A Review of Cardiac Ischemia-Reperfusion Injury Directions

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"Heart attack" or myocardial infarction (MI) is a dangerous condition in which one or more major arteries feeding the heart become mechanically blocked by material that causes reduction in blood flow to the cardiac muscle tissue. This is called an "ischemic event" and depending on how long the muscle is deprived of oxygen and nutrients, it is often fatal. And though the blockade of the artery can be managed in some cases with emergency medical help like balloon angioplasty or stents, and blood clot dissolving enzymes like Streptokinase and tPA, the heart muscle becomes damaged to some extent in almost every instance, sometimes irreversibly. The re-perfusion of nutrients and oxygen back into the temporarily starved muscle is helpful and necessary for the patient to live, but the heart damage upon restoration of flow starts a cascade of inflammation and degradation processes. These processes usually are sub-cellular and operate on fixing small-scale injuries like by doing more-intense-than-usual exercise, but when the damage is extensive, these can interfere with recovery from the event. This is called cardiac Ischemia-Reperfusion Injury (IRI). The search has been ongoing to find cardio-protective agents that will minimize or halt IRI.

IRI is a medically important condition and can be modeled *in vitro*. Because the basic mechanism of ischemia is denial of oxygen and nutrients to cardiac muscle, the initial condition is metabolite buildup and acidosis and then cardiac tissue death by several mechanisms such as necrosis, apoptosis, autophagy, and necroptosis (Ibáñez, 2015). Models of cardiac tissue damage have been established in which transformed cardiomyocytes are grown to confluence on coated plates in media, the media removed and replaced with degassed and nutrient-free isotonic

solution for a given time interval, after which, the cells are analyzed for survival when pre- or post-treated with theoretical protectants or controls (Chen, 2018). Extrapolating from the *in vitro* situation to real world applicability of findings and *vice versa* can be challenging. For example, the hormone-like peptide exenatide (Chang, 2014) has been shown to be cardioprotective *in vitro* at concentrations that would not be found in a living person. "Beta-blocker" drug metoprolol is cardioprotective in the clinic against IRI (Clemente-Moragón, 2020), and was a heart medicine already in use for a long time when it was discovered that in addition to blood pressure lowering, it also interfered with immune neutrophil cells that would cause inflammation after reperfusion during the cellular cleanup phase. Astragaloside IV (Si, 2014), a chemical extracted from a traditional Chinese medicine, operates by a complex independent pathway that eventually interferes with apoptosis caused by IRI.

The amino acid Leucine and its metabolites have been shown to protect against and enhance recovery of damaged skeletal muscle. Roughly 1/3 of all muscle tissue by weight is composed of branched chain amino acids (BCAAs): Leucine, Isoleucine, and Valine. Biochemical mechanisms monitor and conserve muscle amino acids by signaling the need to build up (anabolism) or tear-down (catabolism) large muscle proteins in response to work or damage. Branch chain amino acid Leucine was noticed to have a very different uptake mechanism (L-type vs. A-type), distribution, and concentration in different tissues (Oxender, 1963) and it was speculated that Leucine metabolites may play a role in managing muscle amino acid economy (Hider, 1969) The influence of Leucine in enhancing protein synthesis during heart perfusion studies became apparent (Morgan, 1971) and it was eventually proposed that Leucine itself was the regulatory molecule responsible for controlling overall muscle amino acid turnover (Buse, 1975). However, it was shown conclusively that this control is actually as a

response to the presence of oxidative metabolites of Leucine (Tischer, 1982). Mammal cells block muscle catabolism and direct anabolism using the Leucine metabolites alpha-keto isocaproate (AKIC) and hydroxymethylbutyrate (HMB) (Nissen, 1996; Nissen 1997; Knitter, 2000; Garlick, 2005).

Similar to skeletal muscle, proteolysis of cardiac muscle tissue and resulting inflammation is the primary mechanism by which IRI is caused. When blood supply to the heart muscle is interrupted the cellular pH drops and oxygen available for mitochondrial energy production is eliminated, causing a chain of events that if not corrected will begin to signal to the muscle cells that lysosomal degradation of the dying organelles can begin (Heusch, 2004). This is the well-known normal response to microscopic cellular cleanup that would take place for small metabolic interruptions that happen all the time. But when the destruction of tissue is more than just a pinpoint and instead covers a large amount of working cardiac muscle, these signals cause large inflammation and signaling to get the immune system involved in cleanup (Saini, 2005). The strong chemical signals that call in the immune system and cause inflammation are called “cytokines” and one large contributor is called TNF-alpha. HMB attenuates the global effect of TNF-alpha by interrupting a long series of events that would lead to degradation in the absence of HMB (Eley, 2008).

Based on our knowledge of IRI and skeletal muscle recovery, it is worth investigating whether or not Leucine metabolites, namely HMB, are an important low cost additional strategy to dull the effects of cardiac IRI. HMB testing for in vitro IRI models is worth consideration.

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