REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 4 (2022)
http://www.veterinaria.org
Article Received: 20 August 2022; Revised: 28 October 2022; Accepted: 05 November 2022

Ischemic Post Conditioning Prevents Preterm Cell Death in Rats

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ABSTRACT

Ischemic postconditioning, also known as Postcond, is a sequence of periodic blood flow disruptions that happen in the beginning phases of reperfusion and affect the hydrodynamics of reperfusion. Current research reveals that Postcond lessens the severity of infarcts, which in turn lessens the impact of cerebral ischemia/reperfusion (I/R) damage. The paths of Postcond I/R damage are, however, poorly known. The goal of this research was to evaluate Postcond's effects on I/R damage with blocked arteries of brain. Adult were occluded for 60 minutes before receiving postconversion therapy (beginning of reperfusion). At 24 and 72 hours, infarct dimensions and neurologic state were evaluated. Malondialdehyde test was used to quantify oxidative stress, and Western blotting was used to identify substances linked to apoptosis. Following postmortem treatment, the expression of protein increased, but cyt c levels in cytsol dropped, was not activated. Infarct diameters, oxidative stress, and neurologic ratings were decreased with Postcond therapy results imply induced brain damage.

Keywords: Stroke, Cerebral injury, early cell death, Ischemic postconditioning

INTRODUCTION:

Blood flow disruptions in the brain are related to stroke, brain damage, apoptosis, and ischemic postconditioning.A stroke is significantly correlated with mortality. Reperfusion damage, however, is brought on by radicals free which resultsdue to procedure itself. Apoptosis is preterm death of a cell processwhich activates in response to I/R. In reaction to oxidative stress, the mitochondrial permeability transition pore (PMT) opens, enabling cytochrome c to leave its regular binding location in the mitochondrial intermembrane gap and Bax to flow from the cytosol into the mitochondria[4, 5] The Bcl-2 protein family controls the translocation of proapoptotic proteins in part[6]. Procaspase-9 is the components of the apoptosome, which is formed when cytochrome c is liberated from the cytoplasm[7] Procaspase-9 is the first to be triggered by the apoptosome, followed by procaspase-3. DNA fragments are broken up when caspase-3 is activated[7] At the start of an ischemic infarction, DNA disintegration and apoptosis have been related in several studies[9] Apoptosis targeting would be ideal for preventing or treating I/R-related cell death.The process of making an organ resistant to future ischemic stress is known as "ischemia preconditioning"[10]. According to research, preconditioning the brain to tolerate ischemia lessens cerebral I/R damage; however, this therapy is only useful from a medical viewpoint if the onset of a stroke can be predicted [11]. A sequence of sudden, intermittent interruptions in blood flow that have a mechanical effect on the process' hydrodynamics lead to developmental ischemia postconditioning (Postcond). Postcond lowers infarct size and regulates inflammation and

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apoptosis, according to advancements in cardiac physiology[12–19]. Recent and encouraging clinical studies suggest that if coronary angioplasty and stent implantation are carried out after acute myocardial infarction, human heart tissue may be shielded from further harm[20]. Zhao et alfirst .'s study [21] suggested that cerebral I/R postconditioning could minimise the extent of infarcts. The capacity of Postcond to reduce in vivo apoptosis has not been linked to its protective function. We looked examined how Postcond influenced apoptosis in mice with MCA occlusion in this study (MCAO).

Materials and Method

Rats were selected as per Manual's recommendations. The animal experimentation committee at Delhi Medical College gave its approval to all surgical procedures. Rats received 350 mgper kg body weight of prior to MCAO. To measure the blood flow in the brains of 22 patients, the researchers utilised a laser-Doppler flowmeter with a flexible probe [23]. The procedure was carried out step by step while being supervised via a stereomicroscope.

Post-ischemia

A random selection approach was used to divide the 120 ratscontrol 40, I/R 42, ischemia postcont (40). In the I/R group, MACO administered 1hr to 72 In the Postcond trial, MCA blockade and reperfusion lasted around 30 seconds. We identified five distinct occurrences of this. All signs of the rodents were gone within only 24 to 72 hours.

The neurologic outcomes at 24 and 72 hours were analysed by a blinded observer using a neuroscore(24,25).

Rats were beheaded, and the size of the infarct was assessed in the rats' brains 24 and 72 hours after reperfusion. The necessary exact measurements of the infarct volume were made. 25 To account for cerebral edoema, a modified infarct area was established as follows: It was determined how much infarction 1 there was on the brain's "opposing" side. 26

Rats were administered chloral hydrate 24 hours after the surgery to study the histopathology of the reperfused tissues. TUNEL and immunohistochemistry were used to analyse tissue slices that had been deparaffinized and rehydrated.

To measure DNA damage with TUNEL, the assay-TUNEL technique was createdand given to each group of patients (n = 4).

Using cyt c and HSP 70 antibodies, paraffin slices were immunohistochemically processed.

To assess oxidative stress, the right cortex of four samples from each group was weighed. MDA and SOD levels that are appropriate have been established. [28] The concentrations of malondialdehyde were measured using thiobarbituric acid (MDA). Utilizing spectroscopy, the absorbance at 532 nm was calculated. Utilizing xanthine oxidase, the SOD activity was calculated.

Using an Easy DNA extraction kit (n = 4 per group), DNA was extracted from the right cerebral cortical area (Fermentas Life Sciences). On an agarose gel with 2% salt, ten

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micrograms of DNA were separated by electrophoresis. Ethidium bromide revealed regions of DNA damage after UV exposure.

Caspase-3 was found thanks to the use of a commercial test kit (Beyotime Institute of Biotechnology). Caspase-3 activation was evaluated and contrasted with a control state.

The relative abundance of proteins known to be involved in apoptosis was assessed using Western blotting. Six samples of right cortical tissue were used to extract the proteins. 29,30

Right cortical samples (2 g total RNA; n = 4 per group) were reverse-transcribed. HSP70 was focus of the primer designs.

Statistical tools such as mean. SEM were used to analyse the data.

Results

Physiology

Across the board, all physiological parameters were comparable. I/R and positional rCBF were unchanged following occlusion.

Neurological deficits were reduced by post-contusion therapy that decreased infarct sizes at 24 and 72 hours after reperfusion. In the 24 to 72 hours later than reperfusion, the neurologic scores increased. Rats were often used in sham operated.

Malondialdehyde (MDA), found in elevated concentrations in I/R than that had a sham operation. The incidence of MDA was lower Postconsecond. In contrast to sham-operated animals, SOD restored of brains of operated mice by postoperative care.Compounds that reduce postcond and promote DNA disintegration are also involved in apoptosis. There were less right brain TUNEL+ cells ofPostconsecond.

After I/R as compared to sham, caspase-3 activity was increased in the brain. Procaspase-9 and procaspase-3 levels were considerably lesser. The distribution of cytochrome C expression changed, with more of it being detected in the mitochondria and less of it in the cytoplasm.

Discussion

Postcond, which decreased the size ofinfarct, assisted in the recovery of neurological function after an I/R damage. The results show that Postcond inhibits apoptosis and hence protects neurons. The pathophysiology of postischemic injury cannot occur without prompt reperfusion. Ischemia postconditioning has been shown to have the potential to reduce I/R damage. [21] The evidence backs up this conclusion. The excessive release of reactive oxygen species (ROS) during reperfusion worsens stroke-related brain damage. The brain is susceptible to reactive oxygen species (ROS) produced by I/R damage because antioxidative enzyme activity is lacking in the brain. By messing with the brain, DNA, proteins, and lipids, these ROS trigger cell death and malfunction [1]. As shown by a reduction in MDA and an increment in SOD activity, there was less lipid peroxidation and SO- in the cerebral I/R after the postcond therapy. According to recent research, Postcond suppresses apoptosis both in vivo and in vitro. [18,19, 34,35] The death of cells due to post-contraction reduced I/R is characterised by DNA disintegration and activated caspase-3. Section first activates c-3

before cleaving 116 kDa nuclear protein compound [31]. Our results suggest that Postcond targeted the mitochondrial route directly.

REFERENCES

[1]. Chan PH. Role of oxidants in ischemic brain damage. Stroke. 1996; 27: 1124–1129.CrossrefMedlineGoogle Scholar

[2]. Mattson MP, Duan W, Pedersen WA. Neurodegenerative disorders and ischemic brain diseases. Apoptosis. 2001; 6: 69–81.CrossrefMedlineGoogle Scholar

[3]. Kowaltowski AJ, Castilho RF, Vercesi AE. Mitochondrial permeability transition and oxidative stress. FEBS Lett. 2001; 495: 12–15.CrossrefMedlineGoogle Scholar

[4]. Kroemer G. Mitochondrial control of apoptosis: an introduction. BiochemBiophys Res Commun. 2003; 304: 433–435.CrossrefMedlineGoogle Scholar

[5]. Lim ML, Lum MG, Hansen TM. On the release of cytochrome c from mitochondria during cell death signaling. J Biomed Sci. 2002; 9: 488–506.MedlineGoogle Scholar

[6]. Kuwana T, Mackey MR, Perkins G. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell. 2002; 111: 331–342.CrossrefMedlineGoogle Scholar

[7]. Li P, Nijhawan D, Budihardio I. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997; 91: 479–489.CrossrefMedlineGoogle Scholar

[8]. Green DR, Reed JC. Mitochondria and apoptosis. Science. 1998; 281: 1309–1312.CrossrefMedlineGoogle Scholar

[9]. Li Y, Chopp M, Jiang N. Temporal profile of in situ DNA fragmentation after transient middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metab. 1995; 15: 389–397.CrossrefMedlineGoogle Scholar

[10]. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74: 1124–1136.CrossrefMedlineGoogle Scholar

[11]. Schaller B, Graf R. Cerebral ischemic preconditioning: an experimental phenomenon or a clinical important entity of stroke prevention. J Neurol. 2002; 249: 1503–1511.CrossrefMedlineGoogle Scholar

[12]. Zhao ZQ, Corvera JS, Halkos ME. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol. 2003; 285: 579–588.MedlineGoogle Scholar

[13]. Darling CE, Jiang R, Maynard M. Postconditioning via stuttering reperfusion limits myocardial infarct size in rabbit hearts: role of ERK1/2. Am J Physiol. 2005; 289: H1618–H1626.CrossrefMedlineGoogle Scholar

[14]. Kin H, Zatta AJ, Lofye MT. Postconditioning reduces infarct size via adenosine receptor activation by endogenous adenosine. Cardiovasc Res. 2005; 67: 124–133.CrossrefMedlineGoogle Scholar

[15]. Sun HY, Wang NP, Kerendi F. Hypoxic postconditioning reduces cardiomyocyte loss by inhibiting ROS generation and intracellular Ca2+ overload. Am J Physiol. 2005; 288: 1900–1908.CrossrefMedlineGoogle Scholar

[16]. Bopassa JC, Ferrera R, Gateau-Roesch O. PI3-kinase regulates the mitochondrial transition pore in controlled reperfusion and postconditioning. Cardiovasc Res. 2006; 69: 178–185.CrossrefMedlineGoogle Scholar

[17]. Kin H, Zhao ZQ, Sun HY. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion, Cardiovasc Res. 2004; 62: 74–85.CrossrefMedlineGoogle Scholar

[18]. Tsang A, Hausenloy DJ, Mocanu MM. Postconditioning: a form of 'modified reperfusion' protects the myocardium by activating the phosphatidylinositol 3-kinase–Akt pathway. Circ Res. 2004; 95: 230–232.LinkGoogle Scholar

[19]. Sun HY, Wang NP, Halkos M. Postconditioning attenuates cardiomyocyte apoptosis via inhibition of JNK and p38 mitogen-activated protein kinase signaling pathways. Apoptosis. 2006; 11: 1583–1593.CrossrefMedlineGoogle Scholar

[20]. Staat P, Rioufol G, Piot C. Postconditioning the human heart. Circulation. 2005; 112: 2143–2148.LinkGoogle Scholar

[21]. Zhao H, Sapolsky RM, Steinberg GK. Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats. J Cereb Blood Flow Metab. 2006; 26: 1114–1121.CrossrefMedlineGoogle Scholar

[22]. Longa EZ, Weinstein PR, Carlson S. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke. 1989; 20: 84–91.CrossrefMedlineGoogle Scholar

[23]. Khan M, Sekhon B, Jatana M. Administration of N-acetylcysteine after focal cerebral ischemia protects brain and reduces inflammation in a rat model of experimental stroke. J Neurosci Res. 2004; 76: 519–527.CrossrefMedlineGoogle Scholar

[24]. Garcia JH, Wagner S, Liu KF. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats: statistical validation. Stroke. 1995; 26: 627–634.CrossrefMedlineGoogle Scholar

[25]. Tsubokawa T, Jadhav V, Solaroglu I. Lecithinized superoxide dismutase improves outcomes and attenuates focal cerebral ischemic injury via antiapoptotic mechanisms in rats. Stroke. 2007; 38: 1057–1062.LinkGoogle Scholar

[26]. Jung KH, Chu K, Ko SY. Early intravenous infusion of sodium nitrite protects brain against in vivo ischemia-reperfusion injury. Stroke. 2006; 37: 2744–2750.LinkGoogle Scholar

[27]. Williams AJ, Berti R, Dave JR. Delayed treatment of ischemia/reperfusion brain injury extended therapeutic window with the proteosome inhibitor MLN519. Stroke. 2004; 35: 1186–1191.LinkGoogle Scholar

[28]. Wei X, Liu H, Sun X. Hydroxysafflor yellow A protects rat brains against ischemia-reperfusion injury by antioxidant action. Neurosci Lett. 2005; 386: 58–62.CrossrefMedlineGoogle Scholar

[29]. Matsumori Y, Hong SM, Aoyama K. Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury. J Cereb Blood Flow Metab. 2005; 25: 899–910.CrossrefMedlineGoogle Scholar

[30]. Kao TK, Ou YC, Kuo JS. Neuroprotection by tetramethylpyrazine against ischemic brain injury in rats. Neurochem Int. 2006; 48: 166–176.CrossrefMedlineGoogle Scholar

[31]. Le DA, Wu Y, Huang Z. Caspase activation and neuroprotection in caspase-3-deficient mice after in vivo cerebral ischemia and in vitro oxygen glucose deprivation. Proc Natl Acad Sci U S A. 2002; 99: 15188–15193.CrossrefMedlineGoogle Scholar

[32]. Saraswat. Anuj, Ms. Puja Lal. Demonstrating Mechanisms Of Ischemic Postconditioning. Drugs and Cell Therapies in Hematology, 2021; 10(3), 225–228.

[33]. Sarkar Barun Ranjan, Dr. Rakesh Kumar Sharma. Studying The Significance Of Biopolymers In Wound Healing. Drugs and Cell Therapies in Hematology, 2021; 10(3), 301–305.

[34]. Upadhyay Akash, Shalini Suchita. Significance Of Secondary Prevention Of Cardiovascular Diseases And Related Lifestyle Intervention. Drugs and Cell Therapies in Hematology, 2021; 10(3), 263–267.