Vitamin D signaling pathway plays an important role in the development of heart failure after myocardial infarction

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Bae S, Singh SS, Yu H, Lee JY, Cho BR, Kang PM. Vitamin D signaling pathway plays an important role in the development of heart failure after myocardial infarction (MI) in mice. Vitamin D signaling was activated by administration of paricalcitol (PC), an activated vitamin D analog. Wild-type (WT) mice underwent sham or MI surgery and were treated with either vehicle or PC. Compared with vehicle group, PC attenuated development of heart failure after MI associated with decreases in biomarkers, apoptosis, inflammation, and fibrosis. There was also improvement of cardiac function with PC treatment after MI. Furthermore, vitamin D receptor (VDR) mRNA and protein levels were restored by PC treatment. Next, to explore whether defective vitamin D signaling exhibited deleterious responses after MI, WT and VDR knockout (KO) mice underwent sham or MI surgery and were analyzed 4 wk after MI. VDR KO mice displayed a significant decline in survival rate and cardiac function compared with WT mice after MI. VDR KO mice also demonstrated a significant increase in heart failure biomarkers, apoptosis, inflammation, and fibrosis. Vitamin D signaling promotes cardioprotection after MI through anti-inflammatory, antifibrotic and antiapoptotic mechanisms.

MATERIALS AND METHODS

Adult cardiomyocyte culture and apoptotic stimulation. Primary cultures of cardiomyocytes from 6-wk-old Sprague-Dawley rats were prepared as described (14, 22). Twenty-four hours after PC pretreatment (20 μM), cardiomyocytes were exposed to hydrogen peroxide (H2O2) at 0.1 mM for 24 h to induce apoptosis.

Animal surgery and treatment. Male VDR KO mice (Jackson Laboratory, Bar Harbor, ME) and their littermates in a C57BL/6 background were used as a model for defective vitamin D signaling. To activate vitamin D signaling, 10–12-wk-old male mice were administered with PC or vehicle (Veh) 1 wk before MI and continued until the end of the experiment. Wild-type (WT) mice underwent sham or MI operation with either Veh treatment or PC therapy (15 ng/mouse, i.p., 3 times per week). Coronary artery ligation was performed as described previously (46). After anesthesia with isoflurane (initially 4–5% in an induction chamber then 2% via intubation tube) via a precise vaporizer inhaler, each mouse was intubated, and the heart was exposed and the left anterior descending (LAD) artery ligated proximally with a 7–0 silk suture. We confirmed the occlusion by the pallor of the anterior left ventricular wall. All groups of mice were age- and sex-matched littersmates. For sham operations, the mice underwent the same procedure except for LAD ligation. Euthanasia

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was performed by CO₂ via a gas cylinder followed by heart extraction. All procedures were approved by and performed in accordance with Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee guidelines.

Cardiac function analysis. Cardiac function was evaluated by measuring the left ventricular (LV) pressure-volume (PV) loop to calculate hemodynamic parameters, including cardiac output (CO), stroke work (SW), stroke volume (SV), and isovolumetric relaxation as described previously (3, 13).

Apoptosis assays. The activities of caspase-3 and poly(ADP-ribose) polymerase (PARP)-1 were determined with colorimetric assay kit (R&D Systems, Minneapolis, MN) as described previously (3, 13). Briefly, for caspase-3 activity assay, protein samples were added to substrates of Acetyl-Asp-Glu-Val-Asp-p-nitroanilide, and enzyme-catalyzed release of p-nitroanilide was then measured at 405 nm. For PARP activity assay, activity was measured at 450 nm by incorporation of biotinylated poly (ADP-ribose) onto histone-coated proteins in the plate using colorimetric assay kit. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was performed using in situ fluorescein-based Cell Death Detection Kits (Roche Applied Science, Indianapolis, IN) as described previously (3). Quantitative analysis of apoptotic nuclei were performed in border zones of infarcted hearts. To distinguish cardiomyocyte from noncardiomyocyte nuclei, we triple stained for nuclei [4',6-diamidino-2-phenylindole (DAPI) staining], apoptotic nuclei (TUNEL staining), and cardiomyocytes (α-actinin staining) and analyzed the stained sections using confocal microscopy. A minimum of 200 nuclei/field were counted for each sample.

RT-PCR for mRNA expression. Heart tissues were collected just below the level of papillary muscles and processed for molecular analysis 4 wk after MI. Semiquantitative RT-PCR was performed as described previously (4). The mRNA expression levels were analyzed by RT-PCR by specific primers (see Supplemental Methods; supplemental material for this article is available online at the Journal of Applied Physiology website). Ribosomal 18S primers acted as internal controls, and all RT-PCR signals were normalized to the 18S expression.

Fig. 1. Effect of paricalcitol (PC) therapy after myocardial infarction (MI) in vitro and in vivo. A–B: effect of PC on Caspase-3 activity (A) and poly(ADP-ribose) polymerase (PARP)-1 activity (B) after H₂O₂ (0.1 mM) in adult cardiomyocytes. [N = 4/group; *P < 0.05 vs. vehicle (Veh), †P < 0.05 vs. Veh-H₂O₂]. C–D: heart weight over body weight (HW/BW) ratio (C), and lung weight over body weight (LUW/BW) ratio (D) in sham and MI groups with or without PC. (N = 4–6 mice in each group; *P < 0.05 vs. sham of each group, †P < 0.05 vs. Veh-MI). E: representative image of heart failure markers, atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) in sham and MI groups with or without PC. F: quantitative analysis of heart failure markers, ANF and BNP. (N = 4–6 mice in each group; *P < 0.05 vs. sham of each group, †P < 0.05 vs. Veh-MI). G: representative photomicrographs from histological sections stained with Masson’s Trichrome. H: infarct size analysis of sham and MI with and without PC.
**Immunoblot.** Immunoblot analyses were also performed as described previously (4). Total protein of VDR from heart tissue was probed with an anti-VDR rabbit polyclonal antibody. GAPDH was used as an internal control. Resulting bands were quantified as optical density (OD) × band area by NIH ImageJ software (version 1.38x; NIH, Bethesda, MD).

**Histology.** Hearts were fixed in 10% formalin and paraffin embedded. Sections were stained with Masson-Trichrome at the Histology Core facility at BIDMC. Infarct size was calculated as described previously (25). Briefly, the infarct length was calculated by measuring the endo- and epicardial surface length delimiting the infarcted region. Infarct size percentage was calculated as infarct length divided by the total LV circumference using ImageJ software.

**Statistical analysis.** Data were expressed as means ± SE. Comparisons between and within groups were conducted with unpaired Student’s t-tests and repeated-measures ANOVA using GraphPad Prism 5.0 (San Diego, CA), respectively. Survival curves after MI were created by the Kaplan-Meier method and compared by a log rank test. *P* values of <0.05 were considered significant.

**RESULTS**

**PC therapy attenuates the development of heart failure after myocardial infarction.** We initially determined whether PC treatment exerts a protective effect against H2O2-induced apoptotic cell death in rat adult cardiomyocytes and whether such protection is associated with decreased activation of caspase-3 and PARP-1 activities. H2O2 significantly increased activation of caspase-3 and PARP-1 compared with vehicle control (Fig. 1, A and B). However, PC treatment significantly attenuated activation of caspase-3 and PARP-1. These data suggest that PC protected adult cardiomyocytes against H2O2-induced apoptosis.

Then, to study the effect of activating vitamin D signaling in cardiac apoptosis in vivo, we examined the mice assigned to four groups: 1) Veh + Sham, 2) Veh + MI, 3) PC + Sham, and 4) PC + MI. Mice underwent sham or MI operation with either Veh treatment or PC therapy (15 ng/mouse, i.p. 3 times per week) before MI. We found that PC treatment resulted in significant decrease in heart weight (HW)/body weight (BW) ratio (P < 0.05) and lung weight (LuW)/BW ratio (P < 0.05) in PC + MI group compared with the Veh + MI group (Fig. 1, C and D). No significant differences were observed in the sham-operated mice treated with PC or Veh.

We next sought to determine whether the pathophysiologic findings correlated with other biological and molecular measures. The analyses of mRNA expression levels of atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) of
the LV tissues showed that PC significantly attenuated ANF (~33%) and BNP (~29%), which were both increased after MI (P < 0.05 for all) (Fig. 1, E and F). We also found that the infarct size after MI is significantly decreased in PC + MI group than in Veh + MI group (16.8 ± 4.3 vs. 42 ± 6.0) (Fig. 1, G and H). There were no significant differences observed between the two sham groups.

Effect of PC on fibrosis and inflammation after MI. To examine the potential vitamin D signaling-induced cardio-protective mechanisms after MI, we examined the effect of PC on fibrosis, inflammation, and renin-angiotensin system (RAS). Because studies have shown that vitamin D therapy has an antifibrotic effect (4, 29, 32, 41), the degree of fibrosis was quantified by examining the expression of collagen 1A1 and 3A1, the main forms of collagen in the heart. PC effectively reduced Col1A1 and Col3A1 mRNA levels in the infarct zone compared with the Veh + MI group (Fig. 2, A and B), suggesting that PC treatment attenuates increased fibrosis 4 wk after MI.

Inflammation is known to play an important role in the development and progression to heart failure (34). To establish whether PC suppresses inflammation in the heart, we examined mRNA level of tissue necrosis factor-α (TNF-α) and monocyte chemoattractant protein (MCP)-1. TNF-α and MCP-1 mRNA levels were significantly elevated in Veh operated animals (Fig. 2, C-D). These increases were significantly reduced by PC treatment (P < 0.05 for both).

Activation of circulating and tissue RAS leads to ventricular remodeling after MI, a process that is associated with altering myocardial apoptosis, myocardial hypertrophy, and increasing the content of collagen (33). There were significant increases in mRNA expression of components of the RAS gene 4 wk after MI (Fig. 2, E and F). The mRNA expressions of angiotensinogen, renin, and renin receptors were significantly reduced by PC treatment compared with Veh-treated mice after MI.

Effect of PC on apoptosis and cardiac function after MI. Because adverse LV remodeling after MI has been associated with increased apoptosis in the myocardium (37), we examined the effects of PC on cardiomyocyte apoptosis. The number of TUNEL-positive myocytes in the ischemia border zone were significantly greater in animals subjected to MI (Veh + MI) than in sham-operated animals (Fig. 3, A and B), suggesting that apoptosis is enhanced during cardiac remodeling. Conversely, the number of TUNEL-positive myocytes in ischemia border zone was significantly lower in PC + MI than in Veh + MI groups (0.39% vs. 1.64%, P < 0.05). These data confirmed our notion that PC therapy inhibits apoptosis. Finally, cardiac function was evaluated by measuring the LV PV loop 4 wk after MI. PV loop analysis revealed that PC after MI resulted in a significant improvement of CO, SW, and SV and a significant reduction in isovolumetric relaxation (τ) compared with the vehicle group after MI (Fig. 3, C-F).

Effect of PC on VDR expression after MI. Key components of vitamin D-dependent signaling system are present in the

![Figure 3](http://jap.physiology.org/)

**Fig. 3.** Effect of PC on apoptosis and cardiac function after MI. A: representative terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining of cardiomyocytes in sham and MI groups with or without PC. B: quantification of TUNEL-positive cardiomyocytes/total cells. (N = 4–6 mice in each group; *P < 0.05 vs. sham of each group, †P < 0.05 vs. Veh-MI). C–F: hemodynamic measurements by pressure-volume (PV) loop after MI, cardiac output (CO), stroke work (SW), stroke volume (SV), and tau (τ) in sham and MI groups with or without PC. (N = 4–6 mice in each group; *P < 0.05 vs. sham of each group, †P < 0.05 vs. Veh-MI).
heart (9), and the loss of VDR expression may increase the likelihood of heart failure progression. Thus we investigated mRNA and protein levels of VDR after MI. We confirmed that, 4 wk after MI, mRNA and protein expression of VDR were notably reduced in Veh + MI group when compared with sham controls (Fig. 4, A–D). Consistent with other findings in the kidneys (42), PC amplifies VDR expression in both mRNA and protein expression in cardiac myocytes after MI. In addition, the significant reduction of VDR mRNA and protein levels by MI was abrogated by PC treatment. Together, we conclude that VDR activation by PC may play a cardioprotective role in the progression to heart failure caused by MI.

Effect of defective vitamin D signaling on heart failure after MI. The consequences of VDR disruption on cardiac myocytes were then examined as we compared the VDR KO mice and their WT controls. VDR KO mice did not express any VDR expression in the heart (Fig. 5A). Morphology data showed that, after MI, the HW/BW ratio and the LuW/BW ratio were not only in absolute percentage changes, in HW/BW ratio and LuW/BW ratio of VDR KO sham mice. Thus these findings suggest that VDR KO mice developed a more severe progression to heart failure at 4 wk after MI than WT mice. We also observed an upregulation in mRNA levels of ANF and BNP gene expression in VDR KO mice compared with WT mice after MI (P < 0.05 for all) (Fig. 5, D and E). In addition, inflammatory markers TNF-α and MCP-1 mRNA levels were significantly higher in VDR KO-MI groups than in WT-MI group (Fig. 5, F and G). VDR KO at baseline did not show increases in biochemical and inflammatory markers for heart failure.

DISCUSSION

The key findings of the present study are that 1) the activation of VDR pathway by PC may offer cardioprotection as it attenuates cardiac dysfunction, cardiac myocyte apoptosis, and upregulation of proinflammatory cytokines in mouse models and 2) deficiency of VDR causes fulminant and aggressive heart failure after MI.
progression of heart failure after MI because they lack cardio-
protective signaling through the VDR pathway. These findings
demonstrate that vitamin D therapy could be an effective,
preventive, and therapeutic candidate against progression of
heart failure after MI.

Vitamin D and its biological activities are mediated by a
specific high-affinity receptor, the VDR, a member of the
superfamily of nuclear receptors for steroid hormones (16).
Analogous to other members of the steroid family, VDR acts as
a ligand-activated transcription factor, and, upon activation by
its ligand, VDR forms a heterodimeric complex with its oblig-
atory partner, retinoid X receptor, and binds to the vitamin D
response element. Hence, loss of VDR could lead to an
eradication of vitamin D signaling, even without any reduction
in levels of active vitamin D (42). Several cardioprotective
mechanisms of vitamin D signaling could be postulated from
our study. Earlier studies have stated that there may be a direct
connection between vitamin D and apoptosis (31, 42) although
this connection had not been shown in cardiomyocytes. A
study using an obstructive nephropathy rat model, consistent
with our findings, demonstrated that vitamin D treatment
upregulated VDR levels in vehicle rats and significantly de-
creased the number of TUNEL-positive cells in obstructed
animals (20). Additionally, pretreatment with 1,25(OH)2D3
(calcitriol) increases VDR gene expression in cardiomyocyte
but has no effect in cardiac fibroblasts (10). Collectively, these
results suggest that upregulation/activation of liganded VDR
signaling may protect cardiomyocyte against MI-induced apop-
tosis. Therefore, beneficial effects of PC may be linked to atten-
uation of cardiac dysfunction after MI. The results from our study,
to our knowledge, are the first to suggest that vitamin D therapy
could be a possible approach to the reduction of apoptosis in
cardiomyocytes in MI-induced heart failure.

We showed that vitamin D therapy significantly decreased
cardiac fibrosis and extracellular matrix remodeling, a patho-
logical feature of heart failure, after MI. Tan et al. (42)
observed that dramatic downregulation of VDR in the fibrotic kidney was completely restored by administration of vitamin D, resulting in an increase in VDR expression and further suggesting that deficiency in vitamin D signaling in the diseased kidney is far greater than it was anticipated earlier. Mizobuchi et al. (32) also showed that vitamin D therapy suppresses the progression of perivascular fibrosis and myocardial arterial vessel thickness and inhibits collagen synthesis after MI in vivo, presumably due to the upregulation/activation of VDR signaling. These findings coincide with our data where vitamin D demonstrates a significant increase in VDR expression after MI. Hence, these data along with the findings from this study suggest that cardiac fibrosis after MI may be regulated by the degree of vitamin D signaling in the heart.

Activation of circulating and tissue RAS plays a key role in the pathophysiology of heart failure and ventricular remodel-
ing. In present study, vitamin D therapy significantly attenuated RAS gene activation in the heart, thereby demonstrating additional beneficial effects of vitamin D after MI. Several experimental studies have exhibited that vitamin D therapy effectively reduces renin transcript levels and plasma renin activity in mice (19, 30). Previous findings have also suggested that liganded VDR may play a direct role as a negative regulator of renin gene by specifically interacting with cyclic AMP response element (CRE) binding protein and preventing its association with CRE in the renin gene promoter (48).

Furthermore, as a marker of chronic heart failure, vitamin D signaling downregulated several components of RAS in the kidneys of nephrectomized rats, including renin, renin receptor, angiotensinogen, and angiotensin II type 1 receptor (18, 38). In contrast, VDR KO mice have marked overproduction of renin (30, 47), leading to hypertension, cardiac enlargement, and elevation of natriuretic peptides. Thus these findings, along with our data, demonstrate that vitamin D therapy would be desirable for the treatment of heart failure after MI.

In patients with end-stage heart failure, significantly low levels of serum 25-D levels are an independent risk factor associated with poor clinical outcomes and prolonged decompensated heart failure (7, 24, 26, 49). Distinctive VDR agonists such as calcitriol and its analogs, PC and doxercalciferol, were developed to treat secondary hyperparathyroidism in chronic kidney disease (8), osteoporosis (11), and psoriasis (17). In animal models, PC and doxercalciferol have demonstrated a beneficial effect on preventing cardiac dysfunction (12). However, calcitriol has caused nondesired hypercalcemic actions and contains a narrow therapeutic window. Therefore, correction may be better accomplished by analogs of calcitriol with a wider safety margin (45). Presently, researchers are focused on developing another selective VDR agonist drug candidate with high selectivity and efficacy to treat the heart failure phenotype and high-renin-associated dysfunctions (40).

In summary, we have shown that activation of vitamin D signaling attenuates the progression to heart failure after MI, but defective VDR signaling exacerbates this deleterious effect. Given our evolving understanding of vitamin D and cardiovascular diseases, future studies are needed to determine whether vitamin D therapy could be an effective therapeutic agent for the treatment of heart failure.

DISCLOSURES

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AUTHOR CONTRIBUTIONS


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