

Influence of Spironolactone on Matrix Metalloproteinase-2 in Acute Decompensated Heart Failure

João Pedro Ferreira¹, Mário Santos¹, José Carlos Oliveira¹, Irene Marques¹, Paulo Bettencourt², Henrique Carvalho¹

Centro Hospitalar do Porto¹; Centro Hospitalar São João², Porto – Portugal

Abstract

Background: Matrix metalloproteinases (MMPs) are a family of enzymes important for the resorption of extracellular matrices, control of vascular remodeling and repair. Increased activity of MMP2 has been demonstrated in heart failure, and in acutely decompensated heart failure (ADHF) a decrease in circulating MMPs has been demonstrated along with successful treatment.

Objective: Our aim was to test the influence of spironolactone in MMP2 levels.

Methods: Secondary analysis of a prospective, interventional study including 100 patients with ADHF. Fifty patients were non-randomly assigned to spironolactone (100 mg/day) plus standard ADHF therapy (spironolactone group) or standard ADHF therapy alone (control group).

Results: Spironolactone group patients were younger and had lower creatinine and urea levels (all $p < 0.05$). Baseline MMP2, NT-pro BNP and weight did not differ between spironolactone and control groups. A trend towards a more pronounced decrease in MMP2 from baseline to day 3 was observed in the spironolactone group (-21 [-50 to 19] vs 1.5 [-26 to 38] ng/mL, $p = 0.06$). NT-pro BNP and weight also had a greater decrease in the spironolactone group. The proportion of patients with a decrease in MMP2 levels from baseline to day 3 was also likely to be greater in the spironolactone group (50% vs 66.7%), but without statistical significance. Correlations between MMP2, NT-pro BNP and weight variation were not statistically significant.

Conclusion: MMP2 levels are increased in ADHF. Patients treated with spironolactone may have a greater reduction in MMP2 levels. (Arq Bras Cardiol. 2015; 104(4):308-314)

Keywords: Heart Failure; Spironolactone/therapeutic use; Matrix Metalloproteinase 2/ therapeutic use.

Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent interstitial enzymes important for the resorption of extracellular matrices (ECM) in both health and disease¹. Extracellular matrices are a dynamic structure central to the control of vascular remodeling and repair¹, mostly due to the ability of MMPs to reabsorb and digest excessive amounts of ECM responsible for structural disruption^{2,3}.

Elevated MMPs promote loss of cardiac contractility via cell proteolysis and alterations in the ECM, contributing to cardiac and extra-cardiac remodeling processes⁴. In fact, clinical and experimental heart failure (HF) models of dilated and ischemic cardiomyopathy have demonstrated an increased activity of matrix metalloproteinase-2 (MMP2)^{2,5-7}. In patients with HF, increased levels of MMP2 were associated with all-cause mortality⁸. Concordantly, in the acutely decompensated heart

failure (ADHF) setting a decrease of circulating MMPs has been demonstrated along with successful ADHF treatment^{3,9}. Previous studies have suggested a therapeutic benefit of spironolactone in ADHF setting¹⁰. But no studies had looked to the effect of spironolactone in the ECM remodeling.

In the present study, we aimed to examine the influence of spironolactone on the ECM remodeling in ADHF patients. We hypothesized that MMP-2 plasma levels of ADHF patients will have a steeper decrease if spironolactone is added to standard treatment.

Methods

Study Design

We analyzed data from a previous pilot, prospective, interventional, clinical trial that we performed between February 2012 and February 2013. During that period, we enrolled 100 consecutive patients who presented at a Portuguese tertiary hospital with ADHF. Patients were eligible for enrollment if they had decompensation of chronic HF with symptoms leading to hospitalization. ADHF was diagnosed based on a history of chronic HF and at least one acute symptom (dyspnea, orthopnea, or edema) and one sign (rales, peripheral edema, ascites, or pulmonary vascular congestion on chest radiography).

Mailing Address: João Pedro Ferreira •

Rua Dr. José Oliveira e Silva, nº 52, Salreu-Estarreja. Postal Code 3865-209, Porto – Portugal

Email: jp7ferreira@hotmail.com

Manuscript received June 26, 2014; revised manuscript September 27, 2014; accepted September 30, 2014.

DOI: 10.5935/abc.20140205

Patients were non-randomly assigned in a sequential 1:1 ratio to spironolactone plus standard ADHF therapy or standard ADHF therapy alone, 50 patients in each arm. Patients were alternately assigned to the spironolactone arm or the standard ADHF therapy arm in a sequential manner - the first patient to one arm and the next to the other arm. This sequence was repeated until we reached 100 patients, 50 in the spironolactone group and 50 in the control group. Patients were blinded to the allocation, but the clinicians were not. The recommended spironolactone dose was 100 mg/day. However, the attending physician could decrease that dose to 50 mg/day after 48h upon admission. Furosemide dose and route of administration were clinically adjusted according to the patients' hydration status.

Exclusion criteria were: chronic use of mineralocorticoid receptor antagonists; cardiac surgery within 60 days of enrollment; cardiac mechanical support; cardiac resynchronization-therapy within the last 60 days; comorbid conditions with an expected survival of less than 6 months; acute myocardial infarction at time of hospitalization; uncorrected hemodynamically significant primary cardiac valvular disease; patients requiring intravenous vasodilators or inotropic agents; supine systolic arterial blood pressure < 90 mmHg; plasma creatinine level > 1.5 mg/dL; serum potassium level > 5.0 mmol/L; hemoglobin level < 9 g/dL; and sepsis.

Institutional review board or ethics committee approval was obtained. All patients provided written informed consent to participate in the study.

Clinical assessment of participants

Patients' clinical status, including physical examination, was prospectively recorded by the same attending physician on day 1 and day 3. Medications and respective dosages were prospectively recorded by the investigators according to the attending physician prescriptions.

Blood samples were collected in the first 24 hours after the patient's admission (baseline) to the hospital, and day-3 samples were collected between 72 and 96 hours of hospitalization. Samples were analyzed at a central core laboratory, and included plasma creatinine and urea, electrolytes, NT-pro BNP and MMP2. Clinical assessment and routine analyses were performed daily during hospital stay. Estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation¹¹. All patients performed a transthoracic echocardiography within 72 hours from admission. Left ventricular ejection fraction (LVEF) was calculated according to biplane Simpson method.

MMP2 was measured by using enzyme-linked immunosorbent assays (ELISA) - Quantikine Elisa Human MMP-2 Immunoassay (R&D Systems, Inc®). The normal range of MMP2 values published by the manufacturer, expressed as median [interquartile range₂₅₋₇₅, IQR₂₅₋₇₅], is 199 [161 – 301] ng/mL. The assay sensitivity is 0.047 ng/mL.

Of the 100 studied patients we analyzed baseline (day 1) and day 3 blood samples from 87. Thirteen samples were not analyzed due to transport and/or sampling processing errors. Samples were collected in the morning with patients supine. Serum was separated and stored at -80 °C until sample analysis.

Variable definitions

We classified patients according to spironolactone use and their response to diuretic therapy.

We studied the relationships between baseline characteristics, day 3 and changes (Δ , difference between day 3 and baseline values) in MMP2, NT-proBNP and weight regarding the spironolactone use and diuretic response.

Statistical Analysis

Normally distributed continuous variables are expressed as mean \pm standard deviation (SD), and skewed distributions are presented as median [IQR₂₅₋₇₅].

Categorical variables are expressed in proportions (%).

Comparison between groups was performed using parametric (independent samples t-test), non-parametric (Mann-Whitney test), or Chi-square tests, as appropriate.

Correlations of MMP2 were examined by single variable linear regression and presented as correlation coefficient and 95% confidence interval (95% CI).

A p value < 0.05 was considered statistically significant.

Statistical analysis was performed using SPSS software, version 19 (Chicago, IL, USA).

Results

Baseline Characteristics in Control and Spironolactone Groups

Control group patients were older (78.8 ± 9.3 versus [vs.] 73.2 ± 11.7 years, $p = 0.01$), and had higher creatinine and urea levels (1.15 ± 0.27 vs. 1.03 ± 0.30 mg/dL, $p = 0.026$ and 59.32 ± 22.27 vs. 51.10 ± 18.63 mg/dL, $p = 0.048$). No differences between groups were found regarding the following variables: sex; diabetes mellitus; chronic obstructive pulmonary disease; dementia; sleep apnea; non-invasive ventilation; ischemic heart disease; atrial fibrillation; LVEF; weight; systolic blood pressure; potassium; sodium; hemoglobin; albumin; NT-pro BNP; MMP2; furosemide dose; hospital length of stay; and the proportion of patients on angiotensin converting enzyme inhibitors and beta-blockers (Table 1).

Spironolactone Influence on MMP2, NT-pro BNP and Weight Dynamic Changes

No differences between control and spironolactone groups were observed regarding baseline and day 3 MMP2 levels (Table 2). However, MMP2 decreased from baseline to day 3 in the spironolactone group, while, in control group, MMP2 levels increased, leading to a tendency towards a reduction in MMP2 levels in the spironolactone group ($1.5 [-26$ to $38]$ vs. $-21 [-50$ to $19]$ ng/mL, $p = 0.06$) (Table 2 and Figure 1). The proportion of patients showing a decrease in MMP2 levels from baseline to day 3 was also greater in the spironolactone group. That difference, however, did not reach statistical significance: 21 control patients (50%) vs. 30 spironolactone group patients (66.7%), $p = 0.115$.

Table 1 - Baseline population characteristics, laboratory results, medications, and hospital length of stay in treatment and control groups

	Control Group (n = 50)	Spironolactone Group (n = 50)	p Value
Age (yrs)	78.8 ± 9.3	73.2 ± 11.7	0.010
Male Sex - %	34	44	0.31**
Diabetes Mellitus - %	50	40	0.31**
Chronic Obstructive Pulmonary Disease - %	10	26	0.32**
Dementia - %	16	8	0.22**
Sleep Apnea - %	10	26	0.32**
Non-Invasive Ventilation - %	14	20	0.42**
Ischemic Heart Disease - %	48	52	0.69**
Atrial Fibrillation - %	68	50	0.07**
Left Ventricular Ejection Fraction < 40% - %	56	68	0.22**
Weight (Kg)	75.6 ± 16.3	76.1 ± 16.4	0.89
Systolic Blood Pressure (mmHg)	140.5 ± 23.9	139 ± 27.9	0.80
Plasma Creatinine (mg/dL)	1.15 ± 0.27	1.03 ± 0.30	0.03
eGFR (mL/min/1,73 m ²)	54.5 ± 16.5	68.3 ± 23.6	0.001
Plasma Urea (mg/dL)	59.3 ± 22.3	51.1 ± 18.6	0.05
Serum Potassium (mmol/L)	4.1 ± 0.4	4.0 ± 0.6	0.33
Serum Sodium (mmol/L)	140.5 ± 5.0	140.6 ± 3.7	0.96
Hemoglobin (g/dL)	12.2 ± 1.8	12.7 ± 2.3	0.22
Albumin (mg/dL)	3.7 ± 0.4	3.6 ± 0.4	0.63
NT-pro BNP (pg/mL)	3102 [1797 – 8204]	2701 [1463 – 5004]	0.17*
MMP2 (ng/mL)	260 [226 – 299]	268 [207 – 336]	0.52*
Intravenous Furosemide Dose (mg/d)	75.6 ± 20.7	76.0 ± 25.5	0.93
ACEi/ARB - %	38	50	0.20**
Beta-Blocker - %	42	32	0.30**
Spironolactone - %	-	100	-
Spironolactone Dose (mg/d)	-	94.5 ± 23.3	-
Hospital Length of Stay (days)	9.0 ± 3.7	8.7 ± 3.0	0.59

Continuous variables are presented as mean ± standard deviation [SD], p value or median [interquartile range, IQR], p value. Categorical variables are presented as absolute number (%), p value.

*Non-parametric paired sample test; ** Chi-square test.

eGFR: estimated glomerular filtration rate; NT-pro BNP: N-terminal pro brain natriuretic peptide; MMP2: matrix metalloproteinase -2; ACEi/ARB: angiotensin-converting enzyme inhibitors/angiotensin receptor blockers.

No differences were observed in NT-pro BNP levels at baseline. However, on day 3, the spironolactone group patients showed lower levels of NT-pro BNP (248 [923 – 5502] vs. 1555 [722 – 2554] pg/mL, $p = 0.05$). No differences between groups were observed in the variation of NT-pro BNP levels probably due to the lower levels (although not significantly lower) of NT-pro BNP at baseline in the spironolactone group, leading to a smaller amplitude of variation in this group (Table 2).

A greater weight decrease was also observed in the spironolactone-treated patients (-2.9 ± 2.4 vs. -4.8 ± 2.8 kg, $p < 0.001$) (Table 2).

Δ MMP2, Δ NT-pro BNP and Δ Weight Correlations

No significant correlations were observed between Δ in MMP2, NT-pro BNP and weight (Table 3).

Discussion

In the present study we observed increased baseline levels of MMP2 in patients with ADHF. The patients treated with spironolactone showed a tendency towards a greater reduction in MMP2 levels. These results are consistent with previous findings demonstrating the impact of mineralocorticoid receptor antagonists on the ECM remodeling and highlight the potential interest about

Table 2 - Comparison of MMP2, NT-pro BNP and weight at baseline, day 3, and change (Delta, Δ) between the study groups

	Control Group	Spironolactone Group	p Value
MMP2			
Baseline	260 [226 – 299]	268 [207 – 336]	0.52*
Day 3	266 [227 – 298]	261 [212 – 307]	0.49*
Δ (day 3 – baseline)	1.5 [-26 to 38]	-21 [-50 to 19]	0.06*
NT-pro BNP			
Baseline	3102 [1792 – 8204]	2701 [1463 – 5004]	0.17*
Day 3	2488 [923 – 5502]	1555 [722 – 2554]	0.05*
Δ (day 3 – baseline)	-945 [-2249 to -62]	-816 [-1833 to -106]	0.75*
Weight			
Baseline	75.6 ± 16.3	76.1 ± 16.4	0.89
Day 3	72.8 ± 16.3	71.3 ± 16.2	0.66
Δ (day 3 – baseline)	-2.9 ± 2.4	-4.8 ± 2.8	< 0.001

Continuous variables are presented as mean ± standard deviation [SD], p value or median [interquartile range, IQR], p value, and independent sample t-test or independent sample non-parametric test were used, respectively.

*non-parametric test.

NT-pro BNP: N-terminal pro brain natriuretic peptide; MMP2: matrix metalloproteinase -2; Δ: delta or difference between day 3 and baseline values.

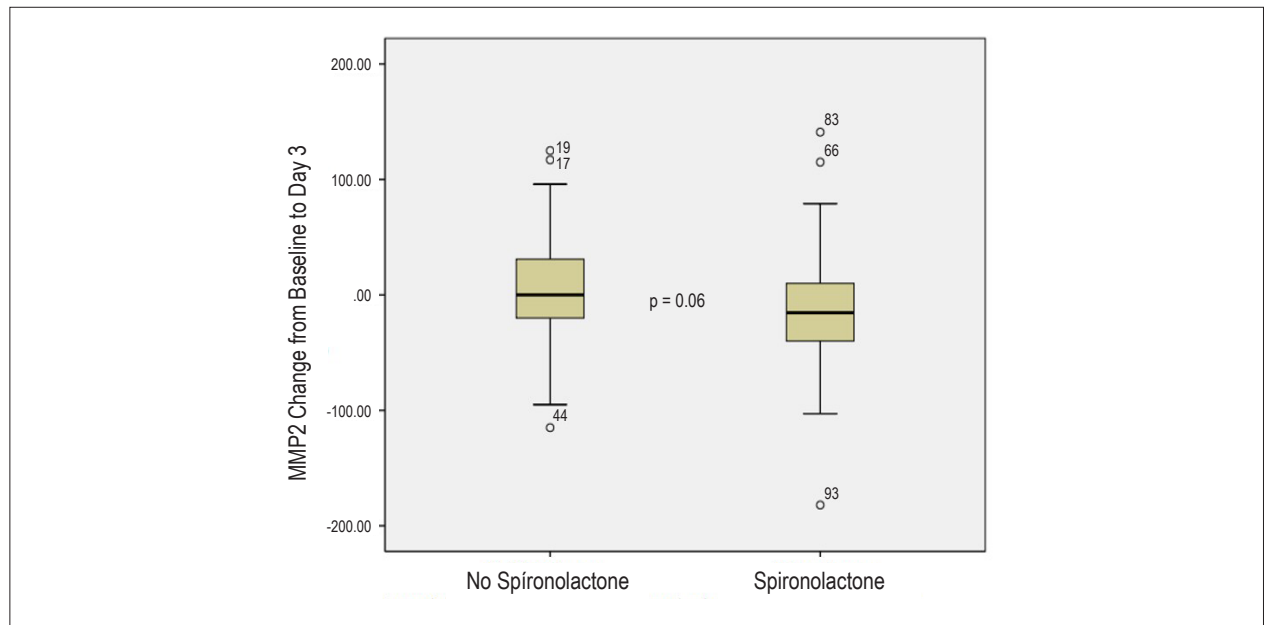


Figure 1 - Comparison of Δ MMP2 Levels from baseline to day 3 between control and spironolactone groups.

A trend towards a more pronounced reduction in MMP2 levels was observed in spironolactone group.

Analysis was performed comparing the variation (Δ, day 3 - baseline) of MMP2 levels between control and spironolactone groups using non-parametric independent sample tests (1.5 [-26 to 38] versus -21 [-50 to 19], p = 0.06).

Legend: MMP2 = matrix metalloproteinase-2.

Table 3 - Correlation coefficients and 95% confidence intervals (CI) between delta (Δ) MMP2, Δ NT-pro BNP, and Δ weight

	Δ MMP2 Correlation Coefficient	95%CI	p Value
Δ NT-pro BNP	0.11	-0.003 to 0.01	0.33
Δ Weight	0.12	-1.97 to 7.05	0.27

MMP2: matrix metalloproteinase-2; NT-pro BNP: N-terminal pro-brain natriuretic peptide; Δ: delta or difference between day 3 and baseline values.

spironolactone for the treatment of ADHF, where those mechanisms are strongly exacerbated.

Increased serum levels of MMP2 have been demonstrated in the ADHF setting^{3,9}. In our study, the median [IQR] MMP2 levels at admission were 260 [225 - 312] ng/mL. These values are above the normal range defined by the manufacturer, 199 [161 - 301] ng/mL, and are in accordance with previous reports on patients with HF decompensation. A previous study by Shirakabe A. et al³ has also shown increased serum MMP2 levels in ADHF, with a rapid decrease along with HF compensation. Furthermore, an interventional placebo-controlled trial performed by Tziakas DN. et al⁹ has shown a significant reduction in MMP2 levels in the group of patients treated with levosimendan. In animal models, exacerbated neurohormonal activation leads to an increase in the levels of several myocardial MMP subtypes¹²⁻¹⁴. MMPs are important for the proteolysis that can affect the composition of ECM and, consequently, myocardial remodeling. Additionally, increased ECM turnover may be associated with pathological myocardial remodeling, which may be accelerated in decompensated HF^{3,15}. Consequently, a reduction in markers of ECM turnover may serve as a surrogate marker for deceleration of myocardial turnover and remodeling. Our study showed a greater decrease of MMP2 in the group of patients submitted to spironolactone treatment. Mineralocorticoid receptor antagonists improve survival and reduce morbidity in patients with HF and reduced ejection fraction and mild-to-severe symptoms, and in patients with left ventricular systolic dysfunction and HF after acute myocardial infarction¹⁶⁻¹⁸. Additionally, used in natriuretic doses, mineralocorticoid receptor antagonists are likely to improve congestion in ADHF with good tolerability and few side effects¹⁹. Several proposed mechanisms explain how mineralocorticoid receptor antagonists improve HF outcomes, and these pathways include a reduction in myocardial remodeling²⁰. Our study provides important information on a better understanding of ECM turnover processes. The steeper MMP2 reduction observed in patients submitted to spironolactone treatment provides a real demonstration of potential mitigation of harmful remodeling through spironolactone use. Interestingly, patients without MMP2 reduction or increase, after an acute HF episode, had poorer prognosis³. Therefore, changes in MMP2 levels are a potentially useful prognostic marker in patients admitted due to ADHF.

NT-pro BNP is a well-validated, widely used, and very accurate biomarker for the diagnosis and risk stratification of HF²¹. Patients submitted to spironolactone treatment had lower levels of NT-pro BNP on day 3, and a more pronounced weight reduction when compared to controls, while patients with slower diuretic response had higher NT-pro BNP levels on day 3 of hospitalization, a tendency towards lower NT-pro BNP reduction, and less weight loss. However, the changes in MMP2 values did not correlate with the variation in NT-pro BNP or weight. The small sample size, the NT-pro BNP and MMP2 elevated variance, and the different mechanisms influencing those biomarkers may all explain the lack of correlation.

Several limitations in our study should be noted. First, this was a single-centre, non-randomized trial with a small number of patients with mixed HF etiologies and treatments. Second, this post-hoc analysis has limitations inherent to observational studies. Third, the decision to withdraw diuretic therapy was based on subjective assessment of congestive signs and symptoms, so we cannot rule out the inter-observer variability. However, in real life, the decision to step down diuretic therapy is also based on subjective clinical evaluation. Fourth, our study excluded HF patients with significant renal impairment, since plasma creatinine level lower than 1.5 mg/dL was an inclusion criterion, leading to a potential selection of a subset of low-risk patients, which can affect the external validity of our results. Fifth, the spironolactone group patients were younger and had lower plasma creatinine and urea levels, which can positively affect the response to that drug. Finally, only MMP2 was evaluated, and other forms of MMPs may have different effects and responses in ADHF patients.

Conclusion

The present study showed that MMP2 levels can be increased in ADHF, and that patients treated with spironolactone may have a greater reduction in MMP2 levels. Whether these findings have prognostic significance requires further investigation.

Acknowledgements

The authors acknowledge the lab technicians, especially Mr. Fernando Santos, for technical assistance, and all physicians collaborating in the study.

Author contributions

Conception and design of the research, Acquisition of data, Statistical analysis and Writing of the manuscript: Ferreira JP; Analysis and interpretation of the data: Ferreira JP, Santos M, Oliveira JC, Bettencourt P; Obtaining financing: Carvalho H; Critical revision of the manuscript for intellectual content: Ferreira JP, Santos M, Marques I, Bettencourt P, Carvalho H.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was partially funded by Johnson & Johnson.

Study Association

This article is part of the thesis of Doctoral submitted by João Pedro Ferreira, from Centro Hospitalar do Porto.

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