

Metabolic cardiac protection

Giacinta Guarini, Cardiovascular Medicine Division, Cardio Thoracic and Vascular Department,
University of Pisa, Italy

Correspondence: Giacinta Guarini, Cardiovascular Medicine Division, Cardio Thoracic and Vascular Department,
University of Pisa, Via Paradisa, 2, 56100 Pisa, Italy.
Tel.: +393494544161, e-mail: giacinta_guarini@yahoo.it

Abstract

Several animal and clinical studies have clearly demonstrated the clinical importance of ischemia reperfusion injury (IRI) in the setting of an acute coronary syndrome (ACS). It is well known that a shift of glucose metabolism from oxidative phosphorylation to substrate level phosphorylation (glycolysis) occurs during prolonged ischemia. As a consequence, protons and lactic acid accumulate within the cells, while ATP, NADH and NAD⁺ dehydrogenase levels decrease. Fatty acid oxidation contribute in the ischemic myocardium to the increased production of reactive oxygen species (ROS), that participate to mitochondrial uncoupling further reducing mitochondrial efficiency and ATP production. These and other changes in cardiac metabolism contribute to the opening of the mitochondrial permeability transition pore (mPTP), responsible for myocytes apoptosis. Varieties of pharmacological approaches have been proposed to minimize IRI and maximize the myocardial salvage, than those achieved by reperfusion alone. This brief review will provide the rationale for the use of metabolic approaches to protect the heart from ischemia and during reperfusion.

Keywords: cardiac ischemia; ischemia/reperfusion injury; cardioprotection; trimetazidine

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Introduction

The process of restoring blood flow to the ischemic myocardium can induce *per se* injury. This phenomenon, known as myocardial ischemia reperfusion injury (IRI), can paradoxically reduce the beneficial effects of myocardial reperfusion. IRI is defined as injury and death of cardiac myocytes that were viable before reperfusion induced by the restoration of coronary blood flow to an ischemic tissue. Therefore, the IRI decreases the beneficial effects that myocardial reperfusion would have on infarct size and prognosis, by independently inducing cardiomyocyte death.

While investigators have progressively identified multiple mechanisms that participate to the IRI, the concept of metabolic protection of the ischemic myocardium is in continuous evolution. Indeed, it is expected that a clear understanding of the mechanisms responsible for myocytes death during myocardial ischemia, and reperfusion, will provide fundamental knowledge in order to generate successful strategies for the treatment of acute ischemic events.

Cardiac metabolism in normal condition

In normal condition, the heart is able to utilize multiple fuel sources (i.e., fatty acids, glucose, and lactate, ketone bodies) to generate high-energy phosphates (particularly ATP), necessary for the daily demands. Fatty acids (FA) constitute the predominant source of energy in the normal heart, supplying from 60 to 80% of energy. Carbohydrate metabolism is controlled by the

rate of FA oxidation (Randle phenomenon), which strongly inhibits the rate of glucose and lactate uptake and oxidation. This inhibition, determined by elevated FA oxidation levels is mediated by increased ratio of NADH/NAD⁺ and of acetyl co-A/free co-A and enhanced activity of pyruvate dehydrogenase kinase (PDHK), which conversely phosphorylates and inhibits pyruvate dehydrogenase (PHD) activity.

Myocardial ischemia and reperfusion alters cardiac metabolism and cell homeostasis

Myocardial ischemia dramatically alters cardiac metabolism, energy production and cellular homeostasis. During ischemia, due to reduced oxygen level, glucose taken up by the myocardium is not efficiently oxidized into mitochondria but is rather converted to lactate. As a consequence, a switch from lactate consumption to lactate production is observed in the ischemic myocardium. These changes lead to disruption of cell homeostasis, with reduction in ATP content within the cells. Accumulation of lactate and H⁺ induce a fall in intracellular pH and decrease in contractile work. Paradoxically, the ischemic myocardium continues to drive most of its energy (50–70%) from the oxidation of fatty acids despite there being a high rate of lactate production. In this condition high rates of FA oxidation further inhibit glucose oxidation (Fig. 1). FA oxidation in this setting contributes to further reduce mitochondrial efficiency and during reperfusion participate to the generation of the IRI.

Mitochondria use oxygen as a substrate, so by default their respiration is inhibited during ischemia. During ischemia, excess protons due to lactic acidosis drive a cytosolic Na⁺ overload via the Na⁺/H⁺ exchanger (NHE) [1]. This Na⁺ exits via the Na⁺/Ca²⁺ exchanger, causing a cytosolic Ca²⁺ overload. The ischemic lack of ATP prevents adequate management Ca²⁺ by the SERCA pump, and subsequently Ca²⁺ is increased in the cytosol. While some Ca²⁺ uptake into mitochondria can occur during ischemia, it is not sufficient to trigger opening of the mPTP, which is also held closed during ischemia due to the acidic pH [2]. Prompt reperfusion will induce a rapid restoration of substrates essential for the generation of ATP, as well as rapid increase in the oxygen supply, and prompt normalization of the extracellular pH. All these events concur to IRI. Rapid normalization of the extracellular pH will instantly create an extreme H⁺ gradient across the plasma membrane that triggers a robust Na⁺/H⁺ exchange and a massive Na⁺ influx. This Na⁺ gradient triggers the passive, inverted action of the surface Na⁺/Ca²⁺ exchanger, called “reverse mode,” which in turn allows intracellular Ca²⁺ overload. Also at this moment a burst of ROS generation occurs, which together with high mitochondrial Ca²⁺ concentration and re-alkalinization triggers mPTP opening [3], leading to cytochrome c release and cell death [4]. Mitochondrial cytochrome c release also inhibits mitochondrial respiratory function increasing ATP deficiency in the post-ischemic heart [5].

Metabolic targets to induce cardioprotection

The concept of metabolic protection of the ischemic myocardium is in constant evolution and has recently been supported by clinical studies. Historically, enhanced glucose metabolism and glycolysis were proposed as anti-ischemic mechanisms of cardioprotection. The metabolic cardioprotection paradigm includes 1) the benefit of improved coupling of glycolysis to glucose oxidation, 2) a more gradual wake-up of mitochondrial function at reperfusion. Consistent with this hypothesis in cardioprotective mechanisms, some studies have demonstrated that only protocols able to induce a significant delay in pH recovery were able to afford cardioprotection. Furthermore, a close correlation between the delay in pH recovery and the magnitude of myocardial salvage was documented [5]. The beneficial effect of coupling glycolysis to glucose

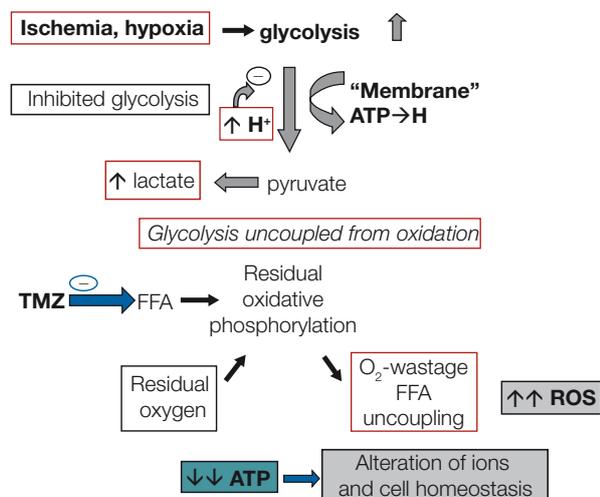


Fig. 1 Ischemia induced alteration in cardiac metabolism. See text for details. Modified from [20].

oxidation explains the efficacy of FA inhibitor such as trimetazidine. This hypothesis is supported by the sub-cellular linkage between key glycolytic enzymes and the activity of two survival-promoting membrane-bound pumps, namely the sodium-potassium ATPase, and the calcium uptake pump of the sarcoplasmic reticulum (SERCA). Indeed, it has been demonstrated that metabolic agents that decrease FA oxidation and increase the rate of glucose and lactate combustion induce enhanced viability after reperfusion. This metabolic strategy affords cardioprotection because glucose oxidation is more “oxygen efficient” than FA oxidation, producing more ATP for mole of oxygen consumed. The same effect might be achieved by administration of insulin-glucose-potassium (GIK) solution, but to date no conclusive results support a wide clinical use of this approach.

Metabolic cardioprotective agents

Mitochondria play a critical role in cardiac function, and are also recognized as crucially involved in signaling cardioprotective pathways. Targets of cardioprotection include Ca²⁺ overload, oxidative stress (ROS), ATP-dependent potassium channels (mK_{ATP}), mechanisms that ultimately lead to disruption of the inner mitochondrial membrane and opening of the nonspecific mitochondrial permeability transition pore (mPTP) complex causing cell death [6].

By increasing NADH and decreasing FAD content, cardiac ischemia conditions mitochondria to be in a relatively reduced state. Consistent with this notion are the observations that during ischemia NADH increases (reduced mitochondria) but on reperfusion NADH decrease and FAD increase, suggesting a more mitochondrial oxidation state [7]. The implications of these results are that a reduced redox state in the cardiac cell could be an important condition for efficient resumption of the H⁺ gradient for ATP synthesis and hence for better functional recovery. Prevention of ATP hydrolysis to maintain mitochondrial membrane $\Delta\Psi$ during ischemia is therefore a valid goal for preserving function. Along with these observations, a recent study has investigated the role of ranolazine in protecting against the IR injury. Its mechanisms of protection are not clearly understood but evidence points to blocking the late Na⁺ current that arises during ischemia, inhibition of mitochondrial complex I activity, or modulating mitochondrial metabolism. Mitochondria isolated from ranolazine-treated

hearts had mild resistance to permeability transition pore (mPTP) opening and less cytochrome c release than control hearts, suggesting that ranolazine was effective in protecting the heart against the IR injury [8]. Other pharmacologic reagents that inhibit complex I activity have now demonstrated to afford protection against IR injury. These include amobarbital [9], rotenone [10], S-nitrosothiols [11], analgesics such as capsaicin [12], volatile anesthetics such as halothane and isoflurane [13], and anti-diabetics such as metformin [14]. Complex I (NADH ubiquinone oxidoreductase) is a key site of electron entry into the respiratory chain in cardiac mitochondria, owing to the heavy reliance of the heart on β -oxidation of fatty acids and subsequent generation of NADH from acetyl-CoA in the Krebs' cycle. In addition to playing a role in cardiac metabolism, complex I is also an important site of ROS generation, and a regulator of NADH/NAD⁺ redox balance in the mitochondrion. Notably, the latter has been shown to be critical in determining the open probability of the mPTP, such that complex I inhibition can directly inhibit pore opening via an increased NADH/NAD⁺ ratio.

Since high fatty acid oxidation rate markedly decreases glucose oxidation, another approach to induce cardioprotection is to increase glucose oxidation, by inhibiting fatty acid oxidation. Pharmacological agents that inhibit fatty acid oxidation include direct beta-oxidation inhibitors. This novel group of compounds includes the 3-ketoacyl-coenzyme A thiolase (3KAT) inhibitor trimetazidine (TMZ). TMZ is a metabolic anti-ischemic agent with cardioprotective properties [15] that selectively inhibits the long chain 3-ketoacyl CoA thiolase (3-KAT) activity. This leads to reduction in fatty acid oxidation and stimulation of glucose oxidation that is independent of any hemodynamic effects that influence myocardial oxygen consumption. Various studies demonstrated that it inhibits mitochondrial oxidation of palmitoyl carnitine, thus only slightly altering oxidation of pyruvate and preserving mitochondrial oxidative functions [16]. It also stimulates PDH activity, the rate-limiting enzyme for glucose oxidation. As a result, TMZ attenuates the adverse effects of free fatty acid-associated oxidative stress [17], lessens oxygen demand by decreasing oxygen consumption [18], and improves mitochondrial metabolism and cardiac performance during ischemia [19]. TMZ has been reported to attenuate neutrophil activation, thereby protecting post-ischemic hearts from

neutrophil-mediated injury. At the cellular level, TMZ conserves ATP production and lowers intracellular acidosis and calcium overload, while maintaining cellular homeostasis. It reduces oxidative damage to the mitochondria and protects the heart from IR induced damage arising from mitochondrial respiration. TMZ has also shown cytoprotective efficacy in several models of myocardial infarction. In addition to its beneficial effects in the treatment of IRI, TMZ has been reported to induce protection in patients experiencing acute periods of ischemia when undergoing PCI. •

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