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STATE-OF-THE-ART REVIEW

Mitochondrial Membrane Permeability Inhibitors in Acute Myocardial Infarction

Still Awaiting Translation

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SUMMARY

Despite therapeutic advances, acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide. One potential limitation of the current treatment paradigm is the lack of effective therapies to optimize reperfusion after ischemia and prevent reperfusion-mediated injury. Experimental studies indicate that this process accounts for up to 50% of the final infarct size, lending it importance as a potential target for cardioprotection. However, multiple therapeutic approaches have shown potential in pre-clinical and early phase trials but a paucity of clear clinical benefit when expanded to larger studies. Here we explore this history of trials and errors of the studies of cyclosporine A and other mitochondrial membrane permeability inhibitors, agents that appeared to have a promising pre-clinical record yet provided disappointing results in phase III clinical trials. (J Am Coll Cardiol Basic Trans Science 2016;1:524–35)

Despite therapeutic advances, acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality worldwide. Sustained research efforts over the years have achieved numerous milestones (1–3). Despite the success stories, the rate of progression to heart failure and related complications remains unacceptably high (3,4). One potential limitation is the lack of effective therapies to optimize reperfusion after ischemia and prevent reperfusion-mediated injury. This has been termed *reperfusion injury*, or alternatively *ischemia-reperfusion injury* (5). Experimental studies indicate that this process accounts for up to 50% of the final infarct size, lending it importance as a potential target for cardioprotection (6). However, multiple therapeutic approaches have failed to translate from the bench to the bedside, or have shown therapeutic potential in early phase II trials (7–9) but have failed to translate into a clear clinical benefit when tested in larger phase III clinical studies (10–12). Here we explore this history of trials and errors, with a particular focus on the studies of cyclosporine A (CsA) and other mitochondrial membrane permeability inhibitors, a therapeutic approach that appeared to have a promising pre-clinical record yet provided disappointing results in phase III clinical trials.

REPERFUSION INJURY

The phenomenon of reperfusion injury was first born out of a demonstration that post-ischemic restoration of blood flow had several potential deleterious effects, including myocardial stunning (13). The idea of harm from reperfusion was later supported by demonstration of smaller infarct size with slower, low-pressure reperfusion over standard abrupt

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reperfusion at normal pressure, a benefit gained from interventions applied after the ischemic period rather than within it (14).

During ischemia, intracellular Na^{+} and Ca^{++} accumulate as downstream results of acidosis from anaerobic glycolysis, ultimately reaching a Ca^{++}-overloaded state. Upon reperfusion, the rapid normalization of pH causes uncontrolled myocyte contraction, intracellular edema, and formation of reactive oxygen species (ROS) (15). Within the context of reperfusion injury, perhaps the most salient component of this process occurs at the inner mitochondrial membrane (IMM). The IMM remains closed throughout ischemia but undergoes an abrupt transition in permeability during reperfusion (16), which collapses the membrane potential and uncouples oxidative phosphorylation (17). As a result, there is increased inorganic phosphate concentration, increased Ca^{++} flux, and mitochondrial edema, ultimately leading to the cytoplasmic release of cytochrome C, a proapoptotic protein that activates caspase-3 and leads to the death of the cardiac myocyte (5,6,15,18) (**Figure 1**).

The biochemical liaison for the increased leakage of the IMM is the mitochondrial permeability transition pore (mPTP). Much remains to be understood about the mPTP, but a leading hypothesis holds that the mPTP forms from F-type adenosine triphosphate synthase dimers within the lipid bilayer of the IMM (19). The channel opens and is forced to remain open in response to high concentrations of calcium, inorganic phosphate, and ROS, or with reduced IMM potential. All of these conditions are active during myocardial ischemia and reperfusion (20).

Many other signaling cascades and processes within and outside the mitochondria are concomitantly activated during ischemia and reperfusion and are likely to contribute to infarct size. For the scope of this review, we focused only on the mechanisms involving a change in permeability in the mitochondrial membrane for which a drug had been tested in both pre-clinical and clinical studies.

ISCHEMIC PRE-CONDITIONING

This discovery that reperfusion could be a double-edged sword (13) spawned a new wave of experiments. One landmark study described how repeating cycles of alternating ischemia and reperfusion performed prior to prolonged coronary artery occlusion significantly reduced final infarct size in dogs (even when total ischemia time was longer); this created the new field of "pre-conditioning" (21), replicated in numerous laboratories around the world (22–24). A "second window" of cardioprotection was also shown to begin 24 h after the initial window of protection. In 1 study, infarct size was reduced in rabbits subjected to four 5-min cycles of coronary artery occlusion prior to 24-h recovery, followed by a 30-min reocclusion (25) or a 90-min reocclusion (26). The clinical translational value of having identified the second window of cardioprotection, however, is still uncertain.

Interestingly, limitations of the pre-conditioning strategy became apparent quite rapidly. Cardiac protection was seen when occlusion/reperfusion cycles were performed prior to 40- or 60-min occlusion, but not prior to 90- (27) or 180-min (21) occlusion. These experiments highlight that the efficacy of pre-conditioning is limited to a specific window of duration of ischemia. Hence the difficulty in translation to humans: it is difficult to determine exactly when a patient starts experiencing ischemia, and usually by the time the patient is seen in the hospital, the ischemia has been ongoing for hours.

ISCHEMIC POST-CONDITIONING

An additional step forward in the field was provided by the demonstration of protective effects of interventions applied after the ischemia and the initial reperfusion: "post-conditioning." Brief serial episodes of controlled reperfusion interrupted by repeated brief bursts of ischemia reduced final infarct size: 3 cycles of 30 s of reperfusion alternating with 30 s of occlusion (for a total of 3 min), after 60 min of ischemia due to coronary occlusion provided a significant reduction in infarct size in dogs by 30% to 40% (28). The finding of beneficial effects of conditioning being applied after reperfusion provided an intense stimulus to the field, as it suggested a time window for intervention that extended beyond reperfusion. The same studies, however, also highlighted the critical time dependency of these approaches. Post-conditioning as means of controlled reperfusion intermittently interrupted by brief ischemia is effective in reducing infarct size if initiated within seconds of reperfusion (29,30) while failing to reduce infarct size if post-conditioning is delayed by more than 1 min (30,31). These considerations may explain the challenge of translating into clinical benefits in recent clinical trials (32).

PHARMACOLOGIC ISCHEMIC CONDITIONING

The study of the events occurring in ischemic pre- and post-conditioning has stimulated a large number of investigations into the signaling and mechanisms.

ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

CsA = cyclosporine A

IMM = inner mitochondrial membrane

mPTP = mitochondrial permeability transition pore

PCI = percutaneous coronary intervention

ROS = reactive oxygen species