ANALYSIS OF NECROPTOSIS AND AUTOPHAGY SIGNALLING IN ACUTE MYOCARDIAL ISCHEMIA/REPERFUSION INJURY: A ROLE OF RIP3

Csaba Horvath[1], Megan Young[2], Izabela Jarabicova[1], Martin Lewis[2], Tatiana Ravingerova[3], M. Saadeh Suleiman[2], Adriana Adameova[1,3]

[1]*Faculty of Pharmacy, Department of Pharmacology and Toxicology, Comenius University in Bratislava, Bratislava, Slovak Republic*
[2]*Faculty of Health Sciences, Bristol Heart Institute, The Bristol Medical School, University of Bristol, Bristol, United Kingdom*
[3]*Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic*

RIP3 is a core mediator of necroptotic cell loss which has been shown to underlie some phenotypes of myocardial ischemia/reperfusion (IR) injury. However, the extent to which necroptosis contributes to such damage under short reperfusion remains elusive. Thus, under conditions of acute myocardial IR we provided a comprehensive analysis of necroptotic and autophagic signalling as there are indications for their interplay. Langendorff-perfused rat hearts subjected to 30-min ischemia and 10-min reperfusion exhibited impaired cardiac function which was not ameliorated by RIP3 inhibition. Immunoblotting analysis revealed that the detrimental effects of IR were unlikely mediated by necroptosis, since neither the canonical RIP3–MLKL nor the non-canonical CaMKIIδ–mPTP and PGAM5–Drp1 pathways were activated. Although the signalling involved in autophagy inhibition was unaffected, autophagy activation was suppressed by IR as evidenced by decreased expression of Beclin-1, pSer555-ULK1, pSer555-ULK1/ULK1 ratio, and LC3-II/LC3-I ratio. RIP3 inhibition prevented the IR-induced plasma membrane rupture and delayed mPTP opening which was associated with modulation of XO and MnSOD. Additionally, LC3-II expression in IR hearts was suppressed by RIP3 inhibition, indicating some effect on autophagosome processing. Conclusively, this is the first study suggesting that early reperfusion of previously ischemic heart is not associated with execution of necroptosis. Furthermore, we showed that RIP3 very likely underlies this cardiac damage via the modulation of oxidative stress- and mitochondrial function, rather than promoting cell loss due to necroptosis. Lastly, the relationship between necroptosis and autophagy under such acute IR settings is unlikely, apart from the potential autophagosome regulation.

Keywords: necroptosis, autophagy, ischemia/reperfusion injury, oxidative stress

Supported by grants APVV-15-0607, 20-0242, 19-0540, VEGA 1/0016/20, 2/0141/18 and MR/N001389/1.