with dilated cardiomyopathy (DCM). Research during the last decade on the single cardiomyocyte level strongly suggested that selective stimulation of β_1 AR activates the proapoptotic signaling pathways, while selective stimulation of β_2 AR is antiapoptotic, but its precise mechanisms remain to be elucidated. Extensive research in the rat model of DCM following induction of myocardial infarction (MI) showed that prolonged treatment with of β_2 AR agonist, fenoterol, in combination with a β_1 AR blocker, metoprolol, is more effective than β_1 AR blocker alone and as effective as β_1 AR blocker with ACE inhibitor with respect to survival and cardiac remodeling. This combined regimen of β_2 AR agonists and a β_1 AR blocker might be considered for clinical testing as alternative or adjunct therapy to currently acceptable CHF arsenal.

Keywords

Chronic heart failure; beta adrenergic receptors; β_2 adrenergic receptor agonists; cardiac remodeling

β_2 adrenergic receptor (AR), one of the first receptors identified by radioligand bindings, belongs to a family of G-protein-coupled receptors and plays important parts in pulmonary and cardiovascular physiology. Additionally, it is also expressed in skeletal muscles, digestive system, eye, uterus, and urinary bladder [1].

β_2 adrenergic receptor (AR) agonists are a necessary part of a long established treatment regimen for bronchial asthma and other pulmonary obstructive conditions [for review see 2,3]. The most often used drugs are a short-acting preparation, albuterol and long-acting, salmeterol. Numerous animal studies indicate that chronic stimulation of β_2 AR at high

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doses can elicit anabolic effects, and research continues with respect to a potential therapeutic application of β_2 AR agonists for skeletal muscle wasting disorders, including neuromuscular diseases, aging and several metabolic conditions [for review see 4]. Additionally, a β_2 AR agonist, clenbuterol is used in conjunction with left ventricular (LV) assist devices and reportedly promotes reverse remodeling [5,6]. Clenbuterol is also widely used as performance enhancer, especially by body builders [7]. Here we review potential therapeutic applications of β_2 AR agonists for chronic heart failure (CHF).

The main clinical manifestations of advanced CHF, e.g. in dilated cardiomyopathy (DCM), are reduced systolic and diastolic functions, increased arterial elastance and arterio-ventricular uncoupling, accompanied and exacerbated by an excessive sympathetic activation [8,9] and extensive abnormalities in the β AR signaling. Sustained pharmacological augmentation of cardiac function using β_1 AR stimulation failed to produce a satisfactory therapeutic effect due to desensitization and reduction in density of β_1 AR receptors in the DCM heart [10–13]. Moreover, down-regulation of β_1 AR is considered to be a protective response against excessive sympathetic stimulation [14,15]; thus β_1 AR activation is contra-indicated in CHF [16–18]. In contrast to β_1 AR, the density and responsiveness of β_2 AR does not seem to change in DCM [10,19], providing some rational for stimulation of β_2 AR to alleviate the progression of CHF. But an interest in using β_2 AR agonists as a therapy for CHF patients, originally expressed in early 1980s [20,21], was short-lived: mounting evidence that β -blockers prolong life of DCM patients and reverse cardiac remodeling [22–26] made the use of β -blockers a mandatory part of the drug arsenal in managing CHF [27,28], and with that the idea of activation of any β AR subtype became counter intuitive.

Mechanistic basis for using β_2 AR for treatment of CHF

Loss of cardiomyocytes due to apoptosis is one mechanism that undoubtedly contributes to cardiac remodeling and functional deterioration associated with DCM [29–31]. Three major advances in research during the last decade on the single cardiomyocyte level have demonstrated distinctly different, even opposite functional roles of two β AR subtypes in regulating cardiac structure and function: (1) dual coupling of most β_2 AR to both G_s and G_i proteins in cardiomyocytes [32–34]; (2) a cardiac protective role of β_2 AR signaling in improving cardiac function and myocyte viability [35,36]; (3) PKA-independent, CaMKII-mediated β_1 AR apoptotic and maladaptive remodeling signaling in the heart [37–39] (Fig. 1). These findings strongly suggest that selective stimulation of β_1 AR activates the proapoptotic signaling pathways, while selective stimulation of β_2 AR is antiapoptotic [35–38]. These findings have rekindled an interest in exploring possibilities of pharmacological stimulation of β_2 AR for treatment of CHF.

Short-term chronic experiments in the rat model of DCM

We provided the initial test of the idea of using β_2 AR agonists in the rat model of DCM following induction of myocardial infarction (MI) in 2004 [40]. Six weeks of treatment with fenoterol (250 μ g/kg/day) or zinterol (1 mg/kg/day in drinking water) starting 2 weeks after the induction of MI by permanent ligation of descending coronary artery reduced left ventricle (LV) dilation, improved LV function and actually arrested the MI expansion. The myocyte apoptosis, assessed at the termination of the study, was significantly reduced. Importantly, the beneficial effects on LV remodeling in animals treated with β_2 AR agonists exceeded those treated with β_1 AR blocker (250 mg/kg/day). While results of this experiment suggested that the entire class of β_2 AR agonists might be beneficial in this experimental model of DCM, experiments on single cardiomyocytes indicate that there could be differences in their efficacies in activating distinct G proteins. Mechanistic studies

have revealed that the additional G_i coupling of β_2 AR delivers a G_s -independent cardioprotective signal through the G_i - $G\beta\gamma$ -PI3K-Akt pathway, which not only counteracts the G_s -mediated apoptotic effect but also protects cells from a variety of apoptosis-triggering assaults (Fig. 1). On the other hand, a β_2 AR agonist, fenoterol, apparently does not couple to G_i , yet has powerful antiapoptotic effect in cardiomyocytes. While additional studies are required to elucidate how fenoterol exerts its antiapoptotic effects in myocytes, it is obviously cardioprotective in vivo. Thus, in our following experiments we concentrated exclusively on fenoterol, a β_2 AR agonist approved for treatment of bronchial asthma in Europe and Canada and marketed there under brand name Berotec (Boehringer-Ingelheim). The LD_{50} for fenoterol in rats had been reported to range 2.5 to 3 g/kg for an oral dose and 42 to 76 mg/kg for intravenous delivery [41]. The dose used in our studies, 250 μ g/kg/day, was 1500 times lower.

Potential chronotropic and proarrhythmic effects [42–44] seem to be an obstacle in the development of the strategy to employ β_2 AR agonists in patients with CHF. These undesirable effects are also known to bodybuilders who use β_2 AR agonist, clenbuterol to gain body mass. There is also an extensive experimental literature on unfavorable effects of long-term use of β_2 AR agonists on the heart [45–50], and some clinical reports of increased mortality related to fenoterol treatment of bronchial asthma [51–53]. An oral dose of 600 mg/kg/day of fenoterol is lethal for rats after more than 30 days of consumption [54]. However, all experimental data on the deleterious effects of β_2 AR agonists on the heart noted above were associated with very high doses of drugs, at least 5–6 fold in excess of those used in our experiments in rats to treat DCM [45–50]. Clinical reports of increased mortality associated with the use of a bronchodilating β_2 AR agonist described above also revealed a drug concentration in the serum greatly exceeding recommended therapeutic doses [55]. We did not observe any adverse chronotropic or arrhythmic effects of fenoterol in our experiment. Nevertheless, to preclude any possibility of these effects, in the next set of experiments we combined a β_2 AR agonist, fenoterol with a β_1 AR blocker, metoprolol.

In the same rat experimental model described above we observed that addition of a β_2 AR agonist, fenoterol to a β_1 AR blocker, metoprolol significantly improved the outcome of a single β_1 AR blocker therapy with respect to LV remodeling, infarct expansion, cardiomyocyte hypertrophy and arterio-ventricular mismatch [56]. Only the combined β_2 AR agonist + β_1 AR blocker regimen improved diastolic function and enhanced the antiapoptotic effect of metoprolol. The superior beneficial effects of combined therapy were associated with a vasodilatory effect of fenoterol. The heart rhythm was analyzed during serial echocardiography tests, and neither an increased heart rate nor arrhythmias were observed. These results of beneficial therapeutic effects of the combined β_2 AR agonist + β_1 AR blocker regimen were confirmed later on another model of CHF induced by isoproterenol infusion [57].

Long-term chronic experiments in the rat model of DCM

In the final analysis a main measure of therapeutic efficacy of the treatment for CHF is its effect on mortality. In our next study [58] we extended the treatment duration in the same experimental model of rat post MI DCM for 1 year. This study compared the efficacy of combined β_1 AR blocker + β_2 AR agonist (fenoterol+metoprolol) therapy and single therapy of either β_1 AR blocker or β_2 AR agonist, with mortality as a primary outcome. The one-year mortality of 67% observed in this experiment among untreated animals was reduced by 34% in combined therapy group, which exceeded the survival benefit of either single therapy (Fig. 2). The beneficial effects of combined therapy on cardiac remodeling and function also exceeded the effects of either single therapy. While MI expansion was attenuated by single therapies, the combined therapy arrested the MI expansion (Fig. 3).

Moreover, in this study we confirmed the previously reported observation that the density of β_1 AR, but not β_2 AR in LV myocardium was reduced with progression of DCM. The reduction of β_1 AR density in LV myocardium was prevented by combined fenoterol-metoprolol therapy.

Since standard therapy for advanced CHF includes RAS inhibition, in another arm of our study we compared the combined (fenoterol+metoprolol) therapy with a combination of β_1 AR blocker and angiotensin-converting enzyme inhibitor [59]. In our model of DCM the combined therapy of β_1 AR blocker and β_2 AR agonist was equipotent to a standard therapy for CHF with the respect to mortality and exceeded the latter with respect to cardiac remodeling and MI expansion.

Long term experiments revealed an additional important finding – prolonged single therapy with fenoterol resulted in tachyphylaxis [58]. All beneficial effects of a single treatment with fenoterol demonstrated in our initial study of 6 weeks in duration [40] were again observed in the 1-year long study, but lasted only during the first 3 months of therapy, after which time the sonographic indices of LV dilatation and function as well as the rate of MI expansion approached that of untreated animals, despite continued treatment with the β_2 AR agonist alone. The loss of efficacy after prolonged single therapy with fenoterol was associated with a reduction of myocardial β_2 AR density, which is usually not reduced in untreated DCM animals [10,19,58]. Importantly, the tachyphylaxis and reduction of β_2 AR density were not observed when therapy with fenoterol was combined with β_1 AR blocker, metoprolol [58,59].

Carvedilol paradox and other considerations

Great effectiveness of combined β_1 AR blocker + β_2 AR agonist (fenoterol+metoprolol) therapy demonstrated in the rat model of post MI dilated cardiomyopathy is somewhat controversial in light of a proven clinical success of carvedilol, a non-selective β_1 β_2 AR blocker, in treatment of CHF patients: while the effectiveness of carvedilol is contributed to the effect of β_2 AR blockade [60], the effectiveness of the fenoterol+metoprolol combination we interpret as a results of β_1 AR blockade and antiapoptotic effect of β_2 AR stimulation. Obviously, the therapeutic effect of these two therapeutic strategies is achieved through different mechanisms the same way as similar survival outcomes were shown in our experiments with respect to two mechanistically different therapies: β_1 AR blocker + β_2 AR agonist combination and β_1 AR blocker + ACE inhibitor combination [59]. Interestingly, both these therapeutic strategies, as well as carvedilol, are accompanied by a vasodilatation, which unloading effect is undoubtedly a contributing factor to their favorable outcomes.

Fenoterol exists as four stereoisomers. Combination of two of them, RR and SS are a racemic mixture (Fig. 4), which represents an actual drug that had been used clinically for treatment of asthma and for our experiments cited above (1,2,39,55–58). In order to refine the therapeutic effectiveness of fenoterol in the CHF model, its stereoisomers have been synthesized and tested with respect to their binding ability to β_2 AR, selectivity, and functional activity [61]. Experiments in isolated rat cardiomyocytes show that the RR isomer of fenoterol exhibits more potent effects on cardiomyocyte shortening and better Gs protein selectivity than other isomers [62,63]. Thus the RR stereoisomer of fenoterol might be a promising new drug. The efficacy of the RR stereoisomer of racemic fenoterol merits testing in whole animal experimental models of DCM.

In conclusion, evidence from experiments on single cardiomyocytes as well as preclinical testing on rat experimental model of post MI DCM indicate that a combination of β_2 AR agonists and a β_1 AR blocker is more effective than β_1 AR blocker alone and as effective as β_1 AR blocker with ACE inhibitor with respect to treatment of DCM. This combined

regimen of β_2 AR agonists and a β_1 AR blocker might be considered for clinical testing as alternative or adjunct therapy to currently acceptable CHF arsenal.

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Figure 1. Subtype-specific G protein coupling and function of β_1 AR and β_2 AR in cardiac myocytes. β_2 AR has dual coupling to G_s and G_i , which activates cell survival signals by the G_s -cAMP-PKA-Akt pathway and the G_i -Gbg-PI3K-Akt pathway, respectively. In contrast, β_1 AR couples exclusively to G_s , which activates PKA-independent, CaMKII-mediated apoptotic signaling. (ECC: excitation-contraction coupling).

Figure 2.

Kaplan-Meier survival curves after induction of MI in a sham-operated group (Sh), an untreated group (nT), and three different treatment groups. MI was induced by a permanent ligation of the left descending coronary artery. Treatment was started 2 weeks after induction of MI. The number of animals at the beginning of treatment was as follows: SH, 10; and nT, β_1 -ACEi, β_1 - β_2 +, and β_1 - β_2 +ACEi, 27 in each. (Ahmet et al, 2009, J Pharmacol Exp Ther, 331: 178–85).

Figure 3.

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. Treatment was started 2 weeks after induction of MI. (Ahmet et al, 2009, J Pharmacol Exp Ther, 331: 178–85).

Figure 4. Fenoterol compound has two chiral centers labeled as R (top structure) and S (bottom structure). Fenoterol used as drug is a 50/50 mixture of the 2 enantiomers shown. (Beigi et al, 2006, Chirality, 18: 822–7).