Left ventricular periostin gene expression is associated with fibrogenesis in experimental renal insufficiency

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Abstract

Background. Cardiovascular diseases are the most important cause of death in patients with impaired kidney function. Left ventricular hypertrophy (LVH), cardiac interstitial fibrosis and cardiovascular calcifications are characteristic of chronic renal insufficiency (CRI). Periostin is a fibrogenesis- and calcification-related matricellular protein re-expressed in adult tissues undergoing remodelling in response to pathological stimuli. The role of periostin in CRI-induced LVH is unknown.

Methods. Rats were 5/6-nephrectomized (NX), and after 15 weeks of disease progression high-calcium, high-phosphate or paricalcitol treatment was given for 12 weeks. Cardiac tissue and blood samples were taken to study periostin gene expression and to determine factors contributing to its reactivation, respectively. Left ventricular (LV) periostin expression was also examined in response to angiotensin II or arginine 8-vasopressin (AVP)-induced pressure overload and in spontaneously hypertensive rats.

Results. CRI resulted in a 6.5-fold increase in LV periostin messenger RNA (mRNA) levels. Positive extracellular immunostaining for periostin was detected in areas of infiltrated inflammatory cells and fibrotic lesions. There was a significant correlation between LV periostin mRNA levels and plasma biomarkers of impaired kidney function, LVH, fibrogenesis-related proteins osteopontin and osteoactivin, and anti-calcific matrix Gla protein. Moreover, LV periostin gene expression in CRI correlated positively with systolic blood pressure (BP) and was activated rapidly in response to angiotensin II or AVP infusions.

Conclusions. Periostin is involved in fibrotic cardiac remodelling in CRI. The re-expression of periostin is localized to the fibrotic and inflammatory lesions and is most likely the consequence of elevated BP.

Keywords: cardiac remodelling; chronic renal insufficiency; fibrosis; hypertension; periostin

Introduction

Cardiovascular mortality accounts for the major part of all deaths in patients with chronic kidney disease [1, 2]. Left ventricular hypertrophy (LVH), cardiac interstitial fibrosis and cardiovascular calcifications are characteristic of chronic renal insufficiency (CRI). CRI-induced LVH differs from cardiac remodelling occurring in non-uraemic hypertension [3, 4]; cardiac structural changes in uraemia include decreased number and hypertrophy of cardiomyocytes, increase in cardiac interstitial cell and nuclear volume and diffuse interstitial fibrosis [4, 5]. Eventually LVH results in the development of congestive heart failure, which together with vascular calcification is a significant cause of death in uraemic patients [6].

In hypertension-induced LVH, a number of inflammation- and stress–response-related genes are activated [7]. One of these stress–response genes is a 90 kDa matricellular protein periostin, which is induced in mice hearts after acute myocardial infarction (AMI) and pressure overload [8]. The lack of periostin expression in mice results in extracellular matrix (ECM) alterations that are reflected as structural valvular and craniofacial anomalies, defective tooth development and smaller overall body weight [9]. Neonatal lethality in periostin-null mice is increased and is proposed to be the consequence of insufficiency of valves characterized with large acellular ECM deposits [10]. Importantly, periostin is responsive to transforming growth factor-β activation and has emerged to be important for collagen fibrillogenesis and overall organization of ECM [9, 11, 12]. Post-infarction, periostin is expressed exclusively by fibroblasts or cells that adopt a fibroblast-like phenotype and is thought to act both as adhesive and regulative molecule in fibrotic remodelling [11, 13]. Periostin can also directly interact with other ECM proteins, such as fibronectin and tenasin-C, and serve as a ligand for select integrins [9]. In addition to fibrous tissue development and maturation, periostin contributes to ECM...
mineralization [14–16]. Moreover, periostin gene expression is significantly increased in myocardium with extensive dystrophic calcification of mice with cardiac myocyte-specific deletion of β1-integrin [17]. However, the role of periostin in CRI-induced heart failure is not known.

In the present study, we examined the mechanisms regulating left ventricular (LV) periostin expression in experimental CRI. CRI rats received different treatments to influence calcium-phosphate-parathyroid hormone (PTH) balance, known to be disturbed and associated with distinct alterations in CRI, including cardiovascular function [18]. To study factors involved in periostin gene expression in CRI, correlations of periostin messenger RNA (mRNA) levels with LVH, blood pressure (BP) and plasma biomarkers of impaired kidney function were assessed. As periostin is involved in fibrotic and soft tissue mineralization processes in the different pathological conditions, we also examined the LV expression of several fibrosis-related genes (osteoclastin, osteopontin and pleiotrophin) and vascular calcification-related genes [bone morphogenetic protein (BMP)-2, BMP-4, bone sialoprotein and matrix γ-carboxyglutamate (Gla) protein] to further characterize the activated pathological processes in uremic cardiac remodelling.

Materials and methods

Animals and experimental design

Chronic renal insufficiency. Sprague–Dawley rats were assigned to 5/6 nephrectomy (NX) or sham operation at the age of 8 weeks [19]. Regular rat chow was provided and contained 0.9% calcium, 0.8% phosphate, 0.27% sodium, 1500 IU/kg vitamin D and 12550 kJ/kg energy (Lactamin R34; AnalyCyto, Lindköping, Sweden). After 15 weeks of disease progression, NX rats were divided into four groups with equal systolic blood pressures (SBPs), body weights, urine outputs and plasma creatinine levels. Then for 12 weeks, the sham, NX and NX + paricalcitol groups continued on control diet (0.3% calcium, 0.5% phosphate), NX + Ca group on high calcium diet (3% calcium, 0.5% phosphate), NX + Pi group on high phosphate diet (0.3% calcium, 1.5% phosphate) and the NX + paricalcitol group received paricalcitol (200 ng/kg of 19-Nor-1,25(OH)2D2, intravenous injection solution, 5 µg/mL, vehicle containing 30% propylene glycol and 20% ethanol) intraperitoneaWea period of 3 days a week. The dose of paricalcitol chosen has previously been shown to decrease efficiently plasma PTH levels and have a beneficial effect on bone, kidney and the vascular system in CRI [20–23]. During the final study week, urine was collected in metabolic cages. The rats were anaesthetized by intraperitoneal urethane (1.3 g/kg), and heart and blood samples were taken and stored at ~70°C. Experimental designs were approved by the Animal Experimentation Committee of the University of Tampere and the Provincial Government of Western Finland Department of Social Affairs and Health, Finland. The investigation conforms to the Guiding Principles for Research Involving Animals.

Analysis of RNA

Total RNA from the heart samples was isolated by the guanidine thiocyanate–cesium chloride method [30]. BMP-2 and -4, bone sialoprotein, periostin, pleiotropin and 185 mRNA levels were measured by quantitative reverse transcription–polymerase chain reaction (qRT–PCR) analyses using TaqMan chemistry on an ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) as previously described [31]. For Northern blot and dot blot analyses, 20 µg samples of total RNA were transferred to nylon membranes (Osmonics Inc.). The complementary DNA probes for rat atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), matrix Gla protein, osteoclastin, osteopontin, periostin and rat ribosomal 185 RNA were randomly prime labelled with [32P]-dCTP, and the membranes were hybridized and washed as described previously [32]. The signals were normalized to 185 RNA in each sample.

Plasma measurements

Plasma creatinine, ionized calcium, phosphate, PTH and urea levels were determined as previously described [33].

Immunohistochemistry

For immunohistochemical analysis, the hearts were fixed in 10% buffered formalin solution, transversal sections of the left ventricle were embedded in paraffin, and 5-µm sections were cut. Masson’s trichrome and von Kossa stainings were used to detect fibrotic and calcific sites, respectively. Rabbit polyclonal periostin antibody (Osteoblast-specific factor 2; BioVendor, Modrice, Czech Republic) at the dilution of 1:3000 was used to determine the localization of periostin in the hearts. Negative control stainings were done by substituting non-immunized rabbit serum and phosphate-buffered saline for the primary antibody.

Statistical analysis

The results are presented as mean ± standard error of the mean. Statistical significance was determined by one-way analysis of variance followed by the least significant difference post hoc test. If the variable was not normally distributed, the Kruskal–Wallis and Mann–Whitney U-tests were used. Analyses were performed by SPSS for Windows (version 16.0, SPSS Inc, Chicago, IL). Differences were considered significant at a value of P < 0.05. The Spearman correlation coefficient (r) was used in the correlation analyses, which were considered significant at two-tailed P-values <0.001.

Results

Cardiac periostin gene expression in response to AMI and pressure overload

Periostin gene expression has previously been shown to be upregulated in the left ventricle after AMI and transverse aortic constriction-induced chronic pressure overload in mice [8]. In agreement with these findings, we observed by qRT–PCR analysis a 5.8-fold increase in LV periostin mRNA levels 24 h post-infarction (Figure 1A). Also in SHR rats with chronically elevated BP, LV periostin mRNA levels were consistently elevated compared to normal age-matched WKY rats (Figure 1B). Interestingly, AVP-induced pressure overload caused a rapid increase in LV periostin mRNA levels already within 4 h (Figure 1C).

Cardiac hypertrophy and biochemical markers of kidney function in CRI

Heart weight-to-body weight ratio and plasma concentration of urea were significantly increased and creatinine clearance decreased in all NX groups when compared with sham-operated rats (Table 1). Plasma concentrations of
PTH and phosphate were also significantly increased in all other NX groups except in the NX + Ca group showing lower levels. Plasma ionized calcium was increased in the NX + Ca and decreased in the NX + Pi group (Table 1).

LV periostin gene expression in CRI

To study the effect of CRI on cardiac periostin gene expression, subtotally nephrectomized rats were used. CRI increased LV periostin mRNA levels 6.5-fold when compared with the sham-operated group (Figure 2A). Periostin LV gene expression correlated positively with plasma concentrations of urea (Figure 2B), creatinine, phosphate and PTH (data not shown), all biomarkers of impaired kidney function. There was a significant correlation between LV periostin mRNA levels and the expression of hypertrophy marker genes ANP and BNP (Figure 3A and B), and the heart weight-to-body weight ratio (Figure 3C).

Influence of pressure overload on periostin LV gene expression in CRI

Because CRI-associated disturbed calcium-phosphate balance affects BP, we studied next the relationship of LV periostin mRNA levels to BP in NX rats treated with high-calcium diet, high-phosphate diet or paricalcitol. Previously, high-calcium diet has been shown to lower BP in NX rats, whereas 12 weeks of high-phosphate diet or paricalcitol treatment had no effect on BP in NX rats [34, 35]. High-calcium diet significantly attenuated the development of hypertension and abolished a NX-induced increase in periostin LV gene expression (Figure 4A). High-phosphate diet did not reduce BP, and periostin mRNA levels were not significantly higher after high-phosphate diet. In agreement with the relationship between BP and periostin, paricalcitol intake was associated with both hypertension and increased periostin LV gene expression (Figure 4A). It is noteworthy that the LV gene expression of periostin significantly correlated with BP (Figure 4B).

Localization of LV periostin in CRI

To study the LV localization of periostin in the rats with CRI, we used immunohistochemical analysis. Both NX and sham-operated rats showed areas of enhanced interstitial and perivascular fibrosis (Figure 5A and C), but no calcification was detected (data not shown). In NX rats, there were also some scattered inflammatory cell infiltrates, usually in the areas of interstitial fibrosis (Figure 5E). Positive extracellular staining for periostin was detected in the areas of infiltrated inflammatory cells (Figure 5F) and/or fibrotic lesions in both groups (Figure 5B and D). Furthermore, local patchy positivity was seen in the endothelium of some blood vessels (Figure 5D) as well as in the endocardium in both groups (data not shown).

Expression of fibrosis-related genes in the left ventricle in CRI

We next assessed the LV mRNA levels of the pro-fibrotic proteins osteopontin and pleiotrophin and the anti-fibrotic osteoactivin. Osteopontin showed similar gene expression pattern as periostin and significantly correlated with it (Figure 6A and B). The NX-induced elevation of osteopontin mRNA levels was abolished by high-calcium diet but not with paricalcitol treatment (Figure 6A). Osteoactivin
LV gene expression increased 4.3-fold in NX rats, which was abolished by all treatments (Figure 6C). A significant correlation was noted between osteoactivin and periostin LV mRNA levels (Figure 6D). Impaired renal function also elevated the LV expression of pleiotrophin, while high-phosphate diet reduced pleiotrophin mRNA levels to those of the sham group (Figure 6E).

**LV expression of calcification-related genes in CRI**

Because cardiovascular calcifications are common complications in CRI [36], we examined whether periostin LV gene expression correlates with vascular calcification-related factors, e.g. pro-osteogenic proteins BMP-2, BMP-4 and bone sialoprotein and anti-osteogenic matrix Gla protein. None of the calcification-related proteins had similar LV expression pattern to that of periostin, this pattern shown for BMP-2 in the Figure 6F. Only the LV mRNA levels of periostin and calcification inhibitor matrix Gla protein correlated positively ($r = 0.604$, $P < 0.001$); however, the pattern of the expression of periostin and matrix Gla protein also differed (data not shown).

**Discussion**

Developmentally regulated periostin is reactivated in adult tissues undergoing remodelling or active stress [11]. The aim of this study was to examine the mechanisms of periostin gene expression in the uraemic cardiac remodelling and LVH. Our results are the first to show that CRI increases periostin gene expression in the left ventricle. Interestingly, periostin gene expression positively correlated with systolic BP and LVH in CRI. In the present study, we also report that ventricular periostin gene expression is activated at a very early stage of pressure overload and post-infarction, thus mimicking the rapid induction of ANP and BNP in response to haemodynamic stress [25, 27]. Moreover, comparison to the LV gene expression of calcification- and fibrosis-related proteins showed that periostin may be primarily involved in fibrotic remodelling of myocardium rather than in mineralization.

Positive immunostaining for periostin in the left ventricles of both sham-operated and CRI rats was seen in the areas of increased fibrosis, whereas in CRI rats enhanced periostin staining was also detected at sites of inflammatory infiltrates. This agrees with previous observations in mice showing periostin expression at sites of injury and inflammation [11]. Interestingly, there was also patchy periostin staining in endothelial cells of endocardium and blood vessels. In agreement with this, periostin mRNA has been shown to be abundant in the endothelium of developing chicken heart [37]. However, since periostin expression has been reported to be absent from endothelial cells in developing mouse heart [38], this issue needs to be studied further.
To examine the factors involved in periostin gene reactivation, we first carried out a series of analyses between LV periostin mRNA levels and biomarkers of impaired kidney function and observed positive correlation. Periostin gene expression correlated also positively with renal insufficiency-induced LVH and the expression of cardiac overload-associated markers ANP and BNP. Because CRI is associated with disturbed calcium-phosphate-PTH balance, we next tested the hypothesis whether the increased LV periostin gene expression is the consequence of changes in levels of calcium, phosphate and PTH or alterations in

Fig. 3. The correlation of LV periostin mRNA levels with the gene expression of ANP (A), BNP (B), and heart weight-to-body weight ratio (C) in experimental renal insufficiency in rats. n = 7–14.

Fig. 4. The effect of experimental renal insufficiency, high-calcium (NX + Ca) or high-phosphate (NX + Pi) diets and paricalcitol (NX + paricalcitol) treatment on LV periostin mRNA levels (left y-axis) and systolic BP (right y-axis) in rats (A). The correlation of BP with LV periostin mRNA levels in experimental renal insufficiency in rats (B). Results are mean ± SEM (A), n = 7–15. *P < 0.05, **P < 0.01, ***P < 0.001 versus sham; #P < 0.05, ###P < 0.001 versus NX.

Fig. 5. Periostin expression in the left ventricle in rats. A and B, adjacent sections of the left ventricle of a sham-operated rat with increased interstitial fibrosis (A) and enhanced periostin staining in fibrotic areas between myocytes (B). C and D, adjacent sections of the left ventricle of a NX rat. There is an artery showing increased perivascular fibrosis (C) with distinct periostin positivity (D). Note the positive staining of the endothelial cells (arrows). E and F, adjacent sections of the left ventricle of a NX rat showing small areas with some interstitial fibrosis as well as inflammatory cells, mainly lymphocytes, (E) that stain positive for periostin (F). G and H, adjacent sections of the left ventricle of a NX rat with normal myocytes (G) that stain negative for periostin (H). Note a small vessel (arrows) with the endothelial cells that stain negative for periostin (H). A, C, E and G Fontana-Masson staining, fibrotic areas with blue colour. B, D, F and H, immunohistochemical staining for periostin. Scale bars correspond to 100 µm.
BP by using high-calcium and high-phosphate diets or treatment with the vitamin D receptor activator, paricalcitol, in CRI rats. A significant correlation between systolic BP and periostin LV mRNA levels existed. On the other hand, the increased gene expression of periostin was probably not due to elevated PTH in CRI. Although both high-phosphate and paricalcitol treatment did significantly influence on PTH levels, periostin mRNA levels were not higher in the NX + Pi group and were not lower in the NX + paricalcitol group than in the NX group. Moreover, increased periostin gene expression could not be attributed to phosphate retention in CRI either, as periostin mRNA levels were not higher in the NX + Pi group and were not lower in the NX + paricalcitol group than in the NX group. Moreover, increased periostin gene expression could not be attributed to phosphate retention in CRI either, as periostin mRNA levels were not higher in the NX + Pi group and were not lower in the NX + paricalcitol group than in the NX group. Altogether, these results along with the experiments in the non-uraemic hypertension strongly suggest that alterations of periostin LV gene expression in CRI were most likely due to cardiac pressure overload.

As myocardial interstitial fibrosis and vascular calcifications are characteristic of CRI, we subsequently examined whether periostin expression is associated with markers of renal insufficiency-induced fibrogenesis or soft tissue mineralization in the heart. All fibrosis-related genes were upregulated in the NX group, proposing that fibrogenesis is activated in the myocardium of uraemic rats. Fibrogenic osteopontin showed a parallel expression profile to periostin in advanced kidney insufficiency, and both osteopontin and anti-fibrotic osteoactivin mRNA levels correlated positively with periostin LV gene expression. Osteopontin has been shown to be involved in the regulation of post-infarction LV remodelling by promoting collagen synthesis [39], and our present results suggest a role for periostin in cardiac fibrotic remodelling in CRI, particularly together with osteopontin. Earlier studies with periostin-null mice have shown that a lack of periostin is associated with better ventricular performance after chronic pressure overload or myocardial infarction, with lesser susceptibility to fibrotic heart disease [11]. Thus, elevated periostin LV gene expression in CRI is most likely detrimental to both cardiac structure and function. It should also be noted that according to the present

![Fig. 6. The effect of experimental renal insufficiency and high-calcium (NX + Ca), high-phosphate (NX + Pi) or paricalcitol (NX + paricalcitol) treatment on LV mRNA levels of fibrosis-related genes osteopontin (A), osteoactivin (C) and pleiotrophin (E) and vascular calcification-related gene BMP-2 (F) in rats. Results are mean ± SEM, n = 7–14. *P < 0.05, **P < 0.01 versus sham; #P < 0.05, ##P < 0.01 versus NX. The correlation of LV periostin mRNA levels with LV gene expression of osteopontin (B) and osteoactivin (D). n = 7–14.](image-url)
results, paricalcitol treatment did not reduce the CRI-induced increase in periostin LV gene expression. However, there is evidence that paricalcitol may attenuate cardiac hypertrophy in the Dahl salt-sensitive rats [40]. The ongoing Paricalcitol Capsules Benefits in Renal Failure Induced Cardiac Morbidity in Chronic Kidney Disease Stage 3/4 (PRIMO) Study is aimed to investigate the effect of paricalcitol on changes on cardiac structure and function in CRI patients with LVH (www.ClinicalTrials.gov).

To study the role of mineralization in periostin gene expression, we assessed the LV mRNA levels of vascular calcification-related genes and compared their expression with periostin. However, none of the calcification-related proteins had similar LV expression pattern to that of periostin, although e.g. BMP-2 and BMP-4 have been suggested to regulate periostin gene expression in other experimental models [12, 41, 42]. Moreover, no myocardial calcification was detected in the immunohistochemical examinations, and LV periostin mRNA levels did not correlate with the expression of pro-osteogenic genes. Interestingly, calcification inhibitor matrix Gla protein, reported to play an important role in the prevention of uremic vascular calcification [43], showed a positive correlation with periostin, although the expression pattern of periostin and matrix Gla protein differed. Clearly, further studies are needed to clarify the role of matrix Gla protein in regulating myocardial remodelling in CRI.

In conclusion, these results suggest that periostin is involved in fibrotic cardiac remodelling in impaired kidney function. Moreover, the re-expression of periostin does not result from the disturbed calcium-phosphate balance but is most likely a consequence of the elevated BP in CRI. Finally, the activation of ventricular periostin gene expression at early stages of pressure overload and post-infarction suggest that periostin may play a role in early remodelling processes in concert with neurohumoral mechanisms. Thus, it would be interesting to study the effect of BP lowering on cardiac periostin expression both in CRI and other experimental models of cardiac overload.

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