The Effect of Hypoxic Cold Storage and Reperfusion Injury on Autophagy and Endothelial Cell Health During Transplantation

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Background

- During the preservation phase of transplantation, transplanted organs are subjected to cold ischemic conditions. During implantation, reperfusion ensues, wherein the transplanted organ incurs significant damage (*i.e.* **ischemic reperfusion injury** - IRI).
- Cold storage and IRI are associated with poor long-term transplant outcomes. [1]
- During ischemia, the reduction in ATP causes cell death and generates harmful reactive oxygen species (ROS), both of which exacerbate inflammatory damage and immunogenicity.[1]
- Immunogenicity is due to inappropriate antigen presentation by endothelial cells (ECs) lining the vessels of transplanted organs, activating circulating effector memory T cells.[2]
- Although this process remains unclear, is believed to be modulated by autophagy.
- **We investigated IRI to better understand its role in EC health following organ transplantation.**

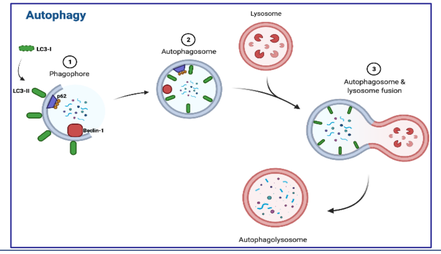


Figure 1: LC3-I: precedes autophagy; LC3-II: phagophore formation; Beclin-1: phagophore and autophagosome formation; p62: autophagolysosome degradation (autophagy)

Hypothesis

Hypoxic cold storage conditions may cause heightened autophagy levels upon reperfusion, ultimately diminishing endothelial cell health.

Methods

- Murine microvascular endothelial cells (MCECs) were transferred into standard organ preservation solution (UW-solution) and either stored at 4ºC in an airtight, oxygen-depleted container, simulating **hypoxic cold storage** (HCS), or incubated at 37ºC in media for normothermia (NT)
- To simulate IRI, cold organ preservation solution was replaced with warm culture medium and the cells were incubated at 37ºC
- Immunoblotting and immunofluorescence assays were performed to detect autophagy, and ELISA was performed to ascertain EC activation as a consequence of autophagy modulation

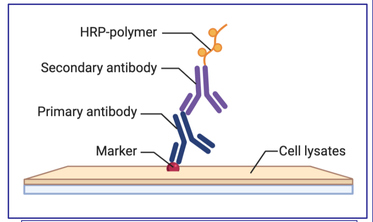


Fig. 2: Depicts binding of antibodies to for immunoblotting

Results

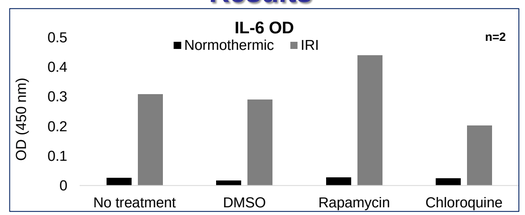
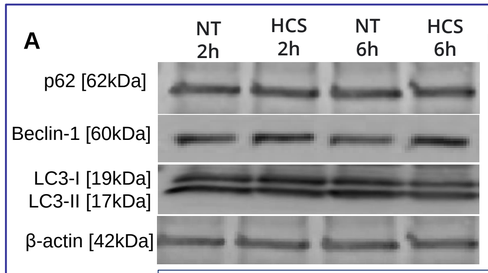
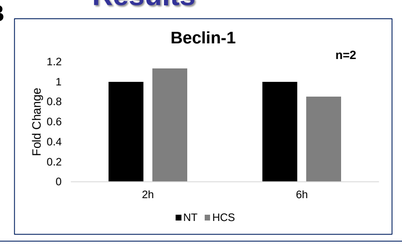


Fig. 6: OD for 24 hour NT and IRI with autophagy-modulating treatments, given with reperfusion after HCS.





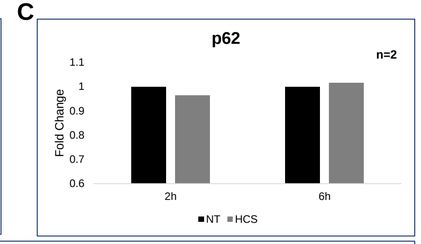
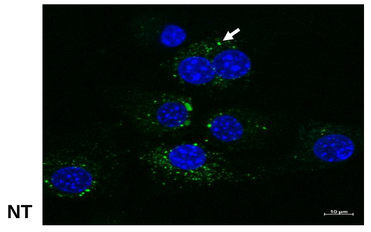
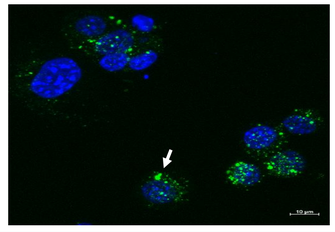


Fig 3: (**A**) Immunoblot of LC3-I and LC3-I, Beclin-1, p62, and beta-actin in NT and HCS conditions for 2 or 6 hours of cold storage. (**B**) Fold change for ratio of Beclin-1/beta-actin. (**C**) Fold change for ratio of p62/beta-actin.





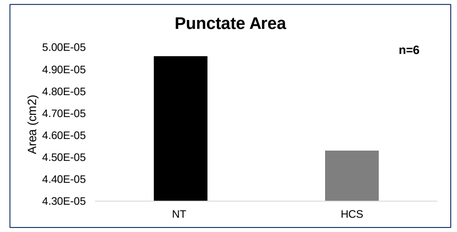
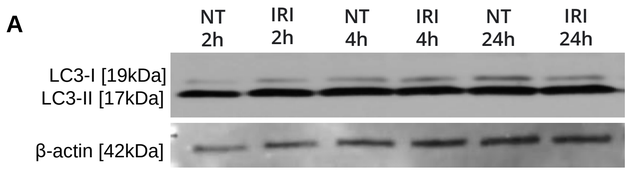


Fig. 4: (**A**) Immunohistochemistry images with Cyto-ID and Hoechst 33342 dyes for 30 min at 37ºC under NT and (**B**) HCS conditions. White arrows indicate puncta**.** (**C**) Average LC3-II puncta per cell following 6 hours of NT or HCS conditions.



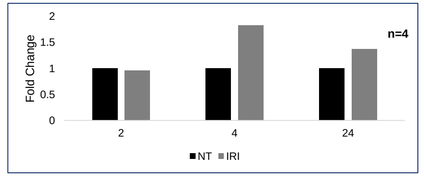


Fig. 5: (**A**) Immunoblot of LC3-II and LC3-I in NT and IRI conditions at 2, 4, and 24 hours post-reperfusion (**B**) Fold change for ratio of LC3-II/LC3-I.

Conclusions

- Beclin-1 is lower during HCS, indicating a potential halt in metabolic processes (Fig. 3B).
- p62 levels rise slightly at end of the cold storage period, confirming reduced autophagosome formation in HCS (Fig. 3C)
- Puncta area is equal in NT and HCS conditions, indicating a potential increase in autophagy until reperfusion, since metabolic processes are reduced (Fig. 4).
- LC3-II levels are highest at 4 and 24 hours post reperfusion, suggesting heightened autophagy following reperfusion (Fig. 5).
- Inducing autophagy via rapamycin may activate ECs more, and blocking autophagy via CQ reduces EC activation (Fig. 6).
- The heightened autophagy levels during reperfusion may be deleterious for EC health, ultimately contributing to immunogenicity and organ transplant rejection

References

1. Saeb-Parsy K, *et al.* Mitochondria as Therapeutic Targets in Transplantation. Trends Mol Med. 2021
2. Tran DT, *et al.* Impact of Mitochondrial Permeability on Endothelial Cell Immunogenicity in Transplantation. Transplantation. 2018