Molecular and cellular characterization of cardiac overload-induced hypertrophy and failure

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CHAPTER 3

Myocardial collagen metabolism in failing hearts before and during cardiac resynchronisation therapy

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Abstract

Background: In patients with heart failure cardiac resynchronization therapy (CRT) leads to reverse ventricular remodelling.

Aim: To evaluate whether myocardial collagen metabolism in patients with heart failure is implicated in adverse ventricular remodelling and response to CRT.

Methods: Collagen synthesis and degradation were assessed from the concentrations of aminoterminal propeptides of type I and type III collagen (PINP and PIIINP) and carboxyterminal telopeptide of type I collagen (ICTP), respectively, in serum of 64 patients with heart failure before and after 6 months of CRT. The pro-form of matrix metalloproteinase-1 (proMMP1) and tissue inhibitor of metalloproteinase-1 (TIMP1) were also assayed at these time points. Forty-six patients (72%) showed a >10% reduction in LV end-systolic volume at follow-up and were classified as responders to CRT, the other 18 patients (28%) were classified as non-responders.

Results: Responders demonstrated a mean (±SEM) increase of serum PINP and PIIINP during follow-up, from 32.9±2.2 to 46.7±4.0 µg/L (p<0.001) and from 4.59±0.24 to 5.13±0.36 µg/L (p<0.05), respectively. In non-responders, serum PINP and PIIINP remained unchanged during follow-up. At baseline, responders had significantly lower serum PINP than non-responders (32.9±2.2 vs. 41.8±4.3 µg/L; p<0.05). ICTP levels of responders at baseline tended to be higher than in non-responders (3.54±0.56 vs. 2.08±0.37 µg/L, p=ns), and in both groups ICTP levels did not change upon CRT.

Conclusion: Reverse LV remodelling following CRT is associated with increased collagen synthesis rate in the first 6 months of follow-up.

Key words: Heart failure, cardiac resynchronisation therapy, PINP, PIIINP, ICTP

Introduction

In addition to myocardial hypertrophy, overloaded hearts frequently demonstrate a reactive fibrosis due to alterations in collagen synthesis and degradation. Accumulation of interstitial collagen type I and type III increases myocardial stiffness resulting in changes in diastolic properties of the left ventricle (LV) [1].
Collagen type I and type III are secreted by interstitial fibroblasts as procollagens followed by splitting off of propeptides by endopeptidases and the release of their aminoterminal propeptides, PINP and PIIINP, and carboxyterminal propeptides, PICP and PIIICP, into the circulation. During degradation of type I collagen fibrils the carboxyterminal telopeptide of type I collagen (ICTP) is formed, which is a 12 kD peptide [2].
Extracellular matrix components, like collagen type I and type III, are continuously synthesized and degraded. With respect to degradation, matrix metalloproteinase-1 (MMP1), a collagenase, and MMP2 and MMP9, two gelatinases, can be upregulated at the mRNA level [3], can be activated at the protein level, for instance by MT_1-MMP (or MMP14)[4], and can be stimulated by lowered concentrations of MMP inhibitors like tissue inhibitor of metalloproteinases (TIMPs) [5].
The myocardial extracellular matrix is implicated in a number of conditions and diseases, such as hypertensive heart disease, dilated cardiomyopathy, and cardiac remodelling. In failing hearts myocardial MMP1 and MMP2 concentrations have been shown to be elevated, while TIMP1 levels were depressed or unchanged [6]. Rapid myocardial collagen synthesis rate and slow collagen degradation rate impair diastolic function and promote LV adverse remodelling, probably due to cardiomyocyte slippage secondary to increased LV diastolic pressures [7].
In the present study, the biochemical markers of collagen synthesis rate (PINP and PIIINP) and collagen degradation rate (ICTP), including proMMP1 and TIMP1, were assayed in patients with heart failure before and after 6 months of cardiac resynchronisation therapy (CRT), to evaluate whether responders and non-responders to CRT differ with regard to serum markers of collagen metabolism at those two time points and whether serum markers of collagen metabolism can predict response to CRT.

Methods

Patients

Sixty-four patients with heart failure, scheduled for implantation of a CRT device, were included in the study. Baseline characteristics of these patients have been reported in an earlier study evaluating the levels of circulating biomarkers of extracellular matrix metabolism [8]. The group had a mean age 64±1.2 years and consisted of 52 men (81%) and 12 women (19%). Ischaemic aetiology of heart failure was present in 45 patients (70%) and non-ischaemic aetiology in 19 patients (30%). Selection criteria for CRT were New York Heart Association (NYHA) functional class III-IV, LV ejection fraction ≤35%, and QRS duration >120ms. Patients with decompensated heart failure and patients with a recent myocardial infarction (<3 months) were excluded. LV dyssynchrony was quantified at baseline. Clinical status was assessed before CRT implantation (baseline) and at 6 months follow-up. At both time points, 2-dimensional echocardiography was performed to determine LV volumes and LV ejection fraction.

Clinical evaluation

At baseline and at 6 months follow-up clinical status was evaluated by (***i***) assessment of NYHA functional class, (***ii***) determination of quality-of-life score using the Minnesota Living with Heart Failure questionnaire, and (***iii***) assessment of exercise capacity using the distance of a 6-minute hall walk test.

Echocardiography

Echocardiography was performed as described previously [8,9]. A commercially available system (Vingmed system Seven, General Electric-Vingmed, Milwaukee, Wisconsin, USA) was used to acquire conventional apical 2- and 4-chamber images, which allow calculation of LV end-systolic and end-diastolic volumes (LVESV and LVEDV) and LV ejection fraction, using the biplane Simpson's technique [10] and commercial software (Echopac version 5.0.1, General Electric – Vingmed). Echocardiographic data were analyzed by two independent observers who were blinded to all other patient data. Inter- and intra-observer variabilities for assessment of LV ejection fraction and LV volumes were 90% and 96%, respectively [11]. Based on recent data [12], patients with a reduction of >10% in LVESV at 6 months follow-up were classified as responders to CRT, whereas patients with a reduction of ≤10% in LVESV or an increased LVESV at 6 months follow-up, were classified as non-responders. For the assessment of LV dyssynchrony, two regions of interest were selected in the basal portions of the septum and the LV lateral wall allowing calculation of the septal-to-lateral delay in time-to-peak systolic myocardial velocity [9]. A septal-to-lateral delay of ≥ 65 ms was considered to represent significant LV dyssynchrony [13]. Additionally, wall motion score index (WMSI) was assessed in all subjects. The LV was divided into 16 segments. A semi quantitative scoring system (1, normal; 2, hypokinesia; 3, akinesia; 4, dyskinesia) was used to analyze each study. Global WMSI was

calculated by the standard formula: sum of the segment scores divided by the number of segments scored [14].

Pacemaker implantation

The LV pacing lead was inserted via the subclavian vein. After a coronary sinus venogram was obtained, the LV pacing lead was introduced into the coronary sinus and placed as far as possible in the venous system, preferably in a (postero-) lateral vein. The right atrial and right ventricular leads were positioned according to standard procedures. Implantation of leads and CRT device was accomplished in all patients without major complications.

Biochemical analysis

Blood samples were obtained before CRT and after 6 months follow-up. Serum and EDTA-plasma samples were stored at -80°C prior to assay.

Serum levels of carboxyterminal cross-linked telopeptide of type I collagen (ICTP) were assayed by a competitive ELISA (Orion Diagnostica, Espoo, Finland) which has a measurement range of 1.0-50 µg/L [15]. Inter-assay variations were 6% both at high (28 µg/L) and low (3 µg/L) ICTP concentrations. Detection limit of the assay was 0.3 µg/L. Intra-assay variability was 11% at low ICTP concentrations, and 7% at high ICTP concentrations. Reference ranges of human serum ICTP concentrations were 1.6-4.2 µg/L for women and 1.5-4.3 µg/L for men.

To measure of rates of synthesis of collagen type I and type III, we chose to use serum levels of aminoterminal propeptides of type I collagen (PINP) and of type III collagen (PIIINP). We acknowledge that there is ample experience with the use of the carboxyterminal propeptide of collagen I (PICP) (see for instance [16]); however, the commercial availability of assays for both PINP and PIIINP and the fact that there is a 1:1:1 stoichiometric relationship between the aminoterminal propeptide, the carboxyterminal propeptide and the collagen molecule formed for either collagen type, influenced our decision to use the assays to quantify PINP and PIIINP. Serum levels of PINP were assayed by radioimmunoassay (Orion Diagnostica) which has a measurement range of 5-250 µg/L [17]. Inter-assay variations were 6% both at high (167 µg/L) and 9.8% at low (12 µg/L) PINP concentrations. Detection limit of the assay was 2 µg/L. Intra-assay variability was 9.8% at low PINP concentrations (12 µg/L) and 10.2% at high (173 µg/L) PINP concentrations. Reference ranges of human serum PINP concentrations were 19-83 µg/L for women and 22-87 µg/L for men.

Serum levels of PIIINP were assayed by radioimmunoassay (Orion Diagnostica) which has a measurement range of 1.0-50 µg/L [18]. Inter-assay variations were 7.2 % at high (12.2 µg/L) and 6.5 % at low (2.7 µg/L) PIIINP concentrations. Detection limit of the assay was 0.3 µg/L. Intra-assay variability was 3.0 % at low PIIINP concentrations (2.8 µg/L) and 4.1% at high (11.9 µg/L) PIIINP concentrations. Reference ranges of adult human serum PIIINP concentrations were 2.3-6.4 µg/L.

The tissue inhibitor of metalloproteinases-1 (TIMP1) was assayed in EDTA-plasma by ELISA (GE Healthcare, Little Chalfont, Buckinghamshire, UK) which has a measurement range of 3-50 µg/L. Inter-assay variations were 15% at low (12.5 µg/L) and 13% at high (47.3 µg/L) TIMP1 concentrations. Intra-assay

variability was 11% at low (10.3 µg/L) and 9% at high (39.4 µg/L) TIMP1 concentrations. Reference range of adult human EDTA-plasma TIMP1 concentrations was 49 – 183 µg/L [19].
Serum levels of pro-matrix metalloproteinase-1 (proMMP-1) were assayed by ELISA (Quantikine, R&D Systems, Abingdon Science Park, Abingdon, UK) which has a measurement range of 0.15 – 10 µg/L. Inter-assay variations were 10.4% at low (0.74 µg/L) and 6.7% at high (4.66 µg/L) proMMP-1 concentrations. Intra-assay variability was 5.6% at low (0.70 µg/L) and 5.4% at high (4.43 µg/L) proMMP-1 concentrations. Detection limit of the assay was 0.02 µg/L. According to the manufacturer, average value of healthy adult human serum proMMP-1 concentration was 3.45 µg/L, with a range of 0.91 – 9.34 µg/L. Mean reference values (± SD) reported in literature are 8.5 ± 5.2 µg/L [20] and 2.43 ± 0.37 µg/L [21].

Statistical analysis

Data were expressed as mean ±SEM unless stated otherwise. Differences between the two groups (responders and non-responders) were tested with two-tailed Student's t-test for paired or unpaired data when appropriate. Univariable and multivariable logistic regression analyses were performed to characterize predictors of good response to CRT. Continuous variables included baseline values of NYHA functional class, quality-of-life score, 6-minute hall walk distance, LVESV, LVEDV, LV ejection fraction, NT-proBNP, PINP, PIIINP, ICTP, TIMP1 and proMMP1. All variables with $p<0.25$ entered the multivariable regression analysis that was performed by stepwise backward deletion. All variables with $p<0.25$ remained in the final model. All statistical tests were performed with SPSS 12.1 software (SPSS Inc, Chicago, IL, USA). For all tests, a probability value <0.05 was considered statistically significant.

Results

None of the patients died during the 6 months follow-up period. CRT was successful in reducing LVESV by >10% in the first 6 months in 46 (72%) patients (responders), whereas in 18 (28%) patients LVESV was reduced by <10% or was even increased (non-responders). The clinical and echocardiographic variables at baseline and after 6 months of CRT in responders and non-responders have been presented previously [8]. At baseline, responders (**1**) were older (65.5±1.4 yrs vs. 59.5±2.6 yrs, p=0.03), (**2**) had more dyssynchrony (100±8 ms vs. 71±11 ms, p=0.05), and (**3**) had longer QRS duration (165±3 ms vs. 135±8 ms, p<0.001), compared to non-responders. There was no difference in baseline WMSI between responders and non-responders (2.30±0.27 vs. 2.18±0.20, n.s.). In responders, NYHA class improved from 3.1±0.1 to 2.0±0.1 (p<0.001), the 6-minute hall walk distance improved from 333±18 m to 427±17 m (p<0.001), and quality-of-life score improved from 35±3 to 17±3 (p<0.001).

Responders had a lower serum PINP level at baseline (32.9±2.2 µg/L vs. 41.9±4.3 µg/L, p=0.04) than non-responders. During follow-up, responders demonstrated an increase in serum PINP level from 32.9±2.2 µg/L to 46.7±4.0 µg/L (p<0.001), whereas non-responders demonstrated no significant change (see Figure). At baseline, plasma levels of PIIINP in responders did not differ from those of nonresponders. In responders, plasma PIIINP levels increased during follow-up (from 4.59±0.24 to 5.13±0.36 µg/L, p<0.05), whereas in non-responders plasma PIIINP levels remained unchanged (see Figure).

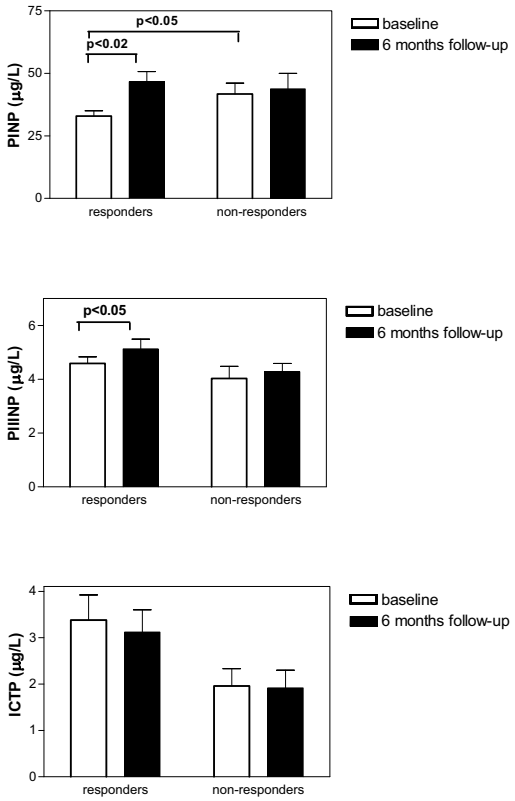

Figure 1. Levels of aminoterminal propeptide of type I procollagen (PINP), aminoterminal propeptide of type III procollagen (PIIINP), and carboxyterminal cross-linked telopeptide of type I collagen (ICTP) measured in serum of 64 patients with congestive heart failure at baseline (□) and at 6 months follow-up (■), divided in a group of patients who were successfully treated by CRT (responders) and a group of patients who responded poorly to CRT (non-responders).

Serum ICTP levels tended to be higher in responders at baseline and at 6 months follow-up than in non-responders at corresponding time points (see Figure). Plasma levels of proMMP1, TIMP1 and proMMP1/TIMP1 did not differ between responders and non-responders at baseline, nor during follow-up (see Table 1).

Table 1. TIMP1 and proMMP1 concentrations in plasma of 64 patients with heart failure determined before CRT (baseline) and at 6 months follow-up, divided according to response to CRT. (mean values ± SEM).

	Responders n=46	**Non-responders n=18**	***p*-value**
TIMP1 (baseline) (µg/L)	124±5.2	111±7.1	0.16
TIMP1 (6 months follow-up) (µg/L)	129±5.8	112±7.7	0.11
proMMP1 (baseline) (µg/L)	7.55±0.72	8.04±1.12	0.71
proMMP1 (6 mo follow-up) (µg/L)	7.89±0.85	8.08±1.11	0.90
proMMP1/TIMP1 (baseline)	0.063±0.006	0.080±0.015	0.20
proMMP1/TIMP1 (6 mo f-u)	0.064±0.007	0.083±0.017	0.21

Abbreviations: CRT, cardiac resynchronisation therapy; TIMP1, tissue inhibitor of metalloproteinases-1; proMMP1, pro-matrix metalloproteinase-1.

Univariable correlation of the following baseline parameters, NYHA functional class, quality-of-life score, 6-minute hall walk distance, LVESV, LVEDV, LV ejection fraction, NT-proBNP, PINP, ICTP, PIIINP, TIMP1 and proMMP1, with good response to CRT demonstrated that PINP scored best (Table 2).
Multivariable correlation of all variables that had an univariable probability value <0.25 demonstrated that PINP, PIIINP and LVEDV at baseline were correlated with good response to CRT, but only PINP to a significant extent (Table 2).

Table 2. Univariable and multivariable correlation of baseline parameters with good response to CRT (reduction of LVESV by >10%).

	Univariable analysis			Multivariable analysis		
	OR	CI	*p*-value	OR	CI	*p*-value
NYHA functional class	0.74	0.28-1.96	0.55			
Quality-of-life score	0.99	0.96-1.01	0.45			
6-min hall walk distance	1.00	0.99-1.00	0.76			
LVESV	1.00	0.99-1.01	0.15			
LVEDV	1.00	0.99-1.01	0.20	1.00	0.99-1.01	0.14
LV ejection fraction	0.94	0.88-1.02	0.15			
NT-proBNP	1.00	1.00-1.00	0.22			
PINP	0.99	0.93-1.00	0.05	0.96	0.93-0.99	0.03
ICTP	1.24	0.93-1.66	0.13			
PIIINP	1.23	0.86-1.76	0.23	1.35	0.94-1.93	0.10
TIMP1	1.01	0.99-1.03	0.16			
proMMP1	0.97	0.87-1.09	0.71			

Abbreviations: OR, odds ratio; CI, 95% confidence limits; CRT, cardiac resynchronisation therapy; NYHA, New York Heart Association; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; NT-proBNP, N-terminal pro B-type natriuretic peptide; PINP, aminoterminal propeptide of type I procollagen; ICTP, carboxyterminal cross-linked telopeptide of type I collagen; PIIINP, aminoterminal propeptide of type III procollagen; TIMP1, tissue inhibitor of metalloproteinases-1; proMMP1, pro-matrix metalloproteinase-1.

Discussion

The present study demonstrated that patients with heart failure who were successfully treated with CRT had relatively low serum levels of PINP at baseline, a measure of collagen synthesis rate, and relatively high serum levels of ICTP at baseline, a measure of collagen degradation rate. After 6 months of CRT, serum PINP and PIIINP levels had increased significantly, whereas serum ICTP had hardly changed. Accordingly, a high serum level of PINP at baseline is associated with failure to respond to CRT, which is in line with reports showing that high levels of markers of collagen synthesis in serum of patients with heart failure are associated with poor outcome [16, 22-24].

In patients with hypertension and LV hypertrophy, elevated PINP levels in serum at baseline are associated with an increased myocardial collagen volume fraction (a measure of myocardial fibrosis) [25], low plasma levels of total and free MMP1, and high plasma levels of total and free TIMP1 [26]. Myocardial fibrosis is often associated with abnormal myocardial stiffness [27], diastolic abnormalities, and a decline in myocardial elastance during contraction, a measure of systolic dysfunction [28]. Perivascular accumulation of collagen may impair the vasodilator capacity of intramyocardial coronary arteries and contribute to the decrease in coronary reserve [29]. In patients with hypertrophic cardiomyopathy, high serum levels of PINP were associated with reduced diastolic function [30]. In patients with essential hypertension, serum levels of PINP and PIIINP were elevated compared to normotensives. In hypertensives, serum PIIINP levels were inversely correlated to diastolic (dys)function, whereas serum PINP levels were correlated positively to LV mass index [24]. In patients with dilated cardiomyopathy, the presence of a restrictive mitral pattern was associated with highest serum levels of PIIINP and high serum levels of PIIINP were related to poor outcome [31]. In patients with dilated cardiomyopathy, serum PIIINP levels were significantly elevated compared to corresponding levels in controls, these serum PIIINP levels were correlated to NYHA functional class, daily diuretics dosage, and mean right atrial pressure, and were inversely correlated to cardiac output [24].

In the present study, the responders to CRT tended to have higher serum ICTP levels than non-responders. Six months therapy with CRT had no significant effect on serum ICTP in responders, nor in non-responders. However, serum levels of proMMP1, a collagenase, did not differ between baseline and follow-up, nor did they differ between responders and non-responders at these two time points.

Previously it has been demonstrated that patients with dilated cardiomyopathy had higher serum levels of MMP1 than controls, which was associated with higher MMP1/TIMP1 ratio, and serum free MMP1 as well as MMP1/TIMP1 ratio which correlated positively with LV end-diastolic volume and negatively with cardiac index [21]. In failing hearts supported by a LV assist device myocardial MMP1 levels decreased, whereas TIMP1 and TIMP3 levels increased [32].

High serum levels of ICTP have also been observed in patients with dilated cardiomyopathy [21] and in patients with hypertrophic cardiomyopathy [30]. Klappacher et al. found that serum ICTP levels beyond a cut-off level of 7.6 µg/L

in patients with dilated cardiomyopathy were associated with increased risk of advanced clinical stage, increased risk of poor haemodynamic condition, increased risk of hyponatraemia (<138 mmol/L), and increased risk of heart transplantation [24].

The increase of serum PINP in responders to CRT (the patients demonstrating reverse LV remodelling) in the present study, is interpreted as an increased myocardial collagen synthesis in responders to CRT during 6 months follow-up. This increased myocardial collagen synthesis in responders may be a reaction of the myocardium induced by improved loading conditions (due to improved synchronicity of segmental wall motion); and thus LVESV and LVEDV were decreased by biochemical forces within the tissue that provide “passive tissue contraction”.

The responders were the group of patients with the lowest collagen synthesis at baseline, which according to the above explanation, associates with adverse LV remodelling by weakening the myocardial structure, thereby allowing progressive LV dilatation. In a study using myocardial biopsies from patients with end-stage heart failure taken before and after 100-600 days of left ventricular assist device (LVAD) implantation, Bruggink and co-workers observed an increase in ECM volume in the first 200 days after LVAD implantation, followed by a gradual decrease of ECM volume in the following 200 days to a level that was still higher than that observed pre-LVAD [33]. Plasma PINP levels increased considerably (≈3-fold) in the first month after LVAD implantation, and remained increased in the first 6 months after LVAD implantation. Thus, reverse LV remodelling induced by LV unloading was associated with increased collagen synthesis and expanded ECM space. Although in our study the tertiles of plasma PINP levels at baseline showed a tendency to associate with response to CRT (lowest tertile: 17/21 responders, 81%; middle tertile: 16/22 responders, 73%; highest tertile 13/21 responders, 62%), our study population was probably too small to find a significant association.

One or more patients with systemic diseases that would induce abnormalities in collagen metabolism may have been included in the study; however, signs and symptoms of such diseases were absent. Furthermore, echocardiography-derived backscatter data indicative of myocardial fibrosis were not available in this study.

In conclusion, elevated collagen synthesis rate at baseline is unfavourable in terms of therapeutic success of CRT. Reverse LV remodelling upon CRT is associated with increased collagen synthesis rate in the first 6 months of follow-up.

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