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# Parathyroid hormone accelerates decompensation following left ventricular hypertrophy

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Abbreviations: FS, fractional shortening; LVH, left ventricular hypertrophy; LVIDs, left ventricular internal dimension at systole; LVPWs, left ventricular posterior wall thickness at systole; PTH, parathyroid hormone; TAB, transverse aortic banding

## Abstract

**Parathyroid hormone (PTH) treatment was previously shown to improve cardiac function after myocardial infarction by enhancing neovascularization and cell survival. In this study, pressure overload-induced left ventricular hypertrophy (LVH) was induced in mice by transverse aortic banding (TAB) for 2 weeks. We subsequently evaluated the effects of a 2-week treatment with PTH or saline on compensated LVH. After another 4 weeks, the hearts of the mice were analyzed by echocardiography, histology, and molecular biology. Echocardiography showed that hearts of the PTH-treated mice have more severe failing phenotypes than the saline-treated mice following TAB with a greater reduction in fractional shortening and left ventricular posterior wall thickness and with a greater increase in left ventricular internal dimension. Increases in the heart weight to body weight ratio and lung weight to body weight ratio following TAB were significantly exacerbated in PTH-treated mice compared to saline-treated mice. Molecular markers for heart failure, fibrosis, and angiogenesis were also altered in accordance with more severe heart failure in the PTH-treated mice compared to the saline-treated mice following TAB. In addition, the PTH-treated hearts were manifested with increased fibrosis accompanied by an enhanced SMAD2 phosphorylation. These data suggest that the PTH treatment may accelerate the process of decompensation of LV, leading to heart failure.**

**Keywords:** fibrosis; heart failure; hypertrophy, left ventricular; parathyroid hormone

## Introduction

Parathyroid hormone (PTH) plays a role in calcium homeostasis, and PTH expression is regulated by the calcium concentration. A low calcium level in the plasma triggers the secretion of PTH from the parathyroid gland. The secreted PTH then raises the calcium level in plasma by promoting the release of calcium from bone, reducing the calcium excretion by the kidneys, and increasing the calcium absorption by the small intestine. In turn, the increased calcium level inhibits PTH secretion from the parathyroid gland (Pocotte *et al*., 1991).

In addition to traditionally known target organs such as bone, kidney, and small intestine, the ventricular cardiomyocytes were also reported to be targets of PTH (Schluter and Piper, 1992). PTH was shown to act as a pro-hypertrophic factor by increasing protein synthesis *in vitro* (Schluter and Piper, 1998) and as a vasodilator by increasing the intracellular cAMP level, leading to a decrease in calcium influx (Clemens *et al*., 2001). The mass of the left ventricle was correlated with the PTH level in patients with end-stage renal disease and secondary hyperparathyroidism (Harnett *et al*., 1988) and in patients with primary hyperparathyroidism (Piovesan *et al*., 1999). However, no significant correlation between the left ventricular mass and PTH level was found in the general population (Saleh *et al*., 2003). The role of PTH in the development of left ventricular hypertrophy (LVH) has remained obscure.

Recently, PTH was shown to exert beneficial effects in a mouse model of myocardial infarction (MI) by improving stem cell mobilization (Zaruba *et al*., 2008). This finding raised an interesting question as to whether or not PTH treatment would be beneficial for other types of cardiovascular abnormalities. This issue may be extremely important considering that an elevated level of PTH may be a risk factor for cardiovascular disorders (McCarty *et al*., 2009).

Cardiac remodeling affects gene expression pro-

files in cardiomyocytes. Notably, the expression levels of sarcoplasmic reticulum $Ca^{2+}$ ATPase (SERCA2a) and $\alpha$-myosin heavy chain ($\alpha$-MHC) are reduced in failing hearts, whereas the levels of brain natriuretic peptide (BNP), TGF-$\beta$1, and collagen 1 are increased. In this study, we performed transverse aortic banding (TAB) in order to induce LVH, which is an established method to generate pressure- overload in the murine heart (Rockman *et al*., 1991). We then evaluated the effects of PTH treatment on pressure overload-induced LVH using echocardiography and molecular analyses. Our data show that PTH treatment accelerated the progression of LVH to heart failure but did not significantly affect the heart under normal conditions.

## Results

### Echocardiographic and histological assessments

The experimental design is summarized in Figure 1. All the mice were examined by echocardiography at baseline, and then TAB or sham operations were carried out. Two weeks later, the TAB-operated mice ($n$ = 36) were re-examined by echocardiography, and the mice ($n$ = 18) with compensated LVH ($>$ 30% increase in interventricular septum thickness at diastole, IVSTd, and $>$ 40% of fractional shortening, FS) were selected and randomly divided into TAB/Saline ($n$ = 9) and TAB/PTH ($n$ = 9) groups. The sham-operated mice ($n$ = 10) were randomly grouped into Sham/Saline ($n$ = 5) and Sham/PTH ($n$ = 5) groups. The mice were then treated with saline or PTH for two weeks as described in Methods. Three mice from the TAB/Saline group and two mice from TAB/PTH group died during the period of PTH or saline treatment. Four weeks later, echocardiographic, histological, and molecular analyses were conducted. We chose this time point for final phenotypic and molecular analyses because a mice strain C57BL/6 used in this study usually showed typical heart failure phenotypes at eight weeks post-banding. Three additional mice from the TAB/ PTH group died under anesthesia during the final echocardiographic analysis. These mice exhibited phenotypes of severe congestive heart failure with abnormally high heart weight to body weight ratio (HW/BW, 8.9, 10.2, and 9.5 mg/g) and lung weight to body weight ratio (LW/BW, 12.2, 13.2, and 12.1 mg/g).

All the remaining viable mice were subjected to echocardiography to assess the progression of LVH. As expected, the prolonged pressure overload induced the typical phenotypes of heart failure (Figure 2). The hearts of saline-treated mice were characterized by reductions in FS, left ventricular posterior wall thickness at systole (LVPWs), and left ventricular internal dimension at systole (LVIDs) following TAB. We observed that the PTH-treated mice exhibited more severe failing phenotypes than the saline-treated mice following TAB, with greater reductions in FS (40.3 $\pm$ 5.3% vs. 26.0 $\pm$ 5.3% after saline or PTH treatment, respectively, $P < 0.05$) and LVPWs (1.8 $\pm$ 0.5 mm vs. 1.2 $\pm$ 0.2 mm after saline or PTH treatment, respectively, $P < 0.05$), and a greater increase in LVIDs (1.9 $\pm$ 0.5 mm vs. 2.8 $\pm$ 0.5 mm after saline or PTH treatment, respectively, $P < 0.05$).

While PTH treatment did not affect the HW/BW in shame-operated mice, it significantly exacerbated the increased HW/BW following TAB (6.0 $\pm$ 0.5 mg/g vs. 7.8 $\pm$ 1.7 mg/g after saline or PTH treatment, respectively) (Figure 3). The increase in the LW/BW, indicative of congestive heart failure, following TAB was also significantly exacerbated in PTH-treated mice (8.9 $\pm$ 3.4 mg/g) compared to saline-treated mice (5.9 $\pm$ 0.7 mg/g).

### Molecular assessments

Quantitative RT-PCR analyses confirmed that the hearts from the saline-treated mice under the prolonged pressure overload underwent an adverse cardiac remodeling in the molecular level (Figure 4). The hearts from the PTH-treated mice under the same conditions exhibited more severe alterations in the gene expression levels. Especially, the expression levels of BNP, a marker for adverse

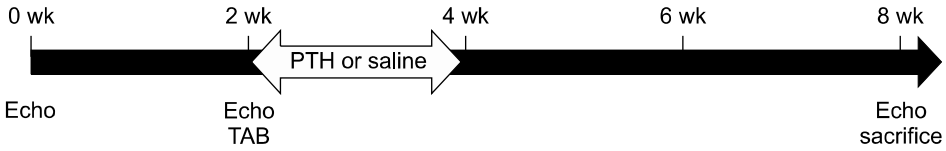
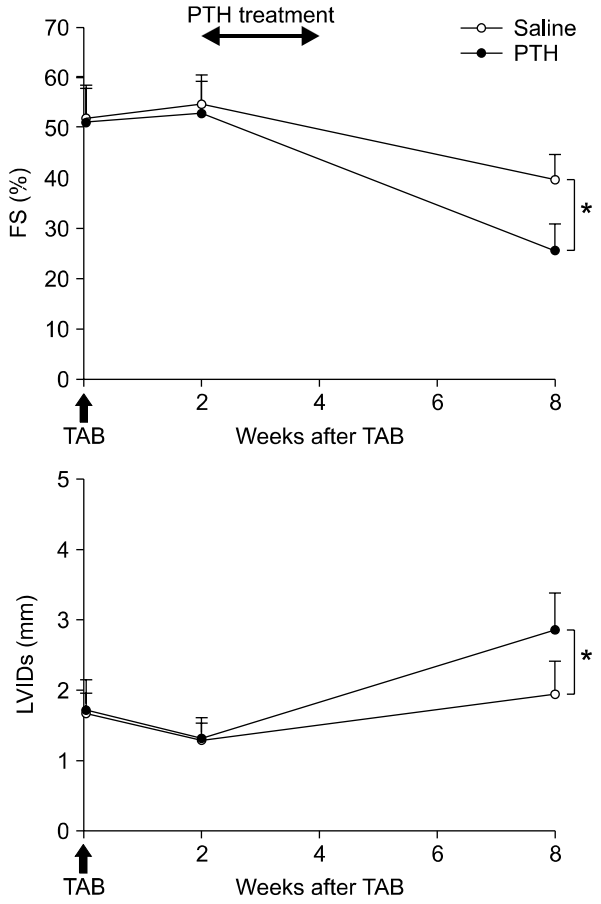



**Figure 1.** Experimental Design. After 2 weeks of transverse aortic banding (TAB), echocardiography was conducted in order to confirm the establishment of compensated left ventricular hypertrophy (LVH). Mice were divided into four groups following TAB or sham surgery (Sham/Saline, Sham/PTH, TAB/Saline, and TAB/PTH) and treated with either saline (0.9% NaCl) or PTH (80 $\mu$g/kg/d) for 2 weeks. After additional 4 weeks, the hearts of the mice were examined by echocardiography and then the mice were euthanized for histological and molecular analyses.



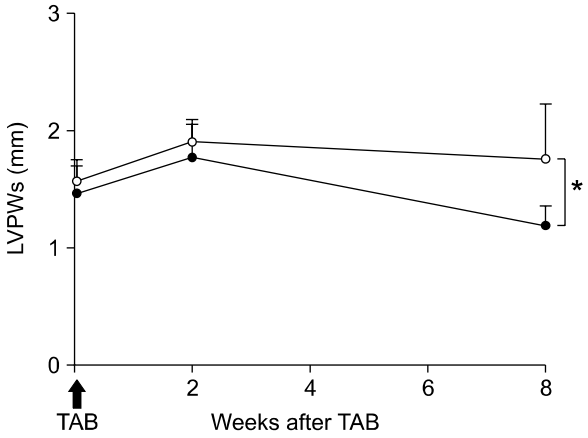




**Figure 2.** Echocardiographic parameters. Shown are the results of the serial echocardiographic measurements on mice following transverse aortic banding (TAB) or sham surgery and subsequent treatment with saline or parathyroid hormone (PTH). Mice of the TAB/Saline group are represented with open circles ($n = 6$) and mice of the TAB/PTH group are represented with closed circles ($n = 4$). Factional shortening (FS), left-ventricular posterior wall thickness at systole (LVPWs), and leftventricular internal dimension at systole (LVIDs) are presented. FS and LVPWs were significantly reduced in mice of the TAB/PTH group compared to mice in the TAB/Saline group, whereas, LVIDs was significantly increased in mice of the TAB/PTH group compared to mice of the TAB/Saline group. Data are presented as mean $\pm$ SD. *$P < 0.05$.

remodeling, and TGF-$\beta$1, a marker for fibrosis, were far more elevated in the hearts from the PTH-treated mice compared to the saline-treated mice following TAB (BNP: 2.1 $\pm$ 0.4 fold vs. 4.1 $\pm$ 1.0 fold after saline or PTH treatment, respectively; TGF-$\beta$1: 1.3 $\pm$ 0.1 fold vs. 1.8 $\pm$ 0.4 fold after saline or PTH treatment, respectively). The expression of VEGF-A was also found to be increased in the PTH-treated mice following TAB. These data indicate that some of signalings associated with heart failure is profoundly expressed in the PTH treated hearts. The expression patterns of SERCA2a and $\alpha$-MHC, markers for reduced contractility in the failing hearts, and collagen 1, a marker for fibrosis, were not significantly altered by the treatment of PTH. This may imply the heterogeneity of heart failure signalings affected by PTH. The heart sections were stained with trichrome to detect fibrotic areas. The fibrosis normally detected following the long-term TAB was significantly exacerbated by the PTH treatment (Figure 5A). Cardiac fibrosis is known to be mediated by the activation of TGF-$\beta$ receptor, which triggers a phosphorylation of SMAD2/3. By Western blotting, we detected that the PTH treatment increases the phosphorylation of SMAD2 by 15% (Figure 5B). These data, combined with the echocardiographic data, suggest that PTH treatment accelerates decompensation of LVH hearts.

## Discussion

LVH appears to be a compensatory response of hearts to a variety of insults, including pressure overload. However, sustained LVH usually leads to the deterioration of heart functions, such as a reduced FS accompanied by wall thinning and chamber dilation. Since LVH is considered to be an independent risk factor for heart failure, intensive efforts have been focused on elucidating the signaling pathways involved in LVH (Hardt and Sadoshima, 2004) and developing strategies to prevent the transition from LVH to heart failure (Anamourlis *et al.*, 2006).

In the present study, we demonstrated that PTH

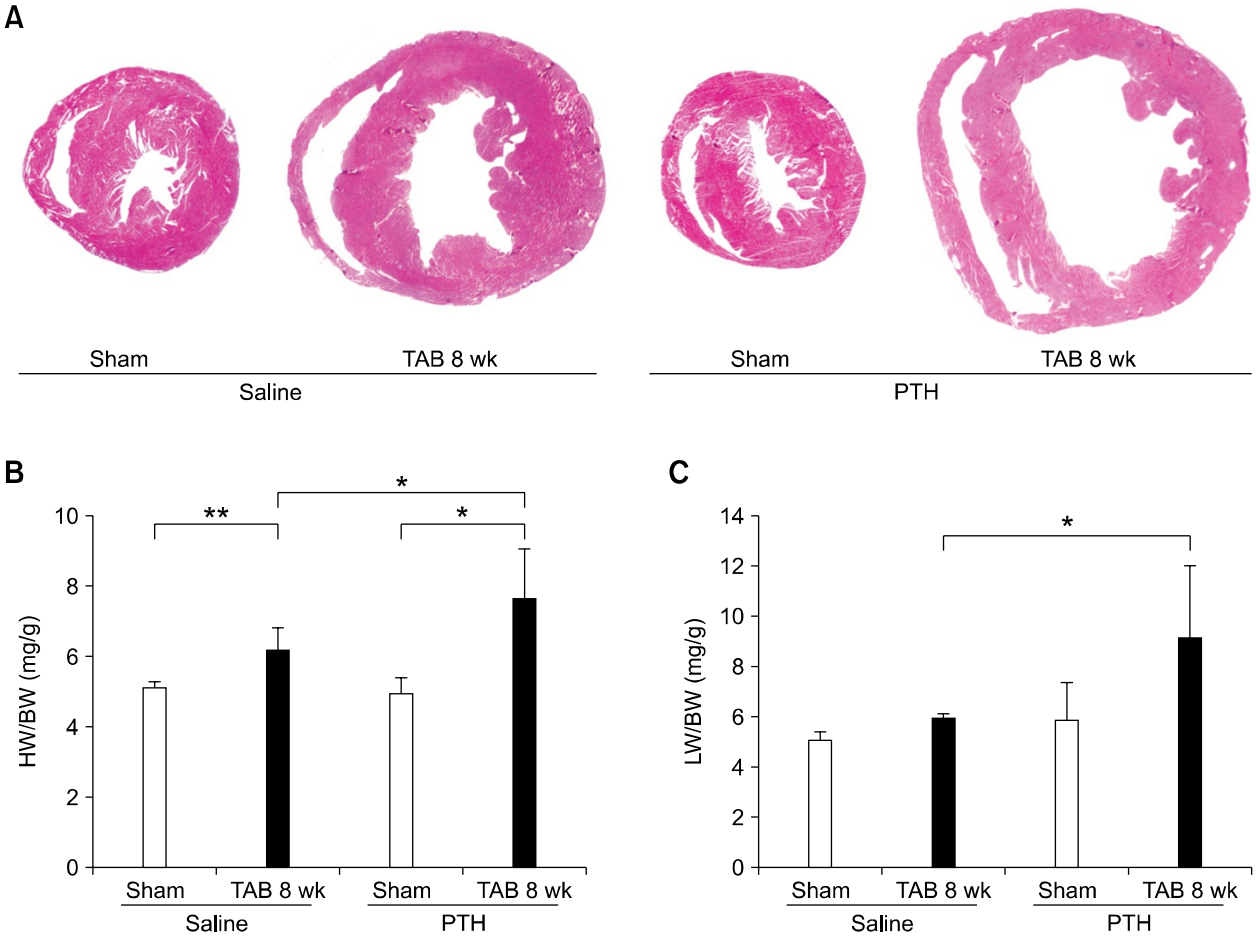



**Figure 3.** Heart weight to body weight ratio (HW/BW) and lung weight to body weight ratio (LW/BW). Mice were euthanized at the end of experiment and their heart and wet lung weights were measured and compared to their body weights. Greater increases in the HW/BW and LW/BW were observed in mice of the TAB/PTH group ($n$ = 6) compared with mice of the TAB/Saline group ($n$ = 4). Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.

treatment exerted detrimental effects on LVH. PTH-treated mice under prolonged pressure overload developed more severe heart failure than saline-treated mice under the same conditions, as assessed by echocardiographic, histological, and molecular analyses. Our finding is consistent with the notion that elevated PTH levels is a risk factor for cardiovascular disorders (McCarty *et al*., 2009). The expression of VEGF in the hearts was increased by PTH treatment. This may contribute to the rapid deterioration of the PTH-treated hearts because it was previously shown that VEGF-induced vascular permeability resulted in extensive injury to ischemic tissues after myocardial infarction (Weis and Cheresh, 2005). The elevated expression of TGF-β may also contribute to the adversary effects of PTH treatment on LVH (Ruiz-Ortega *et al*., 2007).

Our finding is seemingly in contrast with a previous report stating that PTH exerted beneficial effects in a mouse model of myocardial infarction (MI) by improving stem cell mobilization (Zaruba *et al*., 2008). Since a severe loss of cardiomyocytes occurs in MI, a replacement of the dead cells with viable cells is a valuable strategy for the treatment of MI. Supporting this notion, the employment or mobilization of various stem cells has been shown to improve contractile functions in MI (Orlic *et al*., 2001; Laflamme *et al*., 2007). Therefore, it is not so surprising to see that an elevated stem cell mobilization by PTH exerted beneficial effects in MI. In contrast to MI, LVH is not accompanied by a severe cell loss but rather characterized by dysfunctions in calcium metabolism. Therefore, the efficiency of stem cell approaches is unproven in LVH. We suggest that the adversary effects of PTH probably mediated through elevation of VEGF and TGF-β overwhelm the beneficial effect of PTH mediated by improving stem cell mobilization in LVH.

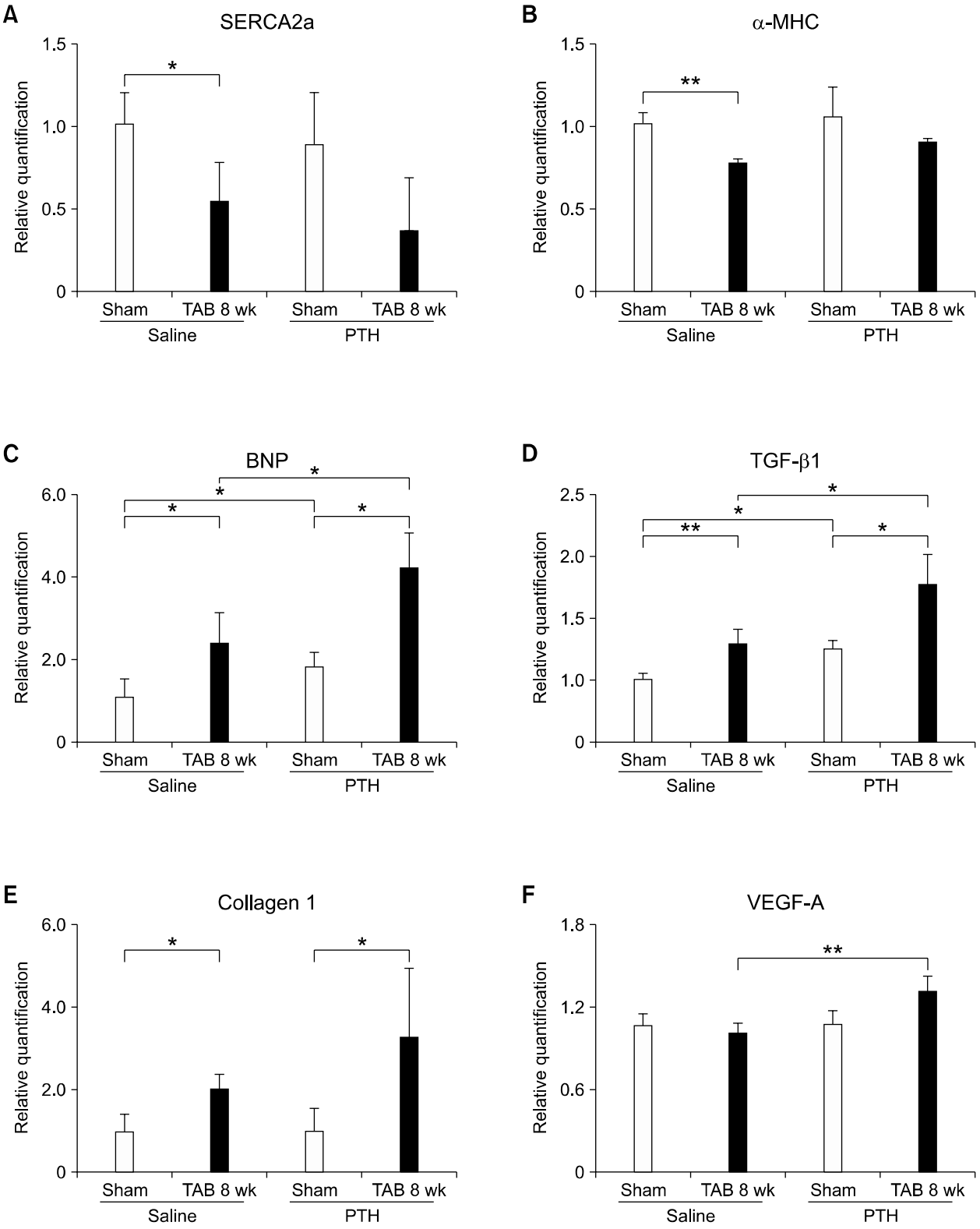



**Figure 4.** Quantitative RT-PCR. RNA was isolated from the hearts of mice from the four experimental groups and subjected to quantitative RT-PCR. The expression levels of sarcoplasmic reticulum $Ca^{2+}$ ATPase (SERCA2a) and alpha-myosin heavy chain (α-MHC) were significantly reduced following transverse aortic banding (TAB), whereas the levels of brain natriuretic peptide (BNP), TGF-β1, and collagen 1 were significantly increased. Note that these changes in the gene expression were more profound with parathyroid hormone (PTH) treatment. The numbers of mice available for this analysis were as follows: Sham/Saline ($n$ = 3), Sham/PTH ($n$ = 3), TAB/Saline ($n$ = 4), and TAB/PTH ($n$ = 4). Data are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$.

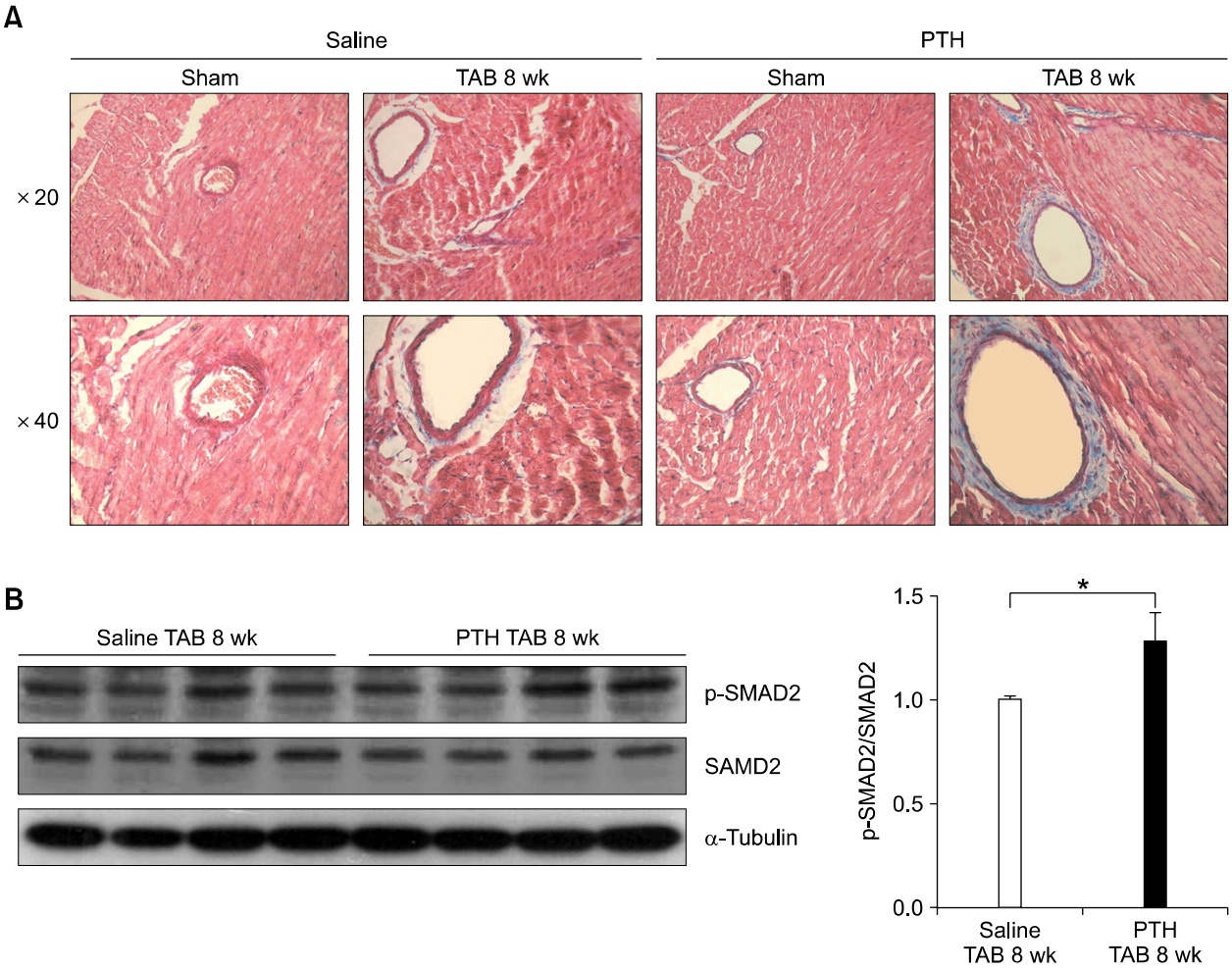



**Figure 5.** Effects on fibrosis. Heart sections were stained with Masson's Trichrome kit. The fibrotic areas stained blue and the normal tissues displayed red. (A) Aggravated fibrosis was observed in mice of the TAB/PTH group compared with mice of the TAB/Saline group. (B) Western blot analysis indicates an increased phosphorylation of SMAD2 protein. The blot was scanned and quantitated using a densitometer. Bar graph represents the density ratio of phosphorylated SMAD2 to total SMAD2. Data are presented as mean ± SD. *$P < 0.05$.

Together, the results of this study suggest that PTH treatment accelerates decompensation of hearts with LVH. PTH is currently approved by the FDA for the treatment of osteoporosis. Considering that the elderly are prone to developing both osteoporosis and LVH (McMurray and Pfeffer, 2005; Dickstein *et al*., 2008), a caution should be taken when PTH is prescribed in clinics for the treatment of osteoporosis.

## Methods

### Subjects

C57BL/6 male mice (22-27 g), 8 weeks of age, were purchased from Japan SLC, Inc. and used in this study. Animal handling and experimentation were performed according to the regulation of Gwangju Institute of Science and Technology.

### Echocardiography

Mice were anesthetized with 0.5-0.7 ml of 1× Avertin solution (mixture of 2-2-2 tribromoethanol and tert-amyl alcohol) by intraperitoneal injection. The chests of the mice were shaved and echocardiography was performed as previously described (Lee *et al*., 2009) using a Powervision 6000 (TOSHIBA) 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 2-dimensional mode. Frozen frames were printed using a video graphic printer (UP-895MD, Sony).

### Transverse aortic banding (TAB)

After 2 weeks of baseline echocardiographic monitoring,

TAB or sham surgery was performed as previously described (Jeong *et al*., 2006). Specifically, the mice were anesthetized with 1 × Avertin by intraperitoneal injection and their breathing was controlled with a ventilator (Harvard Apparatus) while the mice were on heating pad, with a tidal volume of 0.1 ml and a respiratory rate of 120 breaths per minute. In order to visualize the aortic arch, a 2- to 3-mm longitudinal cut was made in the proximal portion of the sternum. The transverse aortic arch was ligated between the innominate and the left common carotid arteries with an overlying 27-gauge needle. The needle was immediately removed, leaving a discrete region of constriction. The operation for the sham controls was conducted in the same way but without the ligation. At 1 week post-operation, mortality was less than 10%.

### Random grouping

Two weeks after the TAB or sham surgery, echocardiography was again conducted in order to identify mice with compensated LVH. We selected eighteen mice from among thirty-six TAB-operated mice; those animals that did not pass our two criteria for inclusion were eliminated from the study. First, animals in which the post-operative increase in the diastolic interventricular septum thickness (IVSTd), compared to the baseline IVSTd for that animal, was less than 30% were eliminated. Second, animals with a fractional shortening (FS) of less than 40% were also discarded. The eighteen remaining animals with compensated LVH following TAB were randomly assigned to a saline treatment group (TAB/Saline, $n = 9$) or a PTH treatment group (TAB/PTH, $n = 9$). The mean FS was 54% in the TAB/Saline group and 53% in the TAB/PTH group. Sham-operated animals were also randomly divided into a saline treatment group (Sham/Saline, $n = 5$) or a PTH treatment group (Sham/ PTH, $n = 5$).

### Saline and PTH (1-34) administration

The saline and PTH treatment were initiated the day following the second echocardiographic monitoring. Sham/saline and TAB/saline groups received daily subcutaneous injection of 0.1 ml of saline (0.9% NaCl) for 2 weeks. Sham/PTH and TAB/ PTH groups received daily subcutaneous injections of PTH 1-34 (80 μg/kg/d) for same period (Bachem), as previously described (Zaruba *et al*., 2008). Echocardiography was again performed 4 weeks after the completion of the PTH and saline treatments.

### Quantitative RT-PCR

After the echocardiographic measurements were completed, the hearts and the lungs were removed, weighted, and frozen with liquid nitrogen. Frozen hearts were grinded to powder with liquid nitrogen. Half of the powder was used to prepare total RNA using the TRI reagent (Sigma) as previously described (Jeong *et al*., 2006). Reverse-transcription was performed using ImProm II reverse-transcriptase (Promega) with oligo-dT priming. PCR was performed using an ABI PRISM Sequence Detector System 7500 (Applied Biosystems) with SYBR Green (TAKARA) as the fluorescent dye and ROX (TAKARA) as the passive reference dye. The PCR primers used were as follows: SERCA2a, 5'-GGT CCT GGC AGA TGA CAA CTT C-3' and 5'-CAG CGC CAA CAT AAC AGC CAA TAG-3'; α-MHC, 5'-ACA ATG CGG AAG TGG TGG-3' and 5'-TCT TGC TAC GGT CCC CTA TG-3'; BNP, 5'-GCT GCT TTG GGC ACA AGA TAG-3' and 5'-GGT CTT CCT ACA ACA ACT TCA-3'; TGF-β1, 5'-GTG TGG AGC AAC ATG TGG AAC TGT A-3' and 5'-TTG GTT CAG CCA CTG CCG TA-3'; Collagen 1, 5'-CGA AGG CAA CAG TCG CTT CA-3' and 5'-GGT CTT GGT GGT TTT GTA TTC CAT-3'; VEGF-A, 5'-CTG GAT ATG TTT GAC TGC TGT GGA-3' and 5'-GTT TCT GGA AGT GAG CCA ATG TG-3' and GAPDH, 5'-TCC GTG TTC CTA CCC CCA ATG-3' and 5'-GGG AGT TGC TGT TGA AGT CGC-3'.

### Histological analysis

Mice of all four groups were sacrificed and fixed in 10% formalin and embedded in paraffin. Paraffin blocks were cut into 5 μm slices and stained with Hematoxylin (Sigma-Aldrich, HHS32)-Eosin (Sigma-Aldrich, HT110116) solution. Masson's Trichrome staining was conducted as a manufacturer's manual (Sigma-Aldrich, HT15 kit).

### Western blot analysis

Heart extracts were homogenated in 50 mM Tris-Cl buffer (pH 7.4) with protease inhibitor cocktail (Calbiochem, 539134) and phosphatase inhibitor (Santa Cruz, SC-45044). Protein homogenates (50 μg/lane) were separated SDS-PAGE gel and transferred to PVDF membrane (Schleicher & Schuell, Germany). After 1 h blocking with 5% non-fat milk, the membrane was incubated with antibodies against Smad2, phospho-SMAD2 (Cell Signaling) and α-tubulin (Santa Cruz, SC-5286). The membrane was incubated with a secondary antibody conjugated to HRP (Jackson ImmnoResearch) and developed using chemiluminescent substrate (PerkinElmer).

### Statistics

All data were reported as the mean ± standard deviation (SD). Statistical significance was analyzed using unpaired Student's *t*-tests. $P < 0.05$ was considered statistically significant.

### Acknowledgements

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