Immediate Mineralocorticoid Receptor Blockade Improves Myocardial Infarct Healing by Modulation of the Inflammatory Response

Daniela Fraccarollo, Paolo Galuppo, Susanne Schraut, Susanne Kneitz, Nico van Rooijen, Georg Ertl, Johann Bauersachs

Abstract—Mineralocorticoid receptor (MR) blockade reduces morbidity and mortality after acute myocardial infarction; however, the underlying mechanisms are still under investigation. This study examined whether MR antagonism promotes healing of the infarcted myocardium. Starting immediately after coronary ligation, male Wistar rats were treated with the selective MR antagonist eplerenone (100 mg/kg per day by gavage) or placebo for 2 to 7 days. At 7 days, eplerenone therapy versus placebo significantly reduced thinning and dilatation of the infarcted wall, improved left ventricular function, and enhanced neovessel formation in the injured myocardium. At 2 days, eplerenone-treated rats displayed lower plasma corticosterone levels, higher circulating blood monocytes, and more macrophages infiltrating the infarcted myocardium. MR blockade led to a transient upregulation (at days 2 and 3 but not at day 7) of monocyte chemotactic protein-1, tumor necrosis factor-α, interleukin-1β, interleukin-6, interleukin-10, and interleukin-4 and an increase in factor XIIIa protein expression in the healing myocardium. Prevention of macrophage accumulation into the infarct zone by treatment with liposome-encapsulated clodronate almost abrogated the protein expression of factor XIIIa and the beneficial effects of eplerenone on infarct expansion. In conclusion, selective MR blockade immediately after myocardial infarction accelerated macrophage infiltration and transiently increased the expression of healing promoting cytokines and factor XIIIa in the injured myocardium resulting in enhanced infarct neovascularization and reduced early LV dilation and dysfunction. (Hypertension. 2008;51:905-914.)

Key Words: aldosterone ■ healing ■ inflammation ■ myocardial infarction ■ heart failure

Cardiac repair after infarction involves inflammatory cell infiltration, the release of multiple neurohumoral stimuli, extracellular matrix remodeling, and adaptive responses of cardiac myocytes.1–5 Several pathways are triggered that can lead to thinning and dilatation of the infarcted wall, paving way for cardiac rupture or aneurysm formation, left ventricular (LV) dysfunction, life-threatening arrhythmia, and heart failure. Therapeutic strategies that target pathophysiologic mechanisms and mediators of early cardiac healing and repair appear to be useful tools to prevent chronic complications.1–5

Recent clinical data6,7 show that mineralocorticoid receptor (MR) antagonism reduces morbidity and mortality among patients with acute myocardial infarction (MI) complicated by LV dysfunction and heart failure. However, the underlying mechanisms are still under investigation. In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study, MR blockade with eplerenone, started between days 3 and 14 after MI in addition to optimal standard therapy, reduced 30-day mortality by 30%. The reduction in mortality, mainly because of a reduction in sudden cardiac death, occurs early after the initiation of MR blockade. Hayashi et al8 showed that immediate MR antagonism with spironolactone for 1 month improved LV remodeling in patients with first anterior MI. We, therefore, hypothesized that MR blockade promotes healing and repair of the infarcted myocardium. Accordingly, this study investigated whether immediate MR blockade after experimental MI provides benefits on hemodynamics, infarct expansion, and neovascularization, with emphasis on underlying cellular and molecular events.

Methods

All of the procedures were approved by the institutional animal research committee.

MI and Study Protocol

Left coronary artery ligations were performed in adult male Wistar rats (200 to 250 g).9 Starting immediately after coronary ligation, surviving rats were randomly selected for eplerenone (100 mg/kg of...
body weight) or placebo treatment (5% arabic gum) administered by gavage once daily for 2, 3, or 7 days. Sham-operated animals received placebo treatment. In additional experimental groups, eplerenone or placebo treatment was started at day 3 after MI. The dosing regimens are not easily comparable between rodents and humans. Eplerenone was used at a dose of 100 mg/kg per day, which is the most commonly used dose for this drug in rats with MI.\textsuperscript{9}

**Liposome-Encapsulated Clodronate Treatment**

Rats received 2 injections of 2-mL liposome-encapsulated clodronate to prevent monocyte accumulation into the infarct zone until 2 days postinfarction. Injections were given 1 day before coronary artery ligations and 24 hours after surgery. Liposome-encapsulated clodronate (Clod-lip) was prepared as described previously.\textsuperscript{10} Clodronate was a gift of Roche Diagnostics GmbH. Clodronate liposomes are efficiently taken up by phagocytic cells, and the intracellular release of clodronate during enzymatic breakdown of the liposomes within the macrophages causes apoptosis and selective depletion of these cells.\textsuperscript{10} Clodronate is not a toxic drug in itself, and clodronate does not cross liposomal or cellular phospholipid membranes. Free clodronate, eg, released from dead macrophages, has an extremely short half-life in the circulation and is removed from the circulation by the renal system (see also www.clodronateliposomes.org).\textsuperscript{10}

**Hemodynamics, LV Volume Measurements, and Infarct Expansion**

LV systolic and end-diastolic pressures, right atrial pressure, and dP/dt were measured 7 days after MI, under light isoflurane anesthesia and spontaneous respiration, using a micromanometer (Millar Instruments). The in vivo LV pressure-volume relationship was analyzed using a conductance catheter (SPR-774, Millar Instruments).\textsuperscript{9} For infarct expansion measurements, the hearts were arrested by intravenous KCl injection and were perfusion fixed with 4% phosphate-buffered formalin. Thin sections (7 μm) were serially cut from apex to base at 1-mm intervals and stained with picrosirius red. MI size (fraction of the infarcted LV) was calculated as the average of all of the slices and expressed as a percentage of length.\textsuperscript{11} The transverse section representing the middle of LV and with the most marked cavity dilatation was used for expansion index determination.\textsuperscript{11} Five evenly spaced radians were passed through the infarct with the center of the LV section as a reference, and the average infarct thickness was calculated. Noninfarcted LV septal thickness was measured similarly. The expansion index was calculated with the following formula: expansion index = (LV cavity area/Total LV area) × (septum thickness/scar thickness).

**Leukocytes, Aldosterone, and Corticosterone**

A blood sample was collected in the morning from the right carotid artery. Peripheral leukocytes were examined using hematology analyzer (XT 2000i, Sysmex). Plasma aldosterone (Sorin Biomedica) and corticosterone (MP Biomedicals) levels were measured by radioimmunoassay.

**Immunohistochemistry**

For immunohistochemical analysis, LV frozen 5-μm sections were stained using primary antibodies against Factor XIIIa (Ab1834, Abcam), CD68 (MCA341R, AbD Serotec), CD4 (550297, BD Biosciences Pharmingen), CD31 (550300, BD Biosciences Pharmingen), α-smooth muscle actin (VPS281, Vector Laboratories), and myeloperoxidase (Ab25989, Abcam). Briefly, sections were fixed in cold acetone for 5 minutes followed by pretreatment with 0.3% hydrogen peroxide for 20 minutes to inhibit endogenous peroxidase activity. Subsequently, sections were blocked with 2% horse serum for 30 minutes and incubated with the primary antibody for 1 hour at room temperature. After rinsing with PBS, the sections were incubated for 30 minutes with a biotinylated antimouse antibody, rat adsorbed, and made in horse (BA-2001, Vector Laboratories). Staining was performed using the Vectastain Elite ABC kit (PK-6100, Vector Laboratories) and diaminobenzidine (SK-4100, Vector Laboratories). Dual immunohistochemical staining to identify neo-vascularature was performed using diaminobenzidine for CD31 and the HistoGreen HRP Substrate kit (E109, Linearis) for α-smooth muscle actin. Sections were counterstained with hematoxylin or eosin. For immunofluorescence double staining, the sections were incubated with the primary antibody against Factor XIIIa for 1 hour, followed by incubation with Texas Red antimonie, made in horse (TI-2000, Vector Laboratories), for 30 minutes. Subsequently, sections were blocked with 5% mouse serum for 1 hour and incubated for 1 hour with mouse antirat CD68 antibody, fluorescein isothiocyanate conjugated (MCA341F, AbD Serotec). Mouse IgG (I-2000, Vector Laboratories) was used as a negative control to verify the staining specificity.

**Tissue Sampling**

A separate group of animals was used for the biochemical analysis. The heart was divided into right and left ventricles, including septum in ice-cold saline. After MI size estimation (the left ventricle was pressed flat on glass plates, and the boundary lengths of the infarcted and noninfarcted epicardial and endocardial surfaces were traced and digitized), the LV was divided into infarcted area and noninfarcted myocardium.\textsuperscript{11}

**Microarray**

RNA from LV samples (infarcted or sham-operated LV myocardium) was extracted using TRizol (Invitrogen) and purified using the RNeasy Mini kit (Qiagen). RNA quality was assessed by Bioanalyzer 2100 (Agilent). RNA samples were converted to biotinylated cRNA and hybridized to Affymetrix GeneChip Rat Expression Array 230 2.0 according to the manufacturer’s directions. Microarray data analysis was performed using R packages from the Bioconductor project (www.bioconductor.org).

**Western Blot Analysis**

For Western blot analysis LV samples (infarcted LV myocardium) were homogenized in ice-cold RIPA buffer. LV extracts were mixed with sample loading buffer and under reducing conditions separated on 10% SDS polyacrylamide gel.\textsuperscript{9} Primary antibodies used recognize Factor XIIIa (Ab1834, Abcam), monocyte chemoattractant protein (MCP-1; 555072, BD Biosciences Pharmingen), and GAPDH (Ab8245, Abcam).

**Human and Rat Monocyte Isolation**

Blood was layered over Histopaque (Sigma). After centrifugation, the mononuclear layer was recovered, washed 5 times with Hank’s balanced salt solution, and suspended in RPMI medium 1640 supplemented with 10% autologous serum and antibiotics. Cells were then adhered to 150-mm tissue culture plates for 2 hours at 37°C in the presence of 5% CO\textsubscript{2}. The nonadherent cells were removed by washing the plates 5 times with PBS. The adhered cells were lysed in ice-cold radioimmunoprecipitation assay buffer.

**Cytokine Levels**

LV samples (infarcted LV myocardium) were homogenized in ice-cold radioimmunoprecipitation assay buffer. Protein levels of MCP-1 were determined by Western blot analysis. A commercially available ELISA kit (RLB800, R&D Systems) was used to quantify interleukin (IL)-1β levels. Tumor necrosis factor (TNF)-α, IL-6, IL-10, and IL-4 protein levels were measured using Bioplex Protein Array system (BioRad), according to the instructions of the manufacturer.

**Zymography and Reverse Zymography**

LV samples (infarcted LV myocardium) were homogenized in ice-cold cacodylic acid buffer.\textsuperscript{11} LV extracts were mixed with loading buffer and electrophoresed on a 10% SDS polyacrylamide gel containing 1 mg/mL of gelatin under nonreducing conditions. Gelatinolytic bands were quantified by Image software Quantity One (Bio-Rad). Reverse zymography was performed in a similar manner, except that purified matrix metalloproteinase-2 (30 ng/mL, Calbio-
Table. Global Parameters of Sham-Operated Rats and of PLA and EPLE Rats 7 Days After MI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>PLA Mi</th>
<th>EPLE Mi</th>
</tr>
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<tbody>
<tr>
<td>No.</td>
<td>10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>ML, %</td>
<td>NA</td>
<td>48±1</td>
<td>48±1</td>
</tr>
<tr>
<td>BW, g</td>
<td>300±5</td>
<td>263±9</td>
<td>270±6</td>
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<tr>
<td>LVSP, mm Hg</td>
<td>132±7</td>
<td>104±3*</td>
<td>107±3*</td>
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<tr>
<td>LVEDP, mm Hg</td>
<td>5.5±0.5</td>
<td>25.5±1*</td>
<td>16.9±1*‡</td>
</tr>
<tr>
<td>RAP, mm Hg</td>
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<td>8.12±0.5*</td>
<td>6.47±0.5†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>343±11</td>
<td>302±10*</td>
<td>314±9</td>
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<tr>
<td>LV dP/dtmax, mm Hg/s</td>
<td>5783±326</td>
<td>3373±156*</td>
<td>4102±165†</td>
</tr>
<tr>
<td>LV dP/dtmin, mm Hg/s</td>
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<td>3112±115‡</td>
</tr>
<tr>
<td>LVESEV, μL</td>
<td>150±17</td>
<td>469±28*</td>
<td>352±26‡</td>
</tr>
<tr>
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<td>421±26</td>
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<td>608±26‡</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>67±3</td>
<td>37±2*</td>
<td>47±3‡</td>
</tr>
</tbody>
</table>

Mean±SEM. BW indicates body weight; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; RAP, right atrial pressure; max, maximum; min, minimum; LVESEV, LV end-systolic volume; LVEDV, LV end-diastolic volume.

Aldosterone Plasma Levels Postinfarction
Aldosterone levels were markedly elevated after MI, with a peak at 1 day (PLA-MI: 822±197, n=7; sham: 122±48, n=5 pg/mL; P<0.01), and at 7 days (PLA-MI: 613±91, n=10; sham: 144±36, n=5 pg/mL; P<0.05).

Immediate MR Blockade Post-MI Reduces Infarct Expansion and Improves LV Dilation
Immediate MR blockade postinfarction versus placebo significantly increased scar thickness and reduced infarct expansion index at 7 days (Figure 1A). Eplerenone ameliorated the rightward shift of the pressure-volume curve (Figure 1B) and significantly decreased the LV filling pressure, end-diastolic volume, and end-systolic volume. Amelioration of early LV dilation by eplerenone was associated with significant improvement of LV ejection fraction and dp/dt (Table).

MR Blockade Promotes Neovascularization
Early MR antagonism significantly enhanced neovascularization in the healing myocardium. We identified capillaries as small lumen vessels lacking smooth muscle layers and positively staining for CD31. As the infarct scar matures, many vessels acquire a muscular coat that stabilizes the neovascularure, whereas uncoated vessels regress. We identified coated vessels as thin-walled α-smooth muscle actin-positive vascular structures. We showed an increased number of capillaries and an enhanced number of coated vessels after eplerenone treatment 7 days postinfarction (Figure 2).

MR Blockade Increases Factor XIIIa Expression in the Infarct Zone
We used a microarray approach to characterize gene expression in the infarcted wall regulated by immediate MR antagonism postinfarction. Microarray analysis of total RNA isolated from the whole infarct zone revealed that eplerenone therapy increased gene expression of coagulation factor XIIIa (1.77-fold; P<0.01) 3 days post-MI. Factor XIII plays an important role in myocardial infarct healing. Factor XIII–
deficient mice suffer from cardiac rupture, infarct expansion, and aggravated LV dilation. Upregulation of factor XIIIa expression in the infarct zone by instant MR blockade was confirmed by Western blot analysis (Figure 3A). Because myocytes, endothelial cells, smooth muscle cells, and fibroblasts do not express factor XIIIa, we next focused on monocytes/macrophages that are known to produce factor XIIIa. We found increased immunoreactivity for factor XIIIa and more factor XIIIa-positive macrophages in the infarcted myocardium 3 days postinfarction after immediate MR blockade (Figure 3B and 3D). Moreover, we investigated the involvement of monocytes/macrophages in factor XIIIa

Figure 2. MR blockade after MI enhanced neovascularization in the healing myocardium. A, Immunohistochemical staining for CD31 and α-smooth muscle actin, showing increased number of coated vessels and of capillaries after eplerenone treatment. (red bar: 50 μm; black bar: 10 μm). B, Dual immunohistochemical staining to identify coated vessels, CD31 (brown) and α-smooth muscle actin (green). C, Numbers of coated vessels and capillaries in placebo- (PLA) and eplerenone-treated (EPLE) rats in the peri-infarct region and scar, 7 days after myocardial infarction. Mean±SEM (n=4). †P<0.05 vs PLA.

Figure 3. MR blockade after MI enhanced factor XIIIa expression in the healing myocardium at day 3 postinfarction. A, Factor XIIIa protein levels, as revealed by Western blot analysis, in the infarcted LV myocardium of placebo (PLA), eplerenone (EPLE), liposome-encapsulated clodronate (Clodr-lip), and Clodr-lip+EPLE treated rats. Mean±SEM (n=4 to 5). B, Immunohistochemical staining for factor XIIIa and CD68, showing increased immunoreactivity for factor XIIIa after EPLE treatment. C, Treatment with Clodr-lip markedly reduced monocyte infiltration into the infarct zone and almost abrogated immunoreactivity for factor XIIIa 3 days after MI. D, Immunofluorescence double staining revealed more factor XIIIa positive macrophages in the infarcted myocardium after EPLE treatment. *P<0.001 vs PLA, EPLE; †P<0.05 vs PLA (B and C, magnification ×200; D, magnification ×400).
expression. Treatment of a subgroup of rats with liposome-encapsulated clodronate markedly reduced monocyte/macrophage infiltration into the infarct zone and concomitantly almost abrogated the protein expression of factor XIIIa (Figure 3A and 3C), indicating that monocytes/macrophages represent the source of factor XIIIa.

We also investigated vascular endothelial growth factor protein expression in the infarcted zone by Western blot analysis 3 days post-MI. Eplerenone therapy tended to increase vascular endothelial growth factor expression (PLA MI: 0.43±0.07; EPLE MI: 0.61±0.05; vascular endothelial growth factor/GAPDH: n=6; P=0.064).

**MR Blockade Induces More Rapid Accumulation of Monocytes**

The number of macrophages infiltrating the healing myocardium was greater in eplerenone-treated rats compared with placebo at 2 days (Figure 4A and 4B). Circulating monocytes were significantly increased by MR antagonism 2 days postinfarction (Figure 4B). Because glucocorticoids decrease circulating blood monocytes (n=7), and in plasma corticosterone levels (n=9 to 12) of placebo- (PLA) and eplerenone-treated (EPLE) rats after myocardial infarction. Mean±SEM. †P<0.05 vs PLA. C, Correlation between plasma corticosterone levels and circulating blood monocytes 2 days after MI.

To test the hypothesis that more rapid induction of monocyte infiltration into the ischemic area at 2 days represents an important mechanism underlying the benefits of immediate MR blockade on infarct expansion, we investigated the effects of liposome-encapsulated clodronate treatment. Depletion of macrophage accumulation into the infarct zone abrogated the beneficial effects of eplerenone 7 days postinfarction on scar thickness (Clodr-lip+PLA-MI: 0.84±0.05; Clodr-lip+EPLE-MI: 0.85±0.06 mm; n=5; P value not significant), infarct expansion index (Clodr-lip+PLA-MI: 1.30±0.07; Clodr-lip+EPLE-MI: 1.21±0.08, n=5, P value not significant), and LV function (maximum dP/dt: Clodr-lip+PLA-MI: 3848±99; Clodr-lip+EPLE-MI: 3675±454 mm Hg/s; minimum dP/dt: Clodr-lip+PLA-MI: 3085±292; Clodr-lip+EPLE-MI: 3086±416 mm Hg; n=5; P value not significant).

Moreover, to show that cellular and molecular effects induced within the first 2 days play a critical role in the benefits of instant MR antagonism on infarct healing, we investigated the effects of eplerenone therapy started at day 3 postinfarction. At day 7, MR antagonism versus placebo did not significantly reduce LV filling pressure (23±1 mm Hg;
n=9; P value not significant), thinning and enlargement of the infarct segment (scar thickness: 0.82±0.03 mm; infarct expansion index: 1.22±0.05; n=9; P value not significant), and early LV dysfunction (maximum dP/dt: 3589±282; minimum dP/dt: 3013±243 mm Hg/s; n=9; P value not significant) when started at day 3 post-MI.

We also studied neutrophil infiltration on day 1 and day 2 postinfarction by myeloperoxidase immunohistochemical analysis (Figure S1). The number of myeloperoxidase-positive cells was similar in placebo rats compared with eplerenone-treated rats (day 1: PLA-MI: 2.7±0.84; EPLE MI: 2.3±0.11; day 2: PLA-MI: 1.67±0.14; EPLE MI: 1.76±0.07; number of low-power fields: n=3; P value not significant) when started at day 3 post-MI.

MR Blockade Transiently Enhances Cytokine Levels in the Infarct Zone

Activated monocytes and T lymphocytes are important sources of cytokines.16,17 We found that accelerated monocyte and lymphocyte infiltration by immediate MR blockade post-MI was associated with transient upregulation of several cytokines in the healing myocardium (Figure 5). The protein levels of the chemotactic cytokine MCP-1 were significantly upregulated by eplerenone compared with placebo at 2 days postinfarction (Figure 5). IL-1β, TNF-α, IL-6, IL-10, and IL-4 protein levels were significantly increased at 3 days. IL-10 was already significantly upregulated at day 2. MR inhibition did not affect cytokine levels 7 days postinfarction (Figure 5).

**MR Blockade Does Not Affect Collagen Deposition**

Microarray analysis of total RNA isolated from the whole infarcted area at 3 and 7 days post-MI showed that MR inhibition did not affect expression of genes involved in collagen turnover (Figure 6A). The expression of collagen was already increased at day 3 and substantially further increased at day 7 postinfarction; placebo- and eplerenone-treated rats showed a comparable upregulation and time course (Figure 6A). Similarly, the same collagen content was found in the infarct zone of placebo- and eplerenone-treated rats 7 days postinfarction (Figure 6B). Moreover, temporal changes in matrix metalloproteinase/tissue inhibitors of metalloproteinase activities (Figure 6C), determined by zymography and reverse zymography, were not significantly different in the infarct zone between placebo and eplerenone treatment.

**Discussion**

The present study demonstrates that immediate MR blockade after MI accelerated macrophage infiltration and transiently increased the expression of healing, promoting cytokines and factor XIIIa in the injured myocardium leading to enhanced infarct neovascularization and reduced early LV dilation and dysfunction.

Macrophages have been shown to promote infarct healing,18–21 phagocytosis and resorption of tissue debris, and granulation tissue and neovessel formation through the release of cytokines and growth factors. Enhanced macrophage infiltration into the ischemic area at the acute stage appears to be an important mechanism of MR blockade, because deple-
tion of macrophage accumulation into the infarct zone by liposome-encapsulated clodronate abrogated the beneficial effects. To study the mechanisms underlying increased monocyte accumulation on day 2 postinfarction, we focused on potential relations between the local responses and humoral effects of MR inhibition. Heil et al.\(^{22}\) showed that circulating monocyte levels correlate with the extent of macrophage accumulation and the enhancement of collateral vessel formation in ischemic tissues. In the present study, circulating blood monocytes were increased by MR antagonism at 2 days. In addition, plasma corticosterone levels were reduced by MR blockade and were negatively correlated with blood monocyte levels. Glucocorticoids have been shown to inhibit the inflammatory process by decreasing the number of circulating and infiltrating monocytes, resulting in impaired wound healing.\(^{14,15}\) Glucocorticoid administration inhibits MCP-1 upregulation in ischemic tissues\(^{23}\) and retards infarct healing, leading to adverse early LV dilation.\(^{24,25}\) Furthermore, glucocorticoids inhibit angiogenesis in the healing myocardium after MI also at physiological concentrations.\(^{26}\) Thus, the reduction in plasma corticosterone by MR antagonism likely contributed to increased circulating and infiltrating monocytes on day 2, leading to acceleration of the healing process. In addition, more infiltrating CD4\(^+\) T cells were observed in the infarcted myocardium of eplerenone-treated rats early post-MI. CD4\(^+\) T cells contribute to monocyte/macrophage accumulation in the ischemic region, which, in turn, trigger neovascularization through cytokines and growth factor secretion.\(^{27}\) CD4\(^+\) T-cell–deficient mice showed impaired macrophage recruitment and collateral vessel growth after the induction of ischemia.\(^{27}\)

To gain insights into the mechanisms by which eplerenone lowers plasma corticosterone levels 2 days post-MI, in additional experiments we investigated the effects of eplerenone in rats without coronary ligation. In these animals also after a 2-day eplerenone treatment, circulating corticosterone levels were reduced. Interrelation between the hypothalamic-pituitary-adrenal axis and the MR may be involved, although previous data reported a stimulatory effect of MR antagonism by spironolactone on the hypothalamic-pituitary-adrenal axis.\(^{28}\) However, the cortisol measurements showed a peak elevation between 2 and 4 hours after spironolactone administration and some decline thereafter. Moreover, unlike spironolactone, eplerenone selectively

Figure 6. MR blockade after MI did not affect expression of genes involved in collagen turnover. A, Cluster analysis of genes involved in collagen turnover, in the LV of sham-operated rats (sham) and in the infarcted myocardium of placebo- (PLA) and eplerenone-treated (EPL) rats 3 and 7 days after MI. Genes involved in collagen turnover were already increased at day 3 and substantially further increased at day 7. PLA and EPL rats showed a comparable upregulation and time course. B, Collagen content at day 7 and (C) representative zymogram and reverse zymogram and temporal changes in matrix metalloproteinase/tissue inhibitors of metalloproteinase activities in the infarcted myocardium. Mean±SEM (n=5 to 10).
blocks the MR and has only little affinity for other steroid receptors (the IC₅₀ of eplerenone for the glucocorticoid, androgen, progesterone, and estrogen receptors is >10 000 nM). Thus, the observed reduction of circulating corticosterone after 2 days of eplerenone therapy is reproducible, but the exact underlying mechanism is not clear.

Acute MR blockade upregulated the expression of proinflammatory and anti-inflammatory cytokines in the injured myocardium at days 2 and 3 but not at day 7. The cytokine cascade after myocardial ischemia allows the myocardium to respond rapidly to tissue injury and orchestrates the manifold cellular activities that underscore inflammation and healing.²⁻⁴ Because persistent expression of inflammatory cytokines can lead to myocardial damage and adverse LV remodeling, the self-limited short-term expression of cytokines by MR antagonism likely provided the heart with a beneficial adaptive response to infarction injury within the first days after MI, without unwanted deleterious effects. MR inhibition transiently enhanced myocardial levels of MCP-1, TNF-α, IL-1β, IL-6, and IL-10 that appear to play a protective and coordinating role for infarct and wound healing.²⁻⁴ MCP-1 is a major chemotactic cytokine that induces the recruitment of monocytes and T lymphocytes at sites of inflammation and plays an important role in stimulating monocyte differentiation into macrophages.²⁹ Infarcted mice with disruption of the MCP-1 gene had delayed and decreased macrophage infiltration, delayed replacement of injured cardiomyocytes with granulation tissue, and reduced myocardial fibroblast accumulation in the healing infarct,¹⁹ whereas cardiac overexpression of MCP-1³⁰ stimulated macrophage infiltration, myocardial IL-6 secretion, and neoangiogenesis, resulting in the prevention of LV dysfunction and remodeling after MI. On the other hand, neutralization of TNF-α promotes ventricular rupture and exacerbates cardiac remodeling after MI,³⁰ whereas anti–IL-1β treatment early postinfarction leads to poor wound healing and delayed collagen deposition.³¹ IL-6 is a pleiotropic cytokine involved in the growth and differentiation of T lymphocytes and in tissue repair.²⁹ Frangogiannis et al²³ demonstrated a marked induction of IL-10 after myocardial ischemia, predominantly localized in lymphocytes infiltrating the reperfused myocardium, suggesting a role for lymphocyte-derived IL-10 in modulating the inflammatory response.

Cytokines not only provide the milieu necessary for monocyte and T-cell recruitment but also for their activation at the site of injury.¹⁶ Macrophages can develop into classically or alternatively activated macrophages.¹⁷ IL-4 induces alternative activation of macrophages, resulting in marked upregulation of factor XIIIa expression.³² We found increased IL-4 protein levels and more factor XIIIa–positive macrophages in the healing myocardium after MR blockade. Treatment with liposome-encapsulated clodronate prevented monocyte infiltration into the infarct zone and concomitantly abrogated the expression of factor XIIIa, indicating that macrophages represent the local source of factor XIIIa. Factor XIIIa plays an important pleiotropic role in wound healing, tissue repair, and angiogenesis.¹³ Factor XIII–deficient mice suffer from cardiac rupture, infarct expansion, and aggravated cardiac remodeling,¹² emphasizing the importance of factor XIII in myocardial infarct healing. In patients with MI, factor XIII activity correlates positively with survival.³³ The angiogenic response after MI is critical for healing and cardiac repair.¹⁻⁵ Thus, upregulation of factor XIIIa by MR antagonism likely contributes to enhanced neoangiogenesis associated with improvement of infarct healing.

The present study demonstrates that MR blockade after MI reduces expansion of the healing infarct and improves early LV dilation and dysfunction. The effect of aldosterone-blocking therapy on infarct expansion may in part explain the effectiveness of eplerenone on mortality 30 days after randomization in patients after acute MI.⁷ Progressive thinning and enlargement of the infarct segment (infarct expansion) are events of the first days after MI. Improvement of scar thickening decreases wall stress and prevents infarct expansion, as well as remodeling, in noninfarcted sites. A reduction in early ventricular remodeling may improve the homogeneity of ventricular conduction and, hence, reduce the likelihood for life-threatening arrhythmias.³⁴ However, other mechanisms, such as prevention of electric remodeling,³⁵,³⁶ and endothelial dysfunction,³⁷ may be of particular importance.

The effect of MR-blocking therapies on healing of the infarct scar apparently depends strongly on their timing relative to pathophysiological stages of cardiac repair. Delyani et al³⁸ showed that MR antagonism did not improve infarct expansion in rats when started 24 hours postinfarction. Finally, we underline that our findings are not in contrast with previously described antiinflammatory effects of long-term MR/aldosterone blocking therapy³⁹–⁴² and do not rule out that aldosterone plays a significant role in the development of vascular and myocardial inflammation via proinflammatory cytokines.⁴¹–⁴⁴ Moreover, it should be noted that long-term MR blockade normalizes the expression of cytoskeleton proteins and matrix metalloproteinases and prevents progressive LV dysfunction and chamber remodeling in experimentally induced heart failure.⁹,⁴⁵ In summary, our study demonstrates that acute MR blockade accelerated inflammatory cell infiltration and induced a very transient upregulation of healing-promoting cytokines in the infarcted myocardium early after MI, leading to enhanced factor XIIIa expression and improvement of infarct neovascularization and expansion.

**Perspectives**

Therapeutic strategies that improve infarct healing and prevent infarct expansion and LV dilatation within the first days after MI are still missing. In the present study, acute MR blockade accelerated macrophage infiltration and transiently increased the expression of healing-promoting cytokines and factor XIIIa in the injured myocardium resulting in enhanced infarct neovascularization and reduced early LV dilation and dysfunction. As in the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study, the patients randomly assigned early (3 to 7 days) to treatment derived greater benefit than those assigned later (7 to 14 days); even better effects may be achieved by early initiation of MR blockade after MI.
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References
32. Fraccarollo et al Aldosterone Antagonism Improves Infarct Healing


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