The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction

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Aims
Myocardial infarction (MI) triggers an intense inflammatory response that is associated with infarct expansion and is detrimental for cardiac function. Interleukin (IL)-1β and IL-18 are key players in this response and are controlled by the NLRP3-inflammasome. In the current study, we therefore hypothesized that selective inhibition of the NLRP3-inflammasome reduces infarct size and preserves cardiac function in a porcine MI model.

Methods and results
Thirty female landrace pigs were subjected to 75 min transluminal balloon occlusion and treated with the NLRP3-inflammasome inhibitor MCC950 (6 or 3 mg/kg) or placebo for 7 days in a randomized, blinded fashion. After 7 days, 3D-echocardiography was performed to assess cardiac function and Evans blue/TTC double staining was executed to assess the area at risk (AAR) and infarct size (IS).

The IS/AAR was lower in the 6 mg/kg group (64.6 ± 8.8%, P = 0.004) and 3 mg/kg group (69.7 ± 7.2%, P = 0.038) compared with the control group (77.5 ± 6.3%). MCC950 treatment markedly preserved left ventricular ejection fraction in treated animals (6 mg/kg 47 ± 8%, P = 0.001; 3 mg/kg 45 ± 7%, P = 0.031; control 37 ± 6%). Myocardial neutrophil influx was attenuated in treated compared with non-treated animals (6 mg/kg 132 ± 72 neutrophils/mm², P = 0.035; 3 mg/kg 207 ± 210 neutrophils/mm²; P = 0.5; control 266 ± 158 neutrophils/mm²). Myocardial IL-1β levels were dose-dependently reduced in treated animals.

Conclusions
NLRP3-inflammasome inhibition reduces infarct size and preserves cardiac function in a randomized, blinded translational large animal MI model. Hence, NLRP3-inflammasome inhibition may have therapeutic potential in acute MI patients.

Keywords
Infarct size • Inflammation • Inhibitor • Myocardial infarction • Inflammasome • Cardiac function

Translational perspective
To protect patients after MI by preserving cardiac function, the development of novel therapeutics is essential. In the current study, we assessed the effect of interference with NLRP3-inflammasome signalling on infarct size and cardiac function in a porcine MI model. For the first time, we show that cardiac function is preserved and infarct size is reduced by attenuating inflammation through NLRP3-inflammasome inhibition in a large animal MI model, making interference with this specific signalling pathway a promising target in MI patients.
Introduction

Myocardial infarction (MI) is one of the most important causes of death worldwide. Improved treatment strategies for MI, including percutaneous coronary intervention, have led to better survival. However, patients with deteriorated cardiac function are at increased risk for heart failure (HF) and the improved survival of MI patients potentiates its incidence. Heart failure is accountable for a large fraction of cardiovascular deaths, is associated with a poor quality of life and extensive health care costs. Novel therapeutics that prevent HF post-MI through preserving cardiac function are therefore crucial.

Cardiac ischaemia-reperfusion triggers a sterile inflammatory reaction. Though essential for wound healing, this intense inflammatory response expands infarct size and deteriorates cardiac function. Infarct size and cardiac function are long-term predictors of adverse remodelling and HF. Immediate damage control by attenuating the post-MI inflammatory response could therefore be of great benefit in MI patients.

Interleukin (IL)-1β and IL-18 are potent mediators in the inflammatory response after MI and have been described to directly impair cardiac contractility in a synergistic way. Moreover, their levels predict the occurrence of adverse events in patients after MI and attenuation of IL-1β or IL-18 signalling has been shown to reduce infarct size and preserve cardiac function in rodent studies.

Interleukin-1β and IL-18 signalling is regulated by the NOD-like receptor, pyrin containing domain 3 (NLRP3)-inflammasome, an intracellular protein complex activated upon tissue injury. Consequently, NLRP3-inflammasome inhibition leads to pronounced infarct size reduction, attenuation of adverse remodelling, and preservation of cardiac function in small animal MI models. Although these studies provide important mechanistic insights, they do not reflect clinical application since they either involved knock-out models or pharmacological treatment preceding MI induction. To successfully translate these findings into clinical applications, preclinical testing in clinically relevant models is mandatory.

Until recently, this was precluded by the lack of selective pharmacological NLRP3-inflammasome inhibitors. MCC950 is a novel, selective small-molecule NLRP3-inflammasome inhibitor with powerful in vitro and in vivo inhibitory effects, enabling clinically relevant testing in large animal models. We therefore hypothesized that pharmacological interference with NLRP3-inflammasome signalling by administration of MCC950 results in infarct size reduction and preservation of cardiac function in a porcine MI model.

Methods

In vitro assay

To measure MCC950 efficacy in pigs, porcine peripheral blood mononuclear cells (PBMCs) were isolated from baseline blood samples (n = 6) using Ficoll density-gradient centrifugation. Cells were stimulated with 10 μg/mL lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA). After 1 h, 5 mM adenosine triphosphate (ATP) (Sigma-Aldrich, St. Louis, MO, USA) and different concentrations of MCC950 (0, 0.3, 7.5, or 450 μM) were added. After 3 h total incubation time, IL-1β release was measured in the supernatant using a luminex immunoassay specific for porcine IL-1β (ProcartaTM Simplex, eBioscience, San Diego, CA, USA), according to the manufacturer’s protocol.

Animal study design

All animal experiments were approved by the institutional animal welfare committee and were executed conforming to the ‘Guide for the Care and Use of Laboratory Animals’. A total of 30 female landrace pigs (body weight 66 ± 4.0 kg) were subjected to transluminal closed-chest left anterior descending (LAD) coronary artery balloon occlusion for 75 min followed by 7 days of reperfusion. One hour prior and 2 h post-occlusion, pigs were subjected to transoesophageal three-dimensional echocardiography (3D TEE). Fifteen minutes before reperfusion, pigs were randomly assigned to intravenous infusion with either a high dose of MCC950 (6 mg/kg in 40 mL phosphate-buffered saline (PBS)), a low dose of MCC950 (3 mg/kg in 40 mL PBS) or PBS alone at a rate of 80 mL/h. Intravenous infusion was repeated daily on Day 1 to Day 6. At the moment of balloon deflation, an additional dose of 6 mg (high dose, in 5 mL PBS), 3 mg (low dose, in 5 mL PBS), or PBS alone (5 mL) was selectively injected into the LAD. On Day 7, animals were again anaesthetized and subjected to 3D TEE and invasive pressure—volume (PV) measurements. This was followed by transthoracic echocardiography and dobutamine stress-echocardiography to assess regional contractility and cardiac reserve capacity. We then performed in vivo determination of the area at risk (AAR) through injection of Evans blue. Finally, the heart was explanted for the determination of infarct size and the myocardial inflammatory response. The investigators were blinded to the treatment group during both the experiments and the analysis of results. Detailed methods of echocardiography, PV measurements, and infarct size assessment are described in Supplementary material online, Methods.

Circulating levels of MCC950

In vivo MCC950 concentrations were measured in plasma samples using mass-spectrometry. We quantified MCC950 content using negative ionization mode and multiple reaction monitoring on a Waters Xevo TQ mass spectrometer (MRM transition: 403.10 > 204.06).

Neutrophil numbers, interleukin-1β assay, and circulating markers

Circulating leukocyte numbers at different time points after reperfusion were measured by whole-blood analysis using an automated haematological cell-counter (Cell-Dyn Sapphire, Abbott, Santa Clara, CA, USA). Plasma samples were obtained by whole-blood centrifugation at 1850 × g and were immediately stored at −80°C. Troponin I, aspartate transaminase (AST), alanine aminotransferase (ALT), and C-reactive protein (CRP) levels were measured using a clinical chemistry analyser (AU5811, Beckman Coulter). Quantification of neutrophils in myocardial tissue is described in Supplementary material online, Methods.

Statistics

All data are expressed as mean ± SD unless stated otherwise. In the current study, we performed a complete case analysis. All other outcomes were compared using a one-way ANOVA followed by post hoc analysis to compare individual groups. Blood parameters and leukocyte levels in the three treatment groups at different time points were analysed using mixed models. Not normally (Gaussian) distributed parameters were transformed with the natural logarithm. The mixed models include group and time point as fixed factors and a
random intercept for each pig. To determine whether the time course of the parameters was different for the groups, the interaction group*time point of measurement was also taken into the model. All statistical analyses were performed in SPSS statistics version 20.0. A two-sided P-value of <0.05 was regarded statistically significant in all analyses.

Figure 1  In vitro reduction of IL-1β secretion and circulating in vivo MCC950 levels. (A) Porcine peripheral blood mononuclear cells secrete IL-1β after administration of lipopolysaccharide and adenosine triphosphate in vitro. Addition of MCC950 after lipopolysaccharide/adenosine triphosphate stimulation dose-dependently reduced IL-1β release (n = 6). P-values represent differences from the previous dosage. (B) In vivo MCC950 levels during the first 24 h of the experiment were within therapeutic range. MCC950 levels were measured at 15 min, 4 and 24 h of reperfusion (n = 8–9 per group). P-values are differences between low and high dose group. Data are depicted as mean ± SD.

Figure 2  MCC950 administration dose-dependently reduces infarct size. (A) Representative pictures of myocardial segments of the three experimental groups. The blue area represents the remote area, the area at risk is stained red and the infarcted myocardium is stained white. (B) The area at risk was similar in all groups. (C) The infarct size/area at risk was lower in the high dose group and the low dose group compared with the control group. (D) The infarct size/left ventricular was lower in the high dose group but not in the low dose group compared with the control group. Data are depicted as mean ± SD.
**Results**

**In vitro assay**

*In vitro* stimulation of isolated porcine PBMCs with LPS and ATP led to a pronounced secretion of IL-1β (*Figure 1A*). Administration of 0.3 μM MCC950 significantly attenuated this response (−45 ± 21%, *P* = 0.002). Increasing MCC950 concentrations further reduced IL-1β secretion (75 μM: −51 ± 24%, *P* = 0.001 compared with control, *P* = 0.023 compared with 0.3 μM; 450 μM: −64 ± 21%, *P* < 0.001 compared with control, *P* = 0.011 compared with 75 μM) (*Figure 1A*).

**Survival, haemodynamics, and MCC950 levels**

Three of 30 pigs died before the follow-up duration of 7 days was completed. Two animals died before reperfusion (1 in control group, 1 in low dose group) due to persistent VF. One pig died on Day 1 after MI induction (high dose group), without preceding clinical signs of acute cardiac failure. Echocardiography prior to MI induction revealed a congenital anomaly (ventricle septum defect) in 1 pig (low dose group), which was therefore excluded from the study. This allowed analysis of nine animals in the control group, eight animals in the low dose group, and nine animals in the high dose group. Heart rate and mean arterial blood pressure were similar during the first 3 h after the induction of MI and subsequent compound administration (Supplementary material online, *Table S1*).

In *vivo* levels of MCC950 were measured at 15 min reperfusion, at 4 h reperfusion and just prior to the second intravenous compound administration (24 h reperfusion) (*Figure 1B*, Supplementary material online, *Table S2*).

**Infarct size**

At 7 days follow-up, infarct size was assessed by Evans blue/TTC double staining (*Figure 2A*). The AAR as a percentage of the LV (AAR/LV) was similar in all three groups (high dose group 21.3 ± 3.2%, low dose group 23.0 ± 2.8%, control group 22.6 ± 4.4%, *P* = 0.7) (*Figure 2B*). Infarct size (IS) as percentage of the AAR (IS/AAR) was significantly higher in the control group compared with both treatment groups (high dose group 64.6 ± 8.8%, *P* = 0.004; low dose group 69.7 ± 7.2%, *P* = 0.038; control group 77.5 ± 6.3%) (*Figure 2C*). The IS as percentage of the LV (IS/LV) of the control group was significantly higher compared with the high dose group, but not to the low dose group (high dose group 13.7 ± 2.8%, *P* = 0.023; low dose group 15.8 ± 4.2%, *P* = 0.5; control group 17.2 ± 3.1%) (*Figure 2D*).

**Global cardiac function**

Baseline cardiac function was similar in all groups. At 2 h reperfusion, global cardiac function did not significantly differ among groups (*Table 1*). At 7 days follow-up, no difference in end diastolic volume was observed between the different groups (*Figure 3A*). End systolic volume (ESV) at 7 days was significantly lower in the high dose group. The low dose group also showed a lower, albeit not statistically significant, ESV than the control group (high dose group 69 ± 12 mL, *P* = 0.042; low dose group 70 ± 13 mL, *P* = 0.085; control group 82 ± 13 mL) (*Figure 3B*). Left ventricular ejection fraction (EF) was higher in both the high dose group and the low dose group compared with the control group: 48 ± 5% (high dose group), 45 ± 7% (low dose group), 40 ± 3% (control group), *P* = 0.001.

**Table 1** Left ventricular geometrical parameters at baseline, 2 h reperfusion, and 7 days reperfusion (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con 3 mg (P-value)</th>
<th>6 mg (P-value)</th>
<th>6 mg (P-value)</th>
</tr>
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<tbody>
<tr>
<td>EDV (mL)</td>
<td>125 ± 14</td>
<td>122 ± 18 (P = 0.7)</td>
<td>121 ± 13 (P = 0.5)</td>
</tr>
<tr>
<td>ESV (mL)</td>
<td>51 ± 7</td>
<td>50 ± 14 (P = 0.8)</td>
<td>46 ± 10 (P = 0.5)</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>74 ± 11</td>
<td>72 ± 7 (P = 0.7)</td>
<td>64 ± 4 (P = 0.5)</td>
</tr>
<tr>
<td>EF (%)</td>
<td>59 ± 4</td>
<td>60 ± 6 (P = 0.8)</td>
<td>61 ± 4 (P = 0.8)</td>
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</tbody>
</table>

EDV, end diastolic volume; ESV, end systolic volume; SV, stroke volume; EF, ejection fraction; 6 mg, 6 mg/kg group; 3 mg, 3 mg/kg group; Con, control group. *P* values are from post hoc tests compared with the control group.}

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*In vitro* stimulation of isolated porcine PBMCs with LPS and ATP led to a pronounced secretion of IL-1β (*Figure 1A*). Administration of 0.3 μM MCC950 significantly attenuated this response (−45 ± 21%, *P* = 0.002). Increasing MCC950 concentrations further reduced IL-1β secretion (75 μM: −51 ± 24%, *P* = 0.001 compared with control, *P* = 0.023 compared with 0.3 μM; 450 μM: −64 ± 21%, *P* < 0.001 compared with control, *P* = 0.011 compared with 75 μM) (*Figure 1A*).
compared with the control group after 7 days follow-up (high dose group 47 ± 8%, P = 0.001; low dose group 45 ± 7%, P = 0.031; control group 37 ± 6%) (Figure 3C). The end systolic pressure–volume relationship, measured by invasive real-time PV loops, showed a higher contractility for the high dose group, but not for the low dose group at 7 days follow-up (high dose group 4.7 ± 2.0 mmHg/mL, P = 0.043; low dose group 2.7 ± 1.4 mmHg/mL, P = 0.8; control group 2.5 ± 1.7 mmHg/mL; Figure 3D).

Regional cardiac function and dobutamine echocardiography

At 7 days follow-up, regional cardiac function was assessed at a mid-ventricular and apical level by transthoracic echocardiography, before and during dobutamine infusion. Systolic wall thickening (SWT), fractional shortening (FS), and fractional area shortening (FAS) were assessed at the AAR and remote myocardium and MCC950-treated animals showed increased regional cardiac function (Figure 4A–H). Dobutamine stress-echocardiography revealed dose-dependent significant differences in cardiac reserve capacity in favour of MCC950-treated animals (Table 2, Figure 5A–D).

Serological and histological read-outs

The extent of cardiac damage was also reflected by systemic cardiac marker concentrations. Troponin I levels (P = 0.047) within the first 24 h after MI and both AST (P = 0.049) and ALT (P = 0.063) levels during the 7-day follow-up period were measured (Figure 6A–D). To assess if inflammasome inhibition also reduced inflammatory markers post-MI, systemic neutrophil numbers (P = 0.056), and CRP (P = 0.030) were measured (Figure 6D and E). Circulating lymphocytes and monocytes did not show differences during the 7-day follow-up period (data not shown).

Both active and inactive intra-myocardial IL-1β, but not IL-18 levels were decreased in the treatment group compared with the control group when measured by luminex (high dose group 131 ± 82 pg/mg, P = 0.076; low dose group 156 ± 64 pg/mg, P = 0.2; control group 211 ± 104 pg/mg; Figure 6F; Supplementary material online, Figure S1A and B). The inhibitory effect of MCC950 on IL-1β activation was confirmed using Western blot for the high dose group (P = 0.015, Figure 6G and H). A dose-dependent effect was observed for the infiltration of neutrophils, but not for macrophages into the infarcted myocardium (high dose group 132 ±

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**Figure 3** NLRP3-inflammasome inhibition preserves global cardiac function. (A) End diastolic volume (EDV) did not differ among the experimental groups. (B) End systolic volume (ESV) was lower in the high dose group and a trend was observed for the low dose group compared with the control group. (C) Ejection fraction was lower in the high dose group and the low dose group compared with the control group. (D) The end systolic pressure–volume relationship (ESPVR) in the high dose group but not the low dose group differed from the control group. Data are depicted as mean ± SD.
Table 2  Left ventricular regional systolic parameters during dobutamine stress-echocardiography (mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mid ventricular</th>
<th>Apex</th>
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<tbody>
<tr>
<td></td>
<td>Con   3 mg (P-value)</td>
<td>6 mg (P-value)</td>
</tr>
<tr>
<td>Septal WT_{ES} (cm)</td>
<td>1.3 ± 0.2 (P = 0.006)</td>
<td>1.5 ± 0.2 (P = 0.005)</td>
</tr>
<tr>
<td>Septal SWT (%)</td>
<td>9 ± 16 (P = 0.1)</td>
<td>26 ± 10 (P = 0.024)</td>
</tr>
<tr>
<td>Lateral WT_{ES} (cm)</td>
<td>1.5 ± 0.1 (P = 0.003)</td>
<td>1.8 ± 0.3 (P = 0.010)</td>
</tr>
<tr>
<td>Lateral SWT (%)</td>
<td>39 ± 22 (P = 0.029)</td>
<td>73 ± 38 (P = 0.039)</td>
</tr>
<tr>
<td>LVID_{ES} (cm)</td>
<td>3.3 ± 0.7 (P = 0.042)</td>
<td>2.7 ± 0.5 (P = 0.041)</td>
</tr>
<tr>
<td>FS (%)</td>
<td>28 ± 8 (P = 0.043)</td>
<td>38 ± 7 (P = 0.015)</td>
</tr>
<tr>
<td>LVIA_{ES} (cm²)</td>
<td>9.9 ± 2.9 (P = 0.023)</td>
<td>7.0 ± 1.8 (P = 0.029)</td>
</tr>
<tr>
<td>FAS (%)</td>
<td>45 ± 9</td>
<td>56 ± 17 (P = 0.1)</td>
</tr>
</tbody>
</table>

WT_{ES}, end systolic wall thickness; SWT, systolic wall thickening; LVID_{ES}, end systolic left ventricular internal diameter; FS, fractional shortening; LVIA_{ES}, end systolic left ventricular internal area; FAS, fractional area shortening; 6 mg, 6 mg/kg group; 3 mg, 3 mg/kg group; Con, control group. P-values reported are from post hoc tests, compared with the control group.
Interestingly, MCC950 administration leads to increased infiltration of CD14$^+$ monocytes and a tendency towards more collagen formation and decreased capillary density (Supplementary material online, Figure S1E–J), whereas TGF-β levels were not significantly changed (data not shown).

**Discussion**

Mechanistic studies have shown an essential role for the NLRP3-inflammasome, not only in IL-18- and IL-1β-driven inflammation in cardiac fibroblasts and circulating inflammatory cells but also caspase-1-dependent cell death (pyroptosis) in cardiomyocytes.25,26 Both IL-18 and IL-1β signalling and pyroptosis induce amplification of the initial ischaemic damage, culminating in infarct size expansion, and decreased cardiac contractility, thereby increasing the risk of HF.27,28 Our study is in line with these findings and for the first time provides in vivo efficacy data in a clinically relevant large animal MI model.

MCC950 has recently been shown to selectively inhibit NLRP3-inflammasome formation and reduce pyroptosis, IL-18 and IL-1β signaling.24 In the current study, we show that daily intravenous administration of MCC950 can maintain pharmacologically active circulating concentrations as evident from our and others’ in vitro studies.24 Importantly, we studied for the first time the effect of MCC950 in an animal MI model.

Transgenic mouse models and pre-treatment protocols (pharmacological preconditioning) in rodent studies have elucidated the mechanisms of the post-MI inflammatory response. However, inflammation-related signaling pathways differ between small and larger mammals.29 Moreover, large animal models allow application of minimally invasive techniques, thereby avoiding major traumatic injury that could confound the possible effect of anti-inflammatory treatment strategies.30 Hence, large animal model testing is an essential step in bringing NLRP3-inflammasome-targeted therapies from bench into clinical application.23,31,32

In the current study, we show that selective inhibition of the NLRP3-inflammasome dose dependently reduces infarct size in a porcine MI model according to a clinically feasible treatment regimen. A pronounced difference in LV function was found in treated compared with non-treated animals at 7 days post-MI. This effect is
markedly higher than those in most large animal cardioprotection studies. At 2 h reperfusion, no differences in cardiac function were detected. This is in line with previous reports, indicating that the primary effect of NLRP3-inflammasome inhibition is to attenuate the inflammatory response in the subacute phase after MI. The reserve capacity of the myocardium was higher in MCC950-treated animals compared with placebo-treated animals after dobutamine infusion. This suggests that NLRP3-inflammasome inhibition attenuates the decrease in exercise capacity that is often observed in patients post-MI.

Our study reveals that circulating markers of damage and inflammation were lower in animals treated with a high dose of MCC950. Myocardial infiltration by circulating neutrophils was lower and myocardial IL-1β levels decreased in the high dose group. To our surprise, MCC950-treated animals showed higher monocyte infiltration, which may be related to increased infiltration of anti-inflammatory macrophages. This hypothesis is supported by a dose-dependent increase in collagen formation (albeit not statistically significant). MCC950-treated animals showed decreased angiogenesis, seemingly contradicting an anti-inflammatory macrophage phenotype. However, this is in line with recent literature on angiogenesis reduction by NLRP3 inhibition or deficiency.

Previous attempts to inhibit inflammation, e.g. by blocking leukocyte transmigration, did not lead to beneficial effects in MI patients. However, the inflammatory response is also necessary for appropriate cardiac wound healing. MCC950 specifically targets cytokines that are notorious for their detrimental effect in cardiac wound healing and cardiac function upon damage. Our observations suggest that MCC950 treatment has more delicate effects in contrast to these previous strategies. Interference of MCC950 with the NLRP3-inflammasome, may fine-tune the inflammatory response rather than completely abolish it and therefore have beneficial effects in MI patients.

**Study limitations**

NLRP3-inflammasome inhibition could possibly prevent post-MI geometrical changes of the LV. However, we did not observe excessive cardiac dilatation in control animals after 7 days. Since infarct resorption occurs at longer follow-up duration, Evans Blue/TTC double staining becomes less reliable, thereby obscuring any effects on infarct size reduction. Moreover, IL-1β levels are mainly elevated during the (sub)acute phase post-MI. A longer follow-up period would therefore preclude the assessment of myocardial

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**Figure 6** Inhibition of NLRP3-inflammasome signaling reduces inflammation. (A) Cardiac troponin levels at the first 24 h of reperfusion. (B) Circulating levels of aspartate transaminase. (C) Circulating levels of alanine aminotransferase. (D) Circulating levels of neutrophils during the 7-day follow-up period in the different experimental groups. (E) Circulating levels of C-reactive protein. (F) Myocardial IL-1β content 7 days post-myocardial infarction. (G) Quantification of active IL-1β levels in the high dose and control group (n = 7 for both groups). (H) Representative western blot of active IL-1β levels in pigs treated with 6 mg/kg MCC950 and control animals. (I) Neutrophils infiltration 7 days after myocardial infarction. Data are depicted as mean ± SEM.
IL-1β content. Hence, we decided to limit the follow-up to 7 days in the current study.

In our model, circulating IL-1β and IL-18 levels after MI were below detection levels. Therefore, we were unable to assess the direct effect of MCC950 on these circulating cytokines. Moreover, due to the lack of reliable porcine-specific techniques that discriminate between the active and inactive form of IL-18 and caspase-1, we were unable to assess their activity in the myocardium. Therefore, no causative link between the known inhibitory properties of MCC950 on caspase-1 or IL-18 levels and the observed cardioprotective effect can be established in the current study. Nonetheless, since we do show that IL-1β levels are effectively reduced in the myocardium, it is conceivable that IL-18 will follow the same pattern.

In conclusion, our study reveals that continuous inhibition of the NLRP3-inflammasome-mediated signaling decreases the post-MI inflammatory response. Daily intravenous infusion of the selective small-molecule NLRP3-inflammasome inhibitor MCC950 for 7 days reduces active myocardial IL-1β levels, culminates in a pronounced cardiac function preservation and infarct size reduction in a randomized, blinded porcine MI study. Interference with NLRP3-inflammasome-mediated signaling therefore has become a promising target to reduce infarct size and preserve cardiac function in MI patients.

Supplementary material
Supplementary material is available at European Heart Journal online.

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Conflict of interest: none declared.

References


