Remote Ischemic Preconditioning Reduces Cerebral Oxidative Stress Following Hypothermic Circulatory Arrest in a Porcine Model

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Remote ischemic precondition has become prominent as one of the most promising methods to mitigate neurological damage following ischemic insult. The purpose of this study was to investigate whether the effects of remote ischemic preconditioning can be seen in the markers of oxidative stress or in redox-regulating enzymes in a porcine model. A total of 12 female piglets were randomly assigned to 2 groups. The study group underwent an intervention of 4 cycles of 5-minute ischemic preconditioning on the right hind leg. All piglets underwent 60-minute hypothermic circulatory arrest. Oxidative stress marker 8-hydroxydeoxyguanosine (8-OHdG) was measured from blood samples with enzyme-linked immunosorbent assay. After 7 days of follow-up, samples from the brain, heart, kidney, and ovary were harvested for histopathologic examination. The immunohistochemical stainings of hypoxia marker hypoxia-inducible factor-1-α, oxidative stress marker 8-OHdG, DNA repair enzyme 8-oxoguanine glycosylase, and antioxidant response regulators nuclear factor erythroid 2-related factor 2 and protein deglycase were analyzed. The level of 8-OHdG referred to baseline was decreased in the sagittal sinus' blood samples in the study group after a prolonged deep hypothermic circulatory arrest at 360 minutes after reperfusion. Total histopathologic score was 3.8 (1.8-6.0) in the study group and was 4.4 (2.5-6.5) in the control group ($P = 0.72$), demonstrating no statistically significant difference in cerebral injury. Our findings demonstrate that the positive effects of remote ischemic preconditioning can be seen in cellular oxidative balance regulators in an animal model after 7 days of preconditioned ischemic insult.

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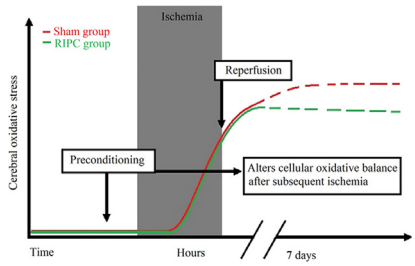

Remote ischemic preconditioning alters cellular oxidative balance after following ischemia.

Central Message

Remote ischemic preconditioning also alters cellular oxidative balance after subsequent ischemic insult.

Perspective Statement

Procedures for repairing complex heart defects and Stanford type A aortic dissection have high rate of neurological complications. RIPC is one of the most promising methods to mitigate neurological damage. In this chronic porcine model, longer lasting alteration of cellular oxidative balance was revealed using this method.

See Editorial Commentary page 103–104.

INTRODUCTION

Stanford type A aortic dissection is extremely life-threatening, with a mortality rate close to 90% without surgical intervention. Even though the operative management of lesions of the transverse aortic arch, techniques used, perioperative care, and intense care have improved during the past few decades, replacement of the aortic arch continues to have an inhospital mortality rate of 8%-15% and following neurological complications from 3%-20%.[1-6]

Deep hypothermic circulatory arrest (HCA) mitigates ischemia-reperfusion damage by decreasing the metabolic rate of the central nervous system (CNS). Various perfusion strategies

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are also used to maintain the supply of metabolic demand of the CNS. Other approaches to suppress the neurological damage consist of drug therapies and remote ischemic preconditioning (RIPC).[7]

RIPC has risen as one of the most promising methods to reduce the neurological damage in ischemia-reperfusion injury. The mechanisms behind the beneficial effects of RIPC are considered in terms of triggers, mediators, and effectors. Protein kinase C mediated pathway is considered to lower the production of reactive oxygen species (ROS) through inhibition of the ATP-sensitive potassium channel. This inhibits the mitochondrial permeability transition pore during ischemia, ultimately resulting in lower production of ROS. The effectors are considered to consist of a neuronal pathway, a humoral pathway or a systemic response, or the combination of both.[8-10]

Both hypoxia and the reintroduction of oxygen to hypoxic cells result in the creation of ROS. The hydroxyl radical is the most unstable ROS, and its interaction with DNA leaves a specific and stable footprint, 8-hydroxydeoxyguanosine (8-OHdG), the expression of which can be reliably assessed with specific antibodies. The 8-oxoguanine glycosylase (OGG1) excises and removes 8-OHdG from the damaged DNA, and thereafter it is secreted to the bloodstream and ultimately to the urine.[11,12] Nuclear factor erythroid 2-related factor 2 (Nrf2) is the key sensor of cellular redox status, and under oxidative stress, it translocates from cytoplasm to nucleus and binds to antioxidant response element in DNA together with small Maf proteins.[13] Nrf2 expression associates with neuronal cell protection during ischemia.[14] Hypoxia-inducible factor-1-α (HIF-1-α) is a constantly produced subunit that stabilises in hypoxic conditions, binding with other subunits, and that regulates hypoxia-inducible gene expression. Both Nrf2 and HIF-1-α target to mitigate ROS production.[15,16] Protein deglycase (DJ-1) is a ubiquitous, multifunctional redox-regulating protein, and it is found in most tissues, including the brain. It is associated with countering oxidative stress.[17]

In our earlier study, leukocyte filtration during cardiopulmonary bypass (CPB) reduced the neurological damage and decreased the number of cerebrocortical adherent leukocytes after HCA. We also demonstrated that RIPC altered in vivo adherent leukocytes in cerebral vessels after HCA.[18,19]

The primary aim of the present study was to investigate whether the effects of RIPC can be seen in the markers of oxidative stress or in the regulators of cellular oxidative balance in an animal model. Therefore, the other principal aim of this study was to investigate whether the mechanism can be explained by reducing the oxidative damage of the DNA and mitochondria, as a previously described trigger. We also sought to take a closer look at the systemic blood count to determine whether the humoral effector can be seen in leukocytes.

MATERIALS AND METHODS

Experimental Setup

A total of 12 female piglets from native stock were randomly assigned to 2 groups. Overall, 6 of the animals were assigned to the intervention group, and 6 animals were assigned to the control group. Both groups underwent a prolonged 60-minute HCA. The animals of the intervention group were preconditioned with 4 cycles of a 5-minute ischemia, followed by a 5-minute reperfusion phase, in the right hind leg. The control group received a sham treatment, but no preconditioning. The randomization was performed using sealed envelopes.

Preoperative Care

All animals received humane care following the instructions of the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Resource Council (Published by National Academy Press, revised 1996). This study was approved by the Research Animal Care and Use Committee of the University of Oulu.

Anesthesia Protocol and Hemodynamic Monitoring

All piglets were sedated with intramuscular injection of ketamine (350 mg), midazolam (35 mg), and medetomidine (1.5 mg). The anesthesia induction consisted of intravenous injection of thiopental (5-7 mg/kg) and fentanyl (50 μg/kg) before endotracheal intubation with 6.5 mm cuffed tube. Anesthesia was maintained with continuous infusion of fentanyl (25 μg/[kg h]), midazolam (0.25 μg/[kg h]), and rocuronium (1.5 mg/[kg h]), with inhalation anesthesia of 1.5% sevoflurane throughout the experiment, excluding HCA. The piglets were ventilated 20 times per minute with positive-pressure of 5 cm H_2O. The end-tidal carbon dioxide in expirated air was kept at 5.0 kPa, and end-tidal oxygen was ensured at 50% in expirated air with end-tidal control of the GE Aisys Carestation (GE Healthcare, Madison, WI). An arterial line was inserted in the left femoral artery for arterial blood pressure monitoring and arterial blood sampling. Cardiac output, pulmonary capillary wedge pressure, central venous