Preventing Connexin 43 Loss in Endothelial Gap Junctions During Ischemia-Reperfusion: A Translational Strategy for Allograft Survival

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Introduction

- Cardiac transplantation is the only treatment for patients with end-stage Heart failure.
- Ischemia-reperfusion injury (IRI) immunologically primes the donor organ resulting in subsequent alloimmune rejection and eventual graft failure[1].
- Endothelial cells (ECs) lining the blood vessels of donor organs are the first to be impacted by IRI[2].
- The EC barrier is compromised during IRI and triggers alloimmune recognition (Figure 1)[3].
- Gap junction (GJ) proteins, specifically connexin 43 (Cx43), play a significant role in regulating the EC barrier[4].
- IRI has been shown to alter Cx43 expression and is increasingly recognized as a therapeutic target for mitigating IRI.
- The effects of IRI on endothelial Cx43 expression in the specific context of heart transplantation are not yet fully understood.

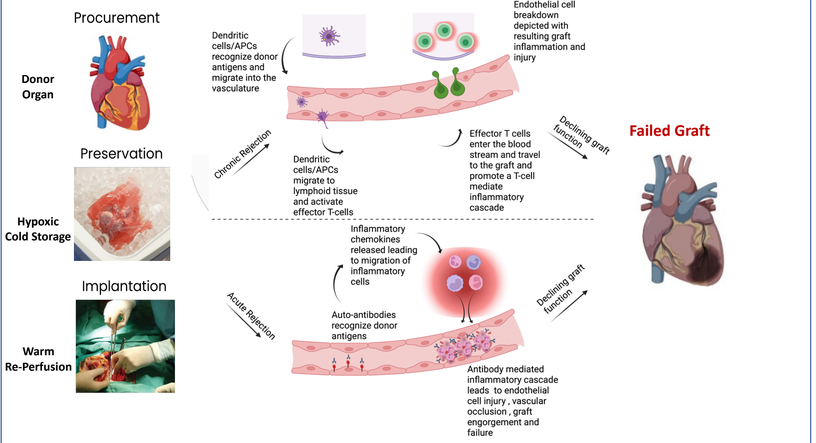


Figure 1: The role of endothelial cells in alloimmune recognition.

Hypothesis

Test if including the aCT1 peptide during cold storage increases Cx43 gap junction expression and enhances endothelial cell barrier integrity in donor hearts.

Results

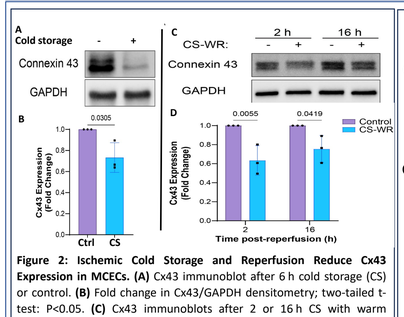


Figure 2: Ischemic Cold Storage and Reperfusion Reduce Cx43 Expression in MCECs. (A) Cx43 immunoblot after 6 h cold storage (CS) or control. **(B)** Fold change in Cx43/GAPDH densitometry; two-tailed t-test: P<0.05. **(C)** Cx43 immunoblots after 2 or 16 h CS with warm reperfusion (WR) or control. **(D)** Fold change in Cx43/GAPDH densitometry; two-tailed t-test: P<0.01.Means ± SD; n=3 independent experiments.

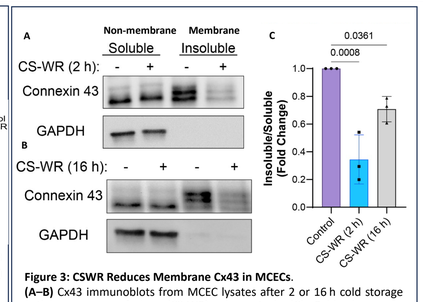


Figure 3: CSWR Reduces Membrane Cx43 in MCECs.
(A–B) Cx43 immunoblots from MCEC lysates after 2 or 16 h cold storage with warm reperfusion (CS-WR) or control. **(C)** Fold change in Cx43/GAPDH densitometry. Two-way ANOVA: P<0.01, P<0.05.Means ± SD; n=3 independent experiments.

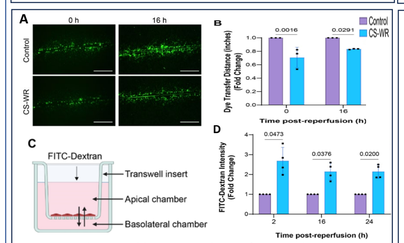


Figure 4: Ischemic Cold Storage and Reperfusion Reduce GJ Activity and Barrier Integrity in MCECs.(A) Representative lucifer yellow dye transfer images; scale bars: 20 µm.**(B)** Fold change in dye transfer distance.**(C)** Schematic of FITC-Dextran permeability assay.**(D)** Fold change in FITC-Dextran intensity post-reperfusion. Two-way ANOVA: P<0.01, P<0.05 (B); P<0.05 (D).Means ± SD from n=3 independent experiments.

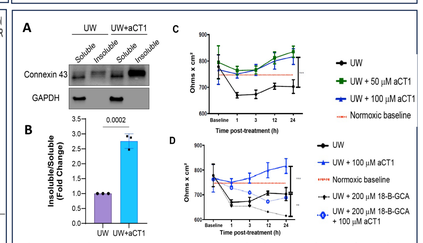


Figure 5: ACT1 Pre-treatment Restores Cx43 Expression and Barrier Integrity During Cold Storage. (A) Cx43 immunoblots from soluble and insoluble fractions after 6 h cold storage with UW or UW+ACT1. **(B)** Fold change in Cx43 insoluble/soluble densitometry; t-test: P<0.001.**(C–D)** TEER measurements post-reperfusion in MCECs treated with UW, UW+ACT1, or 18-β-GCA; control as baseline. Two-way ANOVA: ***P<0.001, P<0.01.Means ± SD; n=3–5 independent experiments.

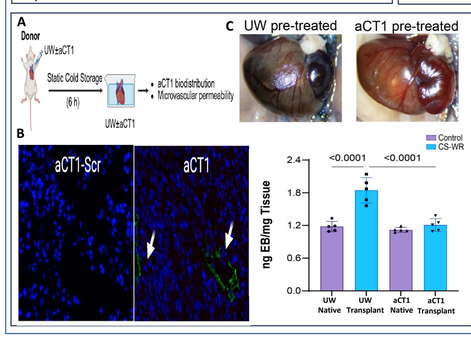


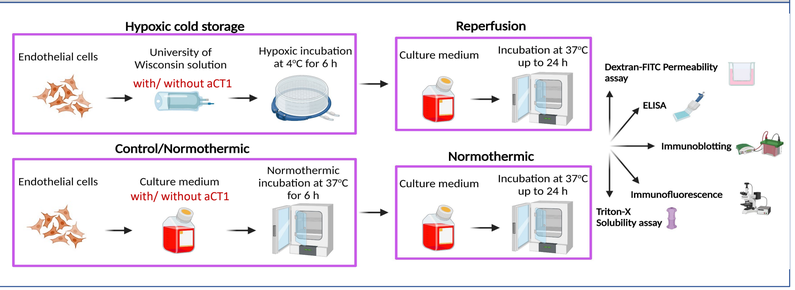
Figure 6: Ischemic cold storage pre-treatment of murine donor cardiac allografts in UW solution augmented with ACT1 peptide improves microvascular integrity.

(A) Experimental schematic of donor cardiac allograft pre-treatment with UW solution augmented with ACT1.

(B) Representative IF staining of ACT1 binding to the microvasculature (white arrowheads) in cardiac allografts after pre-treatment.

(C) Representative photographs of mouse hearts and quantification of extravasated Evan's blue dye in the pre-treated cardiac allografts. Native hearts were used as controls. A two-way ANOVA determined statistical significance: P<0.0001.

Methodology



Results

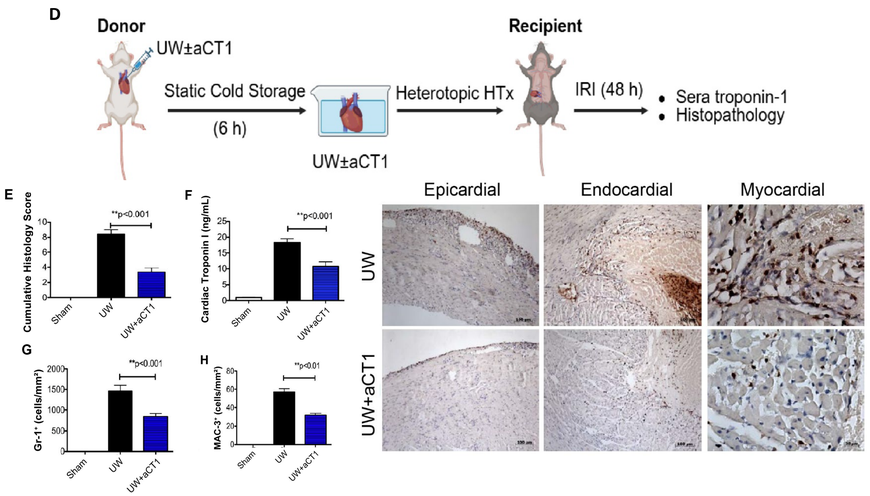


Figure 7: ACT1 Pre-treatment Enhances Microvascular Integrity and Reduces IRI in Murine Cardiac Allografts.

(D) Schematic of donor heart pre-treatment and heterotopic transplantation (HTx).

(E–F) Cardiac injury assessed by histology (E) and serum troponin-I levels (F) at 48 h post-HTx. One-way ANOVA: P<0.001.

(G–I) Immune infiltration assessed by IHC: GR1⁺ (G, I) and MAC-3⁺ (H) cell quantification. Scale bars: 100 µm. One-way ANOVA: P<0.01.Means ± SE; n=6–8 mice/group

Conclusions

- CS reduces Cx43 gap junction expression in MCECs.
- Addition of aCT1 peptide during CS improves Cx43 gap junction expression.
- Preconditioning murine donor cardiac allografts with aCT1 peptide in CS improves microvascular barrier integrity and mitigates IRI.

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