Journal of Steroid Biochemistry & Molecular Biology 132 (2012) 282–289

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

The vitamin D receptor activator paricalcitol prevents fibrosis and diastolic dysfunction in a murine model of pressure overload

Laura M.G. Meems, Megan V. Cannon, Hasan Mahmud, Adriaan A. Voors, Wiek H. van Gilst, Herman H.W. Silljé, Willem P.T. Ruifrok, Rudolf A. de Boer*

Department of Cardiology, University of Groningen, University Medical Center Groningen, P.O. Box 30.001, Postal code AB43, 9700 RB Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 15 February 2012
Received in revised form 21 June 2012
Accepted 24 June 2012

Keywords:
Fibrosis
Heart failure
Paricalcitol
Remodeling
Vitamin D receptor
Renin

ABSTRACT

Background: Activation of the vitamin D–vitamin D receptor (VDR) axis has been shown to reduce blood pressure and left ventricular (LV) hypertrophy. Besides cardiac hypertrophy, cardiac fibrosis is a key element of adverse cardiac remodeling. We hypothesized that activation of the VDR by paricalcitol would prevent fibrosis and LV diastolic dysfunction in an established murine model of cardiac remodeling.
Methods: Mice were subjected to transverse aortic constriction (TAC) to induce cardiac hypertrophy. Mice were treated with paricalcitol, losartan, or a combination of both for a period of four consecutive weeks.
Results: The fixed aortic constriction caused similar increase in blood pressure, both in untreated and paricalcitol- or losartan-treated mice. TAC significantly increased LV weight compared to sham operated animals (10.2 ± 0.7 vs. 6.9 ± 0.3 mg/mm, $p < 0.05$). Administration of either paricalcitol (10.5 ± 0.7), losartan (10.8 ± 0.4), or a combination of both (9.2 ± 0.6) did not reduce LV weight. Fibrosis was significantly increased in mice undergoing TAC (5.9 ± 1.0 vs. sham 2.4 ± 0.8%, $p < 0.05$). Treatment with losartan and paricalcitol reduced fibrosis (paricalcitol 1.6 ± 0.3% and losartan 2.9 ± 0.6%, both $p < 0.05$ vs. TAC). This reduction in fibrosis in paricalcitol treated mice was associated with improved indices of LV contraction and relaxation, e.g. dPdtmax and dPdtmin and lower LV end diastolic pressure, and relaxation constant Tau. Also, treatment with paricalcitol and losartan reduced mRNA expression of ANP, fibronectin, collagen III and TIMP-1.
Discussion: Treatment with the selective VDR activator paricalcitol reduces myocardial fibrosis and preserves diastolic LV function due to pressure overload in a mouse model. This is associated with a reduced percentage of fibrosis and a decreased expression of ANP and several other tissue markers.

1. Introduction

Heart failure (HF) is a progressive condition involving activation of regulatory systems like the sympathetic nervous system (SNS) and the renin–angiotensin system (RAS) [1]. It has become apparent that deregulated vitamin D homeostasis may be of considerable importance for the cardiovascular (CV) system. Patients with CV disease in general, and with HF in particular, often have vitamin D deficiency [2,3]. We and others have reported that vitamin D deficiency is associated with a poor prognosis in HF [2,4]. The vitamin D receptor (VDR) is a nuclear hormone receptor that specifically binds to vitamin D and is expressed in (human, rat and murine) heart tissue [5,6]. Systemic and cardiospecific deletion of the VDR is associated with cardiac hypertrophy [7,8]. Various mechanisms have been proposed to explain the association between VDR signaling and myocardial hypertrophy. It has been shown that vitamin D is a negative transcriptional regulator of renin by binding the VDR [7,8,10]. Furthermore, vitamin D has been linked to pro-hypertrophic pathways [9] and natriuretic peptide expression [11,12]. So, the vitamin D–VDR axis may be a target for therapy in HF. Evidence for this hypothesis was obtained in experimental pharmacological studies, using paricalcitol, a selective vitamin D receptor activator, which was shown to exert anti-hypertrophic effects and to attenuate adverse cardiac remodeling in hypertensive rat models [13–15]. Altogether, these data suggest a potentially important role of the vitamin D–VDR axis in cardiac remodeling and hypertrophy. It remains unclear if VDR activation may reduce LV hypertrophy independent of blood pressure. Furthermore, VDR

Abbreviations: ANP, atrial natriuretic peptide; ARB, angiotensin II receptor blocker; CKD, chronic kidney disease; Combi, combination treatment of losartan and paricalcitol; CSA, cross sectional area; DSS, Dahl salt sensitive; HF, heart failure; HFPEF, heart failure with preserved ejection fraction; Los, losartan; LV, left ventricle; LVEDP, left ventricle end diastolic pressure; LVESP, left ventricle end systolic pressure; MMP, matrix metalloproteinase; Pari, paricalcitol; PTH, parathyroid hormone; RAS, renin–angiotensin system; SHR, spontaneously hypertensive rats; SNS, sympathetic nervous system; TAC, transverse aortic constriction; TIMPS, tissue inhibitors of MMPs; VDR, vitamin D receptor.

* Corresponding author. Tel.: +31 50 361 23 55; fax: +31 50 361 55 25.
E-mail address: r.a.de.boer@umcg.nl (R.A. de Boer).

0960-0760/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2012.06.004

activation has been best studied for its putative effects on myocytic remodeling, however, besides the antihypertrophic effects, beneficial effects on the matricellular matrix have also been observed [14]. We therefore evaluated the effects of paricalcitol in an established experimental model of cardiac hypertrophy, namely transverse aortic constriction (TAC), and focused on the anti-fibrotic effects of paricalcitol.

2. Materials and methods

2.1. Animals

We studied 10-week-old male C57Bl/6J mice (Harlan, The Netherlands). Animals were housed under standard conditions. All animal studies were approved by the Animal Ethical Committee of the University of Groningen, The Netherlands, and conducted in accordance with existing guidelines for the care and use of laboratory animals.

2.2. Experimental procedures

Mice were subjected to transverse aortic constriction (TAC). In brief, mice were anesthetized using isoflurane (2% in O_2). After opening the thorax between the second and third rib, the aorta was constricted at the aortic arch between both carotid arteries and sutured onto a 27-gauge needle with a 7.0 nylon suture. Immediately thereafter the needle was removed, creating a reproducible stenosis of the aorta about 50%. Control mice were subjected to a sham procedure. Sham operations were performed by isolation of the aorta without ligation of the aorta; the suture was removed instead of knotted. All animals were treated with analgesia (carprofen) for 48 h.

2.3. Design of the study

After TAC or sham surgery, a subset of the mice was treated with paricalcitol, a selective vitamin D receptor activator (provided by Abbott, Deerfield, IL, USA), which activates the VDR, at a final dose of 300 ng/kg/day. Paricalcitol was dissolved in a 95% propylene glycol and 5% ethyl alcohol solution. Mice were intraperitoneally injected with paricalcitol (or vehicle only) three times per week on Monday, Wednesday and Friday for five consecutive weeks. We also included an established anti-hypertrophic and anti-fibrotic treatment, namely the angiotensin II receptor blocker (ARB) losartan. Previous experiments have shown it is feasible and efficacious to dissolve losartan in the drinking water at a concentration of 30 mg/kg/day [16]; mice were treated for five consecutive weeks. So, in total eight groups were studied. Sham ($n = 10$), TAC ($n = 10$), Sham + losartan (Sham-los, $n = 10$), TAC + losartan (TAC-los, $n = 10$), Sham + paricalcitol (Sham-pari, $n = 10$), TAC + paricalcitol (TAC-pari, $n = 10$), Sham + paricalcitol + losartan (Sham-combi, $n = 10$) and TAC + paricalcitol + losartan (TAC-combi, $n = 10$).

2.4. Enzyme-linked immunosorbent assay (ELISA)

Parathyroid hormone (PTH) was analyzed in plasma using a commercial Enzyme Linked Immunosorbent Assay according to manufacturer's protocol (mouse intact PTH ELISA Kit, #60-2300, Immunotopics Inc., San Clemente, CA).

Plasma Aldosterone (pg/mL) was analyzed using a commercial Enzyme Linked Immunosorbent Assay according to manufacturer's protocol (mouse intact Aldosterone ELISA Kit, ABIN367767, Antikoerper, Aachen, Germany).

2.5. Echocardiography and hemodynamic measurements

Two (data not shown) and four weeks (before sacrifice) after TAC or sham surgery, echocardiography was performed using a transthoracic echocardiography with a 14 MHz transducer (Vivid 7, GE Healthcare, Diegem, Belgium), as described previously [17]. Mice were anaesthetized (2% isoflurane) and body temperature was maintained by placing the mouse on a heating pad. Short-axis view and M-mode tracings were used to measure cardiac hypertrophy and dimensions. Echocardiographic data at sacrifice are shown in the supplemental data.

Furthermore, prior to sacrifice, hemodynamic function was measured, using an indwelling pressure tip catheter (Millar Instruments, Houston, TX, USA), that is introduced in the right carotid artery and advanced into the LV as described previously [18]. First, blood pressure was recorded in the aortic arch, proximal to the aortic constriction. Then, once advanced into the LV, dPdtmax and dPdtmin, as indices of LV contraction and relaxation, were measured. Furthermore, intracardiac pressures including LV end diastolic (LVEDP) and end systolic (LVESP) were recorded. Tau, an isovolumetric relaxation constant, was measured according to the Glantz method.

After measuring invasive hemodynamics, blood was drawn via cardiac puncture and hearts were rapidly excised and weighed. Myocardial tissue was dissected transversally and processed for immunohistochemistry or snap frozen for molecular analyses.

2.6. Immunohistochemistry

To measure fibrosis score, Masson's trichrome staining was performed on paraffin sections of all experimental animals. Whole stained sections were scanned by a scanning system, ScanScope (Aperio Technologies, Vista, CA, USA). Total fibrosis was calculated automatically by the software under 20× magnification for entire section and expressed as percentage of total area.

To measure capillary density and cardiomyocyte size, endothelial cells were stained with CD31 (PECAM-1, BD-Pharm, #550274). CD31 positive cells stain, depending on fluorescent label, red, cell membranes green and nuclei blue. Tissuefaxs was used to photograph the complete sections. Tissuefaxs software allows standardized counting of nuclei and the amount of capillaries by use of counting and measuring-macros in ImageJ. Number of capillaries was measured per mm^2. Cardiomyocyte size is expressed as cross sectional area (CSA).

2.7. Quantitative real-time PCR

Total RNA from LV tissue was extracted using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA). cDNA synthesis and quantitative real time PCR (RT-qPCR) were performed as previously described with using 0.5 μg total RNA [19]. mRNA levels were expressed in relative units based on a standard curve obtained with serial dilutions of a calibrator cDNA mixture. To normalize expression data, reference genes were used (GAPDH, 36B4). A list of primers is displayed in the supplemental data. We assayed transcript abundance of fibronectin, a connective tissue marker involved in many cellular processes including tissue repair, collagens I and III, and atrial natriuretic peptide (ANP), an established marker of cardiac remodeling.

2.8. Statistical analysis

Results are reported as means ± SEM. Statistical analysis among groups was performed by ANOVA with the Dunnett post hoc test if distributed normally or with the Kruskall–Wallis test followed