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Relationships between plasma levels of matrix metalloproteinases and neurohormonal profile in patients with heart failure☆

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Abstract

Background—Both neurohormonal derangements and alterations in the myocardial extracellular matrix are thought to contribute to adverse ventricular remodelling that results in worsening heart failure (HF). There is also emerging preclinical information to suggest that these signalling pathways mutually regulate in HF.

Aim—To assess the relationships between plasma levels of matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinase (TIMP), and neurohormonal profiles in chronic HF.

Methods and results—In this substudy of 184 HF patients enrolled in the Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) trial, plasma norepinephrine and epinephrine were measured with HPLC; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), angiotensin II, aldosterone, and endothelin-1 were measured with immunoassays; MMP-2, MMP-9, and TIMP-1 were measured with 2-site sandwich ELISA assays. We used Spearman's rank correlation to examine the relationships between plasma MMP and neurohormone levels. Circulating ANP, BNP, and endothelin-1 levels were positively correlated with MMP-2 and TIMP-1 levels. Plasma level of aldosterone showed a weak positive correlation with MMP-9, but there was no significant correlation between angiotensin II, epinephrine or norepinephrine and MMP-2, MMP-9, or TIMP-1.

Conclusions—These findings suggest that specific neurohormones and extracellular matrix modulators may play a coordinated role in the pathogenesis of HF.

Keywords

Heart failure; Matrix metalloproteinase; Neurohormones

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1. Introduction

Left ventricular (LV) remodelling is a critical pathophysiologic process in the development and progression of systolic heart failure (HF). Experimental and clinical studies have demonstrated that neurohormonal derangements mediate maladaptive LV remodelling, [1] while clinical trials have established the efficacy of several neurohormonal modulating therapies in the treatment of HF [2].

Over the past decade, the importance of myocardial extracellular matrix in maintaining intact myocyte alignment, ventricular geometry and mechanical function has become increasingly recognized [3]. Accumulating evidence strongly implicates a pivotal role of matrix metalloproteinases (MMPs) in the pathogenesis of LV dysfunction [3–6]. This family of zinc-dependent proteases regulate the synthesis and degradation of myocardial extracellular matrix proteins. *In vitro* data indicate that neurohormonal stimulation can augment MMP expression and activity in various cell types [7–10]. However, there are a paucity of data on the complex interplay between neurohormones and MMPs in HF patients. Accordingly, our objective was to examine the relationships between MMPs, tissue inhibitor of metalloproteinase (TIMP) and comprehensive neurohormonal profiles using data from the Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) trial.

2. Methods

Details of the RESOLVD trial have been published [11–13]. In brief, RESOLVD was a multicenter, double-blind, randomized, controlled trial with a 3×2 partial factorial design and two-stage randomization. Eligible patients had stable chronic New York Heart Association Functional Class II to IV HF of any etiology, six-minute walk distance <500 m, and LV ejection fraction <40%. Major exclusion criteria were renal impairment and myocardial infarction within the previous 4 weeks. In total, 768 patients were randomized to treatment with candesartan alone, enalapril alone, or candesartan plus enalapril. After 17 weeks, patients eligible for beta-blocker therapy were further randomized to receive metoprolol CR or placebo. LV end-diastolic volume, LV end-systolic volume, and LV ejection fraction were measured by radionuclide angiography according to standardized protocols in the participating institutions, and analyzed in a core laboratory. The present study was based on a random subset of 184 participants in the RESOLVD trial, who had blood samples collected at baseline. Table 1 shows the baseline characteristics of the patients in this substudy (n=184) and of the remaining patients in RESOLVD (n=584). The local institutional review board approved the RESOLVD study protocol, and all participants gave written informed consent.

At baseline, venous blood samples were collected into heparinized tubes from patients in the supine position after an overnight fast. Plasma was then separated, aliquoted, and frozen at −70 °C until analysis. Norepinephrine and epinephrine were measured with HPLC; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), angiotensin II, aldosterone, and endothelin-1 were measured with immunoassays [13]. Data on the neurohormonal profile of the age-matched healthy volunteers without cardiac disease for RESOLVD have been published [13]. We used 2-site sandwich ELISA assays (Amersham Pharmacia Biotech, Buckinghamshire, UK) for measurements of all plasma MMP and TIMP levels, as previously described [14,15]. The MMP-2 assay (sensitivity=0.37 ng/ml) detects the proform of MMP-2 and that complexed with TIMP-2; the MMP-9 assay (sensitivity=0.6 ng/ml) detects the proform of MMP-9 and that complexed with TIMP-1; the TIMP-1 assay (sensitivity=1.25 ng/ml) detects both free TIMP-1 and that complexed with MMPs. We performed duplicate measurements, and the intra-assay coefficients of variation were <10%

for all assays. All MMP and TIMP measurements were performed in aggregates and completed in 2001.

Continuous variables are expressed as mean±standard deviation (SD), and compared by Mann–Whitney *U* test. Because data distributions were not normal, we calculated Spearman's rank correlation coefficients (ρ) for the relationships between plasma MMP and neurohormone levels. We conducted statistical analyses using SPSS version 12.0 (SPSS Inc, Chicago, IL), and considered two-sided p values <0.05 to be statistically significant.

3. Results

Table 1 presents the baseline clinical characteristics of the study population. Most patients were men with ischemic heart disease and in New York Heart Association functional class II. Their neurohormone, MMP, and TIMP measurements are shown in Table 2. There were no significant differences in plasma MMP-2, MMP-9, and TIMP-1 levels according to gender or etiology of HF. Compared to their younger counterparts, older patients (age ≥65) had higher circulating levels of MMP-2 (p=0.001) and TIMP-1 (p<0.001). Patients with more severe HF (functional class III or IV) also had higher plasma TIMP-1 measurements (p<0.001). Plasma MMP-2 level was significantly correlated with TIMP-1 (ρ=0.45, p<0.01), but not significantly with MMP-9 levels (ρ=0.14, p=0.051). There was no significant correlation between MMP-9 and TIMP-1 levels.

Table 3 summarizes the relationships between plasma levels of neurohormones, MMP-2, MMP-9, and TIMP-1. There were significant positive correlations of plasma levels of ANP, BNP and endothelin-1 with MMP-2 and TIMP-1. There was a weak but significant correlation between aldosterone and MMP-9 levels. In contrast, angiotensin II, norepinephrine or epinephrine did not exhibit any significant correlation with MMP or TIMP levels.

4. Discussion

While the relationship between several neurohormones and MMPs in HF has been the subject of prior investigations, [9,14,16–20] the present study is the largest to date and includes the most comprehensive neurohormonal measurements. We demonstrated positive correlations of circulating levels of ANP, BNP, and endothelin-1 with MMP-2 and TIMP-1, whereas only aldosterone was positively correlated with MMP-9 level. These novel findings raise the interesting hypotheses that different proteolytic pathways in extracellular matrix may be modulated through selective pharmacologic targets, and that the complex signalling pathways may mutually regulate in HF.

Previous studies of patients with acute myocardial infarction have reported a significant correlation between plasma BNP and MMP-9 levels [17,19]. Nevertheless, there are considerable differences between early LV remodelling after acute myocardial infarction and ongoing LV remodelling in chronic HF. For example, infiltrating neutrophils in the infarct zone may represent the predominant source of MMP-9, which plays a crucial role in wound healing and infarct expansion [3]. Furthermore, both MMP-2 and MMP-9 may originate from the ruptured atherosclerotic plaques in acute coronary syndromes [21]. Yamazaki and colleagues studied 52 patients with HF and found a significant correlation between plasma BNP and MMP-2 levels (r=0.78, p<0.01) [18]. In a study of 88 patients followed in a HF clinic, George et al. reported only a weak correlation (r=0.19) between circulating levels of BNP and MMP-2 that did not reach statistical significance, [20] possibly due to inadequate power. Our results confirm highly significant and moderately strong, positive correlations of plasma levels of BNP with MMP-2 and TIMP-1.

Furthermore, we found similar correlations with ANP, which are consistent with the notion that both ANP and BNP can bind to natriuretic peptide receptor-A to activate biosignalling cascades [22]. The observed correlations between circulating endothelin-1, and MMP-2 and TIMP-1 levels in HF patients have not been reported previously in the literature, although Coker et al. have demonstrated increased MMP-2 content in isolated porcine LV myocyte incubated with endothelin-1 [7].

Experimental studies suggest that the renin–angiotensin–aldosterone system modulates MMP/TIMP expression and activity [10]. For instance, angiotensin II promotes MMP-9 transcription through activation of nuclear factor-kappa B [10]. Conversely, administration of eplerenone, an aldosterone antagonist, reduces gelatinase zymographic levels [23]. In the present study, plasma MMP-9 level showed only a weak positive correlation with aldosterone but no significant relationship with angiotensin II (ρ=0.11, p=0.14).

In contrast to some previous studies, we did not find any significant correlation between plasma norepinephrine and MMP levels [9,16].Although the precise reasons are unknown, important differences in patient populations, severity or stage of HF, and background pharmacotherapies may account for this discrepancy. However, inadequate power seems less plausible given the larger sample size in this study.

The present study has several limitations. Our modest sample size may have afforded limited power to detect potentially weaker correlations that exist. Although plasma MMP and TIMP levels probably reflect spillover from the myocardium, their exact relationship with myocardial tissue levels are unknown, and other extra-cardiac sources of MMPs cannot be excluded. We only indirectly measured MMP and TIMP abundances rather than their enzymatic activity. Furthermore, the significant correlations reported in this study were only weak to moderately strong, suggesting that MMPs and TIMPs likely are controlled through a myriad of complex pathways, rather than regulated by the measured neurohormones alone. Because we did not measure TIMP-3 in the present study, we could not determine the relationships between MMP-9/TIMP-3 ratio and various neurohormones. Despite corroborative *in vitro* evidence, the significant correlations between neurohormones and MMPs we observed do not necessarily imply a causative pathogenetic mechanism in humans. Finally, because our study sample was derived from a selected population in a clinical trial, the generalizability of our findings needs to be confirmed.

In conclusion, our data demonstrate significant correlations between circulating levels of specific neurohormones and MMPs in HF patients. These findings suggest the presence of intricate relationships between neurohormonal and extracellular matrix regulatory pathways in the pathogenesis of HF.

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Table 1

Baseline characteristics of the patients in the present substudy (*n*=184) and of the remaining patients enrolled in RESOLVD (*n*=584)

	***n*=184 (present substudy)**	***n*=584 (remaining patients in RESOLVD)**	***p* value**
Age, years	66±10	62±11	<0.01
Male, %	85	83	0.82
Etiology of heart failure, %			<0.01
Ischemic heart disease	82	69	
Idiopathic dilated cardiomyopathy	12	19	
Other	6	12	
New York Heart Association functional class, %			0.40
II	61	64	
III or IV	39	36	
Heart rate, bpm	76±12	76±13	0.69
Systolic blood pressure, mm Hg	124±18	120±17	<0.01
Diastolic blood pressure, mm Hg	74±9	73±10	0.29
6-minute walk distance, m	370±86	385±83	0.06
Creatinine, μmol/l	107±24	111±40	0.29
Left ventricular end-diastolic volume, ml	234±91	263±116	<0.01
Left ventricular end-systolic volume, ml	171±83	199±107	<0.01
Left ventricular ejection fraction, %	29±10	27±11	<0.01

Data presented as percentages or mean±standard deviation.

Table 2

Plasma concentrations of MMPs, TIMP and neurohormones

Plasma concentrations	Study patients (*n*=184)
MMP-2, ng/ml	685±271
MMP-9, ng/ml	15±21
TIMP-1, ng/ml	1513±504
MMP-2: TIMP-1 ratio	0.48±0.20
MMP-9: TIMP-1 ratio	0.011±0.014
ANP, pmol/l	1421±1245
BNP, pmol/l	53±61
Angiotensin II, pg/ml	27±16
Aldosterone, pg/ml	107±91
Epinephrine, pg/ml	83±101
Norepinephrine, pg/ml	498±273
Endothelin-1, pg/ml	1.64±0.67

All values are expressed as mean±standard deviation.

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

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Table 3

Relationship between plasma levels of MMP, TIMP, and neurohormones

Spearman's rank correlation coefficient (ρ)	ANP	BNP	Angiotensin II	Aldosterone	Epinephrine	Norepinephrine	Endothelin-1
MMP-2	0.39 *	0.31*	−0.13	0.01	0.09	0.07	0.16¶
MMP-9	0.09	0.04	0.11	0.15¶	0.02	−0.03	0.03
TIMP-1	0.31*	0.22 †	−0.12	−0.09	0.02	0.09	0.23 †
MMP-2: TIMP-1	0.19 †	0.18 †	−0.03	0.03	0.09	−0.01	−0.03
MMP-9: TIMP-1	0.00	−0.03	0.15¶	0.17¶	0.01	−0.05	−0.05

* Statistically significant correlations are marked:$p<0.001$,

† Statistically significant correlations are marked:$p<0.01$,

¶ Statistically significant correlations are marked:$p<0.05$.

ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.