

Mechanisms Involved in the Beneficial Effects of Spironolactone after Myocardial Infarction

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Abstract

Introduction: Our objective was to analyze the effect of spironolactone on cardiac remodeling after experimental myocardial infarction (MI), assessed by matricellular proteins levels, cardiac collagen amount and distribution, myocardial tissue metalloproteinase inhibitor-1 (TIMP-1) concentration, myocyte hypertrophy, left ventricular architecture, and *in vitro* and *in vivo* cardiac function.

Methods: Wistar rats were assigned to 4 groups: control group, in which animals were submitted to simulated surgery (SHAM group; n=9); group that received spironolactone and in which animals were submitted to simulated surgery (SHAM-S group, n=9); myocardial infarction group, in which animals were submitted to coronary artery ligation (MI group, n=15); and myocardial infarction group with spironolactone supplementation (MI-S group, n=15). The rats were observed for 3 months.

Results: The MI group had higher values of left cardiac chambers and mass index and lower relative wall thicknesses compared with the SHAM group. In addition, diastolic and systolic functions were worse in the MI groups. However, spironolactone did not influence any of these variables. The MI-S group had a lower myocardial hydroxyproline concentration and myocyte cross-sectional area compared with the MI group. Myocardial periostin and collagen type III were lower in the MI-S group compared with the MI-group. In addition, TIMP-1 concentration in myocardium was higher in the MI-S group compared with the MI group.

Conclusions: The predominant consequence of spironolactone supplementation after MI is related to reductions in collagens, with discrete attenuation of other remodeling variables. Importantly, this effect may be modulated by periostin and TIMP-1 levels.

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Introduction

Heart failure is a frequent complication of myocardial infarction (MI). Several factors influence the appearance of left ventricular dysfunction after MI. However, cardiac remodeling is a major cause of progressive heart failure following coronary occlusion. Importantly, the consequences of cardiac dysfunction after MI are well established, and cardiac dysfunction increases the risk of death by at least 3-fold. It is well accepted that patients who have heart failure and left ventricular systolic dysfunction are at higher risk for adverse outcomes, including cardiac rupture, stroke, ventricular

arrhythmias, recurrent myocardial infarction, and death, including sudden death [1].

Recent large clinical trials suggest that aldosterone receptor blockade improves survival and reduces morbidity in patients with heart failure and reduced ejection fraction [2-4]. However, to date, there is a poor understanding of the mechanisms involved in the beneficial effects of aldosterone receptor blockade in this scenario. Therefore, the objective of this study was to analyze the effect of spironolactone on cardiac remodeling after experimental MI; the effect was assessed by matricellular proteins, cardiac collagen amount and distribution, myocardial tissue metalloproteinase inhibitor-1 concentration, myocyte hypertrophy, left ventricular architecture,

hemodynamic recording, and *in vitro* and *in vivo* cardiac function.

Materials and Methods

All of the experiments and procedures were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Botucatu Medical School. All efforts were made to minimize suffering.

Male, Wistar rats that weighed 200-230 g were assigned to 4 experimental groups: a control group, in which animals were submitted to simulated surgery (SHAM group; n=9); a group in which animals received spironolactone (20 mg/kg of diet/day) and were submitted to simulated surgery (SHAM-S group, n=9); a myocardial infarction group, in which animals were submitted to coronary artery ligation (MI group, n=15); and a myocardial infarction group with spironolactone supplementation (MI-S group, n=15). An echocardiographic exam was performed 5 days after myocardial infarction, and there was no morphological or functional difference between the MI groups (data not shown). Water was supplied *ad libitum*. The rats were observed for 3 months, after which morphological, functional and biochemical analyses were performed.

Coronary artery ligation

When the animals achieved body weights of 200-250 g, myocardial infarction was induced as previously described [5,6]. Briefly, the rats were anesthetized with ketamine (70 mg/kg) and xylazine (1 mg/kg), and after a left thoracotomy, the heart was exteriorized. The left atrium was retracted to facilitate the ligation of the left coronary artery with 5-0 mononylon between the pulmonary outflow tract and the left atrium. The heart was then replaced in the thorax, and the lungs were inflated by positive pressure as the thoracotomy was closed. The rats were housed in a temperature-controlled room (24°C) with a 12-hour light:dark cycle.

Systolic blood pressure

The systolic pressure in the tails of the animals was measured using a tail plethysmograph with a polygraph (Byo-Sistem PE 300, NARCO), a sensor placed in the proximal region of the tail, and an electrospigmomanometer to enable the recording of tail pressure. The animals were warmed in a wooden box at 37°C for 4 minutes, with the heat generated by 2 incandescent lamps, and were transferred to an iron support, where the tail was exposed. In the proximal region of the tail, a sensor (KSM-microphone) was placed and coupled to the plethysmograph. Blood pressure was recorded on paper with the polygraph at a velocity of 2.5 mm/s.

Echocardiographic analysis

After 3 months, all of the animals were weighed and evaluated by a transthoracic echocardiographic exam [7,8]. All of the measurements were made by the same observer, according to the leading-edge method recommended by the American Society of Echocardiography/ European Association

of Echocardiography [9]. The end-systolic and end-diastolic cavity areas were calculated as the sum of the areas from both the short- and long-axis views in diastole (SumD) and systole (SumS), respectively. The fractional area change (FAC) was calculated from the composite cavity areas as follows: $FAC = (SumD - SumS) / SumD$. Additionally, the left ventricular mass index (LVMI) was calculated using the following equation: $LVMI = \{[(LVEDD + 2 * LVWT)^3 - (LVEDD)^3] * 1.04\} / BW$. The relative wall thickness (RWT) was determined as $2 * \text{posterior wall thickness} / \text{end-diastolic diameter}$. The velocities of transmitral diastolic flow (E and A velocities) were obtained from the apical four-chamber view. The E/A ratio, the isovolumetric relaxation time and the isovolumetric relaxation time corrected by the heart rate (IRT/RR^{0.5}) were used as indices of LV diastolic function.

In vitro left ventricular function analysis

One day after the echocardiographic study, the rats were anesthetized with thiopental sodium (50 mg/kg i.p.) and administered heparin (2000 UI, i.p.). The chest was submitted to a median sternotomy under artificial ventilation. The entire heart was rapidly removed from the chest and transferred to a perfusion apparatus (model 830 Hugo Sachs Electronick-Greenstasse). The ascending aorta was isolated and cannulated for retrograde perfusion with filtered and oxygenated Krebs-Henseleit solution, which was maintained at a constant temperature and perfusion pressure (37°C and 75 mmHg, respectively). All of the hearts were paced at 200 to 250 beats/min. The procedures and measurements were performed following a previously described method [10].

Morphometric analysis

Upon completion of the functional analyses, the right and left ventricles (including the interventricular septum) were dissected, separated and weighed. Transverse sections of the LV were fixed in 10% buffered formalin and paraffin-embedded. Five-micron-thick sections were stained with hematoxylin and eosin (HE). The myocyte cross-sectional area was determined for a minimum of 100 myocytes per H&E-stained cross section. The measurements were obtained from digital images (400 × magnification) that were collected with a video camera attached to a Leica microscope; the images were analyzed with the Image-Pro Plus 3.0 software program (Media Cybernetics, Silver Spring, MD). The myocyte cross-sectional area was measured with a digital pad, and the selected cells were transversely cut so that the nucleus was in the center of the myocyte [11]. The lengths of the infarcted and viable muscle for both the endocardial and epicardial circumferences were determined by planimetry. Infarct size was calculated by dividing the endocardial and epicardial circumferences of the infarcted area by the total epicardial and endocardial ventricular circumferences. The measurements were performed on midventricular sections (5-6 mm from the apex) under the assumption that the left midventricular slice shows a close linear relation with the sum of the area measurements from all of the heart sections [12].

Myocardial hydroxyproline concentration

The myocardial hydroxyproline concentration was utilized for fibrosis estimation. Hydroxyproline (HOP) was measured in the tissue (septum of LV and mid ventricular slice of RV) according to the method described previously [13]. Briefly, the tissue was dried for 4 h using a Speedvac Concentrator SC 100 that was attached to a refrigerated condensation trap (RVT 100) and vacuum pump (VP 100, Savant Instruments, Inc., Farmingdale, NY). The dry weight of the tissue was determined, and the samples were hydrolyzed overnight at 110 °C with 6 N HCl (1 ml/10 mg dry tissue). A 50- μ l aliquot of the hydrolyzate was transferred to an Eppendorf tube and dried in the Speedvac Concentrator. Deionized water (1 ml) was added, and the sample was transferred to a tube with a Teflon screw cap. Potassium borate buffer (1 ml, pH 8.7) was added to maintain constant pH, and the sample was oxidized with 0.3 ml of chloramine T solution at room temperature for precisely 20 minutes. The oxidative process was stopped by adding 1 mL of 3.6 mol/l sodium thiosulfate and mixed thoroughly for 10 s. The solution was saturated with 1.5 g of KCl. The tubes were capped and heated in boiling water for 20 min. After cooling to room temperature, the aqueous layer was extracted with 2.5 ml of toluene. Next, 1 ml of toluene extract was transferred to a 12 x 75-mm test tube. Next, 0.4 ml of Ehrlich's reagent was added to allow for the color to develop for 30 min. Absorbencies were read at 565 nm against a blank reagent. Deionized water and 20 μ g/ml HOP were used as the blank and standard, respectively [14].

Western blot analysis

Left ventricular samples were extracted using Tris-Triton buffer (10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate, 1 nM EDTA, 1 mM EGTA and a mixture of protease inhibitors, 1 mM sodium orthovanadate, 1 mM sodium fluoride and 1% leupeptin, aprotinin, pepstatin) to detect collagen III and I and periostin. The samples were then centrifuged at 12000 at 4 °C for 20 min, and the supernatant was collected. The supernatant protein content was quantified using the Bradford method. The samples were separated on a 10% SDS-polyacrylamide gel, and the proteins were transferred to a nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline containing Tris 1 M (pH 8.0), NaCl 5 M and Tween 20 at room temperature for 2 hours. The membrane was then incubated with primary antibody anti-Collagen III, mouse monoclonal IgG1 (Abcam, Inc., Canada, ab 6310), Collagen I A1, rabbit polyclonal IgG (Santa Cruz Biotechnology, Inc., Europe, sc 8784R), periostin and goat polyclonal IgG (Santa Cruz Biotechnology, Inc., Europe, sc 49480). The membrane was washed with TBS and Tween 20 and incubated with secondary peroxidase-conjugated antibody. A Super Signal® West Pico Chemiluminescent Substrate (Pierce Protein Research Products, Rockford, USA) was used to detect bound antibodies. GAPDH (GAPDH (6C5), mouse monoclonal IgG1, (Santa Cruz Biotechnology, Inc., Europe, sc 32233) was used for collagen III and I and periostin blot normalization.

Metalloproteinase-2 and -9 activity

The metalloproteinase (MMP)-2 and -9 activity was determined as previously reported [10]. In brief, samples for analysis were prepared by dilution in extraction sample buffer consisting of 50mM Tris, pH 7.4 ; 0.2 M NaCl; 0.1% Triton X and 10 mM CaCl₂. Then they were diluted in application sample buffer consisting of 0.5 M Tris, pH 6.8; 100% glycerol, and 0.05% bromophenol blue. The samples were loaded into the wells of 8% SDS-polyacrylamide containing 1% gelatin. Electrophoresis carried out in a Bio-Rad apparatus at 80V for 2 hours, when bromophenol blue reaches the bottom of the gel. The gel was removed and washed 2 times with 2.5% Triton-X-100 and then washed with 50mM Tris pH 8.4. The gel was then incubated at 37°C overnight in activation solution consisting of 50 mM Tris pH 8.4; 5 mM CaCl₂ and Zn Cl₂. The staining was performed for 2 hours with 0.5% comassie blue and destaining in 30% methanol and 10% acetic acid until clear bands over a dark background were observed. Staining and destaining were performed at room temperature on a rotatory shaker. The gels were photographed and the intensity of gelatinolytic action (clear bands) analyzed in UVP,UV, White Darkhon image analyzer.

The evaluation of myocardial tissue metalloproteinase inhibitor-1 concentration

The levels of TIMP-1 in the heart homogenates were evaluated by ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA).

Statistical analysis

The data are expressed as the means \pm SD. Comparisons between groups were performed by two-way ANOVA analysis followed by Holm-Sidak. For infarct size comparison, the Student's t-test was performed. The data analysis was carried out with SigmaStat for Windows v2.03 (SPSS Inc., Chicago, IL). The significance level was set at $P < 0.05$.

Results

There was no difference in infarct size between the MI and MI-S groups (MI: 33.17 \pm 13.39% vs. MI-S: 25.06 \pm 13.64%; $p=0.174$).

The echocardiographic data are listed in Table 1. The animals in the MI group had higher values for left cardiac chambers corrected by body weight, higher LVMI and lower relative wall thicknesses compared with the SHAM group. In addition, diastolic and systolic functions were worse in the MI groups in echocardiographic and *in vitro* analysis. However, spironolactone did not influence any of these variables (Tables 1 and 2). There were no differences in systolic blood pressure between the groups.

The morphological data are listed in Table 3. The BW-corrected left (LVW/BW) and right ventricular weights (RVW/BW) were elevated in the MI groups. No effect of spironolactone was observed. However, the MI-S group had a lower myocardial hydroxyproline concentration (MHOP) and myocyte cross-sectional area (CSA) compared with the MI group.

Table 1. Echocardiographic data.

| | SHAM (n= 9) | SHAM-S (n= 9) | MI (n= 15) | MI -S (n= 15) | p MI | p S | p MixS |
|----------------------------|--------------|---------------|---------------|---------------|---------|-------|--------|
| BW (g) | 486.6 ± 30.3 | 486.1 ± 31.7 | 458.5 ± 30.2 | 482.3 ± 55.2 | 0.190 | 0.336 | 0.318 |
| HR (bpm) | 298 ± 17 | 307 ± 30 | 311 ± 34 | 305 ± 29 | 0.536 | 0.811 | 0.402 |
| LVDD/BW (mm/kg) | 16.9 ± 0.9 | 16.3 ± 1.5 | 23.0 ± 2.5 | 21.8 ± 2.9 | <0.001 | 0.170 | 0.717 |
| LVSD/BW (mm/kg)* | 7.88 ± 0.9 | 6.7 ± 0.9 | 17.4 ± 2.7 | 16.1 ± 3.4 | < 0.001 | 0.019 | 0.480 |
| RWT | 0.34 ± 0.02 | 0.36 ± 0.02 | 0.29 ± 0.03 | 0.30 ± 0.03 | <0.001 | 0.162 | 0.369 |
| LVMI (g/kg)* | 1.72 ± 0.20 | 1.59 ± 0.14 | 3.29 ± 0.96 | 2.90 ± 0.58 | <0.001 | 0.151 | 0.799 |
| IRT/RR ^{0.5} (ms) | 48.0 ± 4.8 | 52.3 ± 6.5 | 51.5 ± 12.9 | 59.8 ± 11.1 | 0.077 | 0.044 | 0.502 |
| E/A* | 1.43 ± 0.17 | 1.38 ± 0.24 | 3.92 ± 3.87 | 2.47 ± 2.21 | 0.141 | 0.507 | 0.839 |
| EDT (ms) | 38.6 ± 6.33 | 43.7 ± 7.52 | 34.8 ± 6.07 | 37.4 ± 6.88 | 0.022 | 0.076 | 0.533 |
| FAC | 73.99 ± 7.85 | 69.84 ± 4.45 | 39.00 ± 11.05 | 34.62 ± 9.37 | <0.001 | 0.120 | 0.966 |
| PWSV (mm/s) | 36.1 ± 3.92 | 33.7 ± 1.90 | 28.1 ± 3.92 | 28.6 ± 4.85 | <0.001 | 0.431 | 0.238 |

S: spironolactone; MI: myocardial infarction; BW: body weight; HR: heart rate; LVDD: LV end-diastolic dimension; LVSD: LV end-systolic dimension; RWT: relative wall thickness; LVMI: left ventricle mass index; IRT/RR^{0.5}: isovolumetric relaxation time corrected for heart rate; E/A: peak velocity of early ventricular filling/ peak velocity of transmitral flow during atrial contraction; EDT: E wave deceleration time; FAC: fractional area change; PWSV: posterior wall shortening velocity. * data normalized for statistical analysis.

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Myocardial periostin and collagen type III were lower in the MI-S group compared with the MI-group. In addition, tissue metalloproteinase inhibitor-1 concentration in myocardium was higher in the MI-S group compared with the MI group (Table 4). On the other hand, in our study, MMP-2 and -9 levels were not affected by spironolactone treatment (Table 4).

Discussion

The objective of this study was to analyze the mechanisms involved in the beneficial effects of spironolactone following myocardial infarction. Our data showed that spironolactone reduced LV myocardium hypertrophy, but without alterations in blood pressure, ventricular enlargement, cardiac geometry, or diastolic and systolic ventricular function. Spironolactone did reduce collagen types I and III, which are associated with alterations in periostin and TIMP-1 levels.

Although the concept that aldosterone receptor blockade improves survival and reduces morbidity in patients with heart failure and reduces ejection fraction is universally accepted, understanding the mechanisms involved in this phenomenon remains a challenge. However, it is well accepted that LV remodeling after MI is associated with increased risk for ventricular arrhythmias and sudden cardiac death. In the EPHEBUS trial, the reduction in cardiovascular death induced by eplerenone was predominantly driven by decreased sudden cardiac death [15]. A recent meta-analysis found the same result [16]. Therefore, attenuation of cardiac remodeling by aldosterone receptor blockade might be associated with decreased malignant arrhythmias. Importantly, there is a strong association between cardiac fibrosis and sudden cardiac death [17].

It is important to note that the effects of aldosterone receptor blockade on cardiac remodeling have been studied previously. Regardless of the complexity of the remodeling process, the

Table 2. In vitro left ventricular function and systolic blood pressure.

| | SHAM (n= 6) | SHAM-S (n= 4) | MI (n= 6) | MI -S (n= 5) | p MI | p S | p MixS |
|---------------------------------|-------------|---------------|------------|--------------|--------|-------|--------|
| + dp/dt max (mmHg/s) | 4041 ± 1111 | 4594 ± 449 | 2437 ± 931 | 2150 ± 736 | <0.001 | 0.739 | 0.298 |
| - dp/dt max (mmHg/s) | 2083 ± 385 | 2344 ± 359 | 1354 ± 490 | 1275 ± 311 | <0.001 | 0.615 | 0.350 |
| Systolic blood pressure (mmHg)# | 130 ± 21.2 | 115 ± 20.3 | 119 ± 15.8 | 126 ± 17.9 | 0.898 | 0.400 | 0.052 |

S: spironolactone; MI: myocardial infarction; + dp/dt max: maximum rate of ventricular pressure rise; - dp/dt max: decreased maximum rate of ventricular pressure rise. # (SHAM=9; SHAM-S=9; MI=15; MI-S=15).

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term is frequently used as a synonym for ventricular enlargement associated with functional alterations after MI [18-21]. Indeed, in experimental models, some, but not all studies showed attenuation of left ventricular dimensions with aldosterone receptor blockade [22-24]. The same fact was observed in clinical trials [25-27]. Therefore, despite hard evidence of the attenuation of collagen levels, the effects of aldosterone receptor blockade on cardiac geometry and function are conflicting.

In the present study, spironolactone attenuated only myocyte hypertrophy but did not change blood pressure, ventricular enlargement, cardiac geometry or diastolic and systolic ventricular function. Therefore, our data suggest that these variables are most likely not involved in the beneficial effects of spironolactone on cardiac dysfunction after MI.

Table 4. Myocardial collagen, periostin and tissue metalloproteinase inhibitor-1 concentrations.

| | SHAM (n= 4) | SHAM-S (n= 5) | MI (n= 6) | MI -S (n= 6) | p MI | p S | PMI xS |
|---------------------------|----------------|----------------------------|------------------------------|---------------|--------|-------|--------|
| Periostin/GAPDH* | 0.001 ± 0.0006 | 0.002 ± 0.001 ^φ | 0.165 ± 0.113 ^{*.f} | 0.045 ± 0.023 | <0.001 | 0.360 | 0.002 |
| Type 1 collagen/GAPDH | 1.366 ± 0.182 | 2.918 ± 1.416 | 4.192 ± 1.183 ^f | 3.001 ± 1.028 | 0.014 | 0.738 | 0.019 |
| Type 3 collagen/GAPDH* | 1.003 ± 0.183 | 1.469 ± 0.937 | 2.618 ± 1.152 ^{*.f} | 1.568 ± 0.391 | 0.005 | 0.548 | 0.045 |
| TIMP-1 (pg/g of protein)* | 21.2 ± 4.3 | 32.9 ± 14.4 | 32.5 ± 14.1 | 150.7 ± 136.7 | 0.010 | 0.008 | 0.149 |
| MMP-9 active/total | 0.48 ± 0.35 | 0.85 ± 0.10 | 0.78 ± 0.23 | 0.84 ± 0.1 | 0.200 | 0.062 | 0.179 |
| MMP-2 active/total | 0.75 ± 0.1 | 0.68 ± 0.1 | 0.57 ± 0.1 | 0.57 ± 0.1 | 0.001 | 0.426 | 0.422 |

S: spironolactone; MI: myocardial infarction; MMP: matrix metalloproteinase; TIMP-1: tissue metalloproteinase inhibitor-1. * data normalized for statistical analysis.

When interactions were observed, symbols show the significant differences (* MI#MI-S; ^f MI#SHAM and ^φ SHAM-S#MI-S)

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Table 3. Morphological data.

| | SHAM (n= 9) | SHAM-S (n= 9) | MI (n= 16) | MI -S (n= 15) | p MI | p S | p MI xS |
|--------------------------------------|-------------|---------------|----------------------------|---------------|--------|-------|---------|
| LVW/BW (g/mg)* | 1.81 ± 0.21 | 1.54 ± 0.40 | 2.04 ± 0.54 ^{*.f} | 1.88 ± 0.51 | 0.038 | 0.115 | 0.695 |
| RVW/BW (g/mg)* | 0.41 ± 0.11 | 0.34 ± 0.11 | 0.69 ± 0.33 | 0.52 ± 0.20 | 0.001 | 0.084 | 0.464 |
| MHPO (mg/g) [#] | 2.95 ± 0.21 | 3.42 ± 0.45 | 5.67 ± 0.70 ^{*.f} | 4.17 ± 0.83 | <0.001 | 0.121 | 0.008 |
| CSA (μm ²) ^{**} | 315 ± 56 | 311 ± 67 | 483 ± 63 ^{*.f} | 370 ± 56 | <0.001 | 0.016 | 0.025 |

S: spironolactone; MI: myocardial infarction; BW: body weight; LVW: left ventricular weight; RVW: right ventricular weight; MHPO: myocardial hydroxyproline concentration; CSA: cross-sectional area. * data normalized for statistical analysis.

#. (SHAM=4; SHAM-S=5; MI=4; MI-S=4.)

** (SHAM=8; SHAM-S=7; MI=6; MI-S=8.)

When interactions were observed, symbols show the significant differences (* MI#MI-S and ^f MI#SHAM)

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Another important issue is that the cardiac extracellular matrix is critical for the remodeling process and is composed of structural elements, such as collagen and other proteins, including fibronectin, proteoglycans and matricellular proteins. An important component of this extracellular matrix is a family of extracellular matrix-degrading enzymes, the metalloproteinases (MMPs). The MMPs are a family of more than 25 species of zinc-dependent proteases that play a pivotal role in collagen degradation. The activity of MMPs in the heart is controlled by the action of specific MMPs inhibitors or TIMPs, particularly TIMP-1. Importantly, it is well accepted that increased TIMP activity is associated with lower collagen degradation and subsequent fibrosis [28]. In the present study,

the decrease in collagen levels induced by spironolactone was associated with higher levels of TIMP-1. On the other hand, in our study, MMP-2 and -9 levels were not affected by spironolactone treatment, suggesting that other MMP could be involved in this process. We therefore might conclude that the effects of spironolactone on cardiac fibrosis are, at least in part, mediated by TIMP-1 action, but not by MMP-2 and -9.

An additional mediator of the remodeling process is a family of structurally unrelated extracellular macromolecules referred to as matricellular proteins; these proteins play a limited role in tissue architecture but serve as links between cells and the matrix. Periostin is one of the most important matricellular proteins and plays a role in the maturation and differentiation of fibroblasts in the developing neonatal heart [29]. After acute MI, during the cardiac repair phase, periostin is released into the infarct and activates signaling pathways that are essential for the reparative process [30-33]. However, the role of periostin in chronic cardiac remodeling after MI is unknown. In the present study, MI induced a dramatic increase in periostin three months after coronary occlusion. Importantly, spironolactone decreased periostin levels. Therefore, considering the role of periostin as a modulator of collagen metabolism, we can infer that the decreased collagen content induced by spironolactone might be regulated by periostin.

In conclusion, we propose that the predominant consequence of aldosterone receptor blockade after MI is related to reductions in collagen, with discrete attenuation of other remodeling variables. Importantly, this effect might be modulated by periostin and TIMP-1 levels.

Author Contributions

Conceived and designed the experiments: SARP PSA LAMZ MFM. Performed the experiments: PPS BPMR AFG RACS FCM BFP KO EJP. Analyzed the data: SARP PSA LAMZ MFM. Wrote the manuscript: SARP PSA LAMZ MFM.

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