Succinate accumulation accounts for greater transplant reperfusion injury induced by warm compared to cold ischaemia in mouse, pig and man

Jack Martin, Ana Costa, Anja Gruszczyk, Mazin Hamad, Andrew James, Nikitas Georgakopoulos, Gavin Pettigrew, Christian Frezza, Michael Murphy, Kourososh Saeb-Parsy
Ischaemia Reperfusion (IR) Injury in Transplantation

• IR injury is inevitable in transplantation

• There has been a rapid expansion in the use of DCD organs

• Warm ischaemia is more detrimental than cold ischaemia

• Mechanism poorly understood

• Unique opportunity for therapeutic intervention

Role of Mitochondria in IR Injury

- Succinate is key source of reactive oxygen species (ROS) in IR injury
- ROS cause tissue damage in IR injury

Aims

1. Characterise and compare the metabolic signature of cold and warm ischaemia

2. Translate findings from mouse to pig to human

3. Demonstrate the efficacy of manipulating the metabolic pathways during ischaemia
Methods: Metabolic Profile of Ischaemia

- Metabolic Profile
- Organ storage
- Clamp frozen
- LC/MS analysis of 80 metabolites

Normoxic control tissue

- Warm ischaemia
- Cold Ischaemia

Organ storage
Methods: Metabolic Profile of Ischaemia

Two-way ANOVA
(n = 5-8)

p < 0.0001

Two-way ANOVA
(n = 4)

p = 0.0006

Two-way ANOVA
(n = 4)

p = 0.006
Succinate Accumulation During Ischaemia

• Succinate accumulates more rapidly during warm ischaemia

• Succinate accumulation is highly conserved metabolic process
Cardiac contraction accounts for a significant proportion of energy requirements.

Temperature directly affects cardiac contraction.

Succinate accumulation is significantly greater during warm ischaemia irrespective of cardiac contraction.
Succinate Accumulation in the Pig Heart

• Succinate accumulates during ischaemia in the pig heart
• Succinate accumulation is dependent on temperature
Inhibition of the Malate / Aspartate Shuttle

Aspartate → Succinate

Succinate → Fumarate

Fumarate → Malate

Malate → Oxaloacetate

AOA

Control

AOA + DMM

Hadacidin

AOA

DMM

DZX

TTF

NADH

NAD⁺
Competitive Inhibition of Succinate Dehydrogenase
Direct Inhibition of Succinate Dehydrogenase (SDH)
Combined Inhibition of the MAS and SDH

Aspartate $\rightarrow$ Oxaloacetate $\rightarrow$ Succinate

Malate $\rightarrow$ Fumarate $\rightarrow$ Succinate

AOA

DMM
Pharmacodynamics of DMM Inhibition

- DMM reduces succinate accumulation during warm ischaemia
Methods: Heart Transplant Model

Donor operation
Reperfusion
Ischaemia
Implantation
Retrieval
Serum troponin
24 hours
Efficacy of Inhibiting Succinate Accumulation

- DMM ameliorates IR injury in transplant reperfusion model
Conclusions

• Succinate is a key metabolite in IR injury in animals and man

• Pathway is amenable to pharmacological intervention

• Ameliorating succinate accumulation is a promising therapeutic approach in both DBD and DCD transplantation

• Potential applications to other IR-related diseases including:
  • Myocardial infarction
  • Cerebrovascular accident
  • Limb ischaemia