Peptides 76 (2016) 108–114

Contents lists available at ScienceDirect

Peptides

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

Cardiorenal fibrosis and dysfunction in aging: Imbalance in mediators and regulators of collagen

S. Jeson Sangaralingham[a,*], Bing H. Wang[b], Li Huang[b], Sirinart Kumfu[b], Tomoko Ichiki[a], Henry Krum[b], John C. Burnett Jr.[a]

[a] Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN, USA
[b] Centre of Cardiovascular Research and Education in Therapeutics, School of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia

ARTICLE INFO

Article history:
Received 12 August 2015
Received in revised form
19 December 2015
Accepted 8 January 2016
Available online 13 January 2016

Keywords:
Fibrosis
Cardiorenal
Natriuretic peptides
Renin–angiotensin–aldosterone system
Gene expression

ABSTRACT

Cardiorenal fibrosis is a biological process that increases with age and contributes to dysfunction of the heart and kidney. While numerous circulating and tissue hormones, cytokines and enzymes have been identified in the development of cardiorenal fibrosis, several reports have suggested that the anti-fibrotic natriuretic peptide system (NPS), pro-fibrotic renin–angiotensin–aldosterone system (RAAS), transforming growth factor-beta 1 (TGF-β1), matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) are fundamental regulators and mediators of this process. However, the simultaneous assessment of these components in the development of age-mediated cardiorenal fibrotic remodeling is not completely understood. Thus, we assessed cardiorenal structure and function, the circulating NPS and RAAS and the cardiorenal tissue gene expression of collagen (Col) I, Col III, TGF-β1, MMP-9 and TIMP-1 in 2 and 20 month old Fischer rats. Our studies determined that aging was characterized by an increase in cardiorenal fibrosis that was accompanied with cardiorenal dysfunction. These alterations were associated with lower circulating atrial and C-type natriuretic peptides and higher angiotensin II and aldosterone levels in the aged rats. Moreover, we observed a decrease in Col I and III and an increase in TIMP- mRNA expressions in the aged heart and kidney, while TGF-β1 expression increased and MMP-9 decreased only in the aged kidney. We conclude that the age-mediated alterations in these fibrotic regulator and mediator profiles favors collagen accumulation due to an imbalance between the NPS and RAAS as well as a decline in the degradative pathway, thus suggesting a therapeutic opportunity to target these components.

1. Introduction

The heart and kidney are hormonally linked via the natriuretic peptide system (NPS), where the cardiac and reno-endothelium derived atrial natriuretic peptide (ANP) and c-type natriuretic peptide (CNP) respectively, mediates the inhibition of cardiorenal fibrosis and adverse remodeling through the particulate guanylyl cyclase receptors [1,2]. The NPS is counter-regulated by the renin-angiotensin-aldosterone system (RAAS) of which angiotensin II (ANG II) via the angiotensin receptor 1 (AT1) and aldosterone through the mineralocorticoid receptor contribute to adverse cardiac and renal remodeling and fibrosis [3]. Notably, combined fibrosis of the heart and kidney are hallmarks of aging [4,5] and indeed in the extreme, contribute to both heart failure and chronic kidney disease. The interstitium of the heart and kidneys are dynamic structures that are reflective of a continuous process of synthesis and degradation of extracellular matrix (ECM) proteins including collagen and of which is influenced by circulating neurohumoral factors [6]. Indeed, an emerging view is that fibrosis of the heart and kidney involves simultaneous remodeling of both organs leading to chronic cardiorenal disease. Furthermore, the important relationship between the NPS and RAAS in the setting of age-related cardiorenal fibrosis is not clearly understood.

To date, ANP and CNP have been shown to inhibit DNA synthesis and fibroblast proliferation [7–9]. Whereas activated RAAS as well as transforming growth factor-beta 1 (TGF-β1) markedly stimulate collagen deposition and the formation of cardiac and renal fibrosis [10,11]. Moreover, the fibroblast is a key regulator collagen

* Corresponding author at: Cardiorenal Research Laboratory, Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA. Fax: +1 507 266 4710.
E-mail address: sangaralingham.jeson@mayo.edu (S.J. Sangaralingham).

http://dx.doi.org/10.1016/j.peptides.2016.01.004
0196-9781/

turnover by a balance of collagen synthesis and degradation [12]. This regulatory process involves the interaction between the matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs) [13–15], of which MMP-9 and TIMP-1 in particular, is altered in models of cardiac and renal disease [16–22]. While fibrosis remains the hallmark of cardiorenal aging and contributes to cardiorenal impairment, the mechanisms for accentuated collagen deposition in the aged heart and kidney remain poorly defined, in part, due to the lack of simultaneous assessment of myocardial and renal fibrosis in the same setting.

Here we wanted to confirm and extend investigations on age-mediated changes in the key regulators and mediators of fibrosis and dysfunction in both the heart and kidney. We utilized an experimental rat model of aging to assess the balance between the anti-fibrotic NPS and pro-fibrotic systems RAAS and TGF-β1 and its relationship with left ventricular (LV), renal cortical and renal medullary fibrosis as well as cardiorenal structure and function. We also defined the gene expressions of collagen (Col) I, Col III, MMP-9 and TIMP-1 in the heart and kidney. We hypothesized that aging would be associated with: (1) a relative deficiency of plasma ANP and CNP as well as an activation of circulating ANG II and aldosterone; (2) parallel increases in LV, cortical and medullary fibrosis coupled with impaired cardiorenal function and, (3) changes in the collagen degrading pathway due to an imbalance in the expression of MMP-9 and TIMP-1. Our study supports the concept that an imbalance between key regulators and mediators of fibrosis, such as the NPS, RAAS and TGF-β1 pathway, characterizes cardiorenal aging and represents a therapeutic opportunity.

2. Methods

2.1. Animals

Studies were performed in 2- and 20-month old male Fischer rats (Harlan Laboratories; $n = 10$ per age group). The Fischer (F344) rat is a widely utilized animal model for aging studies and was developed by the National Institute of Aging [23]. This inbred rat strain closely mimics many characteristics of CV and renal aging seen in humans and also exhibits a lower tumor rate than non-inbred rat strains [24–30]. This experimental study was performed with approval of the Mayo Clinic Institutional Animal Care and Use Committee and in accordance with the Animal Welfare Act.

2.2. Echocardiography

As routinely carried out in our laboratory, standard 2-dimensional echocardiography was performed on lightly anesthetized (1.5% isoflurane in oxygen) rats using the Vivid 7 ultrasound system (GE Medical Systems) and 10S transducer with ECG monitoring. M-mode images and 2-dimensional parasternal short axis images were recorded for off-line analyses of left ventricular structure and function using EchoPAC software (EchoPAC PC BTO 9.0.0, GE Healthcare) are previously described in detail [29]. Diastolic wall strain (DWS), as an index of diastolic stiffness based on the linear elastic theory, was calculated with the following equation: (systolic posterior wall thickness – diastolic posterior wall thickness)/systolic posterior wall thickness [31,32].

2.3. Renal function

Rats were place in metabolic cages with free access to food and water and allowed to acclimatize for 24 h prior to collection. A 24-h urine collection was performed on ice after acclimatization for urinary protein excretion assessment as previously described [28]. Glomerular filtration rate (GFR) was also assessed by inulin clearance on anesthetized (2.0–2.5% isoflurane in oxygen) rats as described previously [28].

Table 1
Cardiorenal structure and function and blood pressure between 2 and 20 months old Fischer rats.

Parameter	2 months	20 months
Body weight (g)	210 ± 3	449 ± 6†
Cardiac structure and function		
LV weight (mg)	470 ± 7	812 ± 22†
LVEDD (mm)	6.74 ± 0.10	7.41 ± 0.08†
LVESD (mm)	3.28 ± 0.10	4.35 ± 0.09†
EF (%)	88 ± 1	79 ± 1†
Diastolic wall strain	0.39 ± 0.01	0.26 ± 0.03†
Renal structure and function		
Total kidney weights (mg)	1623 ± 35	2729 ± 56†
GFR (ml/min/kg)	3.95 ± 0.23	2.52 ± 0.33**
Protein excretion rate (μg/min)	5.6 ± 0.4	15.6 ± 3.6†
Blood pressure		
Systolic BP (mmHg)	101 ± 2	116 ± 3**
Diastolic BP (mmHg)	91 ± 2	102 ± 4*

LV = left ventricular; LVEDD = left ventricular end-diastolic dimension; LVESD = left ventricular end-systolic dimension; EF = ejective fraction; GFR = glomerular filtration rate; BP = blood pressure. Values are mean ± SE.
* $P < 0.05$ vs. 2 months.
** $P < 0.01$ vs. 2 months.
† $P < 0.001$ vs. 2 months.

2.4. Blood pressure and plasma collection

Blood pressure (BP) and plasma collection were performed as previously described [29].

2.5. LV and renal tissue collection

The hearts and kidneys were removed from anesthetized (2.0–2.5% isoflurane in oxygen) rats and the LV, renal cortex and medulla were carefully dissected. A cross-section of the LV and kidney containing both cortex and medulla were preserved in 10% formalin for histological analysis of fibrosis. The remaining LV, renal cortex and medulla were quickly snap frozen in liquid nitrogen.

2.6. Neurohumoral analysis

Plasma ANP [33], CNP [29], ANG II [34] and aldosterone [35] were determined by commercially available radioimmunoassays as described in prior studies.

2.7. Histological analysis for cardiorenal fibrosis

Cross-sections of paraffin-embedded LV and renal tissue (4 μm) were stained with picrosirius red and quantified as previously described [28,29].

2.8. Quantitative mRNA expression

As previously described [36], real-time polymerase chain reaction (RT-PCR) was used to quantify Col I, Col III, TGF-β1, MMP-9, TIMP-1 and 18S gene expression levels. Briefly, total RNA was extracted from ~30 mg cardiac or renal cortical or medullary tissues using Qiagen RNeasy kit (Qiagen, Hilden, Germany). Then the cDNA was reverse transcribed and triplicate cDNA aliquots were amplified using sequence-specific primers (Geneworks, Adelaide, SA, Australia) with TagMan fluorogenic probe for TGF-β1 and 18S (Applied Biosystems) or SYBR Green detection for Col I, Col III, MMP-9 and TIMP-1 (Applied Biosystems) using an ABI prism 7900HT sequence Detection System (Applied Biosystems). The primer pairs