ORIGINAL ARTICLE

Postconditioning with Intralipid emulsion protects against reperfusion injury in post-infarct remodeled rat hearts by activation of ROS-Akt/Erk signaling

MICHAEL ZAUGG, PHING-HOW LOU, ELIANA LUCCHINETTI, MANOJ GANDHI, and ALEXANDER S. CLANACHAN

EDMONTON, CANADA

The clinically used lipid emulsion Intralipid (ILE) reduces ischemia reperfusion injury in healthy rodent hearts. We tested whether ILE is cardioprotective in postinfarct remodeled hearts. Post-infarct remodeled and sham Sprague-Dawley rat hearts were perfused in working mode and subjected to ischemia (15 minutes) and reperfusion (30 minutes). Left ventricular (LV) work was measured in hearts that were untreated or that received ILE (1%) postconditioning administered at the onset of reperfusion, or the reactive oxygen species (ROS) scavenger N-(2-mercaptopropionyl)-glycine (10 μM) alone or in combination with ILE. Mitochondrial O_2 consumption was measured in LV muscle fibers. Acetyl CoA production was calculated from the oxidation of (U-^{14}C)glucose and (9,10-^{3}H)palmitate. ROS production was assessed by loss of aconitase activity as well as by release of hydrogen peroxide. Phosphorylation of Akt, Erk1/2, and STAT3 were used to evaluate protection signaling. Remodeled hearts exhibited LV dysfunction and signs of hypertrophy consistent with significant postinfarct remodeling. ILE postconditioning enhanced the recovery of postischemic LV function in remodeled hearts, preserved energy metabolism in mitochondria, accelerated palmitate oxidation and acetyl CoA production, and activated Akt/Erk/STAT3 in a ROS-dependent manner. Protection by ILE postconditioning evolved rapidly within the first minutes of reperfusion without evidence of additional cardiotonic effects due to provision of supplementary energy substrates potentially released from ILE during reperfusion. ILE represents a novel and clinically feasible cardioprotective strategy that is highly effective in remodeled hearts. Our data provide a rationale for the clinical evaluation of ILE postconditioning where ILE is administered as a bolus at the onset of reperfusion. (Translational Research 2017;■:1–16)

Abbreviations: ADP = adenosine diphosphate; Akt = protein kinase B; ANOVA = analysis of variance; ANP = atrial natriuretic peptide; ATP = adenosine triphosphate; BSA = bovine serum albumin; CAL = coronary artery ligation; COX = cytochrome c oxidase; CS = citrate synthase; DTNB = 5,5′-dithiobis-(2-nitrobenzoic acid); ECL = enhanced chemiluminescence; EF = left ventricular ejection fraction; EGTA = ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; Erk = extracellular signal–regulated kinase; ETC = electron transport chain; FS = left

From the Department of Anesthesiology & Pain Medicine, University of Alberta, Edmonton, Canada; Department of Pharmacology, University of Alberta, Edmonton, Canada.

Submitted for publication January 18, 2017; revision submitted May 2, 2017; accepted for publication May 23, 2017.

Reprint requests: Alexander S. Clanachan, Department of Pharmacology, University of Alberta, Edmonton, Canada T6G2H7; e-mail: sandy.clanachan@ualberta.ca.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2017.05.007

ventricular fractional shortening; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRP = horseradish peroxidase; Hz = hertz; ILE = Intralipid emulsion; IR = ischemia reperfusion; LV = left ventricular; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; MPG = N-(2-mercaptopropionyl)glycine; mPTP = mitochondrial permeability transition pore; NADP = nicotinamide adenine dinucleotide phosphate; NHE = sodium-hydrogen exchanger; NP-40 = nonyl phenoxypolyethoxylethanol; PAGE = polyacrylamide gel electrophoresis; RISK = reperfusion injury salvage kinase; ROS = reactive oxygen species; SDS = sodium dodecyl sulfate; SEM = standard error of mean; SOD = superoxide dismutase; STAT3 = signal transducer and activator of transcription 3; Tris-HCl = 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride

AT A GLANCE COMMENTARY

Zaugg M, et al.

Background

Left ventricular remodeling is one of the major factors contributing to failed clinical translation of many experimental cardioprotective interventions. The clinically-used lipid emulsion Intralipid administered as a bolus at the onset of reperfusion (postconditioning) reduces ischemia reperfusion injury in healthy hearts. However, it is not clear whether Intralipid is cardioprotective in post-infarct remodeled hearts.

Translational Significance

Intralipid postconditioning represents a novel and clinically feasible cardioprotective strategy that is effective in both healthy and post-infarct remodeled hearts. Our data provide a rationale for the clinical evaluation of Intralipid postconditioning.

INTRODUCTION

Coronary artery disease, which leads to coronary artery occlusion and myocardial infarction, is a leading cause of death of men and women. While the effective approach to manage myocardial ischemia is to re-establish coronary perfusion with drugs (fibrinolysis and platelet receptor inhibition), nonsurgical (angioplasty and/or stent placement), or surgical (coronary artery bypass graft) interventions, the associated reperfusion of the myocardium induces additional cellular injury that slows the recovery of left ventricular (LV) mechanical function and initiates a pathologic remodeling process that ultimately leads to LV failure and death.[1] Thus, there have been wide-ranging investigations of the mechanisms underlying ischemia reperfusion (IR) injury and post-ischemic LV mechanical dysfunction, as well as multiple attempts at the identification of potential drug targets for cardioprotective interventions.[2-5] However, their clinical translation has not been successful.[6-9]

Multiple reasons for the lack of cardioprotective effectiveness in clinical reperfusion have been considered.[2,4,10] One important contributor to the marked difference in cardioprotective effectiveness between experimental and clinical studies is the pre-ischemic status of the myocardium. Whereas most experimental studies employ normal hearts from healthy animals, clinical reperfusion is usually applied to diseased hearts that have undergone hypertrophy and remodeling due to chronic ischemia. Indeed, results from experimental studies in animal models of cardiac remodeling indicate that diseased hearts are resistant to many,[10] but not all cardioprotective interventions.[11,12] Thus, mechanisms of protection clearly differ between healthy and remodeled hearts. We have recently shown that the enhanced recovery of post-ischemic LV mechanical function in post-infarct remodeled hearts with limited oxidative capacity is fueled by the acceleration of fatty acid oxidation and acetyl CoA generation.[13] This new finding implies that increasing energy substrate supply and ATP generation by provision of supplementary fatty acids may be beneficial and enhance the recovery of postischemic function, especially in remodeled hearts.

We[14] and others[15-17] have recently reported the profound protective effects of postconditioning with clinically used lipid emulsions in healthy rodent hearts. In our experiments, we were also able to decipher the upstream mechanisms of protection by demonstrating that the fatty acid intermediate, palmitoylcarnitine, which is released from the lipid emulsion Intralipid (ILE), inhibits mitochondrial complex IV, releasing ROS from the electron transport chain (ETC) at the onset of reperfusion and promoting Akt/Erk signaling and cardioprotection.[14] Our experiments suggest that, at least in healthy hearts, protection by lipid emulsions arises in part by protective ROS formation at early stages of reperfusion that activates the reperfusion injury salvage kinase (RISK) pathway. While the well-documented cardioprotection in response to ischemic conditioning[18] or lipid