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ABNORMAL MITOCHONDRIAL FUNCTION DURING ISCHEMIA REPERFUSION PROVIDES TARGETS FOR PHARMACOLOGICAL THERAPY

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Summary

The concept of reperfusion injury has been a subject of intense debate. Some researchers believe that the entire injury develops during the ischemic period, whereas others argue that blood reflow extends tissue injury due to the release of oxygen-derived free radicals. an inflammatory reaction involving influx of various populations of immune cells, and dysregulation of intracellular and particularly mitochondrial calcium concentration.

Mitochondrial calcium overload in the presence of oxygen-derived free radicals can result in the opening of the mitochondrial permeability transition pore (mPTP), which further compromises cellular energetics. The resultant low ATP and altered ion homeostasis lead to a rupture of the plasma membrane and cell death.

Mitochondria have long been proposed as one of the main players in cell death, since the mitochondria are central to synthesis of both ATP and the formation of oxygen-derived free radicals. These mechanisms are centered on mitochondrial calcium overload as a key component of cell death. Pharmacological strategies that are cardioprotective attempt to reduce mitochondrial calcium overload to decrease the likelihood of arrhythmias and cardiac dysfunction elicited by reperfusion.

Introduction

Experimental animal models have shown that the ultimate myocardial outcome of ischemic-reperfusion sequence depends not only on the time delay between occlusion and reperfusion but also on whether lethal reperfusion injury occurs. Despite the early description of reperfusion syndrome and stunned myocardium phenomenon in experimental models,[1] researchers failed to recognize this syndrome in the human heart. Of interest, the occurrence of no-reflow during percutaneous coronary intervention for myocardial infarction was described 30 years ago by Krug et al. in a model of ischemia - reperfusion in which coronary occlusion was achieved by ligature without any thrombotic material. Nevertheless, researchers continued to regard the no-reflow phenomenon as a technical failure caused by thrombus fragmentation and distal embolism (Figure 1).[2,3] This observation was of great importance because it demonstrated that the no-reflow phenomenon occurs in the absence of thrombus; therefore, other mechanisms operate and mediate the no-reflow state.

Based on basic experimental work, the phenomenon of ischemia-reperfusion injury can be attenuated by inhibiting pathways such as the Na^+/H^+ exchange,4 modulation of kinases[5,6] inhibitors of the mPTP. Despite the evidence of molecular mechanisms that mediate cellular injury, most of the clinical strategies used to treat patients with acute coronary syndromes target the anatomical lesion, arterial spasm, and embolism.[7-10] Thus, there is a great opportunity to treat these patients by blocking cellular pathways of injury, for example, those mediated by mitochondrial dysfunction. We will review this specific mode of injury in more detail.

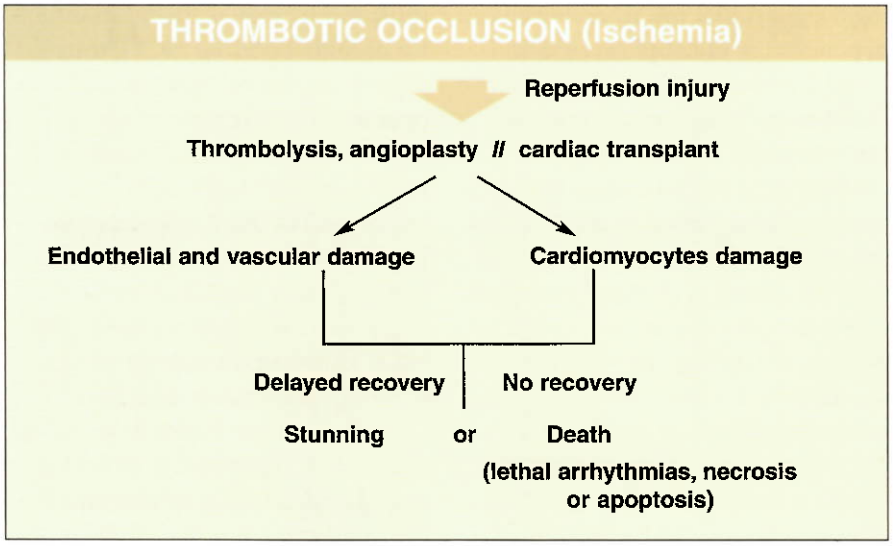

Figure 1. Diagram of the effects of ischemia and reperfusion injury following thrombotic occlusion of a coronary artery. As shown, the initial event is followed by endothelial vascular damage and cardiomyocyte injury. If there is no late recovery, the consequences are left ventricular dysfunction leading to heart failure and potentially death.

Mitochondrial Abnormalities Leading to Cellular Injury: Calcium Overload and Mediation of Apoptosis

Myocardial ischemia leads to various cellular events: altered membrane potential, altered ion distribution, cellular swelling, and cytoskeleton disorganization.[11] Ischemia due to anaerobic metabolism leads to depletion of adenosine triphosphate (ATP), resulting in severe damage to the integrity of heart cells. During reperfusion, abrupt reoxygenation causes further cell damage by oxygen-derived free radicals (ROS).[12] ROS affect the sarcoplasmic reticulum and the sarcolemmal membranes, increasing the cytosolic calcium concentration ($[Ca^{2+}]c$)[13, 14] and therefore the mitochondrial calcium concentration ($[Ca^{2+}]m$).[15] At high $[Ca^{2+}]m$, mitochondria undergo energy-consuming futile cycles through calcium release and reuptake, because the proton-driven energy from the respiratory chain is used for cation transport instead of mitochondrial ATP production. In addition, mitochondrial calcium overload triggers a nonspecific increase in the inner membrane permeability, which contributes to the uncoupling of oxidative phosphorylation and thereby to a diminished ATP synthesis. The ultimate reduction in ATP synthesis is one way by which mitochondrial dysfunction contributes to cell injury.

Another form of mitochondrial injury occurs through nonspecific membrane permeability changes that result in the release of intramitochondrial molecules that participate in apoptotic death signaling, e.g., cytochrome c, Smac/DIABLO, and apoptosis-inducing factor.[16]

The important issue is that cell death during ischemia-reperfusion appears to be an active process that can be inhibited with appropriate interventions. Taken together, the mitochondria emerges as an important mediator and regulator of all forms of cell death during ischemia-reperfusion. In particular, the mPTP appears to be a main regulator of both apoptotic and necrotic cell death. This hypothesis is further supported by the observation that inhibition of mPTP by knockdown of cyclophilin D (a regulator of the mPTP) results in a significant reduction in ischemia-reperfusion infarct size.

Thus, it is clear that mitochondrial dysfunction during ischemia-reperfusion causes cell injury at least by these two mechanisms: a reduction of ATP production and by the release of molecules that stimulate apoptotic pathways.

The Central Role of Calcium Regulation and Membrane Integrity

The central mechanism of injury is the regulation of intracellular calcium. Studies done more than 20 years ago showed that a rise in $[Ca^{2+}]c$ preceded irreversible myocardial injury and that drugs or protocols that reduced or delayed the rise in cytosolic $[Ca^{2+}]$ also reduced myocardial death.[17-20]

Each systole is initiated by Ca^{2+} entry via the L-type Ca^{2+} channel, resulting in Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR), and the combined increase in $[Ca^{2+}]c$ leads to contraction. The Ca^{2+} that enters via the L-type calcium channel is removed from the cell primarily by $Na+/Ca^{2+}$ exchange (NCX), with a very small contribution from the sarcolemmal Ca^{2+}-ATPase.[21] The Ca^{2+} released from the SR is reaccumulated into the SR via SR Ca^{2+}-ATPase (SERCA). Studies show that much of the rise in cytosolic Ca^{2+} during ischemia is due to Ca^{2+} entry by reverse-mode NCX secondary to the rise in cytosolic sodium $[Na^+]i$ during ischemia. This occurs because of increased generation of protons during ischemia are extruded from the cell via Na^+/H^+ exchanger (NHE), which results in an increase in intracellular Na^+.[17] Na^+ has also been shown to enter the cell during

ischemia on non-inactivating Na^+ channels. This rise in intracellular Na^+ coupled with the depolarized plasma membrane results in a reversal of NCX to bring Ca^{2+} into the cytosol. NHE inhibitors, such as cariporide, have been shown in animal studies to attenuate the rise in $[Na^+]i$ during ischemia and the subsequent rise in $[Ca^{2+}]i$ and to reduce ischemia-reperfusion injury. Inhibition of NCX has also been shown to be protective, and hearts from mice lacking the NHE or mice lacking the NCX have been shown to have reduced ischemia-reperfusion injury.[22] This data provides evidence that strategies aimed at the inhibition of the Na^+/H^+ and the Na^+/Ca^+ exchanger may be beneficial.

During the early phases of reperfusion, the intracellular pH is still acidic, and this pH gradient facilitates extrusion of H^+ from the cell in exchange for Na^+ via the NHE. The increased cytosolic Na^+ can be extruded by Na^+/K^+-ATPase or NCX in exchange for Ca^{2+}, thereby raising $[Ca^{2+}]c$, which leads to cell death. Beyond the direct cellular injury mediated by high intracytosolic Ca^{2+}, this event also predisposes the generation of potentially fatal arrhythmias. Also, fluctuations in $[Ca^{2+}]c$ can lead to hypercontracture, which results in ATP decline and deterioration of the myocyte.[23, 24] Improving SR Ca^{2+} handling has been shown to be an important target for reducing ischemic injury.[25, 26]

Ca^{2+} uptake into mitochondria occurs via the mitochondrial calcium uniporter (mCaU), which uses the energy of mitochondrial membrane potential; thus membrane potential used to transport Ca^{2+} is not available to synthesize ATP from ADP. The mCaU is inhibited by ruthenium red and related compounds. Surprisingly, the mCaU has not been cloned, but recent findings suggest that mCaU activity is dependent of uncoupling protein 2 and 3 in the mitochondrial inner membrane.[27] Ca^{2+} uptake into the mitochondria will dissipate the membrane potential unless electron transport is activated to reestablish the gradient. Thus, during ischemia, with inhibition of electron transport due to lack of oxygen, one would expect Ca^{2+} uptake into the mitochondria to dissipate the membrane potential, ultimately limiting Ca^{2+} uptake into the mitochondria. Ca^{2+} is then released from the mitochondria in exchange for Na^+ (on the mitochondrial NCX). The matrix Na^+ level has been reported to be regulated by mitochondrial NHE and thus the pH gradient across the inner mitochondrial membrane. In energized mitochondria, the Na^+ gradient is inwardly directed, and $[Na^+]i$ is reported to be lower at the matrix.[28]

During simulated ischemia, most studies suggest that there is a small rise in mitochondrial Ca^{2+}.[29-31] Interestingly, Griffiths et al.[29] observed that the rise in mitochondrial Ca^{2+} during ischemia was inhibited by clonazepam (an inhibitor of mitochondrial NCX), thus suggesting the mitochondrial NCX operates in the reverse mode to increase mitochondrial matrix Ca^{2+}. It is suggested that the Na^+ gradient decreases due to the decrease in pH gradient, along with the rise in $[Ca^{2+}]i$, may contribute to Ca^{2+} entry into the mitochondria by the NCX. However, clonazepam augmented the rise in mitochondrial Ca^{2+} during reperfusion, suggesting that during reperfusion, when the mitochondrial pH gradient and the Na^+ gradient are restored, the mitochondrial NCX extrudes Ca^{2+} from the mitochondria.

Two lines of evidence suggest that mitochondrial membrane homeostasis is essential during ischemic reperfusion injury. First, Chávez et al.[32] reported for the first time that cyclosporine A (CsA), a classical mPTP inhibitor, decreased myocardial injury following ischemia and reperfusion. CsA does not reduce the Ca^{2+} loading of mitochondria, but reduces the Ca^{2+} sensitivity of the mPTP. Second, we recently reported that ruthe-

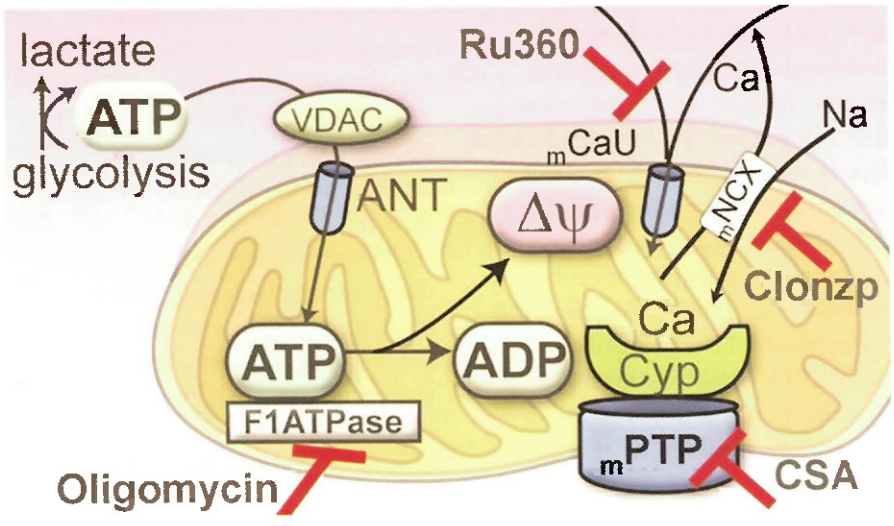

Figure 2. Inhibitors of mitochondrial calcium handling. As shown in the diagram, cyclosporin is an inhibitor of the mitochondrial permeability transition pore (mPTP) that in turn regulates apoptotic and necrotic cell death. Ruthenium 360 inhibits the mithocondrial calcium uniporter (for biological effects of the mCaU, see Figure 3).

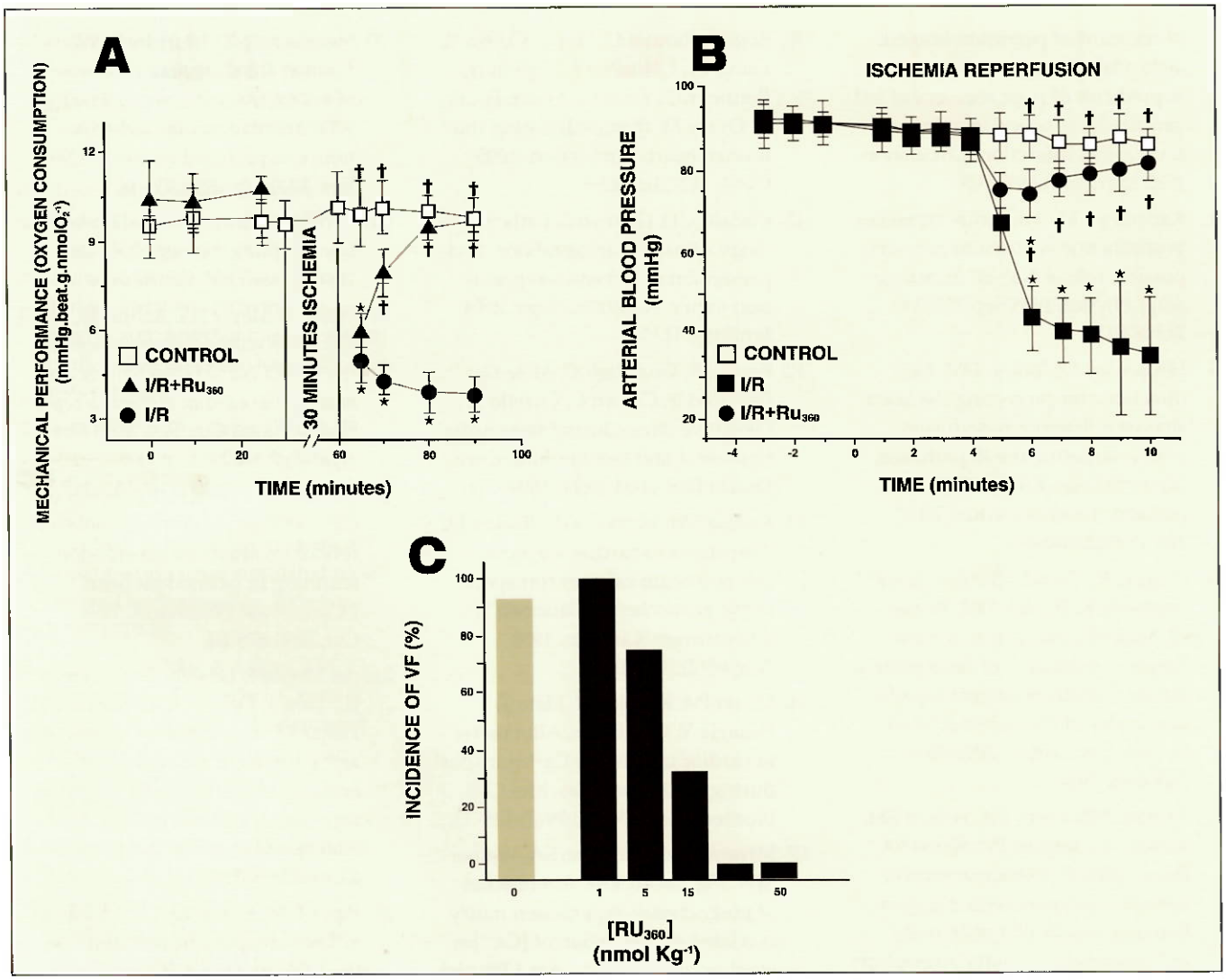

Figure 3. Effect of Ruthenium 360 (an inhibitor of mCaU) on ischemia reperfusion injury. Isolated hearts were treated in a Langerdhoff perfusion system and subjected to ischemia and reperfusion. The hearts were treated with Ruthenium 360, and we measured mechanical performance/oxygen consumption (A) and arterial blood pressure (B). As shown in the graphs, Ruthenium 360 normalized mechanical performance and restored blood pressure.

nium 360 (Ru360, a specific blocker of mCaU) improved recovery of cardiac work during ischemia reperfusion injury (Figure 2).[33] The specific inhibition of the mCaU ultimately prevented mitochondrial permeability transition pore opening. In addition, we showed that Ru360 treatment abolished the incidence of arrhythmias and hemodynamic dysfunction elicited by reperfusion (Figure 3).[34] Ru360 administration also partially inhibits calcium uptake, preventing mitochondria from depolarizing. Taken together, these observations demonstrate that myocardial damage could result from failure of the mitochondrial network to maintain the membrane potential during reperfusion. Strategies that maintain the integrity of the membrane during ischemia and reperfusion injury are exemplified by CsA and Ru360.

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