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Bepridil Suppresses Apoptosis in HL-1 Cardiac Atrial Myocytes Expressing Mutant E334K cMyBPC

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ABSTRACT

Besides its antiarrhythmic effect on atrial fibrillation, bepridil protects tissue, yet its effect on apoptosis has never been fully tested. We examine the effect of bepridil on apoptosis of HL-1 cells expressing E334K myosin-binding protein C (MyBPC), a model cell of apoptosis. Bepridil was compared with amiodarone, and its effects on the expression of pro- and anti-apoptotic protein and apoptosis of HL-1 cells expressing mutant E334K MyBPC-green fluorescent protein (GFP) was analyzed using Western blot and a flow cytometer. Bepridil decreased the protein levels of both Bax and cytochrome c of cells expressing E334K MyBPC-GFP with no changes in p53 and Bcl-2, while amiodarone decreased cytochrome c but did not influence Bax except in its highest concentration. It also decreased the number of Annexin-V positive cells of HL-1 cells expressing E334K MyBPC-GFP, and decreased apoptosis of HL-1 cells expressing E334K MyBPC-GFP.

Key words: amiodarone; apoptosis; bepridil; HL-1 cell

Bepridil is classified as a Ca^{2+} channel blocker according to Vaughn-Williams' classification.[1] This agent also has multi-ion channel blocking effects similar to those of amiodarone and is thought to be effective for atrial fibrillation.[2] Along with its electrophysiological actions on atrial myocytes, bepridil showed beneficial effects in tissue protection from ischemia.[3] However, it remains unknown whether bepridil protects the apoptosis of atrial myocytes. Recently, we proposed cultured mouse atrial myocytes (HL-1 cells) expressing a Glu344Lys (E334K) missense mutation of myosin-binding protein C (MyBPC) as the model to induce apoptosis.[4] These cells exhibit apoptosis with increased pro-apoptotic proteins and decreased anti-apoptotic protein. In the present study, we studied the effect of bepridil on apoptosis in HL-1 cells expressing mutant MyBPC in comparison with amiodarone.

MATERIALS AND METHODS

Cells culture and heterologous expression

HL-1 cardiac myocytes were provided by Dr. Claycomb (Louisiana State University) and cultured according to instructions.[5] cDNA encoding E334K cardiac MyBPC (cMyBPC) with a 6-myc tag at the N-terminus[4] was ligated to pCS^{2+} at *BamHI* and *XhoI* sites to generate plasmid expression vectors pCS-6myc-*MYBPC3*. To visualize the transfected cells, enhanced green fluorescent protein (EGFP) cDNA was added to the carboxy terminus of E334K MyBPC cDNA. Transfection into HL-1 cells was performed using lipofectamin 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

Western blotting

E334K *MYBPC3* was transfected into HL-1 cells in the absence or presence of either bepridil or amiodarone. Protein extracts of cells were prepared 48 h post-transfection, as described elsewhere.[5] Proteins were separated by SDS-PAGE and electrotransferred to polyvinylidene difluoride 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) or Bcl-2 (Santa Cruz). They were developed using an ECL system (Amersham Bioscience, Piscataway, NJ). The intensities of the bands were quantified using National Institute of Health image Software.

Annexin V staining and flow cytometry

Annexin V staining and flow cytometer of E334K MYBPC3-GFP transfected cells were performed in the absence and presence of either bepridil or amiodarone as reported elsewhere.[4]

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Abbreviations: Af, atrial fibrillation; cMyBPC, cardiac MyBPC; EGFP, enhanced GFP; GFP, green fluorescent protein; MyBPC, myosin-binding protein C

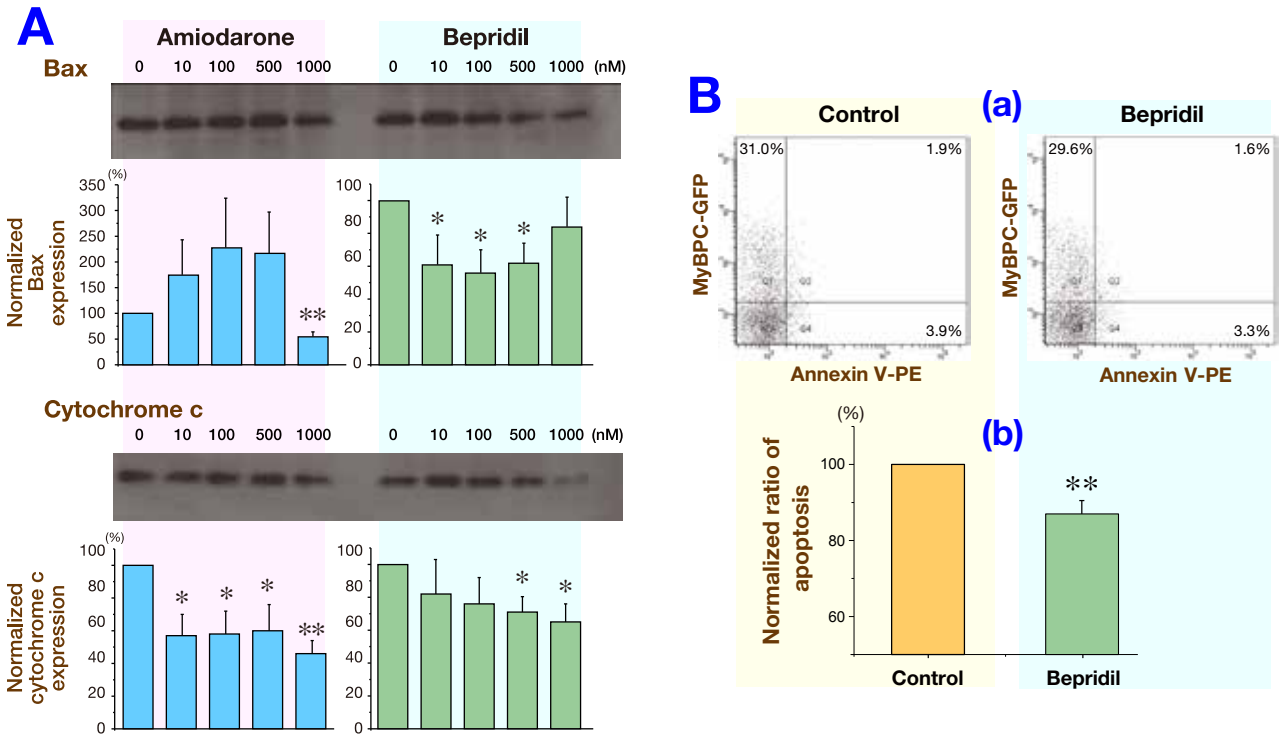

Fig. 1. Effects of bepridil and amiodarone on apoptosis in HL-1 cardiac atrial myocytes expressing E334K cMyBPC.

A: Effect of bepridil and amiodarone on the level of proteins regulating apoptosis in HL-1 cells expressing E334K cMyBPC in a dose-dependent manner. Representative Western blot of pro-apoptotic proteins cytochrome c and Bax in HL-1 cells transfected with E334K cMyBPC in the presence or absence of drugs as indicated. Beta-actin used as a control for protein loading. Summary of quantitative densitometric scan of drugs on the level of Bax and cytochrome c; $n = 8$, $^{*}P < 0.05$; $^{**}P < 0.01$. cMyBPC, cardiac myosin-binding protein C.

B: Effect of bepridil on the apoptotic cells expressing E334K cMyBPC-GFP.

(**a**) The representative immunofluorescence image of annexin V-positive cells in cells expressing E334K cMyBPC-GFP before and after treatment with bepridil.

(**b**) The percentage of annexin V-positive cells ($n = 4$) in those cells. Data are shown as mean (SEM), calculated by unpaired, two-tailed Student's *t*-test. $^{*}P < 0.05$.

GFP, green fluorescent protein.

Used drugs

Amiodarone and bepridil were used in this study. Bepridil was kindly provided by Daiichi Sankyo (Tokyo, Japan) and amiodarone was purchased from Sigma-Aldrich Japan (Tokyo).

Statistical analysis

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

RESULTS AND DISCUSSION

Figure 1A shows the effects of bepridil or amiodarone on the level of proteins that regulate apoptosis in HL-1 cells expressing E334K MyBPC. Bepridil decreased the levels of Bax and cytochrome c, but not those of either p53 or Bcl-2 in cells expressing E334K MyBPC (data not shown), which was also confirmed by quantitative analysis obtained from 6 different experiments. Amiodarone also decreased the level of cytochrome c, while it decreased the level of Bax at the highest concentration. Next, we examined the effect of bepridil on apoptosis of HL-1 cells expressing E334K MyBPC-GFP as shown in Fig. 1B. Bepridil reduced the number of annexin V-positive cells, which was confirmed by quantitative analysis ($n = 4$).

It has been reported that bepridil blocked the several cardiac ion channels and was protective against atrial

fibrillation (Af). In addition to its acute action on ion channels, bepridil reversed atrial electrical remodeling and L-type Ca^{2+} channel down regulation in a canine model of persistent Af,[6] suggesting that bepridil can exert beneficial effects on electrophysiological properties of atrial myocytes via modulating expression of ionic channels. Some research has found that bepridil protects cardiac myocytes from ischemia; impaired mitochondrial function, increasing reactive oxygen species, and apoptosis are involved in age-related atrial remodeling and Af susceptibility.[7, 8] Other research has shown that it reduces reactive oxygen species, and thus, is expected to reduce apoptosis. In the present study, bepridil reduced the level of pro-apoptotic proteins (cytochrome c and Bax) of cells expressing MyBPC-GFP, and this effect was prominent in comparison with that of amiodarone. The reduction of the level of cytochrome c suggested protective effect of bepridil that was mediated through the mitochondrial pathway. Although bepridil inhibited Na^{+}/Ca^{2+} exchanger and suppressed apoptosis of neurons,[9] this is the first report demonstrating the effect of bepridil on apoptosis in a cellular model expressing E334K MyBPC.

The authors declare no conflict of interest.

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