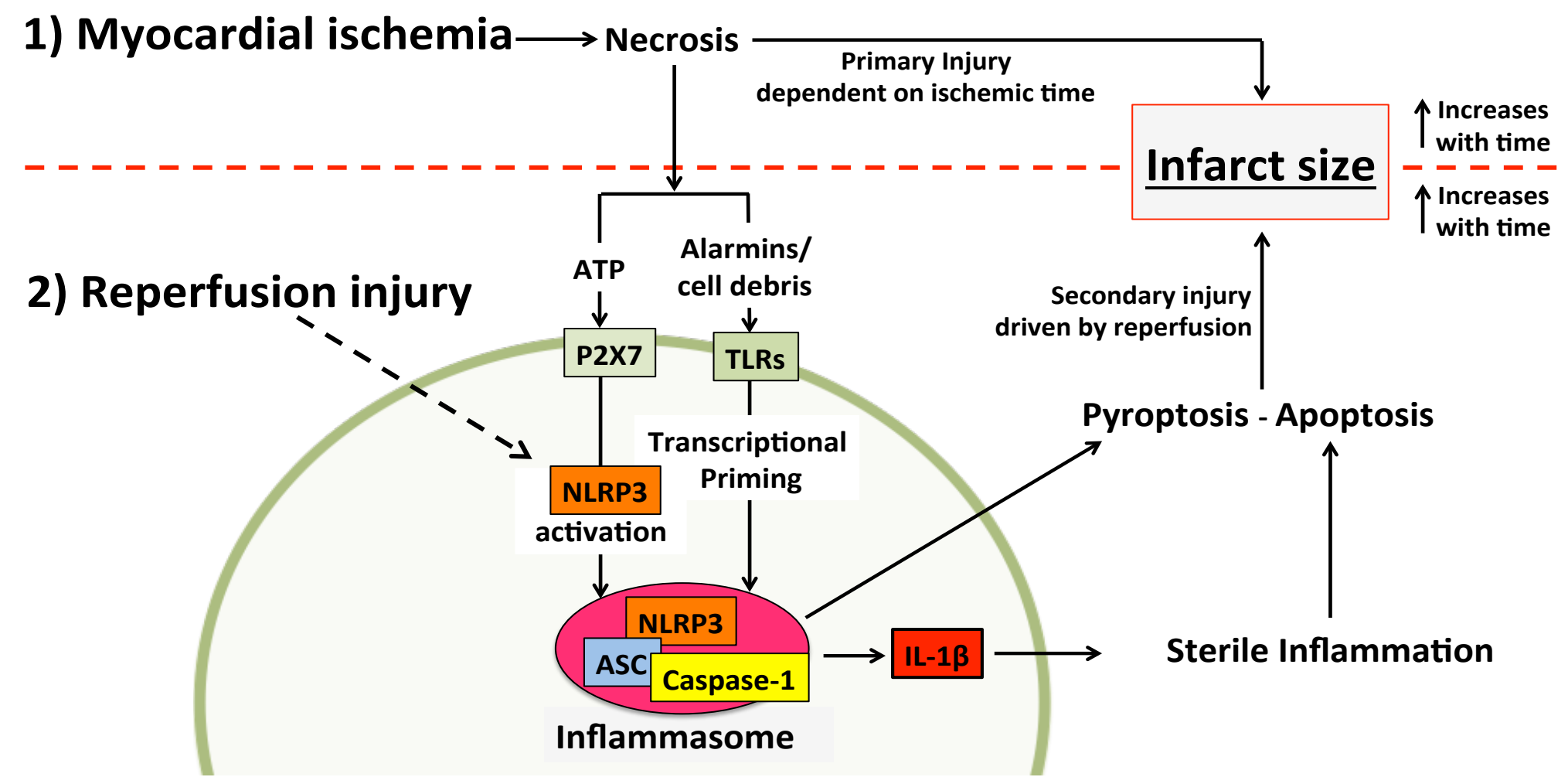


## Background

Activation of the NLRP3 inflammasome during acute myocardial infarction (AMI) mediates further injury after reperfusion.



NLRP3 inflammasome inhibitors have been shown to reduce ischemia/reperfusion injury in preclinical models, yet effective and safe therapies are still not available for patients with AMI.

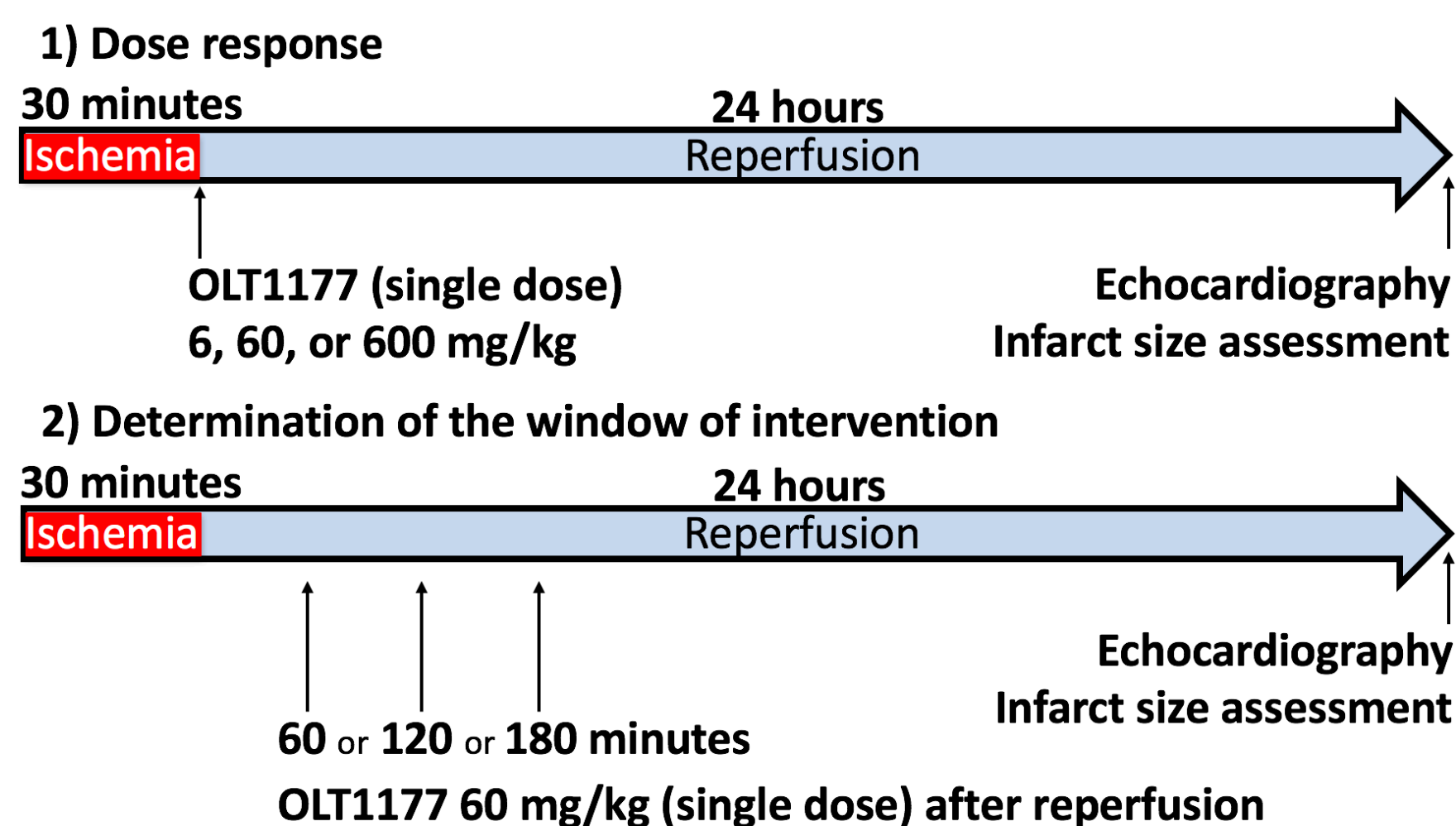
OLT1177, is a novel,  $\beta$ -sulfonyl nitrile synthetic small molecule NLRP3 inflammasome inhibitor, with a favorable Phase 1 safety and tolerability profile in healthy volunteers, and is currently being studied in a Phase 2 clinical trial in acute gout.

## Aim of the Study

To determine the efficacy of OLT1177 in an experimental mouse AMI model.

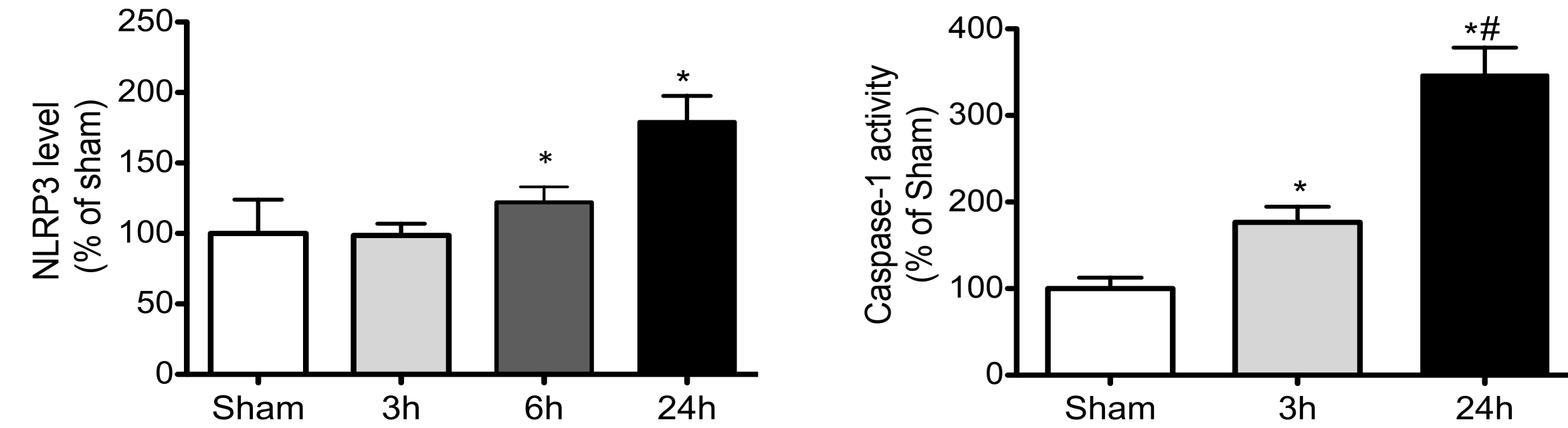
## Methods

Male CD-1 mice underwent transient surgical ligation of the left coronary artery for 30 minutes followed by reperfusion for 24 hours. We administered OLT1177 (6, 60, or 600 mg/kg) or matching vehicle intraperitoneally at time of reperfusion or OLT1177 (60 mg/kg) after a 60-, 120- or 180-minute delay (N=4-8/group). Echocardiography was performed to measure left ventricular (LV) fractional shortening (FS). Infarct size was measured at pathology by triphenyl-tetrazolium-chloride (TTC) and phthalo blue staining and expressed as a percentage of the myocardium at risk.



## Model Validation

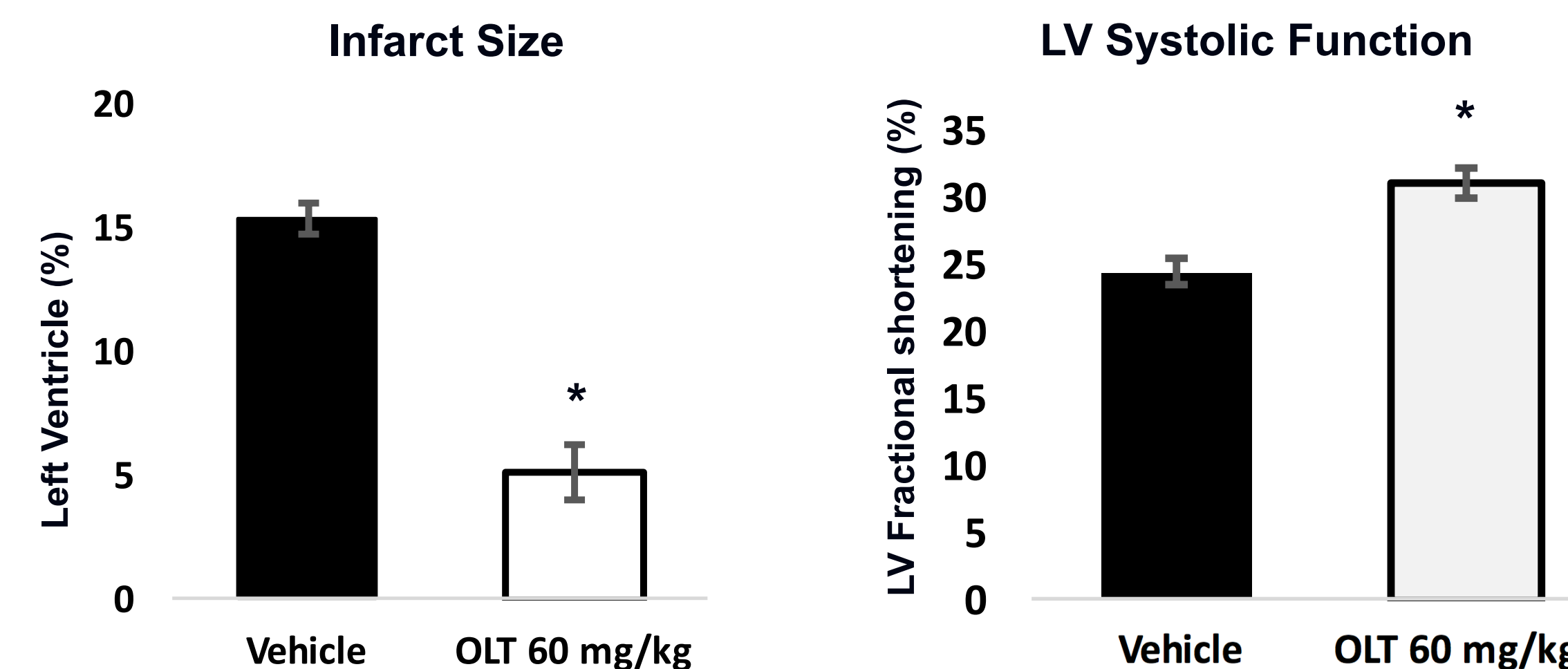
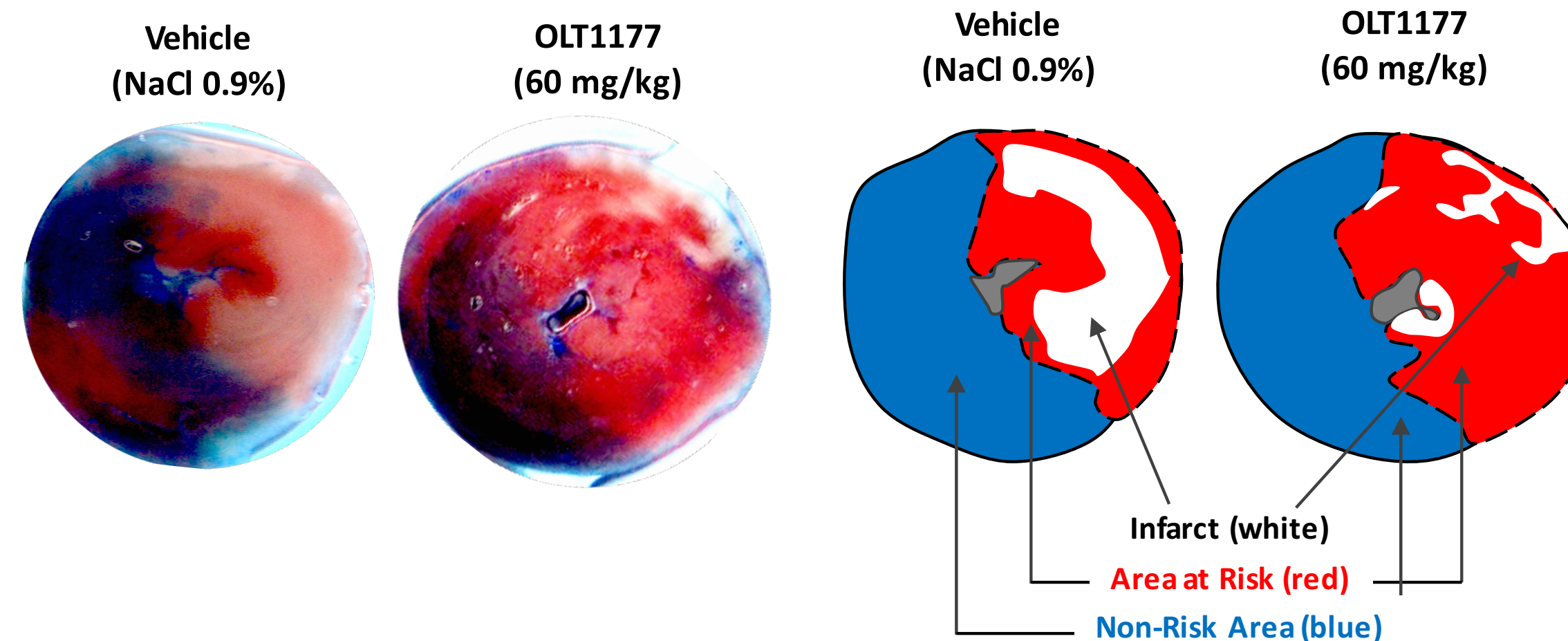
Myocardial NLRP3 expression and caspase-1 activation increase between 3-6 hours following reperfusion and peak at 24 hours.



The left panel shows an increased expression of NLRP3 protein in the myocardium at 6 and 24 hours following reperfusion compared to 3 hours after ischemia-reperfusion injury and the sham surgery. The right panel shows a significant increase in caspase-1 activity, measured using a fluorometric enzymatic assay, reflecting inflammasome activation in the heart, at 24 hours and 3 hours versus sham. \*P<0.05 vs Sham. #P<0.05 vs 3h. Extracted from Toldo S. et al. Int J Cardiol. 2016.

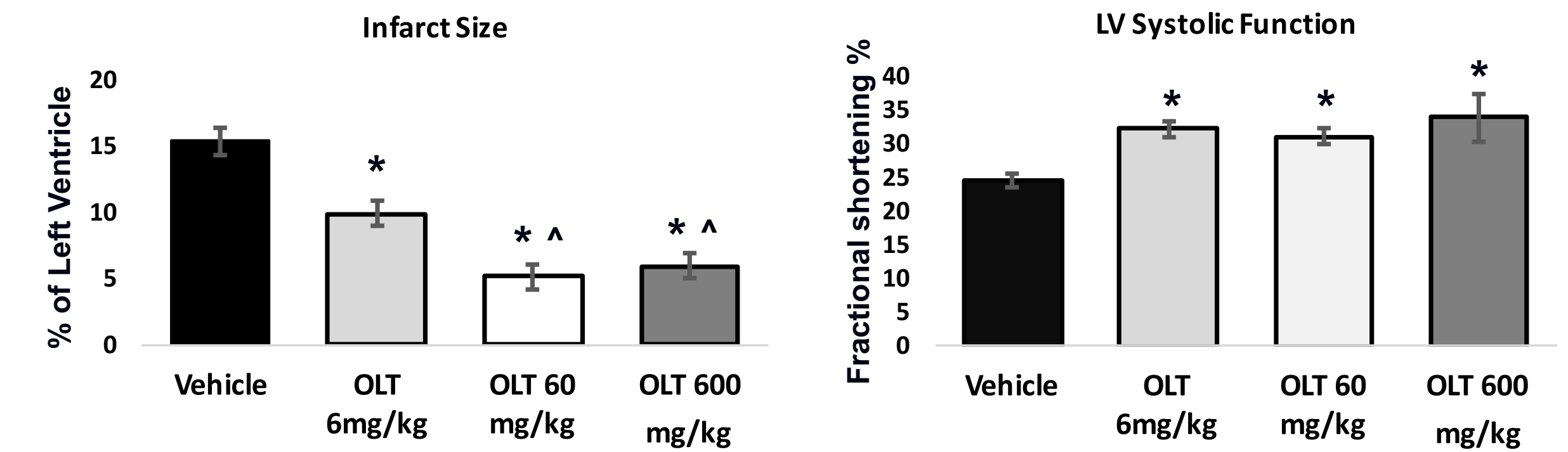
## Results

OLT1177 (60 mg/kg) given at reperfusion provided a significant reduction in infarct size associated with a preservation of left ventricular systolic function.



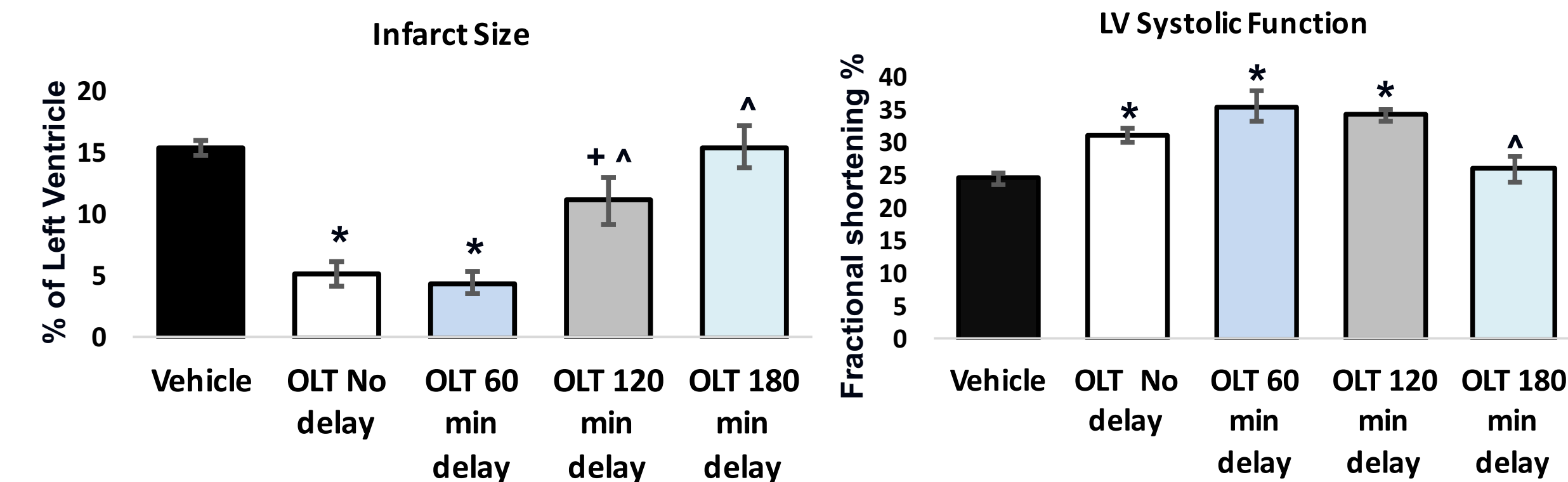
**Effects of OLT1177 on infarct size and LV systolic function.** OLT1177 (60 mg/kg) was given at the time of reperfusion. N=6-8 per group. \* P<0.005

We observed no differences in infarct size between doses of 60 and 600 mg/kg. The effect on infarct size with 6 mg/kg was milder when compared to the 60 mg/kg dose. Fractional shortening was equally preserved at 24 hours with all doses of OLT1177.



**Dose dependent effects of OLT1177.** OLT1177 (6, 60, or 600 mg/kg) was given at the moment of reperfusion. LVFS and infarct size were measured after 24 hours. N= 5-8 per group. \* p<0.005 vs vehicle. ^ p<0.05 vs OLT 6 mg/kg.

OLT1177 given with 60 minutes delay after reperfusion was equally effective as the treatment given with no delay. After 120 minutes treatment delay, LV systolic function was preserved and infarct size was smaller than in vehicle-treated mice, although the difference was not statistically significant (p=0.052). After 180 minutes treatment delay, OLT1177 failed to prevent the drop in fractional shortening or to reduce the infarct size.



**Therapeutic window for delayed OLT1177 treatment.** The effects of OLT1177 (60 mg/kg) at intervals of 0, 60, 120, or 180 minutes after reperfusion were compared to the vehicle control. \*p<0.005 vs vehicle; ^p<0.05 vs no delay and 60 min delay; \*p=0.052 vs vehicle. N=4-7 per group.

## Conclusions

Inhibition of the NLRP3 inflammasome using OLT1177, a novel  $\beta$ -sulfonyl nitrile synthetic small molecule, reduces infarct size and preserves LV function in a mouse model of myocardial ischemia/reperfusion injury. These findings provide proof of concept for a protective effect in patients with acute myocardial infarction.

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