Vascular Smooth Muscle Mineralocorticoid Receptor Contributes to Coronary and Left Ventricular Dysfunction After Myocardial Infarction

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Abstract—Mineralocorticoid receptor (MR) antagonists slow down the progression of heart failure after myocardial infarction (MI), but the cell-specific role of MR in these benefits is unclear. In this study, the role of MR expressed in vascular smooth muscle cells (VSMCs) was investigated. Two months after coronary artery ligation causing MI, mice with VSMC-specific MR deletion (MI-MRSMKO) and mice treated with the MR antagonist finerenone (MI-fine) had improved left ventricular compliance and elastance when compared with infarcted control mice (MI-CTL), as well as reduced interstitial fibrosis. Importantly, the coronary reserve assessed by magnetic resonance imaging was preserved (difference in myocardial perfusion before and after induction of vasodilatation, mL mg⁻¹ min⁻¹: MI-CTL: 1.1±0.5, nonsignificant; MI-MRSMKO: 4.6±1.6 [P<0.05]; MI-fine: 3.6±0.7 [P<0.01]). The endothelial function, tested on isolated septal coronary arteries by analyzing the acetylcholine-induced nitric oxide-dependent relaxation, was also improved by MR deletion in VSMCs or by finerenone treatment (relaxation %: MI-CTL: 36±5, MI-MRSMKO: 54±3, and MI-fine: 76±4; P<0.05). Such impairment of the coronary endothelial function on MI involved an oxidative stress that was reduced when MR was deleted in VSMCs or by finerenone treatment. Moreover, short-term incubation of coronary arteries isolated from noninfarcted animals with low-dose angiotensin-II (10⁻⁹ mol/L) induced oxidative stress and impaired acetylcholine-induced relaxation in CTL but neither in MRSMKO nor in mice pretreated with finerenone. In conclusion, deletion of MR in VSMCs improved left ventricular dysfunction after MI, likely through maintenance of the coronary reserve and improvement of coronary endothelial function. MR blockage by finerenone had similar effects. (Hypertension. 2016;67:717-723. DOI: 10.1161/HYPERTENSIONAHA.115.06709.) • Online Data Supplement

Key Words: coronary vessels ■ myocardial infarction ■ receptors, mineralocorticoid

Chronic heart failure (HF) after myocardial infarction (MI) is steadily increasing worldwide and remains a major cause of death. Mineralocorticoid receptor (MR) antagonists (MRAs) improve survival in patients with HF as illustrated by spironolactone in the RALES (Randomized Aldactone Evaluation Study) trial and by eplerenone in the EPHESEUS (Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study) trial, which only included patients with post-MI. Recently, the EMPHASIS (Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure) study even showed that eplerenone is effective in slowing down the progression of mild-to-moderate HF. However, MRAs are associated with side effects, such as hyperkalemia or gynecomastia; the latter is caused by the antiandrogenic properties of their steroidal structure. These adverse effects are responsible for the underuse of MRAs.

Understanding of the cell-specific contribution of MR to HF and of the effects of MRA on various cell subtypes will be useful for the future development of tissue-selective MR targeting approaches that would improve the benefit/risk ratio. A crucial role of cardiomyocyte MR has been demonstrated in mice with cardiomyocyte-specific MR deletion, which allows improving left ventricular (LV) function after either MI or pressure overload induced by transverse aortic constriction. The deletion of MR specifically in fibroblasts does not affect cardiac failure after aortic constriction. The role of vascular MR has been underlined recently: the MR expressed in vascular smooth muscle cells (VSMCs) is involved in age-related...
increase in blood pressure in mice \(^9\) and activation of MR in VSMCs leads to the stiffening of carotid arteries on hypertension induced by an aldosterone/salt challenge.\(^9\) Although spironolactone limits vascular dysfunction in the rat aorta\(^9\) and mesenteric arteries\(^1\) after MI, whether VSMC-MR is involved in the progressive aggravation of HF post MI is unknown, as well as its role in coronary dysfunction associated with MI.

We used mice with VSMC-specific MR deletion (MR\(^{SMKO}\))\(^9\) to assess the contribution of VSMC-MR to LV and coronary dysfunctions and to impaired myocardial perfusion post MI. To compare the effects of MR inactivation in VSMCs with those of pharmacological MR antagonism, wild-type infarcted mice were treated with finerenone, a highly selective nonsteroidal MRA\(^9\) that mediates end-organ protection with a lower risk of electrolyte disturbances than other MRAs and allows improving LV function in rats with post-MI HF.\(^1\)

**Methods**

Detailed Methods are available in the online-only Data Supplement.

**Mice, MI, and Treatment**

Experiments conformed to the 2010/63 directive of the European Union and the Guide for Care and Use of Laboratory Animals of the US National Institute of Health (number, 85-23). MR\(^{SMKO}\) mice (C57BL6) were generated as described previously.\(^9\) Left coronary artery ligations were performed in 8-week-old MR\(^{SMKO}\) male mice and littermate controls under anesthesia (xylazine [3.6 mg/kg IP] plus 2% isoflurane). Analgesia was induced using buprenorphine (0.05 mg/kg SC just after induction of anesthesia and 6, 12, 24, and 48 hours post coronary artery ligation). The snare was not tied for sham-operated mice. Finerenone (1 mg/kg per day) was administered as food additive for 2 months starting the day after MI.

**Statistics**

Data are presented as mean±SD. For arterial studies, differences between groups were analyzed by 2-factor repeated measures ANOVA. For experiments with apocynin, superoxide dismutase (SOD) or angiotensin-II (AngII), comparisons were assessed by Student [u]test for each contrast, the acetylcholine-mediated relaxation was impaired in the case of VSMC-specific MR deletion.

**Results**

**Cardiac and Coronary Phenotype of MR\(^{SMKO}\) Mice**

The MR gene was inactivated in VSMCs of coronary vessels from MR\(^{SMKO}\) mice. MR expression in the endothelium and global cardiac MR transcription were unaffected (Figure S1 in the online-only Data Supplement).

Cardiac function was examined by magnetic resonance imaging. LV end-systolic and end-diastolic volumes, LV ejection fraction, stroke volume, and cardiac output were similar between MR\(^{SMKO}\) and littermate CTL mice, showing that cardiac function was normal in MR\(^{SMKO}\) mice (Table 1). Moreover, in vivo pressure–volume loops showed that LV end-systolic pressure and pressure–volume relationship (ie, LV elastance; Figure S2A) and LV end-diastolic pressure (ie, LV filling) and LV end-diastolic pressure–volume relationship (ie, LV compliance; Figure S2B) were not different between MR\(^{SMKO}\) and CTL mice.

We next examined the coronary function. Endothelium-dependent relaxation of isolated arteries induced by acetylcholine was similar in MR\(^{SMKO}\) and CTL mice (Figure S2C, left). Preincubation with the nitric oxide (NO)-synthase inhibitor l-NG-nitroarginine abolished the relaxation response to acetylcholine in both groups, indicating that it relies on NO production by the endothelium (Figure S2C, right). Moreover, relaxation induced by the NO-donor sodium nitroprusside (SNP) was similar in both groups, indicating that the response of VSMCs to NO was unaltered in MR\(^{SMKO}\) mice (Figure S2C, middle).

**LV Function on MI**

As expected, 2 months after MI, compared with sham-CTL, MI-CTL mice had increased LV weight and collagen density, higher LV end-systolic and end-diastolic volumes (Table 2), lower LV end-systolic pressure (Figure 1A) and higher LV end-diastolic pressure (Figure 1B), decreased LV end-systolic pressure–volume relationship (Figure 1A), and increased LV end-diastolic pressure–volume relationship (Figure 1B), indicating impaired LV elastance and compliance, respectively. This resulted in decreased stroke volume and cardiac output in association with lower systolic peripheral blood pressure measured in conscious MI-CTL mice (Table 2).

Infarct sizes and LV mass were similar between all MI groups (Table 2). However, interstitial collagen content was decreased in the LV of MI-MR\(^{SMKO}\) mice when compared with MI-CTL mice (Table 2; Figure 1C). The LV end-systolic and end-diastolic volumes in MI-MR\(^{SMKO}\) mice were not different from those in MI-CTL mice (Table 2). Although stroke volume, cardiac output, and peripheral systolic blood pressure were not different in MI-MR\(^{SMKO}\) mice compared with MI-CTL mice (Table 2), both the LV end-systolic pressure–volume relationship (elastance; Figure 1A) and the LV end-diastolic pressure volume–relationship (compliance; Figure 1B) were improved, indicating that impairment of LV hemodynamics was attenuated in the case of VSMC-specific MR deletion.

**Vascular Function and Oxidative Stress on MI**

Two months after MI, both the acetylcholine-mediated and the SNP-induced relaxation showed no difference between sham-CTL, MI-CTL, and MI-MR\(^{SMKO}\) mice in peripheral resistance arteries (mesenteric arteries; Figure S3). In contrast, the acetylcholine-mediated relaxation was impaired in MI-MR\(^{SMKO}\) mice compared with MI-CTL mice (Table 2), both the LV end-systolic pressure–volume relationship (elastance; Figure 1A) and the LV end-diastolic pressure–volume relationship (compliance; Figure 1B) were improved, indicating that impairment of LV hemodynamics was attenuated in the case of VSMC-specific MR deletion.

**Table 1. Comparison of Heart Weight and Function Between Healthy Control and MR\(^{SMKO}\) Mice**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTL</th>
<th>MR(^{SMKO})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>27±0.7</td>
<td>26±0.6</td>
</tr>
<tr>
<td>LV end-diastolic vol, µL</td>
<td>53±4</td>
<td>48±3</td>
</tr>
<tr>
<td>LV end-systolic vol, µL</td>
<td>19±3</td>
<td>16±2</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>64±4</td>
<td>67±3</td>
</tr>
<tr>
<td>Stroke vol, µL</td>
<td>34±3</td>
<td>32±3</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>148±9</td>
<td>133±8</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>106±6</td>
<td>94±8</td>
</tr>
</tbody>
</table>

Body weight: CTL, n=12; and MR\(^{SMKO}\), n=11. End-diastolic volume, end-systolic volume, ejection fraction, and stroke volume: CTL, n=6; and MR\(^{SMKO}\), n=7. CTL indicates control; LV, left ventricle; and MR, mineralocorticoid receptor.
in interseptal coronary arteries isolated from MI-CTL (Figure 2A). This impaired response was improved in interseptal arteries from MI-MRSMKO mice when compared with MI-CTL (Figure 2A). In all groups, SNP-induced relaxation was similar (Figure 2B) and preincubation with \( \text{l}-\text{NG-nitroarginine} \) abolished acetylcholine-mediated relaxation (Figure 2C). Histological study showed that although MI induced an increase in the LV mean media surface of coronary arteries from 20 to 200 \( \mu \text{m} \) in diameter, there was no difference between MI groups (Figure 2D).

We next questioned whether improved coronary function in MI-MRSMKO mice was related to a decrease in oxidative stress–mediated endothelial dysfunction in coronary arteries. Preincubation with the nicotinamide adenine dinucleotide phosphate oxidase inhibitor apocynin or with the antioxidant enzyme SOD, did not affect the acetylcholine-mediated relaxation of coronary arteries from sham-CTL but improved relaxation of those from MI-CTL mice (Figure 3A and 3B). Moreover, at the highest concentration of acetylcholine, apocynin improved the relaxation of arteries from MI-MRSMKO to a smaller extent than in those from MI-CTL mice (Figure 3C and 3D), indicating a lower effect of oxidative stress when MR is absent in VSMCs.

We then assessed whether VSMC-MR is able to promote oxidative stress in the coronary bed. In MI, oxidative stress is partly mediated by AngII.\(^{14}\) Coronary arteries were isolated from noninfarcted mice and exposed in vitro for 1 hour to \( 10^{-9} \text{ mol/L} \) AngII, a dose that did not induce vasoconstriction (Table 3). In arteries from CTL, pre-exposure to low-dose AngII impaired acetylcholine-mediated relaxation (Figure 4A). This effect was abolished by the addition of SOD, underlying a role of oxidative stress production on AngII stimulation (Figure 4B). AngII-mediated endothelial dysfunction was absent in arteries from MRSMKO mice.  

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**Table 2. LV Remodeling and Functional Parameters 2 Months After Myocardial Infarction**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham-CTL</th>
<th>MI-CTL</th>
<th>MI-MRSMKO</th>
<th>MI-Fine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size, %</td>
<td>…</td>
<td>33±2</td>
<td>32±2</td>
<td>37±3</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>29±0.5</td>
<td>30±0.4*</td>
<td>29±0.4</td>
<td>28±0.5</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>106±1.7</td>
<td>94.6±2.3*</td>
<td>94.9±3.2*</td>
<td>99.2±2.4*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>87.2±1.9</td>
<td>76±2.7*</td>
<td>73.7±2.6*</td>
<td>80±2*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>641±17</td>
<td>618±14</td>
<td>567±39</td>
<td>659±6</td>
</tr>
<tr>
<td>LV end-diastolic volume, ( \mu \text{L} )</td>
<td>76±6</td>
<td>172±20*</td>
<td>134±10*</td>
<td>145±13*</td>
</tr>
<tr>
<td>LV end-systolic volume, ( \mu \text{L} )</td>
<td>32±4</td>
<td>144±20*</td>
<td>113±12*</td>
<td>116±13*</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>60±2</td>
<td>19±3*</td>
<td>19±3*</td>
<td>23±2*</td>
</tr>
<tr>
<td>Stroke volume, ( \mu \text{L} )</td>
<td>44±2</td>
<td>28±2*</td>
<td>25±2*</td>
<td>30±1*</td>
</tr>
<tr>
<td>Cardiac output, mL/min</td>
<td>19.1±1.4</td>
<td>13.4±1.3*</td>
<td>10.9±0.8*</td>
<td>13.0±0.6*</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>144±5</td>
<td>190±8*</td>
<td>179±8*</td>
<td>188±12*</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>105±4</td>
<td>139±6*</td>
<td>133±5*</td>
<td>140±8*</td>
</tr>
<tr>
<td>LV collagen density, %</td>
<td>1.5±0.5</td>
<td>6.6±0.7*</td>
<td>4.6±0.3*,†</td>
<td>4.0±0.4*,†</td>
</tr>
<tr>
<td>LV capillary density</td>
<td>1.6±0.0</td>
<td>1.7±0.1</td>
<td>1.6±0.1</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

Body weight: sham-CTL, \( n=22 \); MI-CTL, \( n=25 \); MI-MRSMKO, \( n=15 \); and MI-fine, \( n=11 \). Infarct size: MI-CTL, \( n=19 \); MI-MRSMKO, \( n=14 \); and MI-fine, \( n=13 \). SBP: sham-CTL, \( n=9 \); MI-CTL, \( n=9 \); MI-MRSMKO, \( n=7 \); and MI-fine, \( n=7 \). End-diastolic volume, end-systolic volume, ejection fraction, stroke volume, and cardiac output: sham-CTL, \( n=11 \); MI-CTL, \( n=10 \); MI-MRSMKO, \( n=13 \); and MI-fine, \( n=13 \). Heart and LV weight: sham-CTL, \( n=17 \); MI-CTL, \( n=19 \); MI-MRSMKO, \( n=13 \); and MI-fine, \( n=8 \). Collagen and capillary densities: sham-CTL, \( n=10 \); MI-CTL, \( n=9 \); MI-MRSMKO, \( n=8 \); and MI-fine, \( n=8 \). CTL indicates control; DBP, diastolic blood pressure; LV, left ventricle; MI, myocardial infarction; MR, mineralocorticoid receptor; and SOD, antioxidant enzyme.

*Versus sham-CTL.
†Versus MI-CTL.

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**Figure 1.** Left ventricular (LV) hemodynamics 2 months after myocardial infarction (MI). A, LV end-systolic pressure (LVESP) and pressure–volume relationship (LVESPVPR). B, LV end-diastolic pressure (LVEDP) and pressure–volume relationship (LVEDPVPR) in sham-CTL (white; \( n=9 \)), MI-CTL (black; \( n=14 \)), MI-MRSMKO (down-hatched bars; \( n=12 \)), or fenerone-treated MI-fine mice (up-hatched bars; \( n=6 \)). C, Representative microphotographs after staining with Sirius red for LV fibrosis analyses in sham-CTL, MI-CTL, MI-MRSMKO, and MI-fine (magnification, \( \times20 \)). *\( P<0.05 \) vs sham-CTL; Student \( t \) test. #\( P<0.05 \) vs MI-CTL; ANOVA plus Tukey test. CTL indicates control; and MR, mineralocorticoid receptor.
(Figure 4C), indicating that VSMC-MR is required for the capacity of AngII to promote oxidative stress–induced coronary dysfunction.

### Myocardial Perfusion and Coronary Reserve After MI

Basal myocardial perfusion was impaired after MI as shown in MI-CTL (Figure 5A). Moreover, the adenosine type-2 receptor agonist ATL307, which dilates coronary arteries, increased perfusion in sham-CTL but not in MI-CTL mice, indicating a collapse of the coronary reserve. However, ATL307 still increased myocardial perfusion in MI-MRSMKO mice, revealing the persistence of a coronary reserve (mL mg\(^{-1}\) min\(^{-1}\): MI-CTL: 1.1±0.5, NS; MI-MRSMKO: 4.6±1.6; \(P<0.05\); Figure 5B). Capillary density remained unchanged (Table 2).

### Cardiac and Coronary Effects of the Nonsteroidal MRA Finerenone

To determine the specific contribution of VSMC-MR in the effects of MR antagonism, we compared the results obtained in MI-MRSMKO mice with those obtained in control mice with MI and additional treatment for 2 months with a low-dose of finerenone (1 mg/kg per day; MI-fine).

About LV remodeling and function, less interstitial collagen was accumulated in the LV of MI-fine than in that of MI-CTL mice (Table 2; Figure 1C), without difference in LV weight (Table 2). LV end-systolic and end-diastolic volumes were not different in MI-fine mice from those in MI-CTL mice (Table 2). However, LV end-systolic pressure–volume relationship (Figure 1A) was higher in MI-fine than in MI-CTL mice, and both LV end-diastolic pressure and...
pressure–volume relationship were lower (Figure 1B), indicating that finerenone improved LV hemodynamics.

About coronary function, finerenone improved acetylcholine-mediated relaxation of interseptal arteries (Figure 2A; compare MI-fine with MI-CTL) with a more marked effect in comparison with arteries from MI-MRSMKO, resulting in a restoration of relaxation up to values observed in sham-CTL mice (Figure 2A). There was no difference in SNP-induced relaxation (Figure 2B), and l-NG-nitroarginine similarly abolished acetylcholine-mediated relaxation in all groups (Figure 2C). In coronary arteries from MI-fine mice, acetylcholine-mediated relaxation was not further improved by apocynin or SOD, indicating that finerenone prevented oxidative stress–mediated coronary endothelial dysfunction (Figure 3A–3D). Beyond coronary arteries, finerenone allowed reducing the LV mRNA expression of nicotinamide adenine dinucleotide phosphate oxidase type 2 in MI-fine mice (Figure S4). Moreover, coronary arteries from MI-fine mice treated with finerenone for 4 weeks were protected against the impaired acetylcholine-mediated relaxation observed in the absence of AngII (Figure 4D). Finally, about the coronary reserve, ATL307 still increased perfusion in MI-fine, revealing the persistence of a coronary reserve (mL mg⁻¹ min⁻¹: 3.6±0.7; P<0.01; Figure 5), without modification of capillary density (Table 2).

**Discussion**

MR inactivation restricted to VSMCs allowed diminishing LV interstitial fibrosis (measured at distance from the infarct area in microphotographs that excluded vessels) and allowed improving LV diastolic function, as illustrated by histological and LV pressure–volume loop assessments. It has been already suggested that the MR expressed in coronary arteries affects myocardial fibrosis. Indeed, endothelial-specific MR deletion allows reducing myocardial collagen deposition after deoxycorticosterone/salt treatment in mice. Moreover, the activation of VSMC-MR triggers collagen synthesis in aortas of rats or mice after aldosterone infusion. The activation of VSMC-MR triggers collagen synthesis in aortas of rats or mice after aldosterone infusion. This study indicates that VSMC-MR is 1 additional contributor to the adverse effects post MI, at least next cardiomyocyte MR whose role was already well established. Indeed, in mice with cardiomyocyte-specific MR deletion, there is major improvement of LV function and remodeling after MI, associated with lower production of myocardial/mitochondrial reactive oxygen species. Our results emphasize that blocking MR in myocardium and coronary arteries both benefit cardiac function.

This study demonstrates the implication of VSMC-MR in coronary dysfunction post MI. The involvement of MR in coronary function has been suggested in mice with cardiomyocyte-specific aldosterone synthase overexpression, which prevents altered acetylcholine-mediated relaxation. In this study, the impairment of coronary relaxation post MI was attenuated in MI-MRSMKO mice due to an improved NO bioavailability because the NO-synthase inhibitor l-NG-nitroarginine abolished the relaxation and without changes in the ability of VSMCs to relax because the NO-donor SNP induced equivalent relaxations in all groups. Moreover, the effect of apocynin or SOD to improve NO-mediated relaxation was more efficient in coronary arteries isolated from MI-CTL than from MI-MRSMKO or MI-fine mice. This suggests that a lower oxidative stress within the coronary arteries from MI-MRSMKO and MI-fine mice participated to improve the NO bioavailability.

Nevertheless, reactive oxygen species production does not only depend on VSMC-MR because in MI-fine mice, finerenone also targeted other MR-expressing cells, like endothelial cells and cardiomyocytes, resulting in further improvement of acetylcholine-induced NO-mediated relaxation of coronary arteries. Differential roles for MR-induced oxidative stress have been proposed in the literature, depending on the cell type and the vascular bed. After MI, cardiomyocyte-specific MR deletion allows blunting the increased O₂⁻ production in

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Table 3. **Absence of Contractile Effect Induced by Low Dose AngII on Isolated Coronary Arteries From Noninfarcted Mice**

<table>
<thead>
<tr>
<th>Arteries</th>
<th>Without AngII</th>
<th>+AngII (10⁻⁹ mol/L, 1 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>2.25±0.18</td>
<td>1.94±0.19</td>
</tr>
<tr>
<td>MRSMKO</td>
<td>2.39±0.22</td>
<td>2.36±0.32</td>
</tr>
<tr>
<td>CTL-fine</td>
<td>1.99±0.48</td>
<td>1.71±0.39</td>
</tr>
</tbody>
</table>

Contraction (mN/mm) to 5-HT 10⁻⁵ mol/L of coronary arteries from noninfarcted mice. AngII indicates angiotensin-II; CTL, control; 5-HT, serotonin; and MR, mineralocorticoid receptor.

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Here, we evidenced that VSMC-MR is an important contributor to post-MI oxidative stress in coronary arteries.

On the other hand, the deleterious effect of increased AngII post MI has been shown in mice.18 Indeed, AT1 antagonism allows limiting myocardial hypertrophy and fibrosis.19 Furthermore, MR and AngII signaling pathways are interdependent.20 MR antagonism blunts the oxidative stress induced by AngII,21 and VSMC-MR is mandatory for AngII-mediated increase in blood pressure.4 To establish a link between VSMC-MR and AngII-mediated oxidative stress in coronary arteries, we stimulated arteries isolated from noninfarcted mice with a low dose of AngII, unable to induce vasoconstriction but able to induce endothelial dysfunction because of enhanced oxidative stress. In such conditions, AngII-mediated endothelial dysfunction was blunted in coronary arteries from MRSMKO and finerenone-treated mice. Presumably, VSMC-MR blockage after MI allowed reducing a deleterious cross-talk between AngII and MR signaling pathways.

Besides oxidative stress reduction, other mechanisms might participate to the improvement of coronary function in MRSMKO-MI mice. For example, after aldosterone/salt challenge, arterial stiffness of carotid arteries increases in wild-type mice but remains unchanged in MRSMKO mice, together with prevention of integrin Itgαβ3 upregulation.9 Moreover, in isolated rat muscle arterioles, blockade by Itgαβ3-antibody of α5β1-integrin inhibits the constrictor response to increments of intraluminal pressure.22 However, the implication of this specific integrin in coronary arteries remains to be determined.

The improvement in coronary function of MI-MRSMKO and MI-fine mice also concerned the maintenance of a coronary reserve. Clinically, impaired coronary reserve is associated with a 6-fold increase in the risk of mortality in patients with chest pain and normal angiography.23 In patients with acute ST-segment–elevation MI without HF, the early administration of eplerenone within 24 hours post MI allows reducing the rate of rehospitalization.24 An early improvement of the coronary function/reserve may participate to such benefit. Of note, in patients with diabetes mellitus without clinical cardiovascular disease, receiving an angiotensin-converting enzyme inhibitor and a calcium channel antagonist, adenosine-stimulated coronary reserve is higher after 6 weeks of treatment with eplerenone in comparison with a thiazide diuretic.25 This suggests an important role for MR in the regulation of the coronary reserve in different pathological contexts.

In conclusion, VSMC-MR is involved in the progression of HF post MI, at least through its direct role in oxidative stress–induced coronary endothelial dysfunction and in decreased coronary reserve. Treatment of infarcted mice with the nonsteroidal MRA finerenone21 is associated with cardiac functional benefits, including improvements of endothelial function and maintenance of a coronary reserve. These factors may confer benefits after coronary occlusion, and finerenone may improve clinical outcome in this context.

Perspectives

The link between MR expressed in coronary arteries (ie, VSMC and endothelium) and the myocardial remodeling post MI as a consequence of the modulation of local inflammation remains to be explored. Furthermore, a better understanding of the contribution of MR activation in coronary arteries in pathologies that affect the coronary function and reserve, such as angina, MI, diastolic HF with preserved ejection fraction, or diabetes mellitus, will contribute to address the potential benefits of MR antagonism.

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Disclosures

P. Kolkhof is an employee of Bayer (Germany). The other authors report no conflicts.

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SUPPLEMENTAL MATERIAL

Vascular Smooth Muscle Mineralocorticoid Receptor Contributes to Coronary and Left Ventricular Dysfunction After Myocardial Infarction

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Supplemental Methods:

MRI

Myocardial perfusion was evaluated with MRI (Bruker Biospec 4.7Tesla, France) by arterial spin labeling, as described previously\(^1\). Coronary reserve was calculated as perfusion after coronary dilatation with ATL307 (2µg/kg, IP) minus basal perfusion. Infarct size, LV end-diastolic volume (EDV) and end-systolic volume (ESV) were determined with Intragate sequence (Bruker, France) and FARM-CAAS2.0 (Pie Medical, Netherlands) as described previously\(^2\). Stroke volume, LV ejection fraction ([EDV-ESV]/EDV) and cardiac output (stroke volume x heart rate) were calculated.

Infarct size (excluding the border zone) was averaged from 6 to 9 LV transversal sections under the ligation and was calculated as (infarction perimeter/(epicardial LV perimeter+endocardial LV perimeter)x100).

Hemodynamic and blood pressure

LV hemodynamic was assessed as described previously\(^2\). Mice were anesthetized (chloral 320mg.kg\(^{-1}\), IP) and the carotid artery cannulated with a pressure-volume catheter (SPR839, Millar-Instruments, USA) to record arterial pressure and heart rate, after which the catheter was advanced into the LV. Pressure-volume loops were obtained at baseline and during loading by gently occluding the abdominal aorta. LV end-systolic and end-diastolic pressures were measured, and LV end-systolic and end-diastolic pressure-volume relations were calculated with IOX™ software (EMKA, France). Systolic blood pressure (SBP) was measured in conscious mice by the tail-cuff method as described\(^9\) previously.

Artery assessment

Endothelial function was assessed as described previously for left mouse coronary arteries\(^3,4\). The heart was removed and placed in cold oxygenated Krebs buffer. A 1.5–2mm segment of interseptal coronary artery (internal diameter <120µm), was mounted on a myograph (DMT, Aarhus, Denmark). After normalization, response to acetylcholine (10\(^{-9}\) to 3.10\(^{-5}\) mol/L) and endothelium-independent relaxation to sodium nitroprusside (SNP) (10\(^{-9}\) to 3.10\(^{-5}\) mol/L) were obtained in segments pre-contracted with serotonin (10\(^{-5}\)mol/L). For mesenteric arteries, the vaso dilatory responses were assessed in 2mm segments (diameter <300µm) pre-contracted with phenylephrine (10\(^{-5}\)mol/L). In some experiments, response to Ach was assessed after 35min incubation with the NO synthase inhibitor L-NG-nitro-arginine (L-NNA, 10\(^{-4}\)mol/L), an nicotinamide-adenine-dinucleotide-phosphate (NADPH)-oxidase inhibitor (apocynin 10\(^{-4}\)mol/L) or the anti-oxidant enzyme SOD (10\(^{-4}\)mol/L). In an independent set of experiments, response to Ach was obtained before and after pre-incubation of coronary arteries with angiotensin II (AngII, 1h, 10\(^{-9}\)mol/L).

Histology

For collagen analyses, 7µm cryosections from the mid-heart were stained with pico-Sirius Red as described previously\(^7\). For capillaries and coronary arteries staining, cryosections were post-fixed in acetone and stained according to standard protocols, using biotinylated rat anti-mouse CD31 (PECAM-1, 1:100, BD) plus secondary streptavidin (SA)-Fluorprobe-547 (1:1500, Interchim, France), mouse anti-human αSMA-fluorescein isothiocyanate (1:200; Sigma-Aldrich). Cardiomycocyte number was determined after staining with Wheat Germ Agglutinin-A488 (1:100, Invitrogen). Capillary density was expressed as the number of capillaries/cardiomyocyte. For calculating the media surface of coronary arteries (20-200µm diameter), the lumen surface related to internal perimeter was subtracted from the surface related to the external perimeter.
RNA extraction and real-time PCR

Total RNA was extracted from LV by using TRIZOL® reagent (Life Technologies Corporation, CA, USA), according to manufacturer protocol. The reverse transcription of mRNA (100ng) was performed with Superscript II reverse transcriptase KIT (Life Technologies Corporation, CA, USA). Transcript levels of genes were analyzed by real-time PCR (fluorescence detection of SYBR Green) in an iCycler iQ apparatus (Bio-Rad). For each sample, mRNA levels were normalized to the geometric mean of the amount of two housekeeping genes, 18S and hypoxanthine guanine phosphoribosyl transferase (HPRT). Specific primer sequences (5’-3’): MRL: CCAGAAGAGGGGACCACATA and MRR: GGATTGCAGCTGCTGCTCAT; Nox2L: CGCCCT TTGCTCCATTCTC and Nox2R: CCTTTGCATCTGCTCTCC

Histology

Immunohemical analysis of MR expression was performed as described previously using a 6G1 monoclonal antibody (kindly provided by C. Gomez-Sanchez, Division of Endocrinology, University of Mississippi Medical Center, Jackson, MS, USA). Non-specific signal was assessed by omitting the primary antibody. Image acquisition was made on a DM4000 microscope (Leica, Germany)
Supplemental Reference:

Fig. S1: MR expression in MR SMKO mice. MR is immunolabelled in the nucleus of both endothelial cells (#) and VSMC (*) in coronary arteries in WT mice (left). In coronary arteries of MR SMKO mice, MR is only detectable in endothelial cells (#) but not in VSMC (*) (right). (C) Quantitative real-time polymerase chain reaction in heart from WT (white, n=6) and MR SMKO (black, n=6). Data are presented as mean ± SEM. Statistics: Student’s t-test.
Fig.S2: LV hemodynamics and coronary function in normal MR^SMKO mice. (A) LV end-systolic pressure (LVESP) and pressure-volume relation (LVESPVR), and (B) LV end-diastolic pressure (LVEDP) and pressure-volume relation (LVEDPVR) in control (CTL) (white, n=10) and MR^SMKO mice (gray, n=6). (C) Vasorelaxation of isolated coronary arteries from control (open circles, n=8) and MR^SMKO (solid circles, n=6) induced by acetylcholine (left) or NO-donor SNP (SNP; middle) or acetylcholine after pre-incubation with the NO-synthase inhibitor LNNA 10^{-4} mol/L (right). Statistics: Student’s t-test.
**Fig. S3**: Relaxation in peripheral resistance mesenteric arteries 2 months after MI. (A) Vasorelaxation induced by acetylcholine or (B) SNP of isolated mesenteric arteries from sham-CTL (open circles, n=5), MI-CTL (solid circles, n=6), MI-MR SMKO (triangles, n=6). Statistics: 2-factor repeated measures Anova.
Supplemental figure S4

**Fig. S4:** mRNA expression of NADPH-oxidase 2 (Nox2) at 2 months post-MI. Quantitative real-time polymerase chain reaction in heart from Sham-CTL (n=10), MI-CTL (n=13), MI-MRSMKO (n=17) and MI-fine (n=10). Data are presented as mean ± SEM. *p<0.05 vs. MI-MRSMKO, Anova plus Tukey.