Biochemical and Biophysical Research Communications 421 (2012) 578–584

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The chemical chaperone 4-phenylbutyric acid attenuates pressure-overload cardiac hypertrophy by alleviating endoplasmic reticulum stress

Chang Sik Park, Hyeseon Cha, Eun Jeong Kwon, Pradeep Kumar Sreenivasaiah, Do Han Kim *

School of Life Sciences and Systems Biology Research Center, Gwangju Institute of Science and Technology (GIST), 123 Cheomdan-gwagiro (1 Oryong-dong), Buk-gu, Gwangju 500-712, Republic of Korea

ARTICLE INFO

Article history:
Received 9 April 2012
Available online 14 April 2012

Keywords:
ER stress
PBA
Cardiac remodeling
Fibrosis
TAC

ABSTRACT

Evidence has shown that endoplasmic reticulum stress (ERS) is associated with the pathogenesis of cardiac hypertrophy. The aim of this study was to investigate whether direct alleviation of ER stress by 4-phenylbutyric acid (PBA), a known chemical chaperone drug, could attenuate pressure-overload cardiac hypertrophy in mice. The effects of orally administered PBA (100 mg/kg body weight daily for a week) were examined using mice undergoing transverse aortic constriction (TAC-mice), an animal model to produce pressure overload. TAC application for 1 week led to a 1.8-fold increase in the ratio of the heart weight over body weight (HW/BW) and up-regulation of the hypertrophy markers ANF and BNF accompanied by up-regulation of ERS markers (GRP78, p-PERK, and p-eIF2α). The oral administration of PBA to the TAC-mice reduced hypertrophy (19%) and severely downregulated the fibrosis-related genes (transforming growth factor-β1, phospho-smad2, and pro-collagen isoforms). We conclude that ERS is induced as a consequence of remodeling during pathological hypertrophy and that PBA may help to relieve ERS and play a protective role against cardiac hypertrophy and possibly heart failure. We suggest PBA as a novel therapeutic agent for cardiac hypertrophy and fibrosis.

1. Introduction

The endoplasmic reticulum (ER) plays essential roles in multiple cellular processes such as calcium homeostasis, protein folding, and lipid biosynthesis [1,2]. Various stimuli such as ischemia, hypoxia, free-radical exposure, elevated protein synthesis, and gene mutations can perturb ER homeostasis and cause the pathological accumulation of unfolded/misfolded proteins in the ER—a condition called ER stress (ERS). The unfolded protein response (UPR) is triggered in cells when ER transmembrane protein sensors (PERK, IRE, and ATF6) detect the accumulation of unfolded proteins. The primary response to ERS is the upregulation of ER chaperones such as BIP (GRP78), GRP94, and calreticulin (CRT), which in turn enhance the ability of the ER to maintain homeostasis of the vital cellular processes handling the intracellular Ca^{2+} level and the unfolded proteins [3,4]. The UPR aims to reduce the accumulation of unfolded proteins and restore the normal functions by instilling cytoprotection. However, if the stress is prolonged or overwhelming, the pro-survival effects of UPR switches to pro-apoptotic signaling, which is mostly mediated by transcriptional induction of CHOP or by activation of the JNK/c-JUN and/or caspase-12-dependent pathway [2].

Cardiac hypertrophy is an adaptive response that is triggered by many physiological and pathological conditions. However, chronic cardiac hypertrophy is associated with an increased risk of morbidity/mortality, largely due to maladaptive remodeling and dilatation that progresses into dilated cardiomyopathy. Evidence has shown that several factors, including calcium dysregulation, which is characterized by Ca^{2+} overload; abnormal protein synthesis; and apoptosis; are involved in the development of cardiac hypertrophy [5]. These changes are accompanied by the increased expression of GRP78, GRP94, and CRT. All of these changes delineate ERS in the heart. Furthermore, prolonged ERS overwhelms the protective survival pathways, resulting in maladaptive remodeling, apoptosis with replacement fibrosis, and cardiac failure [6–8].

Because prolonged ERS is deleterious to cells, small molecules such as chemical chaperones that have been shown to alleviate ERS may be beneficial in treating several cardiac diseases. Chemical chaperones are non-selective in their ability to stabilize unfolded proteins and facilitate their proper folding, similar to the chaperonic function of the many intracellular molecular chaperones [9]. Currently, 2 chemical chaperones, namely, 4-phenylbutyric acid

Abbreviations: GRP, glucose related protein; eIF2, eukaryotic initiation factor 2; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PBA, 4-phenylbutyric acid; CHOP, C/EBP-homologous protein; IRE, inositol-requiring enzyme; ATF, activating transcription factor; TAC, transverse aortic constriction.

* Corresponding author. Fax: +82 62 970 3411.
E-mail address: dhkim@gist.ac.kr (D.H. Kim).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.048

(PBA) and tauroursodeoxycholic acid (TUDCA), are approved by US Food and Drug Administration (FDA) for use in humans [10]. PBA is a low-molecular-weight fatty acid and a non-toxic pharmacological compound that has been found to have chaperone-like activity. Its physiochemical properties enable it to stabilize peptide structures, improving the luminal folding capacity and traffic of aberrant proteins [11]. Thus, the use of PBA may provide a therapeutic approach to block the pathological process induced by pressure overload.

In the current study, we investigated the effect of PBA on pressure-overload-induced cardiac remodeling processes such as cardiac hypertrophy, fibrosis, and apoptosis. Our results showed that oral administration of PBA alleviated ERS responses, cardiac hypertrophy, and pathological hypertrophy fibrosis, suggesting that PBA can be a novel and appealing therapeutic agent for cardiac hypertrophy, fibrosis and possibly heart failure.

2. Materials and methods

2.1. Ethics statement

All animal experiments were carried out according to the guidelines for animal care and use approved by the Gwangju Institute of Science and Technology Animal Care and Use Committee.

2.2. Reagents and dosing

The mice were administered PBA (Sigma–Aldrich, St. Louis, MO) at a dose of 100 mg kg^{-1} day^{-1} (dissolved in warm water) by oral gavage. The control-group animals were administered vehicle only (warm water). The treatment started 1 day after the transverse aortic constriction (TAC) operation. The mice were killed after 1 week of treatment.

2.3. TAC

The TAC surgery was performed as previously described [12]. Animals undergoing the sham operation (the same procedure without constriction) were considered the control group.

2.4. Echocardiography

The mice were anesthetized with 0.5–0.7 ml of 1× Avertin solution (a mixture of 2-2-2 tribromoethanol and tert-amyl alcohol) by intraperitoneal injection and their chests were shaved. Echocardiography was performed using a Power vision 6000 (Toshiba, Tustin, CA) instrument with a 12-MHz microprobe (PLM-1204AT; Toshiba). The hearts were scanned using the M-mode guided by a short-axis view of the 2D-mode. Frozen frames were printed using a video graphic printer (UP-895MD; Sony, Tokyo, Japan).

2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western Blot analysis

Western Blot analysis was performed as described previously [13]. The antibodies used were described in Supplementary materials.

2.6. Quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using TRI reagent (Sigma–Aldrich). RT was performed using ImProm II reverse transcriptase (Promega, Madison, WI) with oligo-dT priming. PCR was performed using an ABI PRISM Sequence Detector System 7500 (Applied Biosystems, Carlsbad, CA) with SYBR Green (Takara Bio Inc., Shiga, Japan) as the fluorescent dye and ROX (Takara Bio Inc.) as the reference dye. The PCR primers used were described in Supplementary materials.

2.7. Primary cell culture and immunocytochemistry

Primary neonatal rat ventricular cardiomyocyte culture was prepared as described [14]. Briefly, cardiomyocytes were cultured in serum-free medium for at least 24 h and then treated with 100 μM phenylephrine (PE) with or without 500 μM PBA for 24 h. The cells were then washed 3 times with Dulbecco's phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.5% Triton X-100 in PBS for 5 min, and blocked by incubation with 5% bovine serum albumin for 1 h at room temperature. The cells were incubated with anti-α actinin antibody (Sigma) and further incubated with Alexa 488-conjugated anti-mouse immunoglobulin G (Jackson ImmunoResearch, USA). Immunofluorescence was analyzed using an LSM-700 confocal microscope (Carl Zeiss Inc., Oberkochen, Germany).

2.8. Statistical analysis

Results are given as mean ± standard deviation or standard error. Statistical significance between the multiple samples was determined using analysis of variance. The differences were considered significant when $P < 0.05$.

3. Results

3.1. Cardiac hypertrophy and ERS responses induced by pressure-overload

To investigate whether cardiac hypertrophy and ERS responses are produced by pressure-overload [7], we employed the TAC animal model. Heart weight over body weight (HW/BW) ratio was significantly increased by 1.8- and 2.3-fold at 1 and 5 weeks after TAC, respectively (Fig. 1A). Western Blot results showed upregulation of the ER chaperonic protein GRP78 and the downstream ERS signaling pathway proteins p-PERK and p-eIF2α (Fig. 1B). The extents of upregulation of the tested ERS proteins were significantly higher at 1 week than at 5 weeks after TAC (Fig. 2). Therefore, we chose 1-week TAC for further studies to characterize the effects of PBA on the ERS responses, cardiac hypertrophy, and fibrosis.

3.2. PBA alleviated ERS responses in pressure-overload cardiac hypertrophy

We first examined the effects of PBA on ERS responses by using the HL-1 cell line. Our results showed that 10 μg/ml tunicamycin (TUN) induced significant ERS responses in HL-1 cells and that treatment with 500 μM PBA reduced ERS responses completely (Supplementary Fig. 1). For the *in vivo* study, PBA was orally administrated to sham- and TAC-operated mice for 1 week (100 mg kg^{-1} day^{-1}) and the expression levels of ERS markers were examined (Fig. 1C). The expression levels of the ER chaperon proteins GRP94 and GRP78 and the ERS signaling pathway proteins p-PERK and p-eIF2α were significantly increased in water TAC 1 week (Water TAC) mice compared with water sham 1 week (Water Sham) mice indicative of enhanced cardiac ERS in the hypertrophic heart (Fig. 1C, D). The oral administration of PBA reduced the expression levels of GRP94 and GPR78 as well as p-PERK and p-eIF2α in PBA TAC 1 week (PBA TAC) mice compared with Water TAC mice, suggesting that PBA treatment can attenuate pressure-overload-induced ERS responses in the heart.

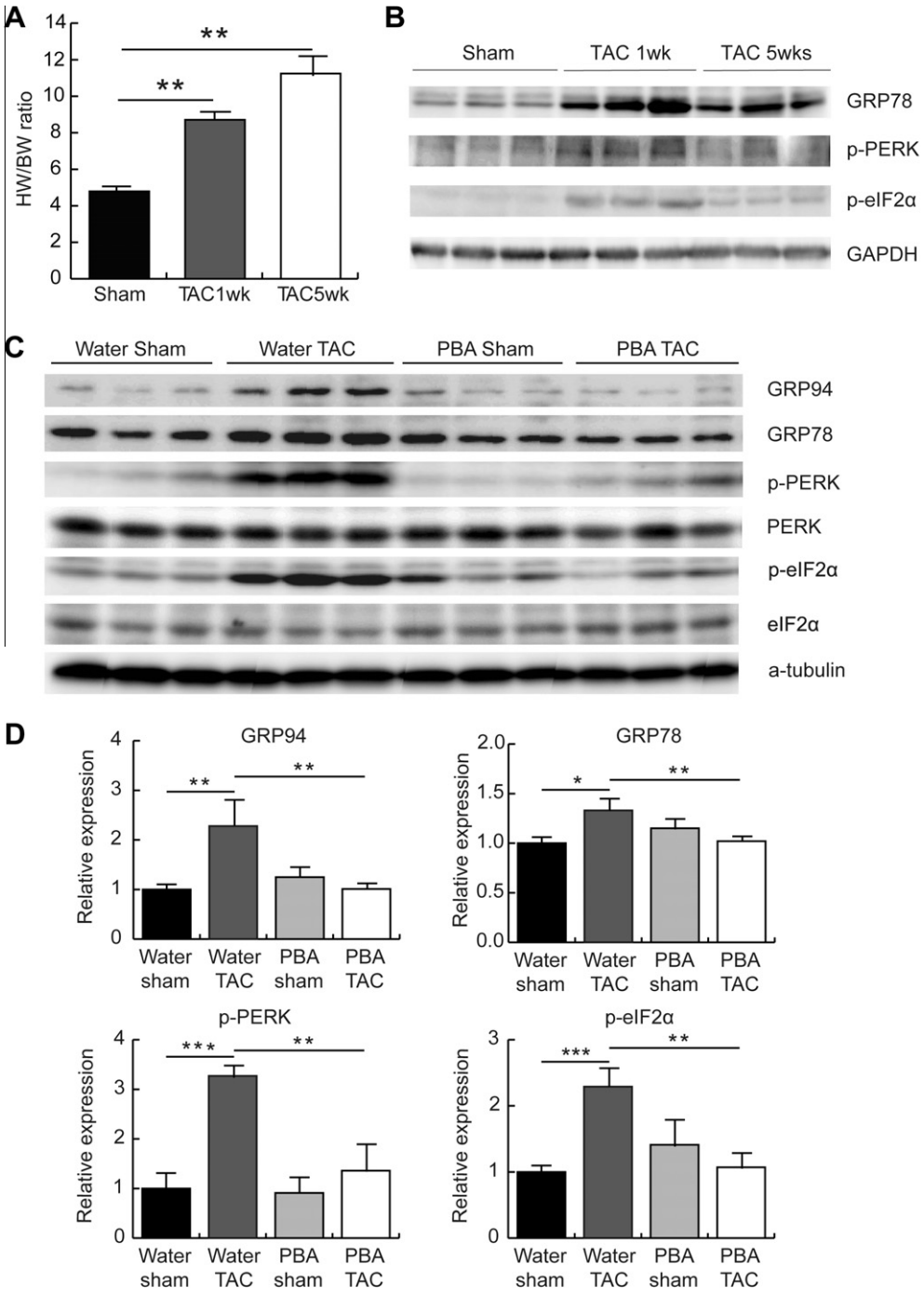

Fig. 1. 4-Phenylbutyric acid (PBA) attenuated endoplasmic reticulum stress (ERS) responses in transverse aortic constriction (TAC)-induced hypertrophic hearts. (A) Quantification of the ratio of the heart weight over body weight (HW/BW), which continuously increased 1–5 weeks after TAC. (B) Expression levels of the chaperonic protein GRP78 and ERS signaling pathway proteins p-PERK and p-eIF2α changed at 1 week and 5 weeks after TAC. Their expression levels were significantly increased at 1 week after TAC. (C) Western blot results of heart whole homogenates obtained from sham and TAC-operated mice treated with water or PBA (100 mg kg^{-1} day^{-1}) for 1 week. The expression levels of chaperon and the ERS signaling pathway proteins were increased in the hearts of Water TAC mice. PBA treatment reversed the process, indicating that it successfully attenuated ERS in hypertrophic hearts. (D) Relative expression levels of the chaperonic proteins GRP94 and GRP78 and the ERS signaling pathway proteins p-PERK and p-eIF2α ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 3–5$).

3.3. PBA reduced pressure-overload cardiac hypertrophy

We initially performed the immunofluorescence study to investigate whether PBA could reduce PE-induced hypertrophy in rat neonatal ventricular cells. PE (10 μM) significantly enhanced cell size, whereas addition of 100 μM PBA reduced cell size (Fig. 2A, B). We then investigated whether the attenuation of ERS responses by PBA could also alleviate pressure-overload-induced cardiac hypertrophy in a mouse model. The oral administration of PBA for a week (100 mg kg^{-1} day^{-1}) significantly reduced the HW/BW ratio and the ratio of the heart weight per tibia length in the PBA TAC mice compared with the Water TAC mice (Fig. 2C, D). Echocardiographic measurements also showed that PBA could attenuate pressure-overload cardiac hypertrophy (Table 1) as attested by the diastolic septum wall thickness (SWTd), diastolic posterior wall thickness (PWTd), and systolic

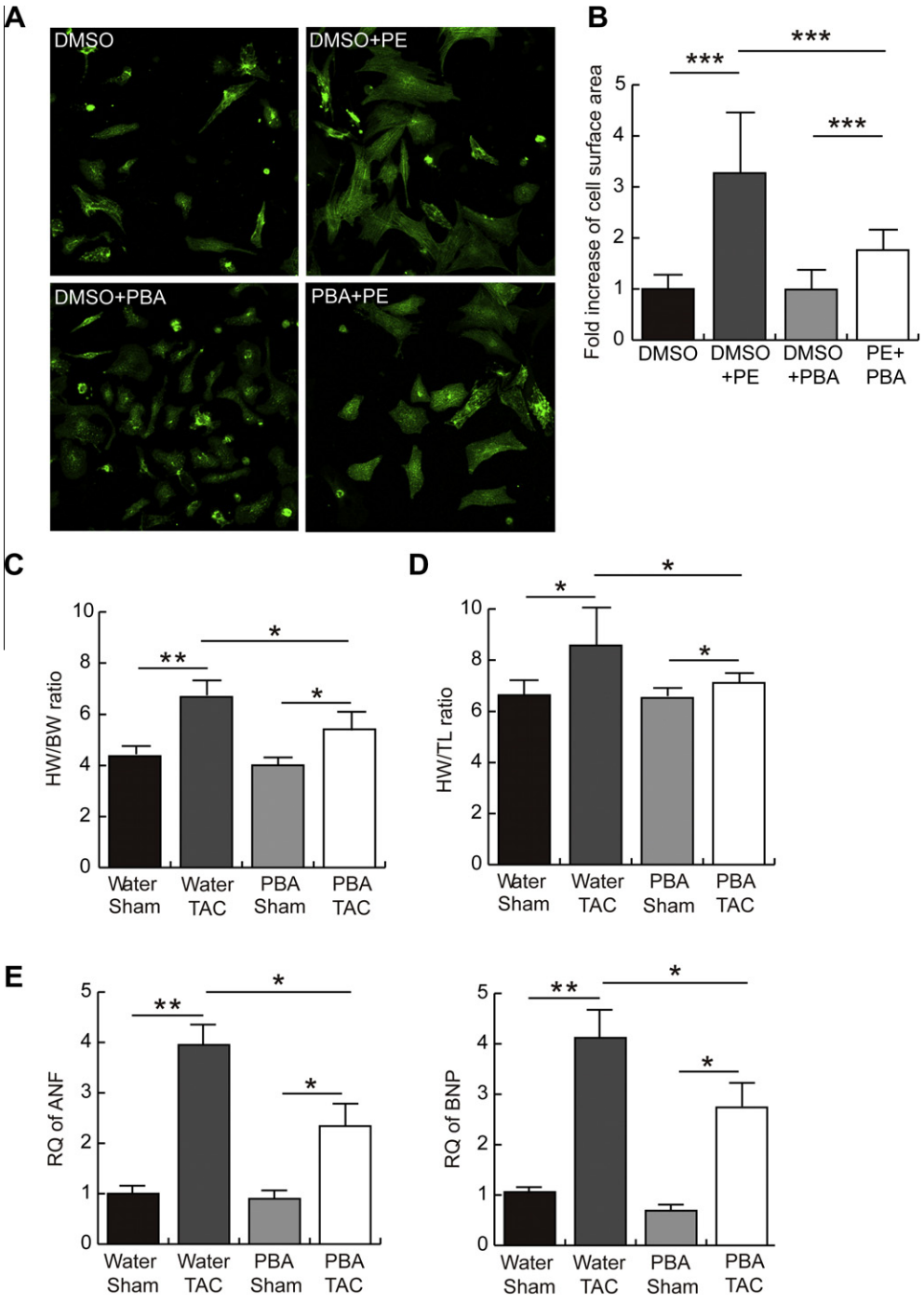

Fig. 2. PBA reduced TAC-induced cardiac hypertrophy. (A) Immunofluorescence imaging to show the cell sizes of neonatal rat ventricular cardiomyocytes treated with 10 μM phenylephrine (PE) in the presence or absence of 100 μM PBA. (B) Fold increase of cell surface area in the immunofluorescence results (sample numbers, dimethylsulfoxide [DMSO]: 65; DMSO + PE: 33; DMSO + PBA: 161; PBA + PE: 58). (C, D) Ratios of HW/BW and heart weight per tibia length as a result of 1-week TAC and treatment with PBA. The protocol for the experiments is described in Fig. 1 legend. (E) Transcripts for ANF and BNP were evaluated by quantitative reverse transcription-polymerase chain reaction using hearts of the sham and TAC-operated mice after treatment with water or PBA. Note that the hypertrophy was significantly reduced after PBA treatment. All data are shown as mean ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $n = 3–5$).

posterior wall thickness (PWTs), which were significantly increased when the pressure-overload returned to control levels (Table 1). Quantitative RT-PCR analysis showed that the enhanced expression levels of the hypertrophic markers ANF and BNP were significantly decreased in the PBA TAC mice compared with the Water TAC mice (Fig. 2E). However, the tested amount of PBA could not completely attenuate TAC-induced hypertrophy to baseline levels.

3.4. PBA downregulated cardiac fibrosis signaling in pressure-overload cardiac hypertrophy

We performed quantitative RT-PCR to study the expression levels of the transcripts for pro-collagen involved in cardiac fibrosis. Our results showed that the transcription of fibrosis marker genes, the pro-collagen isoforms, was greatly enhanced in the Water TAC mice compared with the Water Sham mice. In contrast, the mRNA

Table 1
Summary of echocardiographic parameters from mice with TAC-induced cardiac hypertrophy and partial reversal of the hypertrophic parameters by PBA.

	Water		PBA	
	Sham (n = 3)	TAC (n = 4)	Sham (n = 5)	TAC (n = 5)
SWTd (mm)	0.93 ± 0.06	1.15 ± 0.13*	0.86 ± 0.09	0.96 ± 0.13
LVEDD (mm)	2.83 ± 0.49	2.63 ± 0.44	3.12 ± 0.45	3.46 ± 0.3
PWTd (mm)	0.93 ± 0.25	1.63 ± 0.33*	1.06 ± 0.27	0.94 ± 0.09
SWTs (mm)	1.53 ± 0.06	1.88 ± 0.22*	1.46 ± 0.09	1.68 ± 0.18#
LVESD (mm)	1.27 ± 0.38	1.15 ± 0.26	1.58 ± 0.38	1.62 ± 0.31
PWTs (mm)	1.77 ± 0.35	2.25 ± 0.19*	1.82 ± 0.38	1.96 ± 0.25
FS (%)	56.67 ± 5.86	57.0 ± 6.98	50.75 ± 3.2	55.4 ± 9.24

Echocardiography parameters obtained from water- or PBA-treated mice after 1 week of sham or TAC operation. SWTd, diastolic septal wall thickness; SWTs, systolic septal wall thickness; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; PWTd, diastolic posterior wall thickness; PWTs, systolic posterior wall thickness; FS, fractional shortening. n = 3–5.
* $P < 0.05$ versus Water sham.
# $P < 0.05$ versus PBA sham.

levels were significantly decreased in the PBA TAC mice compared with the Water TAC mice (Fig. 3A). In agreement with the previous findings that showed the TGF-β/SMADs signaling pathway is involved in the pathogenesis of cardiac fibrosis [15,16], our results showed that the expression levels of TGF-β1 and p-Smad2 were highly enhanced in the Water TAC mice compared with the Water Sham mice (Fig. 2B, C). Oral administration of PBA, however, attenuated the expression levels of TGF-β1 and p-Smad2. The complete blockage of the cardiac fibrosis signaling by PBA (Fig. 3) suggests that there is an additional direct effect of PBA on cardiac fibrosis (Supplementary Fig. 4).

3.5. PBA reduced apoptosis in hypertrophic cardiac cells

Prolonged ERS in the hypertrophic heart has been suggested to induce cardiac apoptosis and result in heart failure [7]. In the present study, we investigated the effect of PBA on ERS-induced apoptosis in HL-1 cells. ERS-induced cell death was induced by 10 μg/ml of TUN. Treatment with 500 μM PBA led to decreased expression levels of the pro-apoptotic markers, cleaved caspase-3, and CHOP, and increased expression level of the anti-apoptotic protein Bcl-2 (Supplementary Fig. 2A, B), indicative of attenuation of apoptosis in HL-1 cells. Our terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL) assay results also demonstrated a reduced number of TUNEL-positive cells, suggesting the cytoprotective effect of 500 μM PBA (Supplementary Fig. 2C, D).

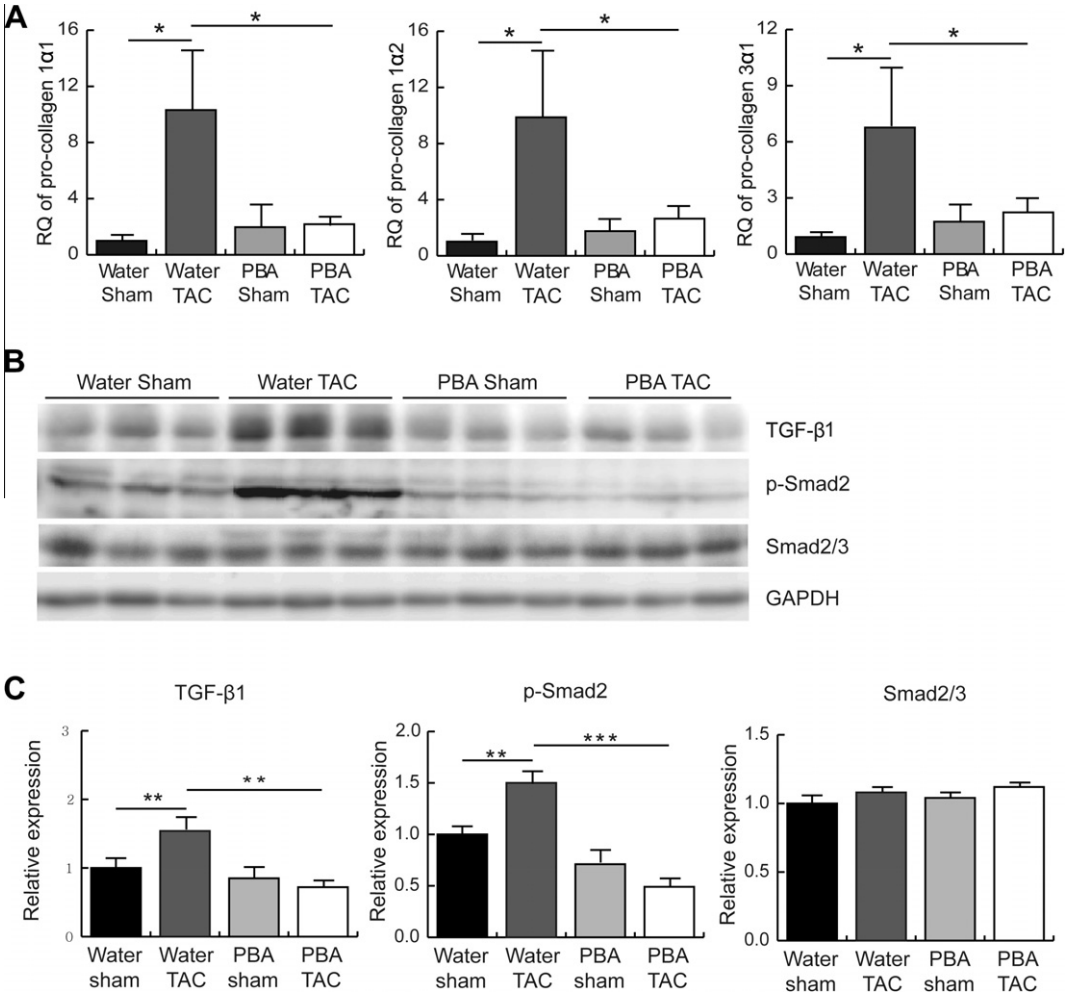

Fig. 3. PBA alleviated TAC-induced cardiac fibrosis. (A) Transcripts for pro-collagen isoforms were evaluated using real-time polymerase chain reaction by using hearts of the sham and TAC mice after treatment with water or PBA. The expressional levels of the transcripts for the pro-collagen isoforms decreased after PBA administration. (B) Western Blot results of transforming growth factor (TGF)-β1-Smad2 signaling pathway proteins in the heart whole homogenates of TAC mice treated with water or PBA. (C) Relative expression levels of TGF-β1-Smad2 signaling pathway proteins. The expression levels of TGF-β1 and p-Smad2 were significantly increased in the hypertrophic heart and were reduced by PBA administration. The protocol for the animal experiments is described in Fig. 1 legend (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 3–5).

4. Discussion

ERS induction has been reported to be correlated with hypertrophy and heart failure [17]. In light of the evidence that chemical chaperones could alleviate ERS in several tissues, including heart [9,18], in the present study, we investigated whether PBA could be used as a therapeutic drug in pressure-overload cardiac hypertrophy by using a mouse model of TAC. The key findings of this study are as follows: (1) the ERS responses were activated in TAC-induced cardiac hypertrophy (Fig. 1); (2) the oral administration of PBA reduced cardiac hypertrophy in the TAC group (Fig. 2); (3) cardiac fibrosis was attenuated by the oral administration of PBA in the TAC group (Fig. 3); and (4) ERS-induced apoptosis was attenuated by PBA treatment of HL-1 cells (Supplementary Fig. 1). Taken together, the present study suggests that the chemical chaperon can be used as a therapeutic drug for the treatment of cardiac hypertrophy and heart failure.

Our data demonstrated a significant reduction of pressure-overload cardiac hypertrophy by oral treatment of PBA, which could also reduce ERS responses (Figs. 1 and 2). The echocardiographic results also showed alleviation of hypertrophic characteristics (Table 1). Earlier studies reported the involvement of multiple pathways in cardiac hypertrophy and heart failure [19,20]. ERS may be a pathogenic factor of hypertrophy and heart failure [21]. The involvement of JNK, c-Jun, NF-KB, and other pathways has been studied in relation to ERS-induced hypertrophy and apoptosis [22,23]. In agreement with the results of these earlier studies, our data also showed that phosphorylation of JNK and c-Jun was significantly enhanced in TAC mice but reduced by PBA. PBA may abrogate deleterious signals such as activation of the JNK-c-Jun pathway that arise from ERS and progress to cardiac hypertrophy. However, because hypertrophy and heart failure are complex diseases, it is reasonable to expect that other ERS-independent mechanisms could also be contributing factors, which PBA treatment may not affect (Supplementary Fig. 4). In support of this, we found that few hypertrophic genes (~16 among 250 hypertrophic genes frequently reported in PubMed) were known to interact with ERS genes [24]. Thus, this could be a potential reason why we noticed only a slight but significant reduction in the hypertrophy instead of a complete recovery.

TGF-β is known to induce proliferation of cardiac fibroblasts, conversion of fibroblasts into myofibroblasts, deposition of extracellular matrix proteins such as collagen, and hypertrophy of cardiac myocytes [25]. Smad isoforms are downstream of TGF-β signaling and are reported to be phosphorylated (Smad2/3), associate with Smad4, and subsequently translocate from the cytosol to the nucleus, where they regulate the transcription of target genes including hypertrophy and fibrosis [26]. In this study, PBA treatment abrogated increased mRNA level of collagen isoforms, protein expression of TGF-β1, and phosphorylation of Smad2 proteins, which was observed in the TAC mice model, indicating inhibited cardiac remodeling and fibrosis. Studies have shown the interdependence between SMAD and JNK signaling in TGF-β-mediated transcription [27]. Our results show that PBA attenuated the phosphorylation of JNK and c-Jun, leading to suppressed JNK signaling (Supplementary Fig. 3), which may affect the SMAD signaling involved in the pathogenesis of cardiac fibrosis. In light of our results, we speculate that pathways involved in cardiac hypertrophy, fibrosis, and ERS responses may cross-talk and influence each other through key signaling molecules such as JNK (Supplementary Fig. 4). Further, it is possible that other systems and mechanisms are involved and act through this complex network of pathways [26].

As a chemical chaperone, PBA may play a direct role in helping cell with proper protein folding meet the increased protein synthesis demand in ER during hypertrophy and, thus influence UPR abrogation [28]. PBA effectiveness may also be attributed to other alternative mechanisms similar to the mechanisms of action of histone deacetylase (HDAC) inhibitors that prevent premature death due to cardiac hypertrophy and cardiac fibrosis [29]. The butyrate short-chain fatty-acid moiety of PBA is known to inhibit HDAC activity, and PBA itself can directly influence the transcription of certain proteins [30–32]. Further, PBA may also protect cells similar to overexpressed ER resident chaperones (e.g., GRP78) that are reported to attenuate cardiomyocyte death by inhibiting proteasome activity [22]. However, the effect of PBA on the proteasomal system has yet to be studied. Thus, we reason that PBA may affect cardiac fibrosis and hypertrophy in a number of already known ways and others that are yet to be discovered, which calls for further investigation.

We further tested whether ERS plays a role in cardiomyocyte apoptosis, a well-known phenomenon that occurs during the transition from cardiac hypertrophy to heart failure. Cardiomyocytes treated with TUN have been observed to develop ERS in as few as 4 h and undergo ERS-induced apoptosis after 24 h [33]. Our data showed that PBA could completely attenuate the expression of CHOP and cleaved caspase 3 as well as ERS-induced apoptosis in TUN-treated HL-1 cells (Supplementary Fig. 2) and activation of the JNK/c-Jun pathways that are necessary for apoptosis [34] in pressure-overload-induced hypertrophic mice (Supplementary Fig. 3), indicating that PBA may suppress the ERS-induced cardiac cell death involved in the transition of cardiac hypertrophy to heart failure. However, investigation into whether PBA can suppress the transition of hypertrophy to heart failure in an *in vivo* model has yet to be performed.

In conclusion, the UPR pathways are connected with other cellular pathways, including cardiac hypertrophy and fibrosis. The mechanism behind physiological and pathological UPR in cells in the presence of different diseases is an important topic for current and future investigation. In this study, we showed the inhibitory effect PBA on cardiac hypertrophy and fibrosis induced by pressure overload in TAC 1 week mice, suggesting the possibility of the use of PBA as a therapeutic agent for cardiac diseases. However, further studies are required to elucidate how different mechanisms of action of PBA can affect ERS and, thus can distinctly influence cardiac hypertrophy and heart failure. Such knowledge will lead to the design of better ERS-targeted therapies against deadly diseases such as heart failure.

Acknowledgments

This work was supported by Korea MEST NRF grant (20110002144), the 2012 GIST Systems Biology Infrastructure Establishment Grant and KISTI-KREONET.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.048.

References

[1] R.J. Kaufman, Orchestrating the unfolded protein response in health and disease, J. Clin. Invest. 110 (2002) 1389–1398.
[2] D. Ron, P. Walter, Signal integration in the endoplasmic reticulum unfolded protein response, Nat. Rev. Mol. Cell Biol. 8 (2007) 519–529.
[3] R.J. Kaufman, Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls, Genes Dev. 13 (1999) 1211–1233.
[4] K.F. Ferri, G. Kroemer, Organelle-specific initiation of cell death pathways, Nat. Cell Biol. 3 (2001) E255–263.

[5] D.G. Krauser, R.B. Devereux, Ventricular hypertrophy and hypertension: prognostic elements and implications for management, Herz 31 (2006) 305–316.
[6] M.A. Brostrom, F. Mourad, C.O. Brostrom, Regulated expression of GRP78 during vasopressin-induced hypertrophy of heart-derived myocytes, J. Cell. Biochem. 83 (2001) 204–217.
[7] K. Okada, T. Minamino, Y. Tsukamoto, Y. Liao, O. Tsukamoto, S. Takashima, A. Hirata, M. Fujita, Y. Nagamachi, T. Nakatani, C. Yutani, K. Ozawa, S. Ogawa, H. Tomoike, M. Hori, M. Kitakaze, Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis, Circulation 110 (2004) 705–712.
[8] C.C. Glembotski, The role of the unfolded protein response in the heart, J. Mol. Cell. Cardiol. 44 (2008) 453–459.
[9] E. Papp, P. Csermely, Chemical chaperones: mechanisms of action and potential use, Handb. Exp. Pharmacol. (2006) 405–416.
[10] L. Ozcan, A.S. Ergin, A. Lu, J. Chung, S. Sarkar, D. Nie, M.G. Myers Jr., U. Ozcan, Endoplasmic reticulum stress plays a central role in development of leptin resistance, Cell Metab. 9 (2009) 35–51.
[11] I. Ben Mosbah, I. Alfany-Fernandez, C. Martel, M.A. Zaouali, M. Bintanel-Morcillo, A. Rimola, J. Rodes, C. Brenner, J. Rosello-Catafau, C. Peralta, Endoplasmic reticulum stress inhibition protects steatotic and non-steatotic livers in partial hepatectomy under ischemia-reperfusion, Cell. Death. Dis. 1 (2010) e52.
[12] H. Cha, J.M. Kim, J.G. Oh, M.H. Jeong, C.S. Park, J. Park, H.J. Jeong, B.K. Park, Y.H. Lee, D. Jeong, D.K. Yang, O.Y. Bernecker, D.H. Kim, R.J. Hajjar, W.J. Park, PICOT is a critical regulator of cardiac hypertrophy and cardiomyocyte contractility, J. Mol. Cell. Cardiol. 45 (2008) 796–803.
[13] C.S. Hong, S.J. Kwon, D.H. Kim, Multiple functions of junctin and junctate, two distinct isoforms of aspartyl beta-hydroxylase, Biochem. Biophys. Res. Comm. 362 (2007) 1–4.
[14] D. Jeong, H. Cha, E. Kim, M. Kang, D.K. Yang, J.M. Kim, P.O. Yoon, J.G. Oh, O.Y. Bernecker, S. Sakata, T.T. Le, L. Cui, Y.H. Lee, H. Kim do, S.H. Woo, R. Liao, R.J. Hajjar, W.J. Park, PICOT inhibits cardiac hypertrophy and enhances ventricular function and cardiomyocyte contractility, Circ. Res. 99 (2006) 307–314.
[15] J. Massague, How cells read TGF-beta signals, Nat. Rev. Mol. Cell. Biol. 1 (2000) 169–178.
[16] D. Javelaud, A. Mauviel, Mammalian transforming growth factor-betas: Smad signaling and physio-pathological roles, Int. J. Biochem. Cell. Biol. 36 (2004) 1161–1165.
[17] J. Groenendyk, P.K. Sreenivasaiah, D.H. Kim, L.B. Agellon, M. Michalak, Biology of endoplasmic reticulum stress in the heart, Circ. Res. 107 (2010) 1185–1197.
[18] P. Ayala, J. Montenegro, R. Vivar, A. Letelier, P.A. Urroz, M. Copaja, D. Pivet, C. Humeres, R. Troncoso, J.M. Vicencio, S. Lavandero, G. Diaz-Araya, Attenuation of endoplasmic reticulum stress using the chemical chaperone 4-phenylbutyric acid prevents cardiac fibrosis induced by isoproterenol, Exp. Mol. Pathol. 92 (2012) 97–104.
[19] J.O. Mudd, D.A. Kass, Tackling heart failure in the twenty-first century, Nature 451 (2008) 919–928.
[20] J. Heineke, J.D. Molkentin, Regulation of cardiac hypertrophy by intracellular signalling pathways, Nat. Rev. Mol. Cell. Biol. 7 (2006) 589–600.
[21] M. Kitakaze, O. Tsukamoto, What is the role of ER stress in the heart? Introduction and series overview, Circ. Res. 107 (2010) 15–18.
[22] T. Hamid, S.Z. Guo, J.R. Kingery, X. Xiang, B. Dawn, S.D. Prabhu, Cardiomyocyte NF-kappaB p65 promotes adverse remodelling, apoptosis, and endoplasmic reticulum stress in heart failure, Cardiovasc. Res. 89 (2011) 129–138.
[23] H.Y. Fu, T. Minamino, O. Tsukamoto, T. Sawada, M. Asai, H. Kato, Y. Asano, M. Fujita, S. Takashima, M. Hori, M. Kitakaze, Overexpression of endoplasmic reticulum-resident chaperone attenuates cardiomyocyte death induced by proteasome inhibition, Cardiovasc. Res. 79 (2008) 600–610.
[24] P.K. Sreenivasaiah, S. Rani, J. Cayetano, N. Arul, D.H. Kim, IPAVS: integrated pathway resources, analysis and visualization system, Nucl. Acids Res. 40 (2011) D803–D808.
[25] S. Rosenkranz, TGF-beta1 and angiotensin networking in cardiac remodeling, Cardiovasc. Res. 63 (2004) 423–432.
[26] I.M. Dixon, J. Hao, N.L. Reid, J.C. Roth, Effect of chronic AT(1) receptor blockade on cardiac Smad overexpression in hereditary cardiomyopathic hamsters, Cardiovasc. Res. 46 (2000) 286–297.
[27] M.E. Engel, M.A. McDonnell, B.K. Law, H.L. Moses, Interdependent SMAD and JNK signaling in transforming growth factor-beta-mediated transcription, J. Biol. Chem. 274 (1999) 37413–37420.
[28] G.H. Yam, K. Gaplovska-Kysela, C. Zuber, J. Roth, Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis, Invest. Ophthalmol. Vis. Sci. 48 (2007) 1683–1690.
[29] D.J. Cao, Z.V. Wang, P.K. Battiprolu, N. Jiang, C.R. Morales, Y. Kong, B.A. Rothermel, T.G. Gillette, J.A. Hill, Histone deacetylase (HDAC) inhibitors attenuate cardiac hypertrophy by suppressing autophagy, Proc. Natl. Acad. Sci. USA 108 (2011) 4123–4128.
[30] K. Nakayama, T. Ohkawara, M. Hiratochi, C.S. Koh, H. Nagase, The intracellular domain of amyloid precursor protein induces neuron-specific apoptosis, Neurosci. Lett. 444 (2008) 127–131.
[31] W.Y. Chen, E.C. Bailey, S.L. McCune, J.Y. Dong, T.M. Townes, Reactivation of silenced, virally transduced genes by inhibitors of histone deacetylase, Proc. Natl. Acad. Sci. USA 94 (1997) 5798–5803.
[32] R.C. Rubenstein, P.L. Zeitlin, Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of DeltaF508-CFTR, Am. J. Physiol. Cell. Physiol. 278 (2000) C259–267.
[33] W.L. Corwin, J.M. Baust, J.G. Baust, R.G. Van Buskirk, The unfolded protein response in human corneal endothelial cells following hypothermic storage: implications of a novel stress pathway, Cryobiology 63 (2011) 46–55.
[34] X.L. Yin, W. Zhang, Y. Yang, H. Shen, Increasing expression of (CCAAT enhancer binding protein) homologous protein induced by endoplasmic reticulum stress in myocardium after cardiac arrest and resuscitation in rat, Resuscitation 83 (2012) 378–385.