Inhibition of the inflammatory injury following myocardial ischemia-reperfusion in the mouse

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Background.

Reperfusion is effective in reducing ischemic injury in acute myocardial infarction (AMI). Ischemia, however, triggers a secondary injury, known as reperfusion injury, contributing to the overall infarct size. Multiple mechanisms are being explored to favorably modify the effects of reperfusion injury. The NOD-Like Receptor family, Pyrin domain containing 3, (NLRP3) inflammasome contributes to tissue injury following ischemia/reperfusion (I/R).

Hypothesis.

We hypothesize that inhibition of the NLRP3 inflammasome limits infarct size following myocardial I/R, by inhibiting the inflammatory component of the reperfusion injury.

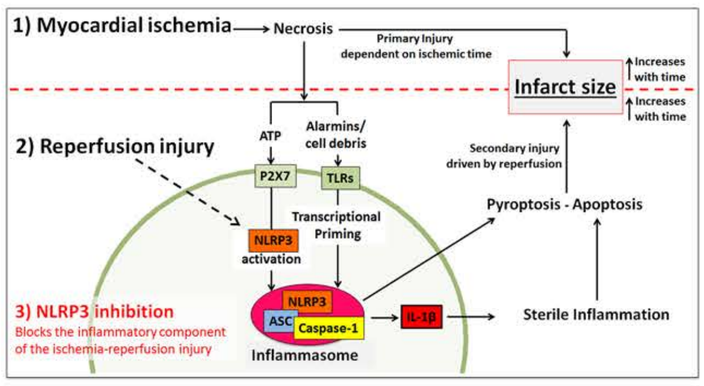

Figure 1. Schematic representation of the hypothesis. Myocardial ischemia induces cardiomyocyte necrosis. The magnitude of the ischemic injury determines the initial infarct size. The reperfusion injury and the cellular byproducts released by the necrotic cells activate NLRP3. NLRP3 induces caspase-1 activation which in turns produces active IL-1β. Active caspase-1 induces cell death (pyroptosis). IL-1β promotes sterile inflammation and apoptotic cell death. This second wave of injury driven by reperfusion contributes to the increase of the infarct size.

Methods.

We induced AMI in adult mice by transient ligation of the left anterior descending coronary artery for 30 or 75 minutes.

We tested 3 different strategies to inhibit the NLRP3 inflammasome: a newly designed small molecule specifically inhibiting the inflammasome (NLPR3inh), plasma derived alpha-1 antitrypsin (AAT) shown to inhibit the NLRP3 inflammasome, and a synthetic oligopeptide (SP16) designed to reproduce the C-terminal peptide of AAT.

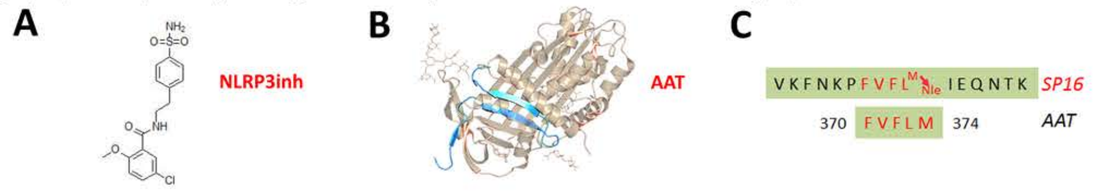

Figure 2. Schematic representation of the 3 different strategies used to inhibit the NLRP3 inflammasome. A) chemical structure of the NLRP3 inhibitor (NLRP3inh); B) tertiary structure of alpha-1 antitrypsin (AAT); C) primary structure of the short peptide SP16, containing AAT's pentapeptide FVFLM, a conserved sequence of several plasmatic protease inhibitors.

Infarct size was measured, using triphenyltetrazolium chloride (TTC) at 1, 3 and 24 hours of reperfusion and expressed as % of area at risk.

Results.

Infarct size increased with duration of ischemia from 43±4% with 30 minutes to 65±3% with 75 minutes if ischemia (P<0.001) showing a wavefront of ischemic injury.

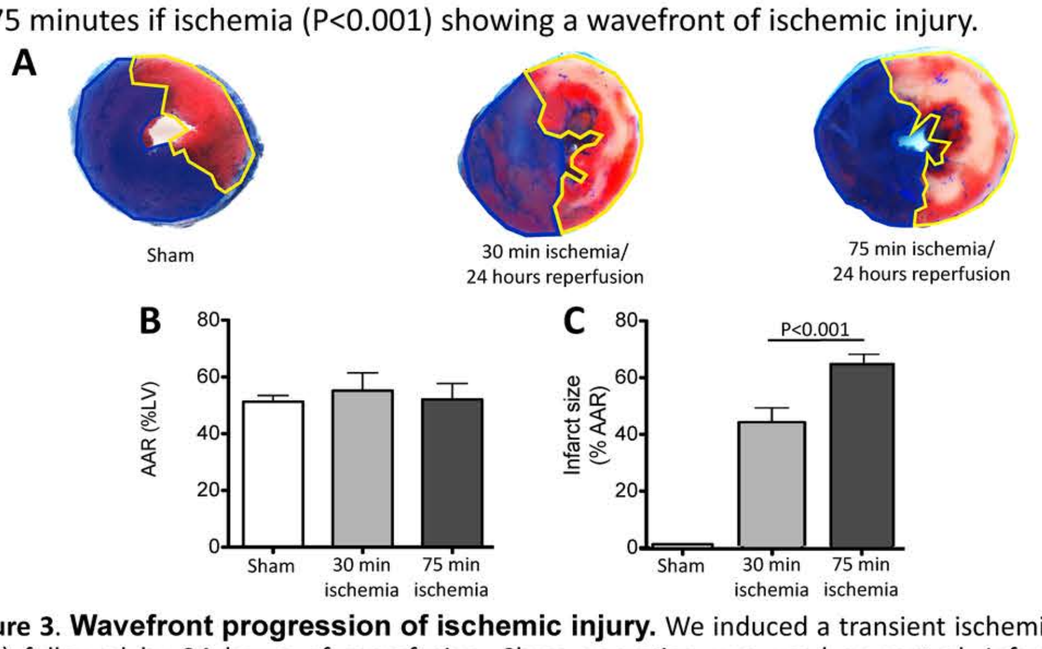

Figure 3. Wavefront progression of ischemic injury. We induced a transient ischemia (30 or 75 min) followed by 24 hours of reperfusion. Sham operation was used as control. Infarct size was measured as percent of area at risk using TTC staining at pathology. Panel A shows representative images of TTC staining. Panel B shows the area at risk expressed as percentage over the left ventricular (LV) area. Panel C shows the infarct size expressed as percentage of the area at risk (AAR).

After 30 minutes of ischemia, however, infarct size progressively increased from 1 to 24 hours after reperfusion (11±2% at 1 hour, 30±5% at 3 hours and 43±4% at 24 hours) showing a wavefront of reperfusion injury.

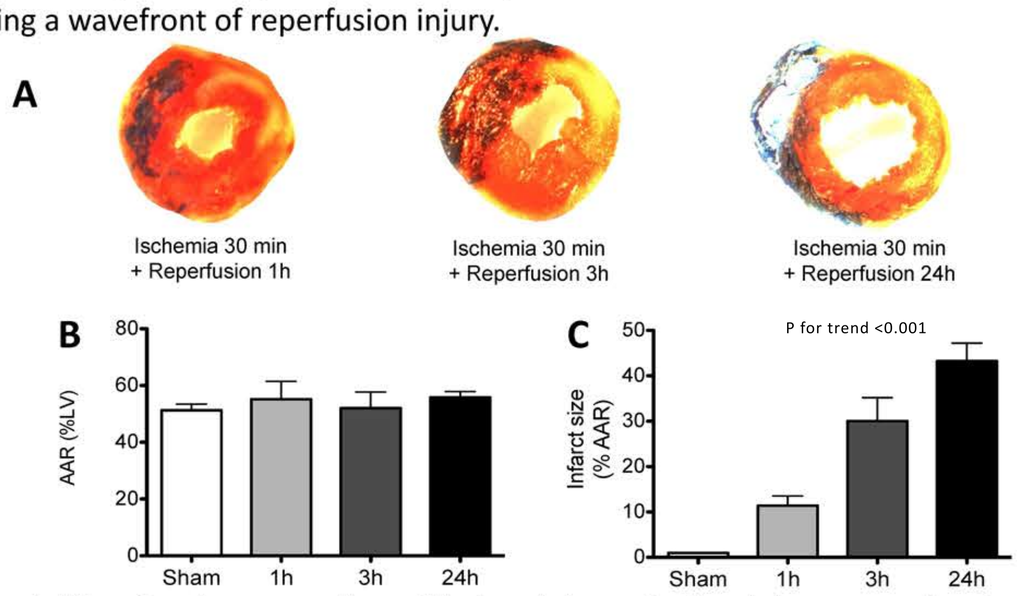

Figure 4. Wavefront progression of ischemia/reperfusion injury. We induced a 30 min transient ischemia followed by 1, 3 or 24 hours of reperfusion. Sham operation was used as control. Infarct size was measured as percent of area at risk using TTC staining at pathology. Panel A shows representative images of TTC staining. Panel B shows the area at risk expressed as % over the left ventricular (LV) area. Panel C shows the infarct size expressed as % of the area at risk (AAR).

Administration of the NLRP3inh, AAT or SP16 given immediately at reperfusion following 30 or 75 minutes of ischemia significantly reduced infarct size at 24 hours (-56%, -44%, -55%, respectively, vs vehicle, all P<0.01).

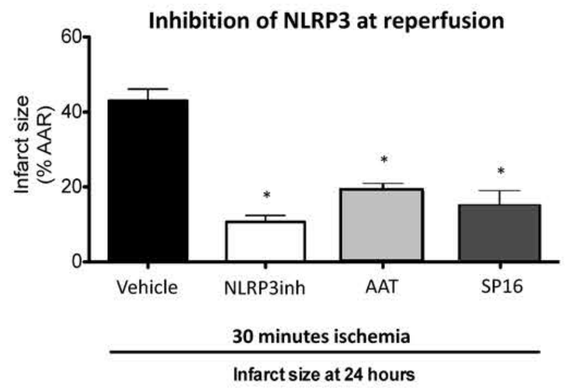

Figure 5. Infarct-sparing effect of NLRP3 inhibition. We induced AMI in adult male mice by coronary ligation and transient ischemia (30 min) followed by 24 hours of reperfusion. Pharmacological inhibition of NLRP3 was achieved using a specific NLRP3 inhibitor, AAT or the SP16, given intraperitoneally at the moment of reperfusion. Infarct size was measured using the TTC staining and comparisons were made between the pharmacological interventions and the vehicle control. N=6-8 per group * p<0.01 vs vehicle.

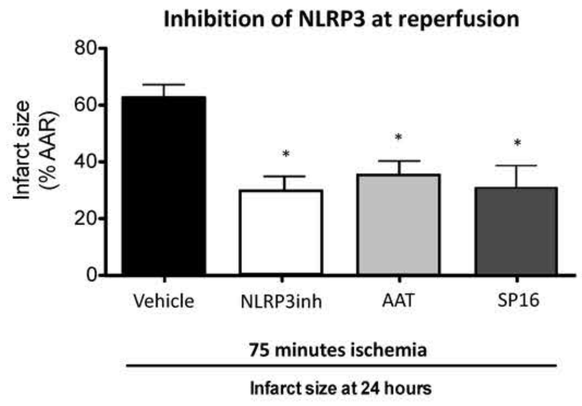

Figure 6. NLRP3 inhibition reduces the infarct size after 75 minutes of ischemia and 24 hours of reperfusion. Pharmacological inhibition of NLRP3 was achieved using NLRP3inh, AAT or the SP16, given intraperitoneally at the moment of reperfusion. Infarct size was measured using the TTC staining and comparisons were made between the pharmacological interventions and the vehicle control. N=5-6 per group * p<0.01 vs vehicle.

Administration of the NLRP3inh, AAT or SP16 given within 30 minutes after reperfusion following 30 of ischemia significantly reduced infarct size at 24 hours.

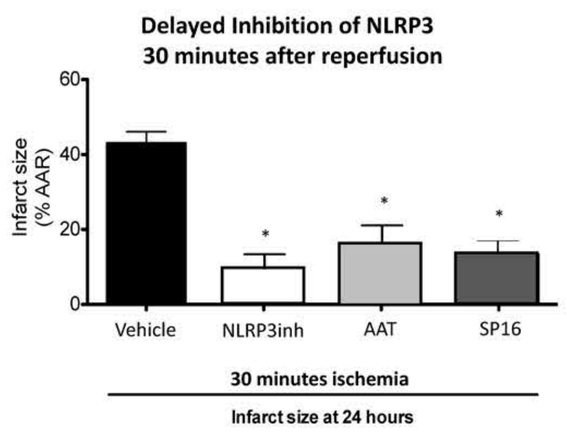

Figure 5. Effects of delayed pharmacological blockade of NLRP3. To simulate a clinical scenario in which treatment may not occur immediately at reperfusion, we tested whether a delay of 30 minutes in treatment with NLRP3inh, AAT or SP16 would affect the results, and found that the cardioprotective effects were maintained and were similar to the treatment at reperfusion. N=5-8 per group. * p<0.01 vs vehicle.

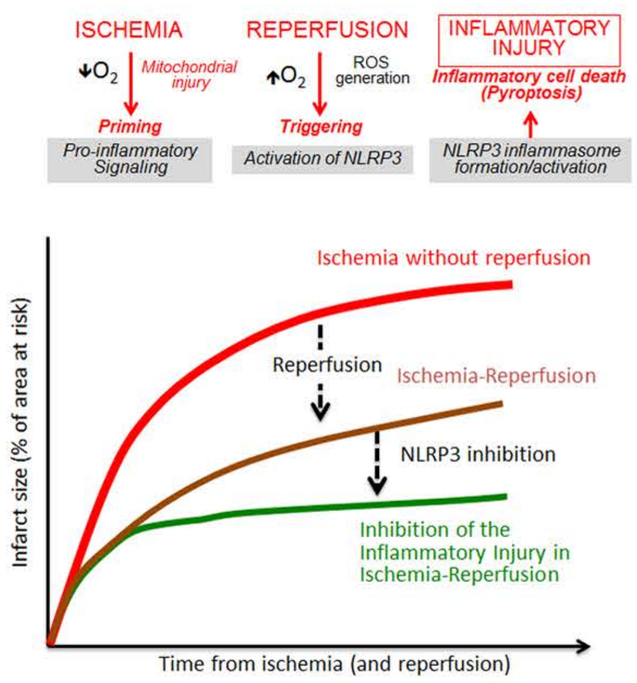

Figure 7. Schematic representation of the proposed benefits of NLRP3 inflammasome inhibition in ischemia-reperfusion injury. Ischemia induces pro-inflammatory signaling to the heart (priming). Reperfusion reduces infarct but also triggers NLRP3 activation leading to an inflammatory injury that limits the benefits of reperfusion. Pharmacological blockade of NLRP3 mitigates the inflammatory injury following reperfusion, thus providing the full benefit of reperfusion on infarct size.

Conclusions.

Pharmacological inhibition of the NLRP3 inflammasome within 1 hour of reperfusion limits the secondary inflammatory injury and infarct size following myocardial ischemia-reperfusion in the mouse. Pharmacological interventions alone or in conjunction with other interventions show promise to significantly further improve outcome post myocardial infarction.

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