ORIGINAL ARTICLES

Reversible blockade of electron transport with amobarbital at the onset of reperfusion attenuates cardiac injury

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Mitochondrial dysfunction contributes to myocardial injury during ischemia and reperfusion. Ischemia damages the mitochondrial electron transport chain. Therapeutic intervention during early reperfusion decreases cardiac injury, which suggests that myocardial injury can be attenuated even though mitochondria were already damaged during the preceding ischemia. Our previous study shows that amobarbital given only before ischemia prevents ischemic damage to the electron transport chain and decreases infarct size measured during reperfusion in Langendorff-perfused Fischer 344 rat hearts. In the current study, amobarbital was given at the onset of reperfusion to test whether the blockade of proximal electron transport only during early reperfusion can decrease myocardial injury. Amobarbital administrated during early reperfusion decreased infarct size compared with untreated hearts, which suggests that the modulation of electron transport during early reperfusion attenuates myocardial injury. The increased generation of reactive oxygen species (ROS) contributes to injury. We tested whether the blockade of proximal electron transport prevents ROS release from the mitochondria that sustained ischemic damage. The blockade of the proximal electron transport chain at complex I attenuates maximal ROS generation from ischemia-damaged mitochondria. Thus, the modulation of oxidative function during reperfusion provides a translationally relevant opportunity to prevent a portion of the mitochondrial-dependent injury. The cardiac protection by amobarbital given during reperfusion may result from decreased ROS generation from the electron transport chain. (Translational Research 2009;153:224–231)

Abbreviations: AMO-AFT = amobarbital during early reperfusion; AMO-PRE = amobarbital before ischemia; ETC = electron transport chain; IFM = interfibrillar mitochondria; i.p. = intraperitoneally; K-H = Krebs-Henseleit; LVDP = left ventricular developed pressure; MPTP = mitochondrial permeability transition pore; ROS = reactive oxygen species; SEM = standard error of the mean; SSM = subsarcolemmal mitochondria

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Supported by the Program Project Grant 2PO1AG15885 from the National Institutes of Health and the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

Submitted for publication October 29, 2008; revision submitted February 9, 2009; accepted for publication February 12, 2009.

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1931-5244/$ – see front matter

doi:10.1016/j.trsl.2009.02.003

AT A GLANCE COMMENTARY

Background

Cardiac ischemia damages the mitochondrial electron transport chain. The modulation of mitochondrial respiration before ischemia protects mitochondria during ischemia and decreases myocardial injury during reperfusion, which suggests that ischemia-mediated mitochondrial damage contributes to myocardial injury. Postconditioning applied at the onset of reperfusion decreases myocardial injury, which indicates that therapeutic intervention at the onset of reperfusion still can attenuate myocardial injury.

Translational Significance

The modulation of mitochondrial respiration with amobarbital at the onset of reperfusion decreases cardiac injury. The reversible blockade of electron transport could be regionally employed to decrease myocardial injury during the treatment of ST-segment elevation myocardial infarction by the direct infusion of pharmacologic agent into the artery at the time of restoration of flow by stent deployment.

Ischemia-reperfusion leads to myocardial infarction and compromises cardiac function. Mitochondrial dysfunction plays a key role in myocardial injury during ischemia-reperfusion.[1,2] Cardiac ischemia damages the mitochondrial electron transport chain (ETC),[3,4] whereas reperfusion does not cause more damage,[5] which indicates that mitochondrial damage mainly occurs during ischemia. However, substantial cardiac myocyte injury occurs during reperfusion.[6] Although mitochondrial damage and myocyte injury can occur in the different time periods, these 2 events are tightly connected. The protection of mitochondria by blockade of electron transport during ischemia with amobarbital[7,8] or ischemic preconditioning[9] decreases myocardial injury measured during reperfusion, which provides strong support that ischemic mitochondrial damage contributes to myocardial injury during reperfusion.[10,11]

Although prevention of mitochondrial damage during ischemia is optimal, this approach has limited therapeutic usefulness because of the unpredictable occurrence of ischemic events. Interventions applied at the onset of reperfusion, including ischemic postconditioning,[12] hypoxia,[13] or pharmacologic intervention,[14] are more practical for therapeutic use. Although postconditioning has been studied intensively, the precise mechanism by which postconditioning decreases myocardial injury remains unclear. It is also unclear whether the blockade of electron transport during reperfusion can protect hearts. The opening of the mitochondrial permeability transition pore (MPTP) is suggested as the final step to induce myocyte death, and prevention of pathologic opening is a potential mechanism for postconditioning protection.[15,16] Cyclosporin A (an MPTP inhibitor) given during reperfusion decreases myocardial injury in isolated rat hearts, which supports the hypothesis that MPTP leads to injury during reperfusion.[15] These results also indicate that proper intervention during reperfusion may still prevent the opening of an MPTP even if the ETC was already damaged during ischemia. The increased production of reactive oxygen species (ROS) is a key factor to induce MPTP.[1,2] The ischemic damage to the ETC increases ROS generation in isolated cardiac mitochondria.[17,18] The blockade of electron transport with amobarbital during ischemia protects mitochondrial oxidative phosphorylation and decreases ROS generation from isolated mitochondria[8,19] and in the isolated heart,[20] and it leads to less cardiac injury during reperfusion.[8,20]

We tested whether the direct blockade of electron transport by amobarbital only at the onset of reperfusion can decrease cardiac injury. At the onset of reperfusion, cardiac mitochondria were already damaged by ischemia.[4,19,21] The inhibition of proximal electron transport decreases ROS generation from the ETC in isolated control mitochondria.[22] We also evaluated whether the inhibition of the proximal electron transport can decrease maximal ROS generation in ischemia-damaged mitochondria relevant to the pathogenesis of reperfusion injury.[10] In the current study, amobarbital inhibition during reperfusion decreases cardiac injury, which indicates that the modulation of mitochondrial electron transport during reperfusion is a clinically relevant approach to protect the heart. Reperfusion therapy of ST-segment elevation myocardial infarction by primary stent deployment[23] provides the cardiologist access to the ischemic-reperfused zone at the onset of reperfusion. The reversible blockade of electron transport at the onset of reperfusion by amobarbital administrated directly into the coronary artery during coronary intervention has the translational potential to decrease cardiac injury during reperfusion in clinical settings.

METHODS

Preparation of rat hearts for perfusion. The Animal Care and Use Committees of the Louis Stokes Cleveland VA Medical Center and Case Western Reserve University approved the protocol. Male Fischer rats [6–8 months of age (350–420 g)] were anesthetized