Myeloperoxidase Mediates Postischemic Arrhythmogenic Ventricular Remodeling

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Rationale: Ventricular arrhythmias remain the leading cause of death in patients suffering myocardial ischemia. Myeloperoxidase, a heme enzyme released by polymorphonuclear neutrophils, accumulates within ischemic myocardium and has been linked to adverse left ventricular remodeling.

Objective: To reveal the role of myeloperoxidase for the development of ventricular arrhythmias.

Methods and Results: In different murine models of myocardial ischemia, myeloperoxidase deficiency profoundly decreased vulnerability for ventricular tachycardia on programmed right ventricular and burst stimulation and spontaneously as assessed by ECG telemetry after isoproterenol injection. Experiments using CD11b/CD18 integrin–deficient (CD11b−/−) mice and intravenous myeloperoxidase infusion revealed that neutrophil infiltration is a prerequisite for myocardial myeloperoxidase accumulation. Ventricles from myeloperoxidase-deficient (Mpo−/−) mice showed less pronounced slowing and decreased heterogeneity of electrical conduction in the peri-infarct zone than wild-type mice. Expression of the redox-sensitive gap junctional protein Cx43 (Connexin 43) was reduced in the peri-infarct area of wild-type compared with Mpo−/− mice. In isolated wild-type cardiomyocytes, Cx43 protein content decreased on myeloperoxidase/H2O2 incubation. Mapping of induced pluripotent stem cell–derived cardiomyocyte networks and in vivo investigations linked Cx43 breakdown to myeloperoxidase-dependent activation of matrix metalloproteinase 7. Moreover, Mpo−/− mice showed decreased ventricular posts ischemic fibrosis reflecting reduced accumulation of myofibroblasts. Ex vivo, myeloperoxidase was demonstrated to induce fibroblast-to-myofibroblast transdifferentiation by activation of p38 mitogen-activated protein kinases resulting in upregulated collagen generation. In support of our experimental findings, baseline myeloperoxidase plasma levels were independently associated with a history of ventricular arrhythmias, sudden cardiac death, or implantable cardioverter–defibrillator implantation in a cohort of 2622 stable patients with an ejection fraction >35% undergoing elective diagnostic cardiac evaluation.

Conclusions: Myeloperoxidase emerges as a crucial mediator of postischemic myocardial remodeling and may evolve as a novel pharmacological target for secondary disease prevention after myocardial ischemia. (Circ Res. 2017;121:56-70. DOI: 10.1161/CIRCRESAHA.117.310870.)

Key Words: Connexin 43 ▪ fibrosis ▪ infarction ▪ inflammation ▪ myocardial ischemia ▪ tachycardia

Sudden cardiac death is the leading cause of death after myocardial infarction, and three quarters of patients dying from an acute arrhythmic event are diagnosed for coronary artery

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Myeloperoxidase (MPO) is released by infiltrating polymorphonuclear neutrophils and has traditionally been viewed as a microbialidal enzyme. Accumulating evidence demonstrates involvement of MPO in cardiovascular disease, including atherosclerosis and myocardial disease. Even with optimal medical therapy according to current standards, life-threatening arrhythmias caused by left ventricular remodeling are a major healthcare concern.

What New Information Does This Article Contribute?

- MPO promotes proarrhythogenic remodeling in different models of myocardial ischemia and increases vulnerability for ventricular tachycardia in this setting.
- MPO augments postinfarct connexin 43 degradation via matrix metalloproteinase 7 (MMP-7) activation, aggraviates fibroblast-to-myofibroblast transdifferentiation via p38 mitogen-activated protein kinase phosphorylation, and increases postinfarct collagen deposition and fibrosis development.
- MPO deficiency decreases heterogeneity of electric conduction and reduces conduction block, thereby reducing the development of reentry circuits.

Despite intensive scientific efforts, ischemic heart disease is still a leading cause of morbidity and mortality in western countries. Electric and structural remodeling of the left ventricle after myocardial infarction is the most common substrate for ventricular tachycardia and subsequent sudden cardiac death. In this context, the discovery of new potential therapeutic targets is urgently required. MPO was recently identified as predictor of cardiovascular disease. Herein, we identify MPO as a promoter for ventricular tachycardia vulnerability via postinfarct connexin 43 degradation and fibrosis and subsequent ventricular conduction inhomogeneity. MPO may therefore emerge as a novel therapeutic target for pharmacological antiarrhythmic therapies after myocardial infarction.

Nonstandard Abbreviations and Acronyms

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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AP</td>
<td>action potential</td>
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<tr>
<td>CD11b</td>
<td>integrin αM</td>
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<td>Cx43</td>
<td>Connexin 43</td>
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<tr>
<td>I/R</td>
<td>ischemia and reperfusion</td>
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<tr>
<td>iPSCM</td>
<td>induced pluripotent stem cell–derived cardiomyocytes</td>
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<tr>
<td>LAD</td>
<td>left anterior descending</td>
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<td>LV</td>
<td>left ventricular</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinases</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteinases</td>
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<tr>
<td>PI</td>
<td>permanent ischemia</td>
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<td>VT</td>
<td>ventricular tachycardia</td>
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<td>WT</td>
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Myocardial ischemia is one of the most powerful triggers of leukocyte recruitment and activation. In particular, neutrophils are among the first cells found in the area at risk, and their activation in the setting of acute myocardial ischemia can be measured systemically. On activation, neutrophils release myeloperoxidase, a heme enzyme abundantly expressed in these cells. Myeloperoxidase until recently was solely viewed as part of the innate immune defense. However, myeloperoxidase has been shown to promote potent proinflammatory vascular properties, facilitating the consumption of endothelial nitric oxide directly or by generation of potent reactive oxygen species. Myeloperoxidase has been demonstrated to be involved in myocardial remodeling after myocardial injury. It increased myocardial collagen deposition after ligation of the left anterior descending (LAD) artery, and myeloperoxidase-deficient (Mpo−/−) mice exhibited less left ventricular (LV) dilatation and attenuated impairment in systolic LV function.

In the atria, myeloperoxidase also increased fibrotic remodeling, which was linked to an increased susceptibility to atrial fibrillation. Apart from ventricular fibrosis, there is indirect evidence that myeloperoxidase promotes the degradation of the gap junctional protein Cx43 (Connexin 43), which has been firmly linked to ventricular arrhythmogenesis. Thus, Cx43 availability has been shown to be essentially determined by matrix metalloproteinase 7 (MMP-7), an enzyme, whose activation has been shown to be regulated by myeloperoxidase.

Herein, we show that systemic levels of myeloperoxidase are associated with a history of ventricular arrhythmias in patients undergoing elective diagnostic cardiac evaluation, and that myeloperoxidase is causally linked to arrhythmogenic ventricular remodeling in mice. A proarrhythmogenic role for myeloperoxidase was observed in vivo using Mpo−/− mice following either myocardial ischemia/reperfusion (I/R) injury or permanent ischemia (PI), revealing a role for myeloperoxidase in both the enzymatic degradation of Cx43 and the propagation of ventricular fibrosis through induction of fibroblast-to-myofibroblast transdifferentiation, which are both hallmarks of proarrhythmogenic myocardial remodeling.

Methods

An expanded Methods section can be found in the Online Data Supplement.

Animal Studies

Male, 8- to 12-week-old wild-type (WT) littermates, myeloperoxidase-deficient (Mpo−/−), and CD11b/CD18 integrin-deficient (CD11b−/−) mice were used for this study. Animals were littermates of C57BL/6J background. The strategy for the generation of Mpo−/− mice has been previously reported. All animal studies were approved by the Universities of Hamburg and Cologne Animal Care and Use Committees and follow ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

LAD Artery Ligation

In brief, 8- to 12-week-old WT, Mpo−/−, and CD11b−/− mice were subjected to left thoracotomy. An 8/0 polypropylene suture was placed through the myocardium into the anterolateral LV wall. For I/R, the ligation was removed after 30 minutes to allow ≤7 days of reperfusion. For I/R of CD11b−/− mice, the ligation was removed...
after 40 minutes to allow ≤2 days of reperfusion. For PI, mice were maintained for ≤21 days without reperfusion. Animals which died during instrumentations or which did not properly recover were excluded from analyses.

**Right Ventricular Stimulation**

After 7 days of reperfusion or 21 days of PI isoflurane anaesthesia, WT, Mpo<sup>−/−</sup> and CD11b<sup>−/−</sup> mice were subjected to a protocol of right ventricular stimulation. VT was defined as a series of repetitive ventricular ectopic beats lasting for >200 ms.

**Implantation of ECG Transmitters, ECG Telemetry, and Arrhythmia Provocation**

ECGs were recorded in freely moving unrestrained mice. Twenty-four hours after LAD ligation, arrhythmia provocation was performed by double injections of isoproterenol (IP 2 mg/kg) separated by an interval of 30 minutes, VT was defined as a series of repetitive ventricular ectopic beats lasting for >200 ms.

**In Vivo Electrophysiological Mapping**

After 7 days IR or 21 days PI, the heart was exposed by thoracotomy. A 32-electrode microelectrode array (Multichannel Systems, Reutlingen, Germany) was positioned on the epicardial surface of the LV. Homogeneity index, absolute inhomogeneity, variation coefficient, and mean velocity of interelectrode conduction were calculated. For pacing studies, the hearts were stimulated with a concentric bipolar electrode (FHC, Bowdoin) with a stimulus rate of 10 Hz.

**In Vitro Electrophysiological Mapping**

Differentiation and purification of murine induced pluripotent stem cell–derived cardiomyocytes (iPSCMs) is described elsewhere. iPSCMs at day 16 of differentiation were cultured on a 120-electrode microarray. After 5 days of settling, cells were treated overnight as indicated. Field potentials were recorded (120pMEA; Multichannel Systems), and activation maps were calculated as described above.

**Echocardiographic Studies**

Transthoracic echocardiography was performed using the Vevo 2100 System (VisualSonics, Toronto, Canada).

**Action Potential Recordings by Sharp Electrode**

Short-axis ventricular slices were prepared from hearts subjected to PI for 3 days as described before. Intracellular action potential (AP) recordings were performed in ventricular slices with sharp electrodes (20–40 mol/L resistance when filled with 3 mol/L KCl) made of borosilicate glass capillaries (WPI, Sarasota). For inhibitor experiments, BaCl<sub>2</sub>, (Sigma Aldrich, Germany) or tetrodotoxin (Abcam, ab120054, Germany) was added, as indicated.

**Determination of Fibrotic Area**

Paraffin sections were stained with picrosirius red following standard protocol. Images were acquired using a DP25 camera (Olympus, Hamburg, Germany) mounted on a BX51 microscope (Olympus). Mean fibrotic area of 12 sections was quantified using Cell A software (Olympus).

**Determination of Infarct Size**

Hearts were excised, cut into cross-sections, and, for IR analyses, incubated in 2,3,5-triphenyl-tetrazolium chloride solution because additional Evans Blue perfusion is unfeasible with reopened LAD. For PI, hearts were excised and injected with Evan blue dye via the aorta ascendens. Infarct area was assessed by planimetry using BZ2-Analyzer software (Keyence).

**Isolation and Treatment of Adult Ventricular Cardiomyocytes**

Hearts were excised and mounted on a constant-flow Langendorff circulation (Radnoti Ltd, Ireland) and retrogradely perfused with Liberase TM (Roche) and trypsin (Invitrogen). Cells were collected and incubated with myeloperoxidase (Planta Natural Products) and H<sub>2</sub>O<sub>2</sub> (Sigma) and an inhibitor to MMP-7 (Calbiochem). Cells were lysed and processed for immunoblotting.

**Murine Myeloperoxidase Plasma Levels**

Heparin plasma was analyzed for myeloperoxidase using Mouse myeloperoxidase-ELISA (Hycult Biotech).

**Staining for Polymorphonuclear Neutrophil Infiltration**

Frozen heart sections (4 μm) were stained with rat antimouse neutrophil Ly6G primary antibody according to standard protocol. Images were acquired using a Prospistica GC camera (Allied Vision Technologies) mounted on a Leica DMLB light microscope.

**Immunofluorescence Staining for α-Smooth Muscle Actin and Myeloperoxidase**

Frozen heart sections (4 μm) were incubated with primary antibody against α-smooth muscle actin (1:200, rabbit IgG) and DDR-2 (discoidin domain-containing receptor 2; 1:50, goat IgG) or myeloperoxidase (1:250, rabbit IgG) after secondary antibody incubation. Nuclei were stained with DAPI.

**Fibroblast Isolation, Stimulation, and Analysis**

Ventricles of WT mice were digested in Liberase/Tyrode solution, and cells were incubated for 8 hours. For the analysis of phospho-p38 mitogen-activated protein kinase (MAPK), incubation time was reduced to 15 minutes. For the analysis of collagen type I incubation, time was extended to 36 hours. Unless otherwise indicated, fibroblasts were treated with 10 μg/mL catalytically active or inactive myeloperoxidase and 20 μmol/L H<sub>2</sub>O<sub>2</sub> or 10 μmol/L p38 inhibitor SB203580. Fibroblasts were incubated with WT and Mpo<sup>−/−</sup> leukocytes for 8 hours. Cells were lysed and processed for immunoblotting.

**Immunoblotting for Cx43**

Protein blotting was performed with a modified standard protocol using primary antibodies to Cx43 (1:2000) or GAPDH (glyceraldehyde 3-phosphate dehydrogenase; 1:5000). Cardiac HL-1 cells were treated with either PBS (phosphate-buffered saline), myeloperoxidase (10 μg/μL)+H<sub>2</sub>O<sub>2</sub> (80 μmol/L), or pro-MMP-7 (1 μg/mL) for 16 hours.

**Immunofluorescence Staining for Cx43**

Staining of frozen heart sections was performed with a modified standard protocol detecting Cx43 and N-cadherin. Images were taken with a Retiga 1300 CCD camera mounted on Leica DMLB fluorescence microscope by iVision v4.0. The infarct region was identified by total absence of Cx43 immunoreactivity.

**Cx43 and Ion Channel mRNA Analyses**

LV heart tissue was collected. RNA was isolated (RNeasy-Kit; Qiagen), and quantitative real-time PCR (Sso Fast Eva Green, BioRad) was performed according to the manufacturer instructions. Target gene mRNA expression was normalized to mRNA expression of GAPDH by ΔΔCT method.

**MMP-7 Activity**

LV tissue was dissected to infer and peri-infarct tissue using a dissection microscope (Leica). Peri-infarct tissue was analyzed for MMP-7 activity using the SensoLyte 520 MMP-7 Assay Kit (Anaspec) following manufacturer instructions protocol.

**Human Myeloperoxidase Plasma Levels**

The study was approved by local ethics committees and was conducted in accordance to the Declaration of Helsinki and the guidelines for good clinical practice. All individuals gave written informed consent before inclusion in the study. Serum myeloperoxidase levels were assessed by CardioMPO (Cleveland Heart Laboratory) assay on autoanalyzer according to manufacturer instructions.
Plasma Cytokines

Plasma was analyzed for cytokines by using a LEGENDplex Mouse Inflammation Panel (13-plex, Biolegend) according to manufacturer instructions.

Statistical Analyses

Results are expressed as mean±SD. Statistical analysis was performed, unless otherwise indicated, using Kruskal–Wallis test followed by Bonferroni post hoc test, Mann–Whitney U test, or unpaired Student t test as appropriate. Univariate and multivariate logistic regression analyses were used to determine independent predictors of arrhythmias, adjusting for traditional cardiac risk factors. Regression analyses were performed using JMP Pro version 10 (SAS Institute, Cary, NC) and R (3.1.2, Vienna, Austria). All other calculations were performed using SPSS version 23.0. *P<0.05, **P<0.01, ***P<0.001.

Results

Myeloperoxidase Plasma Levels, Myocardial Neutrophil Infiltration, and Inflammatory Response

To characterize a potential mechanistic link between myeloperoxidase and the inducibility of VT, different mouse models of myocardial ischemia were applied. WT and Mpo−/− mice were subjected to ischemia and reperfusion (I/R) and PI on ligation of the ramus interventricularis anterior (LAD). Myeloperoxidase plasma levels were increased >2-fold in WT mice after ligation of the ramus interventricularis anterior (LAD). Myeloperoxidase plasma levels were increased >2-fold in WT mice both 7 days after I/R and 5 days after PI compared with sham-operated (sham) animals. After 21 days of PI, myeloperoxidase plasma levels dropped but were still significantly elevated in comparison to sham-operated animals (Figure 1A). Ly6G staining of the infarct and peri-infarct zones revealed a significantly increased infiltration of polymorphonuclear leucocytes in WT animals compared with Mpo−/− animals 7 days after I/R and, to a lesser, not significant extent, 3 days after PI (Figure 1B and 1C). Of note, overall polymorphonuclear leucocytes infiltration was lower after PI than after I/R. Analyses of multiple plasma cytokine levels at multiple time points by flow cytometry revealed no relevant differences between WT and Mpo−/− mice in either model (Online Figure I).

Infarct Size and LV Function

In accordance with previous reports, infarct size after 7 days I/R and 21 days after PI did not differ significantly between WT and Mpo−/− mice. Expectedly, overall infarct size was much larger in hearts subjected to PI than when compared with I/R (Figure 1D). In accordance, more pronounced deterioration of systolic LV ejection fraction was observed after PI than after I/R at multiple time points. No significant differences between WT and Mpo−/− animals were noted at day 3 or 7 of I/R (Figure 1E), whereas on PI, ejection fraction was improved in Mpo−/− mice at day 21 (Figure 1F).

Importance of Neutrophils for Myocardial Myeloperoxidase Accumulation

To investigate the role of altered myocardial neutrophil infiltration and myeloperoxidase release on arrhythmia development after infarction, CD11b−/− mice were subjected to I/R. Expectedly, CD11b−/− mice showed a significantly reduced cardiac neutrophil infiltration when compared with WT littermates 6 hours and 2 days after I/R (Figure 1H and 1J). Importantly, despite elevated plasma levels of myeloperoxidase in CD11b−/− mice, which were similar to WT mice after I/R (Figure 1G), myocardial myeloperoxidase levels were significantly lower than in WT mice (Figure 1I and 1K). Neither neutrophils nor intracardiac myeloperoxidase could be detected after sham operation (data not shown). In line with these observations, supplementation of myeloperoxidase via osmotic mini pumps (data not shown) or daily retro-orbital injections for 7 days did not result in myocardial accumulation of myeloperoxidase within the LV (Online Figure IIA, right) despite supraphysiological plasma levels, suggesting that extravasation of neutrophils into the postschismic tissues is of imminent importance to carry myeloperoxidase into the myocardium.

Inducibility of VT by Right Ventricular Stimulation

At day 7 of I/R and day 21 of PI, WT mice exhibited increased vulnerability to VT (representative ECGs in Figure 2A and 2B) when compared with sham-operated animals (WT sham) with respect to the number of VT episodes (Figure 2C) and the duration of VTs (Figure 2D). In comparison, Mpo−/− mice undergoing I/R (Mpo−/− I/R) or permanent LAD ligation (Mpo−/− PI) showed a significantly reduced vulnerability for VT episodes and duration (Figure 2C and 2D). Over a period of 3 months after permanent LAD ligation, no spontaneous deaths occurred, and, accordingly, no difference in mortality between WT and Mpo−/− mice after PI could be demonstrated (Online Figure III).

CD11b−/− mice, which showed reduced neutrophil infiltration and cardiac myeloperoxidase deposition after I/R subjection, were also significantly protected against VT induction as shown by a lower number and length of VT episodes when compared with WT mice (Figure 2E through 2G). Conversely, intravenous myeloperoxidase infusion in Mpo−/− mice, which was not associated with myocardial myeloperoxidase accumulation, did not re-establish VT vulnerability in Mpo−/− mice (Online Figure IIB and IIC).

Spontaneous VT Development

To assess spontaneous VT development, in vivo ECGs of WT and Mpo−/− mice were recorded 24 hours after LAD ligation and additional challenge with isoproterenol. Mpo−/− mice showed a significantly less frequent development of spontaneous VT than WT (representative ECG traces are shown in Figure 2H). VT probability was lower, VT freedom was longer, and VT number and mean time of VT episodes were lower (Figure 2I through 2L).

In Vivo Epicardial Mapping Studies

Epicardial mapping studies in spontaneously beating and stimulated hearts revealed a disruption of conduction homogeneity in WT animals after I/R and PI, whereas in Mpo−/− mice, it was preserved in both models, as ascertained by respective differences in the inhomogeneity index, absolute inhomogeneity, the variation coefficient of local phase delays, and the mean conduction velocity (Figure 3). Of note, no conduction was detectable within the infarct scar tissue in PI animals (data not shown).

Effects of Myeloperoxidase on Expression and Function of Ion Channels

Transcripts of the sodium voltage-gated channel alpha subunit 5 (Nav1.5), the potassium voltage-gated channel subfamily A member 5 (Kv1.5), subfamily Q member 1 (KVLQT1), subfamily J member 2 (Kir2.1), subfamily J member 11 (Kir6.2),
Figure 1. Infiltration of polymorphonuclear leukocytes (PMN) and accumulation of myeloperoxidase (MPO). A, MPO plasma level of sham wild-type (WT) and Mpo−/− mice or WT mice after left ventricular ischemia and 7 d of reperfusion (I/R) and 5 and 21 d on permanent ischemia (PI) as assessed by ELISA. B, Immunohistochemical Ly6G stainings (brown) of WT and Mpo−/− hearts subjected to I/R or PI. C, Quantitative analysis of Ly6G+ cells into the infarct and peri-infarct zones of WT and Mpo−/− hearts after I/R and PI. Scale bar=200 μm. D, Infarct size in WT and Mpo−/− hearts after 7 d I/R and 21 d on PI. E, MPO plasma levels of WT and CD11b−/− mice subjected to ischemia after 6 h or 2 d of reperfusion. F, Immunofluorescence MPO stainings (light green) and quantitative analysis of MPO immunoreactivity in WT and CD11b−/− hearts subjected to ischemia after 6 h or 2 d of reperfusion. *P<0.05, **P<0.001, Kruskal-Wallis test followed by Bonferroni post hoc test. Mean±SEM is shown.
Figure 2. Vulnerability to ventricular arrhythmias. Representative surface (top panel) and intracardiac (bottom panel) ECG recordings of electrical ventricular stimulation of wild-type (WT) and Mpo−/− mice after (A) ischemia and 7 d of reperfusion (I/R) and (B) 21 d on permanent ischemia (PI). C, Number of episodes of ventricular tachycardia (VT) and (D) total time of VT after I/R and PI. E, Representative ECG recordings of WT and CD11b−/− mice after ischemia after 2 d of reperfusion. F, Number of VT episodes and (G) total time of VT in WT and CD11b−/− mice after ischemia after 2 d of reperfusion. sham: n=4; C and D, WT/Mpo−/− I/R n=8/9; WT/Mpo−/− PI n=6/11; F and G, sham n=11/4, WT/CD11b−/− I/R n=9/8. Analysis of ventricular tachycardia 24 h after left anterior descending (LAD) ligation: (H) representative ECG traces recorded by telemetry investigations (for 2 h) before (left) and 24 h after LAD ligation and 2 isoproterenol injections (2 mg/kg body weight; middle) in WT and Mpo−/− animals. Magnification of the highlighted section is shown in the right. Analyses of (I) VT probability, (J) onset of VT, (K) mean number of VT episodes, and (L) mean time of VT episodes in WT and Mpo−/− animals. n=10/10. *P<0.05, **P<0.01, ***P<0.001, by Kruskal–Wallis test followed by Bonferroni post hoc test. I, χ² test. J, Log-rank test.
the potassium 2 pore domain channel subfamily K member 3 (TASK-1), and the hyperpolarization activated cyclic nucleotide gated potassium channel 2 (HCN2) were detectable within the infarct region 3 days after permanent LAD ligation. Furthermore, the levels of Nav1.5, KV1.5, Kir6.2, TASK-1, and HCN2 mRNA were changed 3 days after LAD ligation when compared with myocardial tissue from healthy mice. Of importance, mRNA levels did not differ between WT and Mpo−/− infarct tissue on PI (Online Table I).

In addition, APs did not differ between WT and Mpo−/− mice as assessed with sharp electrode measurements of living heart tissue slices after 7 days of PI apart from a shortened APD50 in Mpo−/− mice (WT: 13.2±0.3 ms versus Mpo−/−: 9.5±1.4 ms; P<0.05). This is likely irrelevant given the absence of a significant effect on APD90 (WT: 88.8±6.0 ms versus Mpo−/−: 67.9±7.8 ms) or APD50/90 (WT: 15.3±0.9% versus Mpo−/−: 14.2±1.6%). To further characterize myeloperoxidase effects on ion channel function, increasing concentrations of ion...
channel blockers were administered to infarcted tissue slices of WT and Mpo−/− animals. Although the potassium channel blocker BaCl2 revealed no differences between both groups, AP amplitude was significantly decreased in hearts from Mpo−/− animals compared with WT animals after incubation with the sodium channel blocker tetrodotoxin (WT: 73.5±4.0 mV versus Mpo−/−: 47.6±5.5 mV; P≤0.05; Online Figure IV).

Cx43 in the Infarct and Peri-infarct Zone
Because connexins, which allow for ion exchange between cardiomyocytes, are an integral part of myocardial conduction homogeneity and are regulated in a redox-sensitive fashion, we tested the effect of myeloperoxidase on Cx43 expression and function.

Immunostainings for Cx43 revealed a complete absence of Cx43 immunoreactivity in the infarct zone of WT and

![Image](http://circres.ahajournals.org/)

**Figure 4. Connexin 43 (Cx43) integrity.** A, Immunoreactivity for Cx43 in cardiac sections of wild-type (WT) and Mpo−/− mice after left ventricular ischemia and 7 d of reperfusion (I/R) and 3 and 21 d on permanent ischemia (PI; green=Cx43, red=N-cadherin, blue=DAPI; scale bar=200 μm). Immunoreactivity for Cx43 and N-cadherin in the cardiac peri-infarct zone of WT and Mpo−/− animals subjected to (B) sham operation and 7 d I/R or (C) 3 and 21 d PI (red=N-cadherin, green=Cx43, blue=DAPI; scale bar=30 μm). D, Quantitative analysis of Cx43 immunoreactivity in the peri-infarct zone of WT and Mpo−/− mice on I/R or (E) 3 and 21 d PI (WT/Mpo−/− sham n=9/4, WT/Mpo−/− 7 d I/R n=7/11; D, WT/Mpo−/− sham n=6/11, WT/Mpo−/− 3 d PI n=6/7, WT/Mpo−/− 21 d PI n=6/7). F, Analysis of left ventricular Cx43 mRNA expression of sham, I/R, or PI hearts of WT and Mpo−/− mice. n=3 for all groups. *P<0.05, **P<0.01, ***P<0.001, by Kruskal–Wallis test followed by Bonferroni post hoc test. Mean±SEM is shown.
Mpo\textsuperscript{−/−} animals (Figure 4A). In the peri-infarct region, a significantly decreased signal for Cx43 was detected in WT I/R mice compared with sham-operated mice. In contrast, no reduction was recognized in Mpo\textsuperscript{−/−} I/R mice in comparison to sham-operated Mpo\textsuperscript{−/−} mice (Figure 4B and 4C). Accordingly, Mpo\textsuperscript{−/−} hearts revealed significantly higher immunoreactivity for Cx43 in the peri-infarct region 3 days and 21 days after PI induction than WT hearts (Figure 4D and 4E). In both models, I/R and PI, ventricular Cx43 mRNA expression levels did not differ between WT and Mpo\textsuperscript{−/−} animals pointing toward a post-translational effect of myeloperoxidase on Cx43 levels (Figure 4F).}

**MMP-7–Dependent Cx43 Degradation**

Studies in isolated adult cardiomyocytes confirmed a decreased content of Cx43 after incubation with myeloperoxidase. Strikingly, this effect was partly reverted by additional treatment with an inhibitor of MMP-7, an enzyme not only shown to directly bind and degrade Cx43 after myocardial ischemia but also to be activated by myeloperoxidase-derived hypochlorous acid (Figure 5A).\textsuperscript{7,8} In addition, activation of MMP-7 was significantly more abundant in the peri-infarct region of WT than in Mpo\textsuperscript{−/−} mice after I/R in vivo (Figure 5B). To further investigate the effect of myeloperoxidase-mediated Cx43 degradation via MMP-7, conduction patterns of a monolayer of iPSCMs were assessed by in vitro mapping analyses. Importantly, these cells are devoid of MMP-7 as assessed by immunoblotting and quantitative real-time analysis (Online Figure V). Spontaneously beating iPSCM showed homogeneous conduction patterns (Figure 5C) under control conditions. Addition of myeloperoxidase/H\textsubscript{2}O\textsubscript{2} or pro-MMP-7 alone

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**Figure 5.** Myeloperoxidase (MPO)-dependent effect of matrix metalloproteinase 7 (MMP-7) on connexin 43 (Cx43) and electric homogeneity. A, Immunoblot analyses of Cx43 in isolated cardiomyocytes on MPO treatment with and without additional MMP inhibitor treatment (sham: n=4; MPO: n=4; MMP inhibitor: n=4; MPO/MMP inhibitor: n=3, the original immunoblot is shown in Online Figure VIII). B, MMP-7 activity in the peri-infarct region of ischemia/reperfusion (I/R) wild-type (WT) and Mpo\textsuperscript{−/−} hearts (WT/Mpo\textsuperscript{−/−} sham n=5/5, WT/Mpo\textsuperscript{−/−} I/R n=10/8). C, Representative maps of spontaneous conduction of induced pluripotent stem cell–derived cardiomyocytes (iPSCMs) treated with NaCl (control), MPO/H\textsubscript{2}O\textsubscript{2}, pro-MMP-7, or MPO/H\textsubscript{2}O\textsubscript{2}/pro-MMP-7 with analysis of (D) inhomogeneity index, (E) enhanced absolute inhomogeneity, (F) variation coefficient of conduction, and (G) conduction velocity. Control: n=11, MPO/H\textsubscript{2}O\textsubscript{2}: n=5, pro-MMP-7: n=5, MPO/H\textsubscript{2}O\textsubscript{2}/pro-MMP-7: n=7. *P<0.05, **P<0.01, by Kruskal–Wallis test followed by Bonferroni post hoc test. Mean±SEM is shown.
had no effect on the observed patterns. However, concomitant treatment with pro-MMP-7/myeloperoxidase/H₂O₂ resulted in a severe disruption of conduction homogeneity as demonstrated by an increased inhomogeneity index (Figure 5D), an elevated absolute inhomogeneity (Figure 5E), an increased variation coefficient (Figure 5F), and a reduced mean conduction velocity (Figure 5G), indicative of diminished intercellular electric coupling among iPSCM. Conversion of pro-MMP-7 to active MMP-7 by myeloperoxidase-derived HOCl has been described before. Accordingly, incubation of the cardiac muscle cell line HL-1, which also lacks MMP-7 protein expression (Online Figure V), with myeloperoxidase/H₂O₂/pro-MMP-7 resulted in decreased Cx43 levels, whereas single treatment with myeloperoxidase/H₂O₂ or pro-MMP-7 did not induce Cx43 degradation (Online Figure VI).

**Ventricular Fibrosis**

Overall LV fibrosis and interstitial ventricular fibrosis (excluding the infarct scar) 7 days after I/R were profoundly lower in Mpo⁻/⁻ mice than in WT animals, as demonstrated by myocardial picrosirius red stainings for collagen deposition (Figure 6A through 6C; Online Figure VII). Similarly, Mpo⁻/⁻ hearts exhibited significantly less fibrosis after 21 days of PI, albeit overall LV fibrosis was again more pronounced than in the I/R model (Figure 6D and 6E; Online Figure VII).

**Fibroblast-to-Myofibroblast Transdifferentiation**

To determine myofibroblast accumulation in ventricular tissue, the main collagen-producing cell type in ventricular myocardium under pathological conditions, colocalization of the immunoreactivity for the fibroblast marker DDR-2, and the myofibroblast marker α-smooth muscle actin was assessed in cardiac sections 7 days after I/R and 5 days after PI. Indeed, the number of myofibroblasts was significantly lower in the infarct and peri-infarct region of Mpo⁻/⁻ hearts than in WT after I/R or PI. The total number of myofibroblasts was slightly but not significantly lower in WT I/R hearts than in WT PI hearts (P=0.151; Figure 7A and 7B). Immunoblot analyses revealed dose-dependent transdifferentiation of isolated cardiac fibroblasts to myofibroblasts on incubation with myeloperoxidase in vitro. Strikingly, this equaled the effect of PDGF (platelet-derived growth factor) treatment, an established inducer of fibroblast differentiation (Figure 7C). The incubation of fibroblasts with myeloperoxidase leads to a significantly increased expression of collagen type I compared with untreated cells (Figure 7D).

---

**Figure 6. Myeloperoxidase (MPO)-dependent fibrotic remodeling.** Picrosirius red stained cardiac sections of wild-type (WT) and Mpo⁻/⁻ mice (A) on ischemia and 7 d of reperfusion (I/R) or (D) 21 d of permanent ischemia (PI) with red areas indicating collagen deposition and analysis of (B) total left ventricular fibrotic area and (C) interstitial fibrosis; B and C, WT/Mpo⁻/⁻ 7 d I/R n=6/7 and (E) total left ventricular fibrosis after PI of WT and Mpo⁻/⁻ hearts. Mean±SEM is shown; WT/Mpo⁻/⁻ 21 d PI n=7/9. Scale bar=1 mm. *P<0.05, **P<0.01, by unpaired Student t test.
Figure 7. Myeloperoxidase (MPO)-dependent fibroblast transdifferentiation. A, Representative immunofluorescence stainings for fibroblast marker DDR-2 (discoidin domain-containing receptor 2; green) and myofibroblast marker α-smooth muscle actin (α-SMA; red) within the infarct area of wild-type (WT) and Mpo−/− mice after left ventricular ischemia and 7 d of reperfusion (I/R) and 5 d of permanent ischemia (PI; blue=DAPI; scale bar=200 µm). B, Quantitative analysis of myofibroblasts within the infarct and peri-infarct region after 7 d of I/R and 5 d PI; WT/Mpo−/− 7 d I/R n=11/15, WT/Mpo−/− 5 d PI n=5/5. C, Relative α-SMA protein expression of isolated cardiac fibroblasts after 8 h of MPO and PDGF (platelet-derived growth factor) treatment. D, Relative collagen I expression of isolated cardiac fibroblasts after 36 h of MPO treatment. E, Relative α-SMA protein expression of isolated cardiac fibroblasts after coculture with WT or Mpo−/− leukocytes. F, Relative phosphorylation of p38 mitogen-activated protein kinase (p-p38/p38 MAPK) in isolated fibroblasts on 15 min of MPO treatment. Original immunoblots are shown in Online Figures IX through XII. Mean±SEM is shown. C–F, n=4 independent experiments. *P<0.05, **P<0.01, ***P<0.001, by unpaired Student t test for B, D, and E and by Kruskal–Wallis test followed by Bonferroni post hoc test for C and F.
Furthermore, coculture of isolated fibroblasts with isolated WT leukocytes leads to a significantly more pronounced transdifferentiation of fibroblasts when compared with fibroblasts cocultured with leukocytes isolated from Mpo−/− mice (Figure 7E). Next, we tested whether myeloperoxidase induces p38 MAPK phosphorylation in isolated fibroblasts, an inducer of fibroblast-to-myofibroblast transdifferentiation. Indeed, Western blot analyses revealed an augmented amount of p38 MAPK phosphorylation on myeloperoxidase treatment, a process which was completely abolished on additional p38 inhibitor treatment or treatment with catalytically inactive myeloperoxidase (Figure 7F).

**Table 1. Baseline Characteristics of Patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n=2622)</th>
<th>Arrhythmias Negative (n=2448)</th>
<th>Arrhythmias Positive (n=174)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.1±10.6</td>
<td>62±10.6</td>
<td>63.7±11.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>65</td>
<td>65</td>
<td>69</td>
<td>0.311</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>30</td>
<td>31</td>
<td>27</td>
<td>0.364</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>74</td>
<td>74</td>
<td>76</td>
<td>0.569</td>
</tr>
<tr>
<td>Former/current smoker (%)</td>
<td>64</td>
<td>63</td>
<td>70</td>
<td>0.107</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>60 (50–65)</td>
<td>60 (50–65)</td>
<td>55 (50–60)</td>
<td>0.014</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>98 (80–119)</td>
<td>98 (80–120)</td>
<td>96 (78–116)</td>
<td>0.246</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>34 (28–41)</td>
<td>34 (28–41)</td>
<td>35 (28–41)</td>
<td>0.845</td>
</tr>
<tr>
<td>ACE inhibitors (%)</td>
<td>44</td>
<td>43</td>
<td>58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B-blocker (%)</td>
<td>60</td>
<td>60</td>
<td>59</td>
<td>0.988</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>57</td>
<td>57</td>
<td>61</td>
<td>0.314</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>75</td>
<td>75</td>
<td>74</td>
<td>0.861</td>
</tr>
<tr>
<td>MPO, pmol/L</td>
<td>502.5 (287.2–914)</td>
<td>496.7 (284.5–899.6)</td>
<td>593.1 (359.8–1014.4)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LV, left ventricle; and MPO, myeloperoxidase.

**Systemic Myeloperoxidase Levels Are Associated With Ventricular Arrhythmias**

To test whether myeloperoxidase is linked to the occurrence of arrhythmias in humans, we retrospectively measured circulating levels of myeloperoxidase in a high-risk cohort of stable patients undergoing elective diagnostic cardiac evaluations during coronary angiography and who were no candidates for primary prevention with an implantable cardioverter-defibrillator (ejection fraction >35%). Table 1 shows the baseline characteristics of the 2622 subjects included in the study cohort. The mean age of the population was 63.1±11.1 years; 76% of patients had coronary artery disease. Patients with arrhythmias were older, had a lower ejection fraction, and more often received angiotensin-converting enzyme inhibitors. Binary logistic regression analysis revealed that myeloperoxidase plasma levels are associated with a history of ventricular arrhythmias, sudden cardiac death, and/or implantable cardioverter–defibrillator treatment (odds ratio, 1.76; 95% CI, 1.19–2.6, highest versus lowest tertile) and remain correlated after adjustment for pertinent risk factors (odds ratio, 1.83; 95% CI, 1.23–2.73, highest versus lowest tertile; Table 2).

**Discussion**

Herein, it is shown that myeloperoxidase promotes pro-arrhythmic remodeling and ventricular arrhythmias after myocardial ischemia. Studies in different animal models of ischemia-related myocardial damage using Mpo−/− mice and studies using spontaneously beating iPSCMs reveal that myeloperoxidase augments arrhythmogenic LV remodeling as manifested in (1) breakdown of Cx43 by activation of MMP-7, and (2) enhanced ventricular fibrosis by transdifferentiation of fibroblasts, which ultimately leads to (3) pronounced electric conduction slowing and disruption of conduction homogeneity, and (4) increased susceptibility to VT.

The mechanisms underlying myocardial damage in I/R and PI are diverse, but neutrophils have long been regarded as a crucial component. The neutrophil-derived enzyme myeloperoxidase has been investigated in the context of ischemic myocardial damage. Vasilyev et al showed that myeloperoxidase depletion had no effect on infarct size after myocardial I/R, whereas Askari et al demonstrated preserved LV end-diastolic diameter and LV function in Mpo−/− mice.

**Table 2. Unadjusted and Adjusted OR for History of Ventricular Arrhythmia, Sudden Cardiac Death, and/or Implantable Cardioverter–Defibrillator**

<table>
<thead>
<tr>
<th>Serum MPO</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>&lt;354.1</td>
<td>354.1–736.23</td>
<td>≥736.23</td>
</tr>
<tr>
<td>Unadjusted OR</td>
<td>1</td>
<td>1.33 (0.88–1.99)</td>
<td>1.76 (1.19–2.6)*</td>
</tr>
<tr>
<td>Adjusted OR</td>
<td>1</td>
<td>1.38 (0.92–2.09)</td>
<td>1.83 (1.23–2.73)*</td>
</tr>
<tr>
<td>Event rate</td>
<td>43/864=4.98</td>
<td>58/893=6.49</td>
<td>73/865=8.44</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, systolic blood pressure, low-density lipoprotein, high-density lipoprotein, smoking, diabetes mellitus, coronary artery disease, and left ventricular ejection fraction. MPO indicates myeloperoxidase; and OR, odds ratio.

*P<0.01, by binary logistic regression analysis.
Instigated by these studies, we were able to show that myeloperoxidase also exerts proarrhythmogenic effects in the context of postischemic myocardial remodeling. We identified Cx43 degradation and increased LV fibrosis as underlying mechanisms for this observation. Cx43 is the principal gap junctional protein in the LV, which is critically linked to ventricular homogeneity of electric conduction and ventricular arrhythmias. As outlined above, an important mechanism of peri-infarct disruption of gap junctional integrity is MMP-7-dependent degradation of Cx43. MMP-7 has previously also been shown to be specifically activated by myeloperoxidase. Indeed, we were able to demonstrate reduced peri-infarct MMP-7 activity in Mpo−/− mice and abrogation of myeloperoxidase-dependent Cx43 degradation by inhibition of MMP-7 in isolated adult murine cardiomyocytes. The dependence of myeloperoxidase effects on MMP-7 is strongly underlined by in vitro mapping studies of iPSCMs. These cells do not express MMP-7, and indeed conduction homogeneity was not affected by myeloperoxidase and H2O2, alone but only after addition of myeloperoxidase/H2O2 and pro-MMP-7.

Fibrosis is regarded as a critical substrate for ventricular arrhythmias, and myeloperoxidase profibrotic effects, for example, by oxidative inactivation of plasminogen activator inhibitor-1, have previously been demonstrated. We therefore assessed ventricular fibrosis in our animal models and found that Mpo−/− mice were protected. Because activation of MMP-7 is not involved in profibrotic remodeling after infarction, we assessed fibroblast-to-myofibroblast transdifferentiation. Myofibroblasts maintain their secretory activity of collagen ≤90 days and are a significant component of progressive adverse cardiac remodeling. As evidenced by immunostainings of myocardial sections and functional in vitro studies, we could demonstrate myeloperoxidase-dependent fibroblast transdifferentiation and could link this effect to myeloperoxidase-derived HOCl-dependent p38 MAPK activation, thus disclosing a novel, additional profibrotic property of myeloperoxidase.

Ion channel alterations in the peri-infarct region have been demonstrated to be of relevance for ischemia-related cardiac arrhythmias. We have previously shown that myeloperoxidase does not affect AP characteristics in isolated cardiomyocytes. Because studies on isolated cardiomyocytes only crudely approximate the conditions of cells integrated in the myocardial cell network within the peri-infarct region, we performed sharp electrode measurements on living tissue slices of infarcted murine hearts and found no relevant differences between hearts from WT and Mpo−/− mice. However, Mpo−/− hearts showed an increased susceptibility to voltage-gated sodium channel inhibition by tetrodotoxin as manifested by a decreased AP amplitude. This points toward post-translational modifications of voltage-gated sodium channels by myeloperoxidase, which could be responsible for differences in susceptibility to arrhythmias. Although further investigation of this interesting observation is clearly warranted, the absence of an effect of myeloperoxidase deficiency on APs in untreated tissue slices and the strong effects of myeloperoxidase deficiency on Cx43 and fibrosis together with the reduced epicardial conduction velocity suggest a more dominant role for these latter mechanisms in the context of this study.

It is of great interest for myeloperoxidase mode of action that infiltration of neutrophils seems to be required for accumulation of myeloperoxidase in the ventricular myocardium as suggested by our studies on CD11b−/− mice and myeloperoxidase supplemented Mpo−/− mice. Indeed, this observation could provide an additional explanation for the limited usefulness of myeloperoxidase as a biomarker for myocardial infarction because myeloperoxidase plasma levels might only partially correlate with local abundance of myeloperoxidase.

Certainly, this study bears some limitations. It remains open whether the mechanisms revealed herein will finally affect mortality in human pathophysiology. As shown previously, this animal model is not yielding significant mortality—in fact, we and others did not observe any deaths of mice on 3 months after PI. Moreover, the complex pathogenesis of ventricular arrhythmias on myocardial ischemia, which is based on electric remodeling of ion channels and calcium kinetics, can only be in part appreciated in a mouse model. However, mouse models have been widely used and have allowed broad insight into the pathogenesis of VTs when focusing on connexin-dependent electric homogeneity and fibrosis. Given the fact that myeloperoxidase is much less expressed in murine polymorphonuclear leukocytes when compared with human neutrophils, the observed effects potentially underestimate the role of myeloperoxidase in this disease in humans. In addition, it clearly has to be assumed that next to the specific effects of myeloperoxidase on Cx43 and fibroblast transdifferentiation, which were evident in the absence of neutrophils in our in vitro experiments, myeloperoxidase promotes perpetuation of local inflammation by its chemoattractant and neutrophil-activating properties. In addition, other pathways, such as oxidative inactivation of plasminogen activator inhibitor-1, are known to contribute to the observed effects of myeloperoxidase on fibrotic remodeling, as has previously been demonstrated. Many limitations also apply to our clinical observational data. Some baseline parameters were not balanced between both groups, which can only partly be corrected by multivariate analysis. In addition, myeloperoxidase levels were only examined in subjects subjected to coronary angiography at 1 point in time at a single tertiary referral center: therefore, we cannot exclude selection bias for patients undergoing diagnostic cardiac catheterization. Given these limitations, the data can only be regarded as a pilot study and should be confirmed in independent cohorts. In addition, whether the prognostic value of myeloperoxidase would be further increased on serial myeloperoxidase assessments remains unknown. Furthermore, the implementation of myeloperoxidase as a biomarker to predict VT seems problematic because of the fact that plasma myeloperoxidase levels will only in part reflect myocardial myeloperoxidase activity. Thus, the clinical usefulness of myeloperoxidase as a biomarker for arrhythmic events cannot be derived from the present data and needs to be further studied. However, the current data set comprises the largest population tested for an inflammatory biomarker indicating ventricular arrhythmogeneity, which in our view confirms the mechanistic data reported herein.

In conclusion, the current data reveal that myeloperoxidase affects electric conduction in the ischemic LV and...
thereby increases the vulnerability for ventricular arrhythmias. Mechanistically, myeloperoxidase impairs CX43 integrity through the activation of MMP-7 and induces fibrotic remodeling by stimulating fibroblast transdifferentiation to myofibroblasts through activation of p38 MAPK. These results not only indicate that the innate immune system and leukocytes exert proarrhythmogenic properties, but also point toward myeloperoxidase as a potential pharmacological target in this disease.

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Disclosures

S.L. Hazen reports being named as coinventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics; having been paid as a consultant for the following companies: Esperion and P&G; receiving research funds from Abbott, P&G, Pfizer Inc, Roche Diagnostics, and Takeda; and having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland Heart Lab, Siemens, Esperion, and Frantz Biomarkers, LLC. The other authors report no conflicts.

References


Myeloperoxidase Mediates Postischemic Arrhythmogenic Ventricular Remodeling

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Online Methods

Human MPO plasma levels
The study was approved by local ethics committees and was conducted in accordance to the Declaration of Helsinki and GCP requirements. All individuals gave written informed consent prior to inclusion in the study. Sequential stable subjects undergoing elective diagnostic coronary angiography were eligible. Subjects with recent myocardial infarction (within preceding 1 month, or positive troponin I testing of baseline sample) were excluded. Blood was taken at time of coronary angiography prior to administration of heparin. Samples in serum separator tubes were maintained at room temperature for 45 min to permit clot formation, and then placed in ice/water bath until processing, aliquotting and freezing at -80C within 4 hours of sample collection. All event data was adjudicated using medical records as source. Serum myeloperoxidase levels was performed by CardioMPO (Cleveland Heart Lab, USA) assay on autoanalyzer according to manufacturer instructions.

Animal studies
Male, 8- to 12-week old Mpo−/− mice (C57bl/6J background, Jackson Laboratory, Bar Harbor, ME, USA), CD11b−/− mice (Igam tm1-Myd/J, C57bl/6J background, Jackson Laboratory, Bar Harbor, ME, USA) and wildtype littermates (WT) were used for all animal studies. The strategy for the generation of Mpo−/− mice and CD11b−/− mice has been previously reported1,2. All animal studies were approved by the Universities of Hamburg and Cologne Animal Care and Use Committees and follow ARRIVE guidelines. Animals underwent permanent ligation of the left anterior descending artery (LAD) or LAD ligation and reperfusion followed by investigation or treatments described below. Animals subjected to sham surgery were used as healthy controls.

Left anterior descending artery ligation
Mice were anaesthetized by intraperitoneal injection of 0.5 mg/g bodyweight avertin (2-2-2-tribromoethanol, Sigma-Aldrich, St. Louis, MO, USA) with temperature kept constant using a rectal thermometer and an electric warming pad. Animals were placed in a supine position, intubated under direct laryngoscopy with a 22 gauge Angiocath and ventilated using a small animal respirator (Harvard Apparatus, USA; tidal volume: 0.1 ml per 10 g mouse body weight, ventilation rate: 170/min). Surgical procedures were carried out using a dissecting
microscope (Leica MZ6, Leica Microsystems, Wetzlar, Germany). After lateral thoracotomy of the fourth intercostal space, a suture (8/0 polypropylene suture, Polypro, CP Medical, USA) was placed around the left coronary artery after retraction of the left atrial appendage and the artery was ligated with a bow tie. For I/R experiments the ligation was removed after 30 min to allow for reperfusion, the thorax was closed and the animals were allowed to recover. For PI experiments the LAD was ligated permanently without reperfusion. For I/R experiments with CD11b-/− mice the ligation was removed after 40 min to allow for 2 days of reperfusion.

**Right ventricular stimulation**

Mice were anaesthetized with isoflurane and placed in supine position on a heating pad. An octapolar electrophysiological catheter (1.1 F, Scisense) was inserted via the right jugular vein to the right atrium and ventricle. Surface ECG was analyzed under stable baseline conditions for at least 3 min. Electrophysiological investigation with induction of arrhythmias was performed as described previously3. Intracardiac atrial and ventricular recording and ventricular stimulation maneuvers were performed using a CardioTek EPTracer (Biotronik). Bipolar field potentials were obtained from each electrode pair during the whole procedure. Programmed ventricular stimulation was performed at a pacing stimulus amplitude of 1.0 mA with 7 stimuli fixed rate at S1S1 cycle length of 120 ms, 110 ms and 100 ms, respectively, with one short coupled extra stimulus with a 10 ms-stepwise S1S2 reduction starting at cycle length of 80 ms down to 10 ms. Ventricular burst stimulation was performed for 1 sec (three times consecutively) at S1S1 stimulation cycle lengths starting at 50 ms with 10 ms stepwise reduction down to 10 ms at pacing stimulus amplitudes of 1.0 mA and 2.0 mA. Between these stimulation procedures, a 10 sec recovery period was maintained. Ventricular tachycardia (VT) was defined as a series of repetitive ventricular ectopic beats lasting for more than 200 ms.

**Implantation of ECG transmitters, ECG-telemetry, arrhythmia provocation**

Mice were anaesthetized with isoflurane via mask ventilation and placed on a heating pad (37°). The abdominal skin was depilated and disinfected and a 2 cm long median incision was made. The ECG-transmitter (Data Sciences International, ETA-F10) was placed into the peritoneal cavity and the electrodes were lead through the peritoneum. The negative electrode was fixed to the right pectoralis fascia and the positive electrode was fixed 1 cm left to the xiphoid. The abdomen was closed with resorbable sutures. Buprenorphine 0.01 mg/kg s.c.
was used before and for postoperative analgesia (30 min). For baseline investigations recordings were started after a recovery time of 5 days for 3 hours. After baseline recordings mice were subjected to LAD ligation as described above. Recording and analysis parameters were set according to the manufacturer’s instructions using P3 Plus software (DSI).

24 hrs after LAD ligation arrhythmia provocation was performed by double injections of isoproterenol (ISO, i.p. 2 mg/kg) separated by an interval of 30 minutes; analyses were performed after a recovery time of 15 min for 2 hours after the first ISO injection. Ventricular tachycardia was defined as a series of repetitive ventricular ectopic beats lasting for more than 200 ms and were identified by two independent cardiologists according to Walker et al.4.

**In-vivo electrophysiological mapping**

After 7 d of reperfusion mice were anaesthetized with isoflurane and placed in supine position on a heating pad. The heart was exposed by thoracotomy. A 32-electrode microelectrode array (MEA, Multichannel Systems, Reutlingen, Germany) was positioned on the epicardial surface of the left ventricle apico-septally of the region of I/R damage (periinfarct region). To do so, the infarcted area, which was identified by tissue swelling and darkening adjacent to the site of suture, was microscopically determined for each mouse after careful lateral reopening of the chest. The mapping electrode was placed between ventricular septum and the infarct region. For stimulated mapping analysis the pacing electrode was placed directly laterally of the mapping electrode next to the border of the infarcted region in fiber direction5,6. Field potentials were recorded using a 128-channel, computer-assisted recording system (Multichannel Systems) with a sampling rate of 25 kHz (25,000 samples per second). Data were bandpass filtered (50 Hz), digitized with 12 bit and a signal range of 20 mV. Activation maps were calculated from these data using custom-programmed software (Excel). The first derivative of each unipolar field potential was evaluated and maximal slope of dV/dt activation was defined as timepoint of local activation for spontaneous stimulation and the minimum of dV/dt activation for pacing studies respectively7. To obtain an index of local conduction slowing for each electrode, the activation time differences to neighbouring points were normalized to interelectrode distance (300 µm for vertical and horizontal, 424.26 µm for diagonal adjacent electrodes). The largest difference at each site was defined as local phase delay. These phase delays were used for further calculation of the inhomogeneity index in global conduction, the absolute inhomogeneity, velocity and the variation coefficient as described before8–10. For pacing studies the hearts were stimulated with a concentric bipolar
electrode (FHC, Bowdoin, USA) with a stimulation rate of 10 Hz at the left ventricular lateral wall next to the microscopically determined I/R region (see above).

**In-vitro electrophysiological mapping of iPSCM**

Murine iPSCM differentiation and purification is described elsewhere. IPSCM of day 16 were cultured on a collagen-coated 120-electrode microelectrode (120pMEA200/30iR-Ti, Multichannel Systems, Reutlingen, Germany) for 5 days at high density (50000 cells / µl). Viability and contraction were checked microscopically. Cells were treated with either PBS, MPO (10 µg/ml) + H2O2 (80 µM) or MPO (10 µg/ml) + H2O2 (80 µM) + pro-MMP-7 (1 µg/ml, MMP-7, proenzyme, human, recombinant, E.coli, Merck Millipore, Cat.No: 538540) for 24 hrs. Reagents were incubated for 3 hrs at 37°C to ensure full MMP-7 activation, before they were added to the cells. Field potentials were recorded using a 128-channel, computer-assisted recording system (Multichannel Systems) with a sampling rate of 25 kHz (25,000 samples per second). Data were bandpass filtered (50 Hz), digitized with 12 bit and a signal range of 20 mV. Activation maps were calculated from these data using custom-programmed software (Excel). The first derivative of each unipolar field potential was evaluated and minimum of dV/dt activation was defined as timepoint of local activation for spontaneous stimulation. Electrodes, which received non-analysable signals, were excluded automatically by the recording system (white circles). To obtain an index of local conduction slowing for each electrode, the activation time differences to neighbouring points were normalized to interelectrode distance (200 µm for vertical and horizontal). The largest difference at each site was defined as local phase delay. These phase delays were used for further calculation of the inhomogeneity index in global conduction, the absolute inhomogeneity, velocity and the variation coefficient as described before.

**Echocardiographic studies**

Transthoracic echocardiography was performed using the Vevo 2100 System (VisualSonics, Toronto, Canada). B-mode recordings were performed using a MS 400 transducer (18–38 MHz) with a frame rate of 230–400 frames/s to assess left ventricular (LV) dimensions. All images were recorded digitally and analysis was performed using the Vevo 2100-software. Ejection fraction and LV end diastolic dimension were calculated as described before.

**Determination of fibrotic area**
Hearts were excised, fixed in 3.7% formaldehyde solution for 2 days and embedded in paraffin. Consecutive cross sections of 2 µm were cut from apical to base every 200 µm. Sections were stained with picrosirius red following standard protocols. Images were acquired using a DP25 camera (Olympus, Hamburg, Germany) mounted on a BX51 microscope (Olympus). The area of fibrosis in percent of left ventricular wall area was quantified using Cell A software (Olympus). Mean fibrotic area of 12 sections was calculated, respectively. Interstitial fibrosis was calculated as: [fibrosis of left ventricular wall area - fibrosis of infarct scar area].

**Determination of infarct size**

Upon right ventricular stimulation, hearts were harvested and cut into 1 mm thick cross sections and incubated in 1.0% 2,3,5-triphenyl-tetrazolium chloride solution (TTC, Sigma-Aldrich) for 30 min at 37°C. Images of stained sections were acquired using a Prosilica GC camera (Allied Vision Technologies, Stadtroda, Germany) mounted on a dissecting microscope (Leica MZ6, Leica Microsystems, Wetzlar, Germany). Infarct area was assessed by planimetry using BZ2-Analyser software (Keyence, Osaka, Japan).

**Isolation and treatment of adult ventricular cardiomyocytes**

Mice were sacrificed by cervical dislocation after pretreatment with 10 IU of intraperitoneal heparin and inhalative general anaesthesia with isoflurane. Hearts were excised and mounted on a constant-flow (3 ml/min) Langendorff circulation (Radnoti Ltd., Ireland) and retrogradely perfused with calcium-free solution with a constant temperature between 36°C and 37°C. Subsequently, the solution was switched to an enzyme solution containing collagenase I and II (1.5 mM Liberase TM, Roche, Mannheim, Germany) and 0.24 mM trypsin (Invitrogen, Darmstadt, Germany). Hearts were then transferred into a stop solution containing 10 mM fetal bovine serum and filtered through a 250 µm mesh cell collector at room temperature. Subsequently, the calcium-content of the solution was uptitrated to a final concentration of 1.6 mM.

Cells were incubated with MPO (10 µg/ml, Planta Natural Products, Vienna, Austria) and H₂O₂ (40 µM, Sigma) and/or an inhibitor to MMP-7 (100 nM, Calbiochem, USA) as indicated for 30 min. Cells were washed twice with PBS, centrifuged and lysed in 0.1% Triton X-100 in PBS, supplemented with 10x EDTA-free Protease Inhibitor Tablets and 10x PhoSTOP (Roche Diagnostics, Basel, Switzerland) and lysates were processed for western blotting as described below.
MPO plasma level
Blood was drawn in deep isoflurane anaesthesia of mice by heart puncture into heparinized syringes and centrifuged for 10 min with 1,300 x g. Plasma was analyzed for MPO using Mouse MPO ELISA (Hycult biotech, Uden, The Netherlands) according to manufacturer’s instructions.

Staining for polymorphonuclear neutrophil infiltration
Hearts were frozen in OCT compound and cut to 4 µm sections. Frozen heart specimen were fixed with acetone. Sections were incubated with rat anti-mouse neutrophil Ly6g primary antibody (1:40, Hycult biotech) and endogenous peroxidase activity was blocked. Secondary antibody was horseradish peroxidase (HRP)-labeled rabbit anti-rat (1:100, Dako, Glostrup, Denmark) and tertiary antibody was HRP-labeled goat anti-rabbit (1:500, Vectorlabs, Burlingame, USA) in 3% normal mouse serum, respectively. PMN were stained with AEC solution and tissue was counterstained with hematoxyline. Images were acquired using a Prosilica GC camera (Allied Vision Technologies) mounted on a Leica DMLB light microscope. Results were shown as Ly6G⁺ area in % of the LV tissue area.

Immunofluorescence staining for α-SMA
Hearts were frozen in OCT compound and cut to 4 µm longitudinal sections. Sections were thawed, fixed with 3.7% formaldehyde solution and were blocked with 10 % mouse serum. Slides were treated with 0.1% Triton X-100 and incubated with primary antibody against α-SMA (1:200, rabbit IgG, ab5694, Abcam, Cambridge, UK) and DDR-2 (1:50, goat IgG, sc7555, Santa Cruz, Texas, USA) respectively for 1 hr at RT in PBS with 0.1 % Triton-X100 and 10% mouse serum. Secondary antibody was Alexa Fluor-594 chicken-anti-rabbit IgG and Alexa Fluor-488 chicken-anti-goat IgG (Invitrogen) and nuclei were stained with DAPI. Images were taken with a Retiga 1300 CCD camera mounted on Leica DMLB fluorescence microscope by iVision v4.0.

Fibroblast isolation and stimulation
WT mice were sacrificed, ventricles were removed and washed in sterile HEPES-buffered Tyrode's solution (135 mM NaCl, 4 mM KCl, 0.3 mM NaH₂PO₄, 1 mM MgCl₂, 10 mM HEPES, 2 g/l glucose, pH = 7.3, Sigma-Aldrich, St. Louis, USA). Ventricles were minced
and digested in 0.1 g/l Liberase/Tyrode solution (Liberase TM research grade, Roche, Basel, Switzerland) for 10 minutes at 37°C. The supernatant was collected and the digestion step repeated 6 times. The supernatant was filtered (40 µm cell strainer, Thermo Fisher Scientific, Waltham, USA) centrifuged and the fibroblasts resuspended in DMEM supplemented with 10% FCS. The cells were again centrifuged and incubated with growth medium (DMEM high glucose (4.5 g/l) without L-glutamine (GE-Healthcare, Munich, Germany) containing penicillin (100 U/ml, Invitrogen, Darmstadt, Germany), streptomycin (100 µg/ml, Invitrogen, Darmstadt, Germany) and 2% FBS) for 18 hrs at 37°C with 5% CO₂. The medium was removed and cells were washed with DPBS (Gibco, Darmstadt, Germany) at room temperature. Next, cells were incubated with growth medium plus various stimulants for 8 hrs at 37°C with 5% CO₂. For the analysis of p38 mitogen-activated protein kinase (MAPK) incubation time was reduced to 15 min with or without 10µM p38-Inhibitor (SB203580, Sigma, Cat.No: S8307) incubation. For the analysis of collagen type I incubation time was extended to 36 hrs.

Unless otherwise indicated fibroblasts were treated with 10 µg/ml catalytically active or inactive MPO, and 20 µM H₂O₂ for 8 hours. For leukocyte co-culture heparinized full blood samples of WT and Mpo⁻/⁻ mice were treated with erythrocyte-lysis buffer (0.83% NH₄Cl, 0.1% KHCO₃, 0.1 mM EDTA pH = 7.4) for 8 min at 4°C and leukocytes were collected after centrifugation in growth medium. Cultured fibroblasts were incubated with leukocytes for 8 hrs at 37°C with 5% CO₂.

**Western blot for fibroblast analyses**

Cultured cells were washed with DPBS (Gibco) and collected in homogenization buffer (20 mM Tris-HCl pH=7.5, 250 mM sucrose, 20 mM EDTA, 3 mM EGTA, supplemented with 10x EDTA-free Protease Inhibitor Tablets and 10x PhoSTOP (Roche Diagnostics) containing 0.1% SDS using cell scrapers. The cell lysates were vortexed every 1 min for 10 min at 4°C. The lysates were centrifuged at 4000 rpm for 10 min at 4°C. Supernatants were collected, diluted with Laemmli loading buffer and heated at 95°C for 5 min. Protein levels were determined using BCA Assay (Thermo Scientific).

Protein samples were diluted in Laemmli buffer and loaded on a 10% SDS-polyacrylamide gel. Electrophoresis was performed at 100 V and samples were transferred to a nitrocellulose membrane at 200 mA for 2 hrs. The membrane was blocked for 1 hr at room temperature. The primary antibody was incubated overnight at 4°C and the corresponding secondary antibody for 1 hr at room temperature. Primary antibodies were α-SMA (ab5694, 1:200, Abcam),
Western blot for connexin43

HL-1 cells\textsuperscript{16} were lysed in lysis buffer (20 mM Tris-HCl pH 7.5, 250 mM sucrose, 20 mM EDTA, 3 mM EGTA, 0.1\% Triton X-100, supplemented with 10\% EDTA-free Protease Inhibitor Tablets and 10\% PhoSTOP (Roche Diagnostics) using the Tissue Lyzer (Qiagen). Homogenates were centrifuged at 14,000 g (4\(^{\circ}\)C, 10 min) and the supernatant was recovered. Proteins of heart homogenates or cardiomyocyte lysates were separated by SDS-PAGE and transferred to nitrocellulose membranes. After blocking with either 5\% nonfat milk or 5\% BSA in TBST (20 mM Tris-HCl pH 7.5, 137 mM NaCl, 0.1\% (v/v) Tween 20), membranes were incubated with primary antibodies to connexin43 (C6219-2ML, 1:2000, Sigma-Aldrich, Mannheim, Germany) or GAPDH (1:5,000, Cell Signaling Technology), followed by HRP-conjugated secondary antibodies (1:10,000, Vectorlabs, Burlingame, USA) and chemiluminescence signals were detected on film and analyzed densitometrically with ImageJ. Cardiac HL-1 cells were treated with either PBS, MPO (10 \(\mu\)g/\(\mu\)l) + \(\text{H}_2\text{O}_2\) (80 \(\mu\)M) and/or pro-MMP-7 (1 \(\mu\)g/ml) respectively for 16 hours. Cells were lysed and protein was collected for Western blot analyses as described above.

Immunofluorescence staining for connexin43

Hearts were frozen in OCT compound and cut to 4 \(\mu\)M longitudinal sections. Sections were thawed, fixed with 3.7\% formaldehyde solution and were blocked with 10\% mouse serum. Slides were treated with 0.1\% Triton X-100 and incubated with primary antibody against connexin43 (1:250, rabbit IgG, Sigma-Aldrich) and N-cadherin (1:70, goat IgG, Santa Cruz, Texas, USA) for 1 hr at RT in PBS with 0.1\% Triton-X100 and 10\% mouse serum. Secondary antibody was Alexa Fluor-488 chicken-anti-rabbit IgG and Alexa Fluor-594 chicken-anti-goat IgG (Invitrogen) and nuclei were stained with DAPI. Images were taken with a Retiga 1300 CCD camera mounted on Leica DMLB fluorescence microscope by iVision v4.0. The infarct region was identified by total absence of connexin43 immunoreactivity. The evaluation of connexin43 integrity was performed in direct proximity analysing one field of view in 40x magnification around the edge of the infarct, respectively. The person evaluating the images was blinded to the experimental groups.
Connexin43 and ion channel mRNA analyses

For connexin43 analyses left ventricular heart tissue of sham, I/R or PI mice was collected. RNA was isolated using an RNA isolation kit (RNeasy Mini Kit (250), Qiagen, Cat.No: 74106) according to manufacturer’s instruction. First strand synthesis with a High Capacity cDNA Reverse Transcription Kit (8200rcn, Applied Biosystems, Cat.No: 7368814) was performed according to manufacturer’s instructions and qRT-PCR was performed with a Sso Fast(TM) Eva Green Supermix with Low ROX ((500rcn), BioRad, Cat.No: 172-5211) at an annealing temperature of 60°C in a C1000 Touch Thermal Cycler (CFX96 Real-Time System, BioRad). CT-values of Cx43 were normalized to CT-values of GAPDH.

Primers:
Fwd GAPDH:  5´-tccactcttccaccttc-3´
Rev GAPDH:  5´-ctgtagccgtattcattg-3´
Fwd Cx43:  5´- gagagccgcaactctcttt-3´
Rev Cx43:  5´- tggagtagctgtggaccttg-3´

For ion channel analyses left ventricular heart tissue of sham or PI mice (3 days) was collected and analyses were performed as described above.

Primers
Scn5a  Fwd: gacccagaggactgttcac, Rev: gagcageatcetcaacacaa
Cacna1c Fwd: aggtcactgtgggcaagttc, Rev: ccactctcctcacagcagtc
Cacna1g Fwd: ggcaagtcccaactatgagct, Rev: tccaggtgctcagaggaact
Kcnd2 Fwd: gggaagccgaactctcat, Rev: ttttgtctcctcactagcag
Kcnd3 Fwd: gcagaagccacactctcat, Rev: agggtgctgacagagaagga
Kcna4 Fwd: gcagagacacagaggtgtt, Rev: gttccttcctcagacact
Kcna7 Fwd: gatcaggtcagacagcag, Rev: atgcagactagcagagcagt
Kcn4 Fwd: aatcgcctctctcacta, Rev: agctccctctcagacact
Kena5 Fwd: cgtactctgatcctcttgag, Rev: cttctgctctgagcctgaggt
Knc1 Fwd: tggcaggggaagagactag, Rev: tattcttctccagaggttgagg
Kchn2 Fwd: tctgggaggaagaagcaatc, Rev: gcctctctctctctctcag
Kcnq1 Fwd: atggctttctggtcttat, Rev: gcgcagagcagagaggaag
Kcnj2 Fwd: gtcgctctctctcagcact, Rev: tccgctctctctcagcacta
Kcnj12 Fwd: ctgctctagcctcttcctttcgag, Rev: atgggctcagcagttt
Kcnj11 Fwd: ctgctcttcctcgctctct, Rev: tccaggtgctcagaggaag
Kcnj3 Fwd: tccgctctctctcagcact, Rev: gcgcctcctctcagcacta
Kcnj5 Fwd: aagatcagcagcagcaagaa, Rev: gagaagatcagagggaggaaga
Kcnk1 Fwd: caagcagggctcagcagag, Rev: tcaagcagcagcagcagag
Kcnk6 Fwd: aggctcagggcttcagcag, Rev: gagaagatcagagggaggaaga
Kcnk3 Fwd: gcaggtgtgtctcactggtc, Rev: gttgagggatgtaagcagt
Kcnk4 Fwd: ggtcgactgtcgaagcgagctgta, Rev: gagaattggtggcagagtaag
Hcn2 Fwd: tccaccaggtggagagagtt, Rev: aagagcagcagagcagag
**Ventricular slices and AP recordings by sharp electrode**

Short axis ventricular slices were prepared as described before. After excision of the heart, ventricles were separated from the atria, perfused with ice cold Ca\(^{2+}\)-free tyrode solution (composition in mM: NaCl 136, KCl 5.4, NaH2PO4 0.33, MgCl2 1, glucose 10, Hepes 5, 2,3-butanedione monoxime (BDM) 30; adjust pH to 7.4 with NaOH) and embedded in a 4% low-melt agarose block. The block is placed on a microtome with vibrating blade, and tissue slices with a thickness of 300 µm were prepared. Slices were stored in ice cold tyrode solution with 0.9 mM Ca\(^{2+}\) (composition in mM: NaCl 136, KCl 5.4, NaH2PO4 0.33, MgCl2 1, glucose 10, Hepes 5, BDM 30, Ca2Cl 0.9; adjust pH to 7.4 with NaOH) at 4 °C for 30 minutes. Afterwards, slices are transferred to 37°C warm DMEM for 30 min to wash out the BDM before further use.

Intracellular action potential recordings (APs) were performed in ventricular slices with sharp electrodes (20-40 MΩ resistance when filled with 3 mol/l KCl) made of borosilicate glass capillaries (WPI, Sarasota, USA) as described before. Signals were amplified with a SEC-10LX amplifier (npi electronic, Tamm, Germany) and recorded with the Pulse software (HEKA, Lambrecht/Pfalz, Germany). Obtained data were analyzed offline with the Mini Analysis program (Synaptosoft, Fort Lee, USA). All measurements were performed at 37°C in DMEM bubbled with carbogen (5% CO\(_2\) and 95% O\(_2\)). For inhibitor experiments DMEM was further supplemented either with BaCl\(_2\) (Sigma Aldrich, Germany) or Tetrodotoxin (Abcam, ab120054, Germany) as indicated.

**MMP-7 activity**

Mice were sacrificed by cervical dislocation, hearts were excised and left ventricular tissue was immediately dissected to infarct and periinfarct tissue using a dissection microscope (Leica). Periinfarct tissue was analyzed for MMP-7 activity using the SensoLyte 520 MMP-7 Assay Kit (Anaspec, Fremont, CA, USA) following manufacturer’s instructions protocol B without pro-MMP activation.

**Retroorbital MPO injection and MPO staining**
Mpo−/− mice were anaesthetized with isoflurane and subjected to I/R as described above. Still under anesthesia the mice received a first retroorbital saline or MPO injection (7µg in 100µl saline) directly after initiation of reperfusion. Each day, the MPO injection was repeated under isoflurane anaesthesia for 7 days and electrophysiological investigation was performed as described above.

For investigations of intraventricular MPO deposition WT and Mpo−/− mice were subjected to I/R for 3 days. WT mice received retroorbital saline injection, Mpo−/− mice were daily treated with saline or MPO (7 µg) for 3 days. Hearts were frozen in OCT compound and cut to 4 µM longitudinal sections. Sections were thawed, fixed with 3.7% formaldehyde solution and were blocked with 10% mouse serum. Slides were treated with 0.1% Triton X-100 and incubated with primary antibody against MPO (1:250, rabbit IgG, Calbiochem) for 1 hr at RT in PBS with 0.1% Triton-X100 and 10% mouse serum. Secondary antibody was Alexa Fluor-488 chicken-anti-rabbit IgG and nuclei were stained with DAPI. Images were taken with a Retiga 1300 CCD camera mounted on Leica DMLB fluorescence microscope by iVision v4.0.

**MMP-7 Western Blot**

iPSCM cells were cultured and cells were lysed at day 16 and protein was collected as described above. MMP-7 protein was detected with an Anti-MMP-7 antibody (1:500 Abcam, Cat.No: ab4044).

**Plasma cytokines**

Blood was drawn in deep isoflurane anaesthesia of mice by heart puncture into heparinized syringes and centrifuged for 10 min with 1,300 x g. Plasma was analyzed for cytokines by using a LEGENDplex Mouse Inflammation Panel (13-plex) (Biolegend, San Diego, USA) according to manufacturer’s instructions.

**Statistical analyses**

Results are expressed as mean ± SEM. Statistical analysis was performed, unless otherwise indicated, using Kruskal Wallis test followed by Bonferroni post hoc test, Mann-Whitney U test or unpaired Student’s t-test as appropriate. Univariate and multivariate logistic regression analysis were used to determine independent predictors of arrhythmia (defined as history of ventricular arrhythmia, sudden cardiac death and/or implantable cardioverter defibrillator placement), adjusting for traditional cardiac risk factors such as age, sex, systolic blood pressure, low-density lipoprotein, high-density lipoprotein, and cigarette smoking. All
analyses were performed using JMP Pro version 10 (SAS Institute, Cary, North Carolina) and R (3.1.2, Vienna, Austria). A p-value < 0.05 is considered statistically significant. All other calculations were carried out using SPSS version 23.0.* = P < 0.05, ** = P < 0.01, *** = P < 0.001.

References


Supplemental Material

Online Figures
Online Figure I: Plasma cytokine levels of WT and Mpo$^{-/-}$ mice upon ischemia and reperfusion (I/R) or permanent ischemia (PI) determined by FACS multiplex analyses. n=3/4/3/4/5/5. Bars without error bar indicate measurements below the detection limit.
Online Figure II: (A) Representative pictures of the infarcted regions of $Mpo^{-/-}$ and WT (right panel) mice subjected to 3 days I/R. Immunohistochemical MPO stainings (green) revealed the absence of MPO and MPO-containing cells within the infarct regions of $Mpo^{-/-}$ hearts after 3 days of retroorbital MPO injection (7 µg/day) as compared to WT hearts. Nuclei were stained with DAPI (blue). Scale bar=50 µm. n=5. Electrophysiological investigation of $Mpo^{-/-}$ animals subjected to ischemia and 7 days of reperfusion (I/R) with retroorbital MPO (7 µg/day for 7 days) injection. (B) Analysis of ECGs revealed no difference in number of VT episodes or (C) total length of episodes. n=5.
Online Figure III: Kaplan-Meier curves of WT and Mpo⁻/⁻ animals after permanent LAD occlusion revealed no differences of survival after 100 days. Animals which died during operation were excluded from the analysis. n=5.
**Online Figure IV:** Sharp electrode measurements of living cardiac tissue slides in the periinfarct region of WT and *Mpo*⁻/⁻ mice subjected to 7 days of permanent LAD ligation. Slides were measured before treatment (native) and incubated with the announced concentration of the K⁺-channel inhibitor BaCl₂ or the voltage-gated Na⁺-channel inhibitor tetrodotoxin (TTX). Mean ± SEM is given. * = $P (WT \, vs. \, Mpo^{-/-}) < 0.05$. n=6-3.
Online Figure V: Western blotting of induced pluripotent stem cell-derived cardiomyocytes (iPSCM) (d16) and HL-1 revealed very low levels of matrix metalloproteinase 7 (MMP-7) protein. Additionally, no MMP-7 mRNA expression could be detected by quantitative real-time PCR in iPSCM (data not shown). n=3.

Online Figure VI: Representative Western blottings of murine cardiac HL-1 cells treated with PBS, MPO + H₂O₂, pro-MMP-7, pro-MMP-7 + MPO + H₂O₂ revealed reduced connexin43 (Cx43) levels upon MPO-activated MMP-7 treatment. n=5; * = P < 0.05.
Online Figure VII: Picrosirius red staining for ventricular fibrosis after ischemia and 7 days of reperfusion (I/R) and 21 days of permanent ischemia (PI). Scale bar=50 µm.
**Online Figure VIII:** Representative original immunoblots of Cx43 in isolated cardiomyocytes upon MPO treatment with and without additional MMP-inhibitor treatment as indicated in Figure 5A.
**Online Figure IX:** Representative original immunoblots of α-SMA protein expression of isolated cardiac fibroblasts after 8 hours of MPO and PDGF treatment (Figure 7C).
Online Figure X: Representative original immunoblots of collagen I expression of isolated cardiac fibroblasts after 36 hours of MPO treatment (Figure 7D).
**Online Figure XI:** Representative original immunoblots of α-SMA protein expression of isolated cardiac fibroblasts after co-culture with WT or Mpo−/− leukocytes. (Figure 7E).
Online Figure XII: Representative original immunoblots of phosphorylation of p38 MAP-kinase (p-p38/p38 MAPK) in isolated fibroblasts upon 15 minutes of MPO treatment (Figure 7F).
Online Table I: Quantitative mRNA expression analyses with real-time PCRs of pertinent myocardial ion channels (Grant, Circ Arrhythmia Electrophysiol 2009) of isolated healthy heart tissue and infarct tissue of the left ventricle 3 days after LAD ligation. mRNA levels of Nav1.5, Cav1.2, KV1.5, KVLQT1, Kir2.1, Kir6.2, TASK-1 and HCN2 were detectable within the infarct region 3 days after permanent LAD ligation (1 equals 100% relative mRNA expression of WT baseline). Furthermore, the mRNA expression of Nav1.5, KV1.5, Kir6.2, TASK-1 and HCN2 was regulated after 3 days LAD ligation as compared to healthy hearts but there were no significant mRNA expression differences in WT as compared to Mpo⁻/⁻ hearts after infarction. Mean ± SEM is given.