CLINICAL IMPLICATIONS OF BASIC RESEARCH

Targeting Succinate Metabolism in Ischemia/Reperfusion Injury

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Timely reperfusion is critical for salvaging ischemic tissue in myocardial infarction (MI), stroke and during resuscitation. Paradoxically, the reperfusion of blood into the ischemic organ is damaging in itself, leading to ischemia/reperfusion (IR) injury. Current best clinical practice is to reperfuse rapidly to limit ischemic time. Despite this, there is still extensive IR injury which is a major driver of pathology, making the prevention of this damage a clear unmet clinical need[1]. Recent work has shown a role for mitochondrial succinate metabolism in IR injury that opens up exciting new therapeutic approaches.

SUCCINATE METABOLISM IN IR INJURY

IR injury was long thought of as a random and chaotic series of damaging events resulting in reactive oxygen species (ROS) production from reperfusing ischemic tissue. A different picture is now emerging, suggesting that metabolic mechanisms within mitochondria are central to IR injury, providing a rational basis for therapies. During ischemia, the citric acid cycle metabolite, succinate builds up[2]. Upon reperfusion, this succinate is rapidly oxidised by succinate dehydrogenase (SDH), driving a burst of ROS production by mitochondrial complex I[2]. This ROS pulse, together with calcium dysregulation and ATP depletion, initiates a cascade of damaging events that culminate in cardiomyocyte death termed reperfusion injury[1]. While cell death from ischemia contributes to the infarct, reperfusion leads to damage over and above that from ischemia alone, thus reperfusion injury provides a therapeutic window to reduce organ damage.

Many clinical trials have targeted different facets of IR injury following heart attack but translation to the clinic has been unsuccessful so far. Here, we highlight the emerging therapeutic strategy of targeting succinate metabolism.

SUCCINATE DEHYDROGENASE (SDH) INHIBITORS FOR CARDIOPROTECTION

SDH is a key enzyme in succinate formation during ischemia and its oxidation upon reperfusion[1]. Malonate, a competitive inhibitor of SDH, has emerged as a candidate therapy for selective SDH inhibition to diminish reperfusion injury. This was first shown using the malonate prodrug, dimethyl malonate (DMM), where it was protective when administered prior to and throughout ischemia[2]. Also, disodium malonate (DSM) has a cardioprotective effect when administered intracoronary at reperfusion in a pig model of IR injury[3]. Both approaches alter succinate metabolism, either preventing its accumulation during ischemia (thus less succinate is available to be oxidised during reperfusion) or directly blocking its oxidation during reperfusion.

Not all the succinate that accumulates in the heart during ischemia is oxidised by mitochondria upon reperfusion. A significant proportion is released selectively into the circulation, as has been demonstrated in patients with an ST-segment elevation MI. In this situation, succinate was released into the blood stream following reperfusion by primary percutaneous coronary intervention (PPCI)[4]. The mechanism of succinate release from the cardiomyocytes is currently unclear, but once released it may be detected by the succinate receptor (SUCNR1) which is involved in inflammation. Thus, by targeting succinate accumulation during ischemia, subsequent release and action in the circulation, malonate may provide additional therapeutic strategies for the heart damage underlying chronic heart failure.

SDH INHIBITION FOR OTHER IR INJURY PATHOLOGIES

In addition to heart attack, ischaemic stroke and kidney IR injury may benefit from malonate therapy[2]. Furthermore, disrupting succinate metabolism during predictable periods of ischemia, such as that occurring in elective surgery or organ transplantation is another potential therapeutic target. Cessation of circulation in these scenarios leads to ischemia and a rise in succinate. Upon reperfusion, the succinate will be rapidly oxidised driving IR injury, tissue damage and inflammation. Treatment with malonate to blunt succinate accumulation prior to elective surgery or transplantation thus provides a promising therapeutic opportunity. DMM has also been shown to decrease the brain damage associated with resuscitation after cardiac arrest in rats[5]. Infusing DMM intravenously prior to cardiopulmonary resuscitation improved neurological performance post-cardiac arrest, suggesting SDH inhibition by malonate in the brain decreased the production of mitochondrial ROS and downstream damage – though whether DMM's action is during ischemia or at reperfusion is unclear. IR injury to the brain, either during cardiopulmonary resuscitation or stroke is clinically important and there are no pharmacological interventions available. Thus, therapies modulating SDH may help address this unmet clinical need.

TRANSLATING SDH INHIBITORS FOR IR INJURY

Despite clear *in vitro* and animal model efficacy of many compounds in preventing IR injury, translation has proved difficult[1]. High failure rates in human trials are often due to low drug

exposure at the target site or clinical safety problems. Translation failure in IR injury is likely due to delivery difficulties or insufficient knowledge of the pathological mechanisms, leading to inappropriate drug targets, such as the broad use of antioxidants. Additionally, target specific drugs such as cyclosporine A may suffer from being too far downstream of the damage initiating mechanism. Furthermore, inappropriate trial design ranging from poor inclusion criteria to insufficient powering has led to disappointing outcomes.

Malonate use nullifies a number of barriers to clinical translation. Importantly, malonate can enter mitochondria by endogenous transport mechanisms thus, allowing the compound to reach the target site in a timely manner. Furthermore, malonate has limited toxicity, well established metabolism and has been used as an excipient in pharmaceutical development.

For the successful translation of malonate therapy, some hurdles remain. Firstly, as malonate is a competitive inhibitor, high concentrations are required for protection. Strategies to improve the cellular delivery of malonate may therefore enable a dose reduction to overcome this issue. Secondly, the correct timing of malonate administration is critical. For clinical use to reduce reperfusion injury, the drug must be at the right concentration in the affected tissue at the time of reperfusion. Therefore, understanding the pharmacokinetics of malonate during administration will be important to know the timing of malonate delivery. Thirdly, selectivity to the ischaemic tissue by malonate is unlikely. However, strategies to limit off-target malonate delivery are possible and may enable rapid cardioselective malonate delivery to prevent IR injury. Overcoming each hurdle presented here may lead to successful translation of malonate-based therapeutics from bench to bedside in the coming years.

Disclosures

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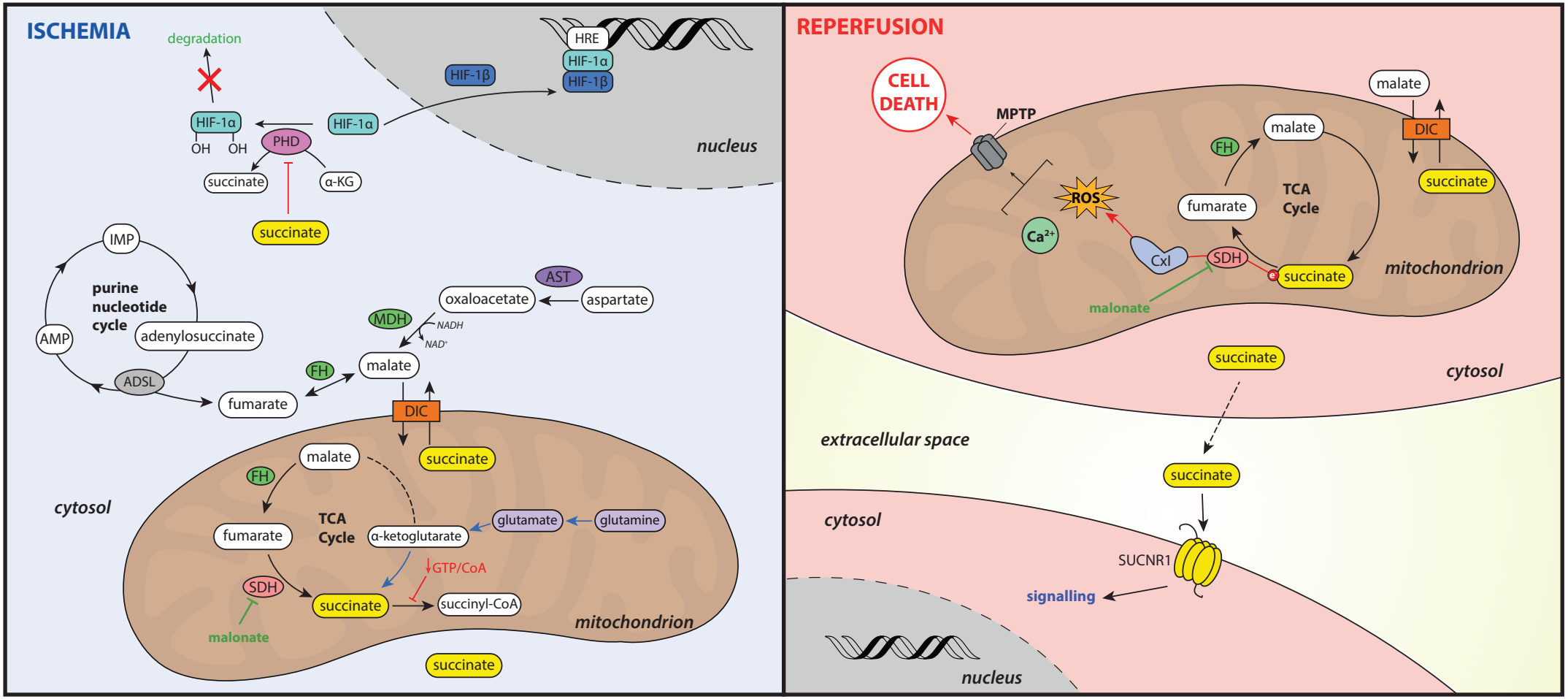
Figure Legend

Targeting succinate metabolism to protect against IR injury.

(a) During ischemia, succinate is extensively accumulated via reversal of SDH to reduce fumarate. Fumarate supply is maintained by either (i) the degradation of purine nucleotides by the purine nucleotide cycle or (ii) arising from upstream aspartate transamination and translocated to the mitochondria via the dicarboxylate carrier (DIC). With the depletion of the GTP and CoA pool in mitochondria, succinate activation to succinyl-CoA is inhibited, thus succinate is a terminal metabolite during ischemia. The canonical TCA cycle may also contribute to succinate accumulation via glutaminolysis to alpha-ketoglutarate (α-KG). Due to the extent of succinate accumulation during ischemia, succinate is exchanged from mitochondria into the cytosol, where it can act to inhibit prolyl hydroxylases (PHD) involved in the degradation of hypoxia-inducible factor 1α (HIF-1α). As HIF escapes degradation, it can translocate to the nucleus and promote the transcription of a number of genes involved in the hypoxic response. Inhibiting SDH with DMM prevents the accumulation of succinate during ischemia. (b) During reperfusion, the accumulated succinate has two fates: oxidation by SDH or efflux from the cell. Succinate is rapidly oxidised by SDH, generating a highly reduced CoQ pool and large mitochondrial membrane potential. These conditions drive reverse electron transport through complex I (CxI), generating superoxide, the proximal reactive oxygen species (ROS). Together with calcium dyshomeostasis, these events support the opening of the mitochondrial permeability transition pore (MPTP) and subsequent cell death associated with IR injury. By inhibiting SDH with malonate during reperfusion, succinate oxidation is slowed, reducing ROS production. In addition to oxidation, succinate is exported from the cell and able to enter the systemic circulation, thus has the potential to carry out a signalling role by interacting with the succinate receptor, SUCNR1. HRE, hypoxia response element; IMP, inosine monophosphate; AMP, adenosine monophosphate; ADSL, adenylosuccinate lyase; FH, fumarate hydratase; MDH, malate dehydrogenase; AST, aspartate transaminase; DIC, dicarboxylate carrier.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NADH
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glutamine
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malonate
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