AUTOPHAGY REGULATION IS DEPENDENT ON THE ISCHEMIC DURATION AFTER RENAL ISCHEMIA-REPERFUSION INJURY IN RATS

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Introduction

Renal ischemia-reperfusion injury (IRI), unavoidable during kidney transplantation, is a major cause of delayed graft function and poorer graft outcome. IRI results from the lack of oxygen and energy after the loss of blood supply (ischemia) in the donor, which pushes the cells towards anaerobic metabolism. This sets the stage for massive formation of reactive oxygen species (ROS) during the oxygenated blood reflow at the time of transplantation (reperfusion). The pathophysiology of IRI includes direct cellular damage due to the ischemic insult and indirect damage resulting from the activation of the inflammatory pathway and the innate immune response. The stress signals during renal IRI will induce necrotic cell death, further enhancing the inflammation response. To avoid necrosis, cells can move towards alternative cell death (**apoptosis**) or induce the survival pathway **autophagy**. However, it is still unclear how apoptosis and autophagy are regulated during renal IRI and what their exact contributions are to the injury. Moreover, the interplay between the cell death and survival pathways has not been investigated.

Aims

Recently, we hypothesized that the dynamics and role of autophagy are dependent on the length of ischemia and reperfusion (Decuypere *et al.* 2014 Am J Transplant).

Therefore, we aimed to analyze autophagy and apoptosis in different female rat renal IRI models subjected to mild (45 min) and severe (60 min) of ischemia and various time points of reperfusion.

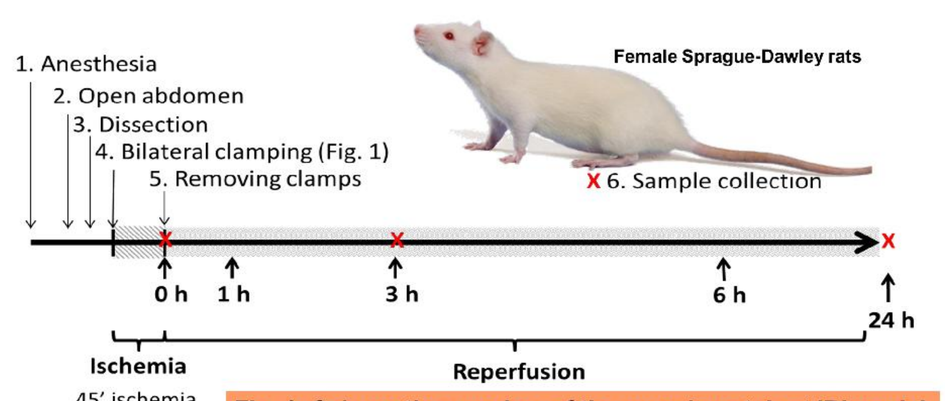

Fig. 1: Schematic overview of the experimental rat IRI model

Results

Reduced survival and increased renal injury post-reperfusion following 60 min (I60) *versus* 45 min (I45) of renal ischemia

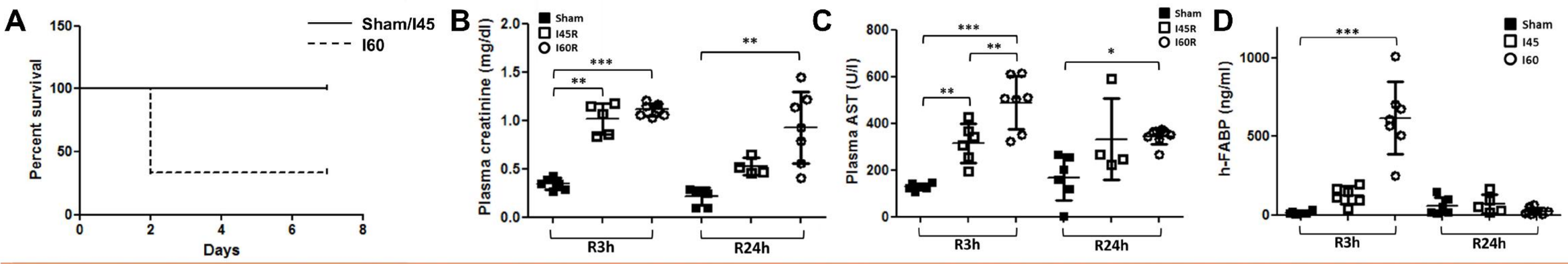

Fig. 2: Reduced survival and increased renal injury in I60 *versus* I45. A) Survival analysis of rats subjected to Sham operation, 45 min of ischemia (I45, straight line) or 60 min of ischemia (I60, dashed line). Survival was followed until 7 days post-reperfusion (*N*=4). B-D) Plasmatic kidney function markers in Sham-operated rats (full squares), I45 rats (open squares) and I60 rats (open circles): B) Plasma creatinine ; C) Plasma aspartate aminotransferase (AST); D) Plasma heart-type aftty acid binding protein (h-FABP). * $p < 0.05$; ** $p < 0.01$; *** $p < 0,001$ (One-Way ANOVA, Tukey *post-hoc*)

Increased apoptosis and autophagy post-reperfusion following 60 min (I60) *versus* 45 min renal ischemia (I45)

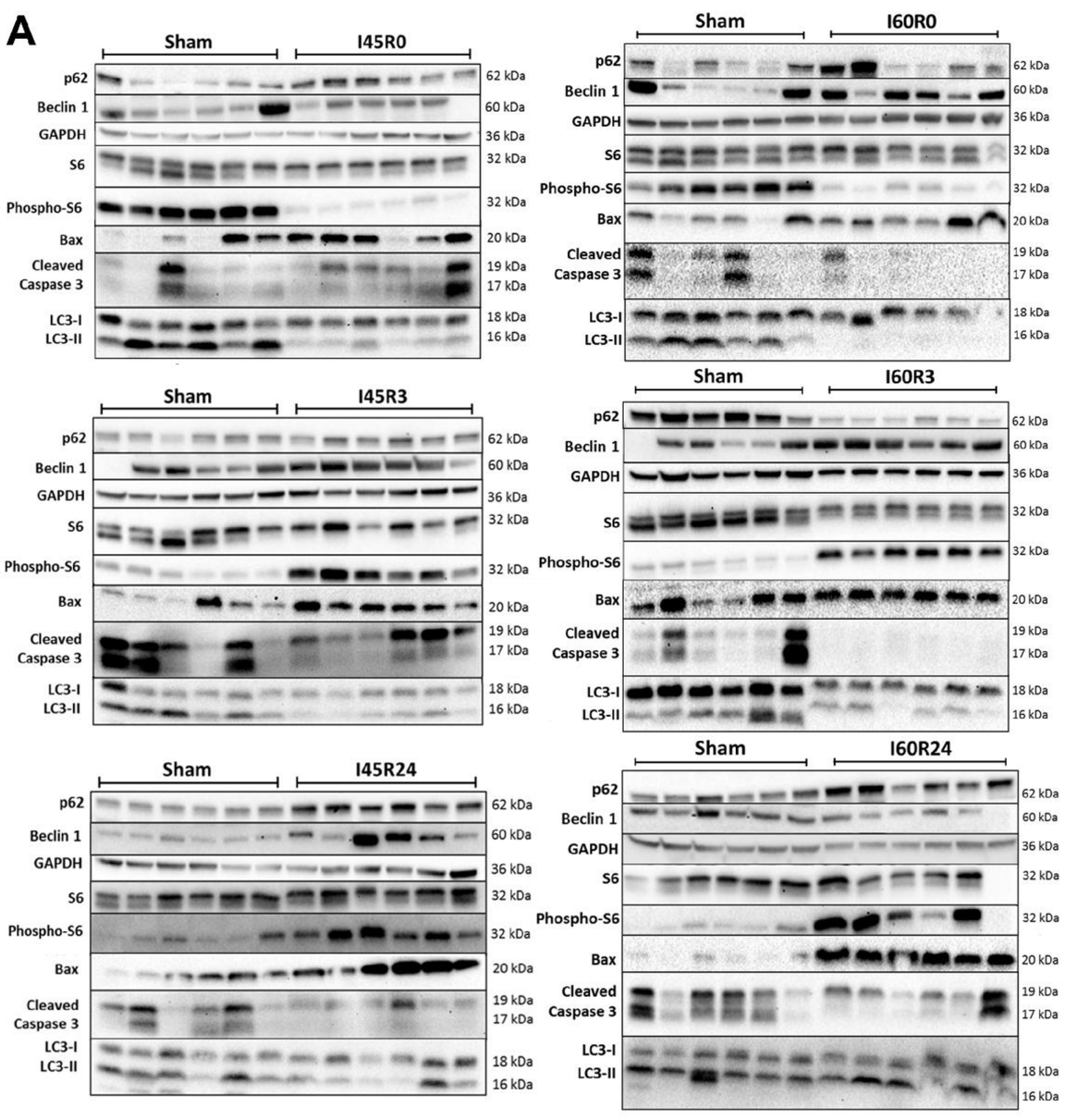

Fig. 3: Inreased apoptosis and autophagy in I60 *versus* I45. A) Western blotting analysis of kidney tissues from rats subjected to Sham operation (Sham), 45 min of ischemia (I45) or 60 min of ischemia (I60), followed by 0h (R0), 3h (R3) or 24h (R24) of reperfusion . B) Quantitative analysis of apoptosis markers Bax and Cleaved Caspase 3 and autophagy markers LC3-II, p62, Beclin 1 and mTOR activity marker phospho-S6. Values were first normalized for the mean of the correspondingSham group and than compared in the I45 and I60 groups. The combination of increased LC3-II and decreased p62 signifies increased autophagic flux. C) qPCR analysis of apoptosis marker Bax and autophagy marker LC3. LC3 expression is higher in I60 than in I45, corresponding to the Western blotting data. * $p < 0.05$; ** $p < 0.01$; *** $p < 0,001$ (*N*=6; Unpaired *t* test or Mann-Whitney)

Conclusions

1) Autophagy is decreased post-reperfusion after 45 min of mild ischemia (increased LC3-II and increased p62)
2) However, autophagy is increased after 60 min of severe ischemia (increased LC3-II and decreased p62); This difference in mTOR-independent (phospho-S6 is not different)
3) Apoptosis increases in both models

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DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.
52nd ERA-EDTA CONGRESS LONDON 2015 UNITED KINGDOM MAY 28
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