The role of NLRP3 inflammasome as pharmacological target in *in vivo* models of diabesity and related myocardial ischemic injury

F. Chiazza[1], R. Mastrocola[2], E. Benetti[1], A. Couturier-Maillard[3], D. Nigro[2], M. Cocco[1], J.C. Cutrin[4], M. Bertinaria[1], C. Penna[2], P. Pagliaro[2], B. Ryffel[3], C. Thiemermann[5], M. Aragno[2], R. Fantozzi[1], M. Collino[1].

[1]Department of Drug Science and Technology, University of Turin, Italy; [2]Department of Clinical and Biological Sciences, University of Turin, Italy; [3]CNRS, UMR7355 INEM, Immunologie et Neurogénétique Expérimentales et Moléculaires, University of Orléans, Orléans, France;[4]Department of Biotechnology and Sciences for the Health, University of Turin, Italy; [5]Queen Mary University of London, William Harvey Research Institute, London, UK

Diet-induced metabolic overload initiates a low-grade, chronic inflammatory response, known as 'metaflammation', which significantly contribute to the development of obesity and diabetes ('diabesity') and related cardiovascular disorders [1]. Although the molecular mechanisms underlying metaflammation are not yet clear, compelling evidence suggests an involvement of the recently identified danger-sensing platform NLRP3 inflammasome, that promotes autocatalytic activation of the cysteine protease caspase-1 and mediates the cleavage of inactive pro-IL-1β and pro-IL-18 into their active forms [2]. Here we report our most recent findings on its potential role in *in vivo* models of cardio-metabolic diseases and the effects of its pharmacological modulation.

Male C57/BL6 wild-type mice were fed standard diet (SD) or high-fat high-fructose diet (HD) for 12 weeks. A sub-group of HD-fed mice was exposed to cardiac *ex vivo* ischemia/reperfusion (I/R) injury.

HD-feeding increased lipids concentrations in the plasma, liver and heart and impaired glucose homeostasis and renal function. These effects were associated to significant increase in NLRP3 inflammasome expression and activation in the liver, kidney and heart. Interestingly, when exposed to myocardial I/R, HD mice hearts showed greater infarct size and lactic dehydrogenase release in comparison with SD mice. The exacerbation of myocardial injury was due, at least in part, to a stronger I/R-induced upregulation of the NLRP3 inflammasome pathway in mice fed HD when compared to SD mice.

To assess whether the pharmacological modulation of NLRP3 pathway could affect metainflammation and thus improve systemic metabolic profile, a sub-group of HD-fed mice was treated with the NLRP3 inflammasome inhibitor BAY 11-7082 (3 mg/kg, i.p.) for the last 7 weeks. HD diet was also provided to $NLRP3^{-/-}$ littermates. None of the diet-induced metabolic abnormalities were detected in HD-fed $NLRP3^{-/-}$ mice and they were dramatically reduced by BAY 11-7082 administration. The improved glucose tolerance by NLRP3 pharmacological and genetic inhibition was, at least partially, mediated by enhancing the insulin-related signaling pathway in the liver of HD-fed mice. Treatment of WT mice with BAY 11-7082 attenuated also the renal injury (histology) and dysfunction (albuminuria) caused by HD to a degree that was very similar to that seen in mice in which the NLRP3 inflammasome had been deleted.

The effects of the pharmacological inhibition of NLRP3 inflammasome were also tested in a rat model of cardiac *ex vivo* ischemia/reperfusion (I/R) injury by administering a selective NLRP3 inflammasome inhibitor, INF, recently synthetized by members of the research team [3]. Administration of INF exerted protection against myocardial I/R, as shown by a significant reduction in infarct size. Moreover, INF treatment attenuated the I/R-induced increase in caspase-1 activity, thus confirming drug's ability to affect NLRP3 inflammasome complex activation.

Overall, our results confirm the involvement of NLRP3 inflammasome in the developing of metabolic and cardiovascular diseases. Most notably, they demonstrate that the pharmacological modulation of NLRP3 inflammasome complex protects against the diet-induced metabolic anomalies and the organ ischemic injury, by affecting multiple levels of the insulin and inflammatory cascades.

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