# Carvedilol suppresses apoptosis

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# Carvedilol Suppresses Apoptosis and Ion Channel Remodelling of HL-1 Cardiac Myocytes Expressing E334K cMyBPC

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Keywords
- carvedilol
- bisoprolol
- apoptosis
- Cav 1.2
- E334K MyBPC

## Abstract

**Background:** Besides its antiarrhythmic action, carvedilol has an activity to suppress cardiac tissue damage. However, it is unknown whether it has any effect on cellular apoptosis and ion channel remodelling.

**Purpose:** To know whether carvedilol has any effect on apoptosis and ion channel remodeling of HL-1 cells expressing E334K MyBPC, and comparing it with bisoprolol.

**Method:** We examined effects of carvedilol and bisoprolol on the levels of pro- and anti-apoptotic proteins and ion channels as well as apoptosis of HL-1 cells transfected with E334K MyBPC using Western blot and flow cytometry.

**Results:** Carvedilol decreased the protein levels of p53, Bax and cytochrome *c* and increased that of Bcl-2 in HL-1 cells expressing E334K MyBPC. Bisoprolol failed to affect the protein levels. Both carvedilol and bisoprolol increased the protein levels of Cav1.2 but not that of Nav1.5. Carvedilol was stronger than bisoprolol at decreasing the number of annexin-V positive cells in HL-1 cells expressing E334K MyBPC.

**Conclusion:** Carvedilol suppressed apoptosis of HL-1 cells expressing E334K MyBPC through modification of pro- and anti-apoptotic proteins, whose was associated with an increase of Cav 1.2 protein expression.

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## Abbreviations

| | |
|---|---|
| E334K | Glu344Lys |
| MyBPC | cardiac myosin-binding protein C |

## Introduction

Activation of β-adrenergic response may predispose to apoptosis of cardiac myocytes [1] leading to heart failure, suggesting a beneficial action of β-adrenergic receptor antagonists. Clinical studies showed that β-blockers could reduce cardiovascular events as well as mortality of patients with chronic heart failure [2]. Carvedilol is classified as a non-selective β-blocker [3]. This agent has anti-apoptotic, antioxidant, anti-proliferative and anti-arrhythmic effects in experimental studies [4–7]. Although these effects were demonstrated in various models of ischemia, ischemia-reperfusion or atrial fibrillation, it has never been tested whether carvedilol has protective actions on hypertrophic cardiomyocytes. Recently, we proposed cultured mouse cardiac myocytes expressing cardiac myosin-binding protein C (MyBPC) with a Glu344Lys (E334K) missense mutation as a cellular model of hypertrophic cardiomyopathy [8–10]. Cardiac HL-1 cells expressed the increased levels of pro-apoptotic proteins and decreased levels of anti-apoptotic proteins and underwent apoptosis [9,10]. They also impaired ion channel expression leading to arrhythmias [9]. In the present study, we studied effects of carvedilol on levels of pro- and anti-apoptotic proteins as well as ion channel proteins and apoptosis in HL-1 cells expressing the E334K MyBPC, and comparing it with bisoprolol.

## Material and Methods

### Cells culture and heterologous expression

HL-1 cardiac myocytes were provided by Dr. Claycomb (Louisiana State University) and were cultured as described [11]. cDNA encoding E334K cMyBPC with a 6-myc tag at the N-terminus [8] was ligated to $pCS^{2+}$ at *BamHI* and *XhoI* sites to generate an expression plasmid pCS-6myc-*MYBPC3*.

To visualize transfected cells, EGFP cDNA was added to the carboxy terminus of E334K MyBPC cDNA. Transfection into HL-1 cells was performed using lipofectamin 2000 (Invitrogen) according to the manufacturer's instructions.

### Western blotting

E334K *MYBPC3* was transfected into HL-1 cells in the absence or presence of calvedilol or bisoprolol. Protein extracts of cells were prepared 48 h post-transfection, as described elsewhere [8]. Proteins were separated by SDS–PAGE and electrotransferred to PVDF membrane. Membranes were probed with antibodies to actin (Calbiochem, La Jolla, CA), p53 (Santa Cruz Biotechnology, Santa Cruz, CA), Bax (Santa Cruz), cytochrome *c* (BD Biosciences, Franklin Lakes, NJ), Bcl-2 (Santa Cruz), Nav1.5 (Abcam, Cambridge, MA) or Cav1.2 (Santa Cruz Biotechnology). They were developed using an ECL system (Amersham Bioscience, Piscataway, NJ). The intensities of the bands were quantified using NIH image Software.

### Annexin V staining and flow cytometry

Annexin V staining and flow cytometry were conducted as described elsewhere [8].

### Drugs

Carvedilol was kindly provided by Daiichi Sankyo, Japan, and bisoprolol was purchased from Sigma-Aldrich Japan.

### Statistical analysis

OriginR for Windows software version 7.0 (OriginLab Corporation, Northampton, MA, USA) was used for statistical analysis. Differences between 2 groups were assessed using the 2-sample *t*-test. One-way ANOVA with the Bonferroni test for post-hoc analysis was used for multiple comparisons. All experimental data are expressed as the mean ± SEM. Differences with *p*-values < 0.05 were considered significant.

## Results

**Fig. 1** shows effects of either carvedilol or bisoprolol on the level of proteins regulating apoptosis in HL-1 cells expressing E334K MyBPC. Carvedilol (1 µM) decreased the levels of p53, Bax and cytochrome *c* and increased that of Bcl-2 significantly, which was confirmed by quantitative analysis of data obtained from 8 different experiments. Bisoprolol (1 µM) did not influence the levels of these proteins. In control HL-1 cells, both agents failed to induce any changes in protein levels of these proteins, although carvedilol significantly decreased the protein level of cytochrome *c*.

Next, we examined the effects of either carvedilol or bisoprolol on the protein levels of Nav1.5 and Cav1.2 in HL-1 cells expressing E334K MyBPC (**Fig. 2**). Neither carvedilol nor bisoprolol influenced the level of Nav1.5, whereas both agents decreased

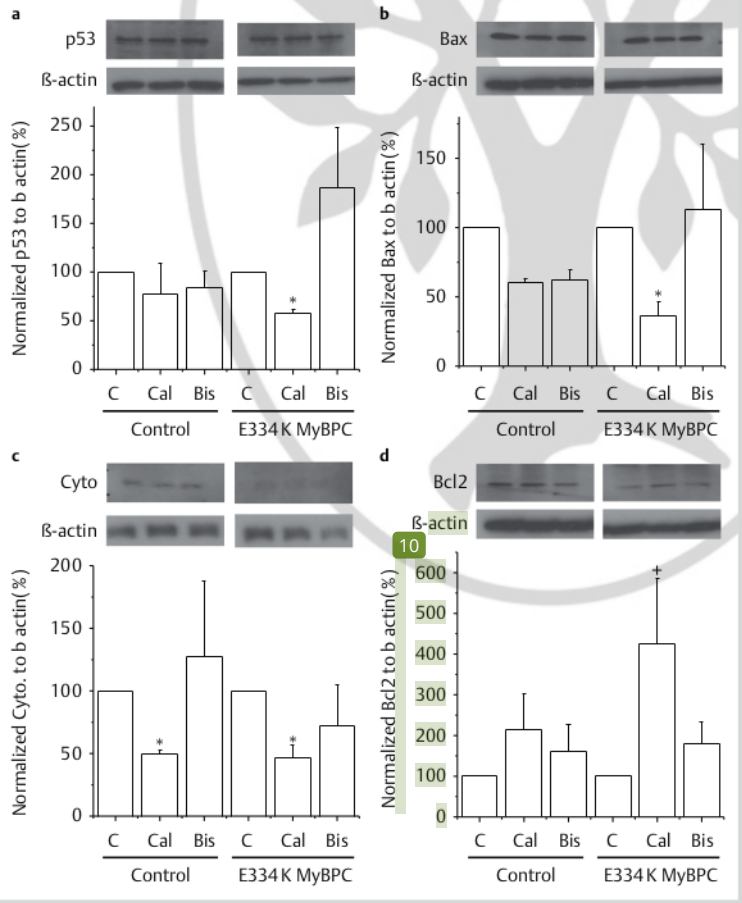


**Fig. 1** Effects of either carvedilol or bisoprolol on the pro- and anti-apoptotic proteins in HL-1 cells expressing E334K MyBPC. Western blot of protein level of p53 **a**, Bax **b**, cytochrome *c* **c**, and Bcl-2 **d** in HL-1 cells with or without E334K MyBPC expression in the presence of carvedilol (1 uM) or bisoprolol (1 uM) as indicated. The bar graph show summary of quantitative densitometric scan of protein levels as indicated, $n = 8$ for each group. $^{*}p < 0.05$. $^{+}p < 0.001$ cyto, cytochrome *c*; C, control; Cal, carvedilol; Bis, bisoprolol.

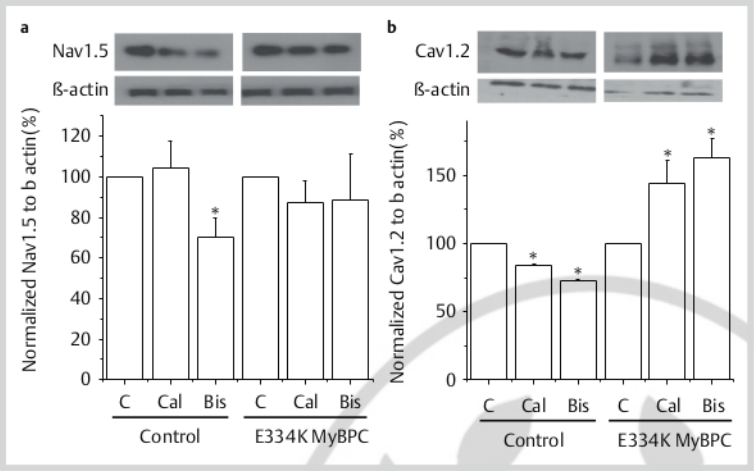


**Fig. 2** Effects of either carvedilol or bisoprolol on cardiac ion channel proteins in HL-1 cells expressing E334K MyBPC. Western blot of protein level of Nav1.5 **a** and Cav1.2 **b** in HL-1 cells. $n=4$ for each group. Please see the **Fig. 1** legend for further explanation.

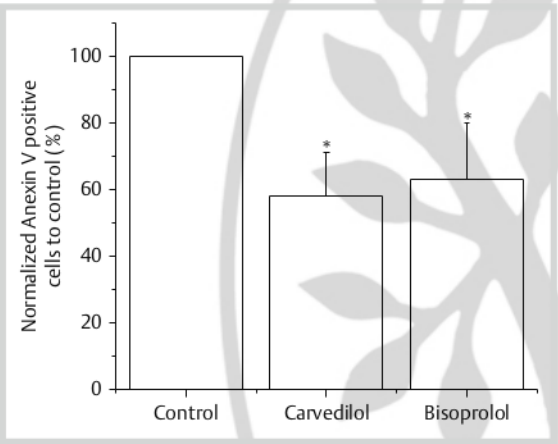


**Fig. 3** Effects of either carvedilol or bisoprolol on the number of annexin-V-positive cells in HL-1 cells expressing E334K MyBPC. The graph show summary of the normalized number of annexin-V-positive cells in HL-1 cells, $n=4$ for each group. $^{**}p<0.001$, $^{*}p<0.05$. Please see the **Fig. 1** legend for further explanation.

the protein level of Cav1.2. Bisoprolol also decreased the level of Nav1.5 in control HL-1 cells. Both carvedilol and bisoprolol decreased the level of Cav1.2 in control HL-1 cells, whereas both agents increased the level of Cav1.2 in HL-1 cells expressing E334K MyBPC.

Finally, we examined effect of carvedilol and bisoprolol on apoptosis of HL-1 cells expressing E334K MyBPC-GFP (**Fig. 3**). Although both agents reduced the number of annexin-V-positive cells, the effect of carvedilol was stronger than that of bisoprolol. This was confirmed by quantitative analysis ($n=4$).

## Discussion

In the present study, carvedilol decreased pro-apoptotic proteins of p53, Bax and cytochrome *c*, increased anti-apoptotic protein of Bcl-2 and attenuated apoptosis of HL-1 cardiac myocytes expressing E334K MyBPC. These actions were not observed in the cells treated with bisoprolol. Clinical and experimental studies reported that carvedilol supresses apoptosis in heart failure, ischemic reperfusion, and acute myocardial infarction models [12–15]. This drug delayed oxidative stress-induced apoptosis in ischemic hearts and this effect was independent from β-adrenergic receptors [12, 13]. It has anti-oxidative actions, inhibitory actions against inflammation and NF-κB and suppress apoptosis [4, 5, 13, 14]. However, it has never been reported whether carvedilol could suppress the apoptosis of cardiac myocytes in hypertrophic cardiomyopathy. A gene mutation, E334K *MYBPC3*, was discovered in the patients with hypertrophic cardiomyopathy [8]. Overexpression of E334K MyBPC induced apoptosis of cardiac cells through increases in protein expression of p53, Bax and cytochrome *c* and decreases in that of Bcl-2 [8]. We also demonstrated that accumulation of Cav1.2 played a pivotal role in apoptosis of HL-1 cells expressing E334K MyBPC, which could be attenuated by an L-type $Ca^{2+}$ channel blocker azelnidipine [9]. This is the first report to show that carvedilol reduced pro-apoptotic proteins such as p53, Bax and cytochrome *c* and increased anti-apoptotic protein Bcl-2 leading to suppression of apoptosis in HL-1 cardiac myocytes expressing E334K MyBPC. It has been reported that carvedilol showed a mild $Ca^{2+}$ channel blocking action in hearts [16], suggesting an involvement of $Ca^{2+}$ antagonizing effect of carvedilol in its anti-apoptotic action.

Interestingly, in the present study, carvedilol as well as bisoprolol increased the protein level of Cav1.2 in HL-1 cells expressing E334K MyBPC, although both did not altered the protein level of Nav1.5. It has been reported that carvedilol has antiarrhythmic effects in the patients with chronic heart failures in CAPRICONR study [7]. Kishihara et al. [17] reported that carvedilol prolonged atrial effective refractory period and atrial fibrosis in canine atrial fibrillation (AF) models and prevented induction of AF. Li et al. [18] demonstrated that carvedilol restored mRNA and protein levels of L-type $Ca^{2+}$ channels and its activity in a rabbit myocardial infarction model. These results indicated that carvedilol could improve electrical remodelling and reduce $Ca^{2+}$ channels in hypertrophied cardiomyocytes. This increased expression of Cav1.2 induced by carvedilol might counteract its inhibitory action on apoptosis in HL-1 cardiac myocytes expressing E334K MyBPC. However, carvedilol suppressed the cellular apoptosis, suggesting that carvedilol could suppress apoptosis and improve

electrical remodelling of heart in patients with hypertrophic cardiomyopathy. In conclusion, we demonstrated that carvedilol suppressed apoptosis of HL-1 cells expressing E334K MyBPC through modification of pro- and anti-apoptotic proteins, whose was associated with an increase of Cav 1.2 protein expression. These findings provide a new insight into the pharmacological treatment of patients with hypertrophic cardiomyopathy.

## Acknowledgements

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## Conflict of Interest

IH received a research grant from Daiichi-Sankyo Inc., Japan.

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