

# The NLRP3 inflammasome as a new pharmacological target in myocardial ischemia/reperfusion injury in the adult rat

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The NLRP3 inflammasome is a large multimeric danger-sensing platform that promotes autocatalytic activation of the cysteine protease caspase-1 and mediates the cleavage of inactive pro-IL-1 $\beta$ , among other proteins, into its active form [1]. We and others have recently documented that the NLRP3 inflammasome is a central mediator in the inflammatory response to tissue injury [2,3,4]. Besides, we have shown that the activation of the NLRP3 inflammasome amplifies myocardial ischemic injury in diabetic mice [5]. Here, we present preliminary data on the effects of a small electrophilic molecule, INF-4E, bearing substituted  $\alpha,\beta$ -unsaturated nitrile and  $\alpha,\beta$ -unsaturated carbonyl-derived functionalities, in an ex-vivo model of myocardial ischemia/reperfusion injury in the rat heart. We have recently demonstrated that INF-4E inhibits NLRP3 ATPase and caspase-1 activities in THP-1 cells [6]. Isolated rat hearts from male Wistar (5–6 month old, body weight 450–550 g, n=20) underwent perfusion without ischemia (Sham) or ischemia/reperfusion (30-min ischemia plus 20-min or 60 min reperfusion) with and without INF-4E treatment (50  $\mu$ M in the perfusate for 20-min before ischemia). Administration of INF-4E exerted protection against myocardial IR, as shown by a significant reduction in infarct size and improvement in left ventricular pressure. Western blot analysis demonstrated that the formation of the NLRP3 inflammasome complex is induced by myocardial IR injury. Interestingly, INF-4E treatment attenuated the IR-induced increase in caspase-1 activity, thus confirming drug's ability to affect NLRP3 inflammasome complex activation. Besides, the ischemic hearts of the INF-4-pretreated animals displayed a marked modulation of the so-called Reperfusion Injury Salvage Kinases (RISK) pathway.

In conclusion, the results demonstrate that the small molecule INF-4E inhibits the formation of the NLRP3 inflammasome in the rat heart and limits myocardial IR injury. The present study also increases our understanding of the role of the NLRP3 inflammasome as key player in myocardial infarction, pointing out to a new pathway for the development of novel pharmacological approaches for cardioprotection. However, further studies are warranted to better elucidate the effects of pharmacological inhibition of NLRP3 inflammasome during myocardial IR injury and to clarify the potential clinical relevance of our findings.

## References

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