The impact of FPRs on Annexin A1 cardioprotection in vivo.

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In Acute Myocardial Infarction (AMI) inflammation is a prerequisite for healing but it can paradoxically also extend tissue injury, hence it needs to be modulated. Appreciation of the mediators operative in the area of resolution of inflammation for AMI is emerging, and these include the protein Annexin A1 (AnxA1; 1) and its receptor, the GPCR termed FPR2 (2), though there is no in-vivo evidence. Here we investigated the role of FPR2 in Annexin A1-induced protection in AMI, focusing on the heart and the organ most vulnerable to secondary injury, the lung.

The Left Anterior Descending Coronary Artery (LADCA) of male Fpr1-/-, Fpr2/3-/- and littermate controls (WT) (25±5 g body weight) was occluded for 25 min and re-opened for 90 min. At the end of the reperfusion period myocardial tissue injury was quantified with Evan's blue dye (2%, to distinguish the area at risk) and p-nitroblue tetrazolium (0.5mg/ml, to determine the infarct size). In separate experiments, the staining procedure was omitted and tissues were harvested and analyzed by ImmunoHistoChemistry, Immunofluorescence, ELISA or extracted for mRNA analysis (RT-PCR).

The AMI procedure led to necrosis of ~55% of the area at risk (AAR). In WT mice, administration of AnxA1 (1μg/mouse; i.v.)(3) before LADCA occlusion afforded significant cardioprotection (~30% reduction of infarct size, $p<0.01$) as compared to vehicle (PBS). AnxA1 administration at the beginning of reperfusion afforded a much lower degree of protection (~10%). Deletion of Fpr1 did not significantly alter the cardioprotective effect of AnxA1, whilst the protein was no longer effective in Fpr2/3 KO mice ($p<0.05$). Intriguingly, a significant proportion (~50%) of Fpr2/3-/- mice perished during the procedure, and those that did survive up to 90 min post-reperfusion exhibited a larger infarct size (~15%, $p>0.05$) than WT animals. After AMI, RT-PCR analysis showed a 2-fold increase of Fpr2 mRNA in the heart and in lung (3 fold increase) of WT mice. Corresponding results were obtained with immunostaining for Fpr2 protein. Pronounced signs of local inflammation were found in the myocardium (KC, TNFα and IL-6) and in lungs (higher MPO, IL-6, IL-1β and less IL-10) of Fpr2/3 null mice in compare with WT mice.

In summary these pharmacological and pathological investigations indicate that the AnxA1/FPR2 pathway is operative in AMI. On one hand, AnxA1 delivery affords cardioprotection - partly through Fpr2 - on the other hand Fpr2 sustains non-redundant protective actions both in the injured heart and in distant organs. We propose that the AnxA1 pathway can be harnessed for the development of novel therapeutics to prevent primary and secondary tissue damage caused by AMI.

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