

Cardiac β ARK1 inhibition prolongs survival and augments β blocker therapy in a mouse model of severe heart failure

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Chronic human heart failure is characterized by abnormalities in β -adrenergic receptor (β AR) signaling, including increased levels of β AR kinase 1 (β ARK1), which seems critical to the pathogenesis of the disease. To determine whether inhibition of β ARK1 is sufficient to rescue a model of severe heart failure, we mated transgenic mice overexpressing a peptide inhibitor of β ARK1 (β ARKct) with transgenic mice overexpressing the sarcoplasmic reticulum Ca^{2+} -binding protein, calsequestrin (CSQ). CSQ mice have a severe cardiomyopathy and markedly shortened survival (9 ± 1 weeks). In contrast, CSQ/ β ARKct mice exhibited a significant increase in mean survival age (15 ± 1 weeks; $P < 0.0001$) and showed less cardiac dilation, and cardiac function was significantly improved (CSQ vs. CSQ/ β ARKct, left ventricular end diastolic dimension 5.60 ± 0.17 mm vs. 4.19 ± 0.09 mm, $P < 0.005$; % fractional shortening, 15 ± 2 vs. 36 ± 2 , $P < 0.005$). The enhancement of the survival rate in CSQ/ β ARKct mice was substantially potentiated by chronic treatment with the β AR antagonist metoprolol (CSQ/ β ARKct non-treated vs. CSQ/ β ARKct metoprolol treated, 15 ± 1 weeks vs. 25 ± 2 weeks, $P < 0.0001$). Thus, overexpression of the β ARKct resulted in a marked prolongation in survival and improved cardiac function in a mouse model of severe cardiomyopathy that can be potentiated with β -blocker therapy. These data demonstrate a significant synergy between an established heart-failure treatment and the strategy of β ARK1 inhibition.

The β -adrenergic receptor (β AR) signaling pathway is one of the key pathways regulating cardiac function. However, chronic stimulation of β ARs, which occurs in heart failure, leads to chronic desensitization and impaired β AR responsiveness. This process of agonist-induced β AR desensitization requires phosphorylation of the agonist-occupied receptor by the cytosolic enzyme β AR kinase 1 (β ARK1), which is recruited to the plasma membrane through its interaction with dissociated membrane-bound $\text{G}\beta\gamma$ -subunits (1).

Although the molecular mechanisms involved in the pathological progression to decompensated heart failure are not well understood, a leading candidate is impaired β AR signaling. Abnormalities in β AR signaling that characterize human heart failure include a 50% reduction in β AR density selectively involving the β_1 AR subtype, marked uncoupling of remaining β_1 and β_2 ARs, and an ≈ 3 -fold increase in β ARK1 levels and activity (2, 3).

Whether down-regulation and desensitization of β AR function are adaptive or maladaptive in the failing heart remains controversial. In this regard, an important role of β ARK1 in the pathogenesis of heart failure was recently demonstrated in a mouse model of cardiomyopathy wherein mice overexpressing a cardiac-targeted peptide inhibitor of β ARK were significantly protected from the development of myocardial failure (4). To inhibit the β ARK- $\text{G}\beta\gamma$ interaction, a strategy of $\text{G}\beta\gamma$ sequestration was achieved by overexpression of the C-terminal 194 aa of β ARK1 (β ARKct), which effectively inhibits the action of β ARK1 and augments β AR responsiveness (5). In a similar approach, β ARKct expression through adenoviral gene delivery

in rabbit hearts at the time of myocardial infarction significantly delayed the development of heart failure (6). Thus, β ARKct expression and subsequent β ARK1 inhibition seem to positively affect the failing heart.

Because sudden cardiac death is a prominent feature of the clinical syndrome of human heart failure, we wanted to test whether normalizing β AR function through β ARK inhibition would improve survival. To test this possibility, we used a model of severe heart failure generated by cardiac overexpression of the sarcoplasmic reticulum Ca^{2+} -binding protein calsequestrin (CSQ); this model is characterized by an aggressive phenotype of dilated cardiomyopathy and premature death by 16 weeks of age (7, 8). In addition to using a β ARKct transgenic mouse crossbreeding strategy to attempt to rescue CSQ heart-failure mice, we tested how β ARK1 inhibition compared with chronic β AR-antagonist treatment—a standard therapy for heart failure—and whether β ARK1 inhibition could provide additional benefit to β -blocker therapy.

Methods

Experimental Animals. Transgenic mice overexpressing either CSQ or the β ARKct peptide were generated as described (5, 7). Briefly, full-length canine cardiac CSQ cDNA was fused to the α -myosin heavy-chain promoter to drive cardiac-targeted expression. For the β ARKct, the coding sequence for the last 194 aa of bovine β ARK1 was fused to the α -myosin heavy-chain promoter. F₁ pups were generated from the crossbreeding of CSQ transgenic mice with β ARKct transgenic mice. Wild-type, CSQ transgenic, β ARKct transgenic, or CSQ/ β ARKct hybrid transgenic littermates of either sex were used for all studies.

In separate experiments, mice were chronically treated with the selective β_1 AR antagonist metoprolol (350 mg/kg of body weight per day) administered in the drinking water (2 mg/ml) starting from 1 week of age and continuing until death. The animals in this study were handled according to approved protocols and the animal welfare regulations at Duke University.

Transthoracic Echocardiography. Serial echocardiography was performed on conscious mice with an HDI 5000 echocardiograph (ATL Ultrasound, Bothell, WA) at 7 and 11 weeks of age, as described (9).

Cardiac Catheterization. Hemodynamic evaluation in intact closed-chest anesthetized mice was performed as described (4).

Abbreviations: β AR, β -adrenergic receptor; β ARK1, β AR kinase 1; β ARKct, C-terminal of β ARK1; CSQ, calsequestrin; LV, left ventricular; MLP, muscle LIM protein.

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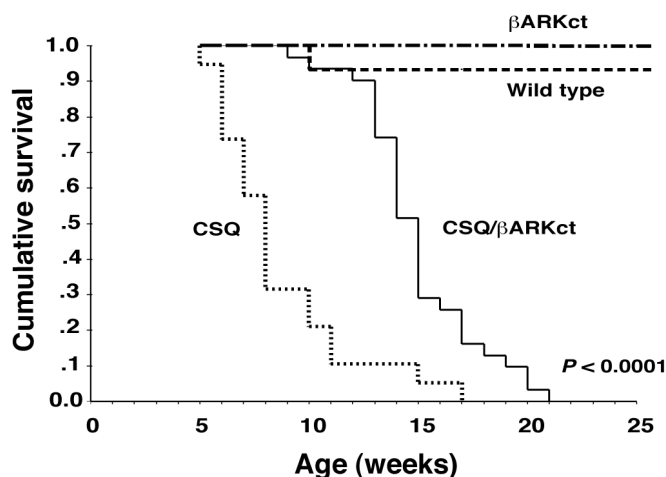


Fig. 1. Survival analysis in CSQ/ β ARKct mice and CSQ mice. Kaplan–Meier survival analysis was used to determine the survival probability between the different genotypes of mice from the CSQ- β ARKct cross. Mean survival age of the CSQ mice was 9 ± 1 weeks vs. 15 ± 1 weeks in the CSQ/ β ARKct mice ($P < 0.0001$). Wild type, $n = 15$; β ARKct, $n = 23$; CSQ, $n = 14$; CSQ/ β ARKct, $n = 31$.

Membrane Preparation and Immunoblotting. Briefly, left ventricles were homogenized and sarcolemmal membranes were prepared as described (5, 10). Cytosolic extracts were then used for immunoblotting; membranes were used in the adenylyl cyclase, β AR density, and β AR competition binding assays. For protein expression, $100 \mu\text{g}$ (β ARK1 and β ARKct) or $10 \mu\text{g}$ (CSQ) of cytosolic extracts was immunoblotted by using rabbit polyclonal antibodies raised against either an epitope on the C terminus of GRK2 (1:500) (Santa Cruz Biotechnology) or CSQ (1:2000) (L.R.J.), β ARK1 protein levels were quantified by densitometry.

Adenylyl Cyclase Activity, β AR Density, and Radioligand Binding. For cyclase activity, membranes ($15 \mu\text{g}$ of protein) were incubated for 20 min at 37°C with [α - ^{32}P]ATP under basal conditions or indicated agonists and cAMP was quantified (5, 10).

Competition binding isotherms in sarcolemmal membranes ($25 \mu\text{g}$) were done in triplicate with 12 concentrations of isoproterenol (10^{-10} M to 10^{-4} M). Assays were performed at 37°C for 60 min (5, 10). Competition isotherms were analyzed by

nonlinear least-square curve fit to determine the percentage of β ARs in a high-affinity state (GraphPad PRISM).

Statistical Analysis. Data are expressed as mean \pm SE. Survival data were analyzed by using a Kaplan–Meier survival analysis with a log rank method of statistics. Statistical significance for echocardiographic variables was performed with a one-way ANOVA for 7-week data and a repeated-measures ANOVA for the serial echocardiographs. β ARK1 levels were compared with an unpaired Student's t test.

Results

To determine whether normalizing β AR function by inhibiting the β ARK- $G\beta\gamma$ interaction would improve survival in heart failure, we crossed transgenic mice with cardiac-targeted overexpression of the β ARKct with transgenic mice overexpressing CSQ. Single transgenic CSQ mice and binary transgenic CSQ/ β ARKct mice were monitored for survival and compared with their wild-type littermates. Whereas the CSQ mice had a mean survival of only 9 ± 1 weeks, the CSQ/ β ARKct mice showed a significant increase in lifespan, with a mean survival age of 15 ± 1 weeks ($P < 0.0001$; Fig. 1). This result demonstrates that β ARK1 inhibition has a significant positive effect on survival.

To determine whether overexpression of the β ARKct would affect the dilated cardiomyopathy phenotype in the CSQ mice, transthoracic echocardiography was performed in conscious mice at 7 weeks of age. Compared with wild-type and single β ARKct transgenic mice, CSQ mice have enlarged cardiac chambers, as shown by the increased left ventricular (LV) end-diastolic and end-systolic dimensions, and severe cardiac dysfunction, as shown by the markedly reduced fractional shortening and mean velocity of circumferential fiber shortening (mVcfc) (Table 1 and Fig. 2 *A* and *B*). In contrast, the CSQ/ β ARKct mice had significantly less cardiac dilation (25%, $P < 0.0001$) and significantly improved cardiac function (2-fold, $P < 0.0001$), as compared with their CSQ littermates.

To further evaluate the effects of β ARKct expression on the progression of cardiac failure in CSQ mice, serial echocardiography was performed in the surviving transgenic and wild-type mice. Individual data points are plotted for LV end diastolic dimension and fractional shortening from echocardiograms recorded at 7 and 11 weeks. Cardiac function in CSQ mice was severely depressed at 7 weeks and did not significantly change from 7 to 11 weeks (Fig. 2 *C* and *D*); however, most CSQ mice

Table 1. Echocardiographic and physiologic parameters at 7 weeks of age

| Parameter | Wild type $n = 15$ | β ARKct $n = 23$ | CSQ $n = 14$ | CSQ/ β ARKct $n = 31$ | CSQ (metoprolol) $n = 18$ | CSQ/ β ARKct (metoprolol) $n = 18$ |
|----------------------------|-----------------------|---------------------------|-------------------|--------------------------------|---------------------------------|--|
| LVEDD, mm | 2.98 ± 0.10 | 3.26 ± 0.08 | $5.60 \pm 0.17^*$ | $4.19 \pm 0.09^{+\S}$ | $5.15 \pm 0.14^{\parallel}$ | $4.01 \pm 0.12^{\parallel}$ |
| LVESD, mm | 1.02 ± 0.07 | 1.26 ± 0.07 | $4.79 \pm 0.25^*$ | $2.71 \pm 0.11^{+\S}$ | $4.03 \pm 0.18^{\parallel}$ | $2.46 \pm 0.15^{\parallel}$ |
| FS, % | 66 ± 2 | 61 ± 1 | $15 \pm 2^*$ | $36 \pm 2^{+\S}$ | $22 \pm 2^{\parallel}$ | $39 \pm 2^{\parallel}$ |
| SEpth, mm | 0.78 ± 0.04 | 0.76 ± 0.02 | $0.55 \pm 0.02^*$ | $0.75 \pm 0.03^{+\S}$ | $0.63 \pm 0.03^{\parallel}$ | 0.70 ± 0.03 |
| PWth, mm | 0.80 ± 0.06 | 0.76 ± 0.03 | $0.76 \pm 0.03^*$ | $0.79 \pm 0.03^{+\S}$ | 0.62 ± 0.02 | $0.73 \pm 0.02^{\parallel}$ |
| HR, beats/min | 565 ± 11 | 588 ± 16 | 579 ± 33 | 564 ± 14 | 525 ± 8 | 527 ± 16 |
| mVcfc, circ/sec | 4.83 ± 0.16 | 4.68 ± 0.16 | $1.6 \pm 0.18^*$ | $3.02 \pm 0.16^{+\S}$ | $1.79 \pm 0.16^{\parallel}$ | $3.51 \pm 0.24^{\parallel}$ |
| dP/dt_{max} basal | $10,054 \pm 824$ | $10,239 \pm 722$ | $3,443 \pm 404^*$ | $4,812 \pm 347^{\ddagger}$ | | |
| dP/dt_{max} iso | $17,973 \pm 712$ | $20,074 \pm 892$ | $4,641 \pm 589^*$ | $6,261 \pm 345^{\ddagger}$ | | |
| LVW/BW, mg/g | 3.53 ± 0.06 | 3.32 ± 0.05 | $8.53 \pm 0.35^*$ | $6.20 \pm 0.14^{+\S}$ | 7.95 ± 0.15 | $6.75 \pm 0.33^{\parallel}$ |
| (n) | (16) | (11) | (11) | (20) | (17) | (14) |

Analysis of *in vivo* cardiac size and function by echocardiography in gene-targeted mice. LVEDD, LV end diastolic dimension; LVESD, LV end systolic dimension; FS, fractional shortening; SEpth, septal wall thickness; PWth, posterior wall thickness; HR, heart rate; mVcfc, heart rate corrected mean velocity of circumferential fiber shortening; dP/dt_{max} , first derivative of LV pressure; LVW, LV weight; BW, body weight. (n), at bottom of table, the number of animals used for the hemodynamic study and calculation of LVW/BW. *, $P < 0.005$, CSQ vs. wild type; \ddagger , $P < 0.005$; \S , $P < 0.05$ CSQ/ β ARKct vs. CSQ; \parallel , $P < 0.01$, CSQ/ β ARKct vs. CSQ metoprolol; \parallel , $P < 0.05$, CSQ metoprolol vs. CSQ; \parallel , $P < 0.005$, CSQ/ β ARKct metoprolol vs. CSQ metoprolol.

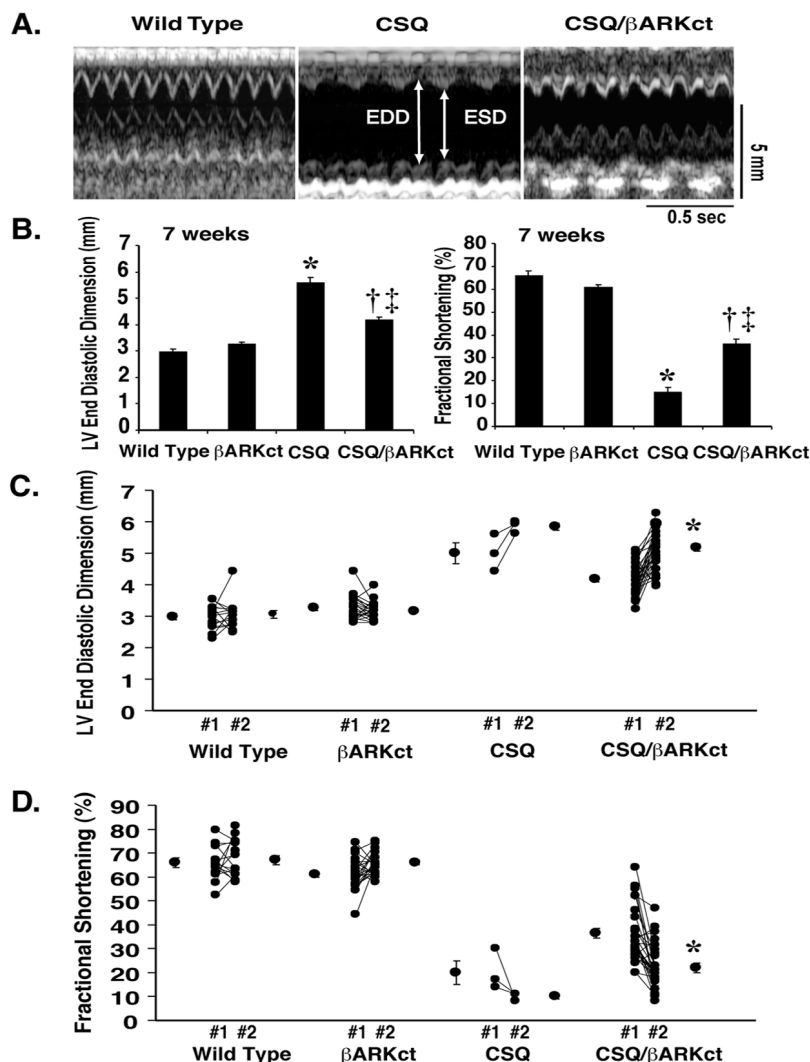


Fig. 2. Analysis of cardiac function by noninvasive echocardiography in conscious mice. (A) Transthoracic M-mode echocardiographic tracings in 7-week-old wild-type (Left), CSQ (Center), and CSQ/βARKct (Right) mice. LV dimensions are indicated with the double-sided arrows. EDD, end diastolic dimension; ESD, end systolic dimension. Wild-type mice have normal chamber size, whereas the CSQ mice have chamber dilation and depressed cardiac function. The CSQ/βARKct mice have only moderate chamber dilation and slightly reduced cardiac function as compared with the wild-type mice. (B) Echocardiographic findings in 7-week-old wild-type and transgenic mice. LV EDD (Left) and percent fractional shortening (Right) are shown. Wild type, $n = 15$; βARKct, $n = 23$; CSQ, $n = 14$; CSQ/βARKct, $n = 31$. *, $P < 0.0001$, CSQ vs. wild type; †, $P < 0.0001$, CSQ/βARKct vs. CSQ; ‡, $P < 0.0001$, CSQ/βARKct vs. wild type. (C) Data from serial echocardiograms in the same mouse at 7 weeks (#1) and 11 weeks (#2) of age for LV EDD. (D) Percent fractional shortening in the same mouse. Wild type, $n = 14$; βARKct, $n = 22$; CSQ, $n = 3$; CSQ/βARKct, $n = 28$. *, $P < 0.001$, CSQ/βARKct (#2) vs. CSQ/βARKct (#1).

did not survive to the 11-week time point because of the already severe stage of myocardial failure in the mice at 7 weeks of age. In contrast, cardiac function in the CSQ/βARKct mice at 7 weeks of age was significantly improved in comparison to CSQ mice, and although there was a decline in cardiac function from 7 to 11 weeks, it was still significantly better in the CSQ/βARKct mice (Fig. 2 C and D). The improvement in function was also associated with a significant decrease in the LV weight/body weight in the CSQ/βARKct mice compared with the CSQ mice (Table 1). These data indicate that the βARKct not only improves survival but also markedly impacts and lessens the progression of cardiac failure in this aggressive model of cardiomyopathy.

To determine whether inhibition of βARK1 through overexpression of the βARKct peptide could restore normal βAR signaling, we evaluated receptor–effector coupling in sarcolemmal membranes from 7-week-old transgenic hearts. Total βAR density and the percentage of receptors exhibiting high-affinity

β-agonist binding were significantly reduced in the CSQ mice as compared with wild-type mice (Table 2 and Fig. 3A). Consistent with lowered βARK1-mediated βAR desensitization, the percentages of βARs in the high-affinity state were restored to normal in the CSQ/βARKct myocardial membranes (Fig. 3A). Postreceptor defects have been well characterized in endstage human heart failure. We found a similar defect in CSQ mice, as shown by isoproterenol- and NaF-stimulated adenylyl cyclase activity in the CSQ mice (Table 2). Interestingly, overexpression of the βARKct did not reverse the abnormality in adenylyl cyclase activity compared with the CSQ mice. These data show that the βARKct functions to prevent agonist-induced phosphorylation and desensitization of βARs to maintain normal receptor/G protein coupling; however, overexpression of the βARKct does not alleviate apparent postreceptor defects in the CSQ mouse.

Because βARK1 protein levels and activity are increased in human heart failure, we sought to determine whether myocardial

Table 2. β AR signaling characteristics

| Mice | β AR density, fmol/mg protein <i>n</i> = 4 | Adenylyl cyclase activity, pmol/min per mg protein <i>n</i> = 5 | | | Fold Iso induction over basal |
|--------------------|--|--|-----------------------------|-------------------------------|-------------------------------|
| | | Basal | Iso 10^{-4} M | NaF | |
| Wild type | 32.8 \pm 5.1 | 24.3 \pm 2.5 | 45.0 \pm 5.0* | 208.8 \pm 34.0 [†] | 1.85 |
| β ARKct | 32.5 \pm 9.4 | 19.3 \pm 1.8 | 35.6 \pm 4.4* | 132.0 \pm 10.0 [†] | 1.85 |
| CSQ | 22.2 \pm 2.5 | 16.6 \pm 1.2 | 22.3 \pm 1.6 [†] | 75.0 \pm 7.0 [†] | 1.34 [§] |
| CSQ/ β ARKct | 23.5 \pm 3.4 | 15.6 \pm 1.2 | 21.9 \pm 1.1 [†] | 84.4 \pm 6.6 [†] | 1.40 [§] |

Iso, isoproterenol. *, $P < 0.01$; †, $P < 0.05$ Iso vs. basal; ‡, $P < 0.0001$ NaF vs. basal; §, $P < 0.05$ CSQ or CSQ/ β ARKct vs. wild type.

β ARK1 levels decrease in the CSQ/ β ARKct mice as compared with the typically high levels in CSQ mice. Cytosolic myocardial β ARK1 protein levels were higher in the CSQ mice than in the wild-type mice (1.3-fold over wild type, $P = 0.05$; $n = 6$), with no significant difference between the CSQ and CSQ/ β ARKct mice (1.3-fold over wild type vs. 1.5-fold over wild type, respectively; $n = 6$) (Fig. 3B). Because of the use of the same promoter in the transgene constructs, CSQ and β ARKct expression levels were monitored for potential promoter competition when expressed together. The CSQ and β ARKct protein levels assessed by immunoblotting were the same in the single vs. binary transgenic animals (Fig. 3B).

Because a marked postreceptor defect persisted in the CSQ/ β ARKct mice and cardiac function still improved, we tested

whether overexpression of the β ARKct would affect β AR-stimulated inotropy. We performed cardiac catheterization in intact anesthetized mice. LV contractility (assessed by LV dp/dt_{max}) at baseline in the CSQ/ β ARKct mice was slightly greater than in the CSQ mice, although it was still significantly less than in wild-type mice (Table 1). As expected, isoproterenol (1 picogram) stimulation gave only a small but significant effect on LV dp/dt_{max} in both the CSQ ($P < 0.05$) and CSQ/ β ARKct ($P < 0.005$) mice, in contrast to the pronounced wild-type response ($P < 0.0001$). These data are consistent with the biochemical data that show a marked postreceptor abnormality.

Recent clinical data in human heart failure have shown that the addition of a β -blocker to standard therapy can significantly improve survival in patients with severe heart failure (11–13). To determine whether β -blocker therapy would act in a synergistic fashion with the β ARKct, we chronically treated all mice from birth with the selective β_1 AR antagonist metoprolol. In a manner consistent with the clinical data, metoprolol also improved survival from 9 ± 1 to 14 ± 1 weeks ($P < 0.0001$) in the CSQ mice (Fig. 4). Remarkably, the combination therapy of β ARKct expression and metoprolol treatment in the CSQ mice gave an ≈ 3 -fold increase in the mean survival age. Thus, there was a dramatic lengthening of survival if CSQ mice were treated with both the β ARK inhibitor and metoprolol. Metoprolol treatment did provide some functional benefit to the CSQ mice, as seen by a small but significant decrease in chamber size ($P < 0.05$) and increase in cardiac function ($P < 0.05$). Importantly, cardiac function in metoprolol-treated CSQ/ β ARKct mice was significantly better than in metoprolol-treated CSQ mice, indicating an important positive effect on long-term cardiac function with the β ARKct (Table 1). Taken together, these data show that the action of β -blockade is synergistic with the action of the β ARKct in failing myocardium.

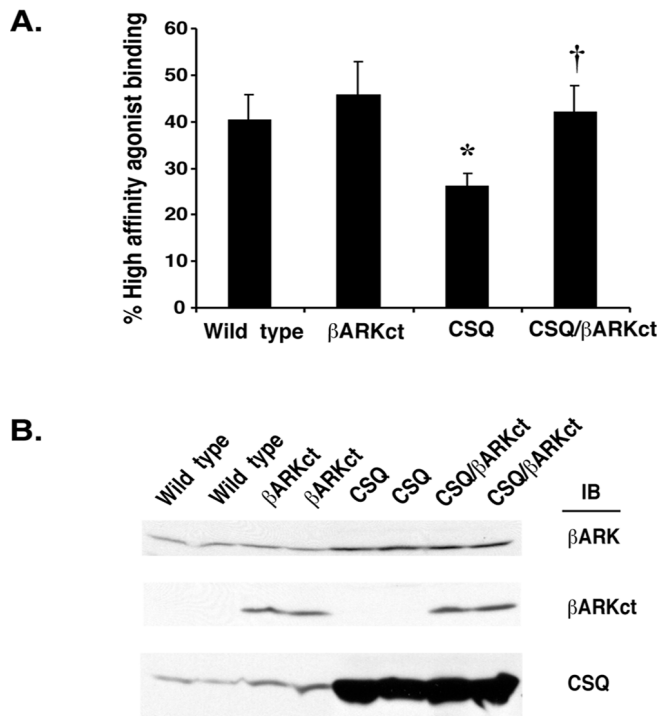


Fig. 3. β ARK1 high-affinity β -agonist binding and β ARK1 protein expression in CSQ and CSQ/ β ARKct mice. (A) Membrane preparations from left ventricles were used to measure % high-affinity β AR binding. *, $P < 0.05$, CSQ vs. wild type; and †, $P < 0.05$, CSQ/ β ARKct vs. CSQ. Wild type, $n = 7$; β ARKct, $n = 7$; CSQ, $n = 8$; CSQ/ β ARKct, $n = 6$. (B) Immunodetection of β ARK1 in cytosolic extracts from wild-type and transgenic hearts at 7 weeks of age. Shown is a representative experiment with two hearts from each gene-targeted mouse. Each heart was immunoblotted (IB) for levels of β ARK1 and expression of the transgenes β ARKct and CSQ. Protein expression was quantitated by densitometry.

Discussion

The present study demonstrates that inhibition of β ARK1 through cardiac-targeted expression of the β ARKct peptide results in a marked improvement in survival in the CSQ model of severe cardiomyopathy. In addition to the significant increase in survival, the β ARKct also was able significantly to improve cardiac function, suggesting a mechanism of action that positively affects (i.e., limits) the progression of the myopathic disease. The receptor uncoupling of β AR from G protein usually seen in heart failure was ameliorated through expression of the β ARKct, although its expression was not able to reverse the defects observed in adenylyl cyclase activity. Most dramatic, however, was the synergistic action of β -blocker therapy with β ARK inhibition, which resulted in a nearly 3-fold increase in survival of the CSQ mice.

Two standard therapies known to increase survival in human heart failure are angiotensin-converting enzyme (ACE) inhibition and β -blocker treatment (14, 15). We demonstrate here that chronic use of the β -blocker metoprolol in addition to β ARK

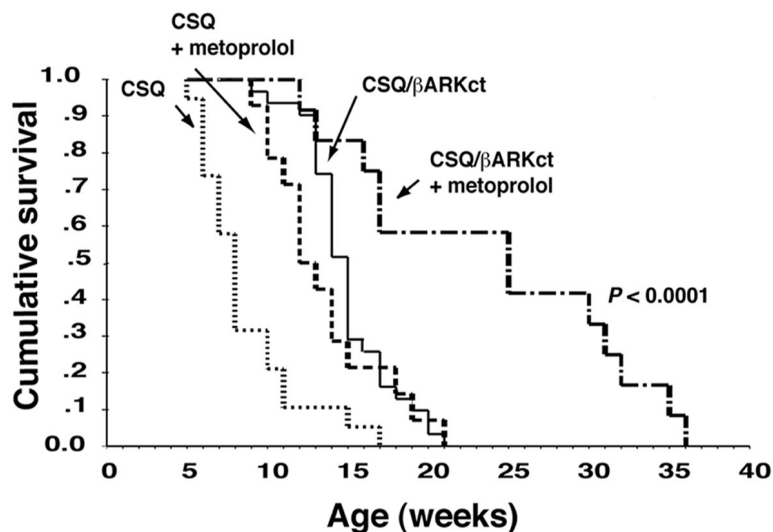


Fig. 4. Survival analysis of the CSQ and CSQ/ β ARKct mice treated with the β -blocker metoprolol. Kaplan–Meier survival analysis was used to determine the survival probability between the specified genotypes of mice while chronically treated with metoprolol in the drinking water. Mean survival age of the CSQ mice ($n = 14$) receiving no drug was 9 ± 1 weeks vs. 14 ± 2 weeks in the metoprolol-treated CSQ mice ($n = 14$; $P < 0.0005$). Mean survival age of the CSQ/ β ARKct mice ($n = 31$) receiving no drug was 15 ± 1 weeks vs. 24 ± 3 weeks in the metoprolol-treated CSQ/ β ARKct mice ($n = 14$; $P < 0.0001$). Curves for wild-type ($n = 20$) and β ARKct ($n = 13$) mice treated with metoprolol are not shown but, collectively, they had only one death throughout the course of the study.

inhibition acts synergistically in the CSQ model of cardiomyopathy to increase mean survival age from 9 weeks (with no treatment) to a mean of 24 weeks (with treatment). The increase in survival found when both therapeutic modalities are administered is comparable to the increase in survival observed with β -blocker and ACE therapy in humans (14, 15), and indicates that therapies relating to the β ARKct peptide might also provide additional benefits to heart-failure patients who are currently on β -blocker therapy.

In this study, the β ARKct peptide delays progression of cardiac dysfunction in the CSQ model of severe cardiomyopathy, as evidenced by the reduced LV dilation and increased cardiac function at 7 weeks in the CSQ/ β ARKct mice compared with the CSQ mice. Given the aggressive myopathy of the CSQ mice, it is not surprising that the CSQ/ β ARKct mice eventually progress to myocardial failure and death. This finding is in contrast with the MLP^{-/-}/ β ARKct model of cardiomyopathy, wherein the β ARKct prevented completely the progression of the MLP^{-/-} phenotype (4). Reasons for this contrast may be (i) the severity of the CSQ phenotype and (ii) the inherent differences between the CSQ and MLP^{-/-} models of cardiomyopathy. The lifespan of the MLP^{-/-} model (4) is not appreciably shortened, and survival rate was never studied. In another model of murine cardiomyopathy (cardiac overexpression of G_q), crossbreeding with mice expressing adenylyl cyclase VI could improve heart function; however, survival was never studied (16). Although chronic β -blocker treatment of mice with cardiac overexpression of G_s α diminished the premature death associated with that phenotype (17), our study shows a dramatic increase in survival with the addition of β ARK1 inhibition to standard β -blocker therapy.

An interesting finding in our study is that the β ARKct was able to decrease some of the hypertrophy in the CSQ/ β ARKct mice, as seen by the significantly decreased LV weight/body weight at 7 weeks. This finding was especially interesting considering we have recently shown that the β ARKct has no effect on preventing the development of pressure-overload hypertrophy (10). Interestingly, ablation of phospholamban in CSQ-expressing mice is capable of rescuing the diminished contractility and myocyte hypertrophy that is typically seen in the CSQ mice (18).

These findings may be indicative of the favorable effects on overall cardiac function caused by these manipulations.

One of the most interesting findings of our study was that the β ARKct improved survival without affecting the postreceptor defect in the CSQ mice. This defect was evident by the persistent abnormality in isoproterenol-stimulated adenylyl cyclase and LV dp/dt_{max} in the CSQ/ β ARKct mice. Considerable controversy continues concerning whether inotropic therapies are detrimental when used in heart failure cases, especially considering the dismal results of trials in patients with inotropic agents (19). Our data clearly show that, whereas the β ARKct acts to normalize β AR G protein coupling as shown by the improvement in high-affinity agonist binding, it only acts mildly to enhance the inotropic state of the heart in this model. This result is not surprising, given the significant postreceptor defect and the inability of the CSQ mice to effectively release Ca²⁺ (necessary for an increase in contractility) from the sarcoplasmic reticulum (7). However, the biochemical data demonstrate that the β ARKct functions to inhibit β ARK1-mediated receptor effects, as shown by the increased β AR density and the percentage of high-affinity agonist binding sites. Nevertheless, β ARKct expression through noninotropic means significantly improved cardiac function, delayed overt failure, and improved survival of the CSQ mice.

One possible mechanism for the beneficial effect of the β ARKct is decreased desensitization of other G protein-coupled receptors, such as endothelin and angiotensin receptors. Although previous studies have shown the benefits of blocking both angiotensin (20) and endothelin (21) receptors, inhibiting β ARK1, as we have done here, would act to enhance angiotensin and endothelin signaling; therefore, inhibiting β ARK1 on these receptors does not seem to be the likely mechanism of action. Because the β ARKct peptide inhibits β ARK1 through the sequestration of G $\beta\gamma$ -subunits, it is possible that the mechanism of action of the β ARKct for delaying the progression of the CSQ phenotype is caused, in part, by the inhibition of other G $\beta\gamma$ -dependent pathways such as adenylyl cyclase (22), phospholipase C- β (PLC β) (23), mitogen-activated protein (MAP) kinase (23), or phosphatidylinositol (PI) 3-kinase (23). MAP kinase and PI 3-kinase activities have recently been shown to be influenced by

activated $G\beta\gamma$ - β ARK1 (24, 25), which, in this model of $G\beta\gamma$ sequestration, would be inhibited by the overexpression of the β ARKct.

Whereas chronic metoprolol treatment markedly improved survival in CSQ/ β ARKct mice, it had no additional benefits to cardiac function. These data suggest that the mechanism of action of the β ARKct is distinctly different from that of metoprolol. β -blocker therapy is known to be antiarrhythmic, and the CSQ model has very prominent arrhythmias with marked abnormalities in ion-channel function (7, 26). It is likely that the synergistic action of the two therapeutic approaches is related to the positive influence on cardiac function of the β ARKct and the reduction in arrhythmias with metoprolol. Additionally, the combination of β ARK inhibition and β -blockade may differentially affect β_1 and β_2 AR subtype signaling, given that it has recently been shown in transgenic mouse models that enhanced β_1 AR signaling is deleterious (27), whereas enhanced β_2 AR signaling can be beneficial (28).

In summary, this study demonstrates that the inhibition of β ARK1 through the expression of the β ARKct peptide is able to increase survival and delay the progression of myocardial failure in the CSQ model of cardiomyopathy. That the β ARKct is able to significantly improve multiple and different models of cardiomyopathy and that its benefits are synergistic with standard β -blocker therapy suggest that drugs which inhibit β ARK1 might serve as an important new class of therapeutic agents for the treatment of human heart failure.

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