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Laboratory Study

Neuronal protective effects of focal ischemic pre- and/or postconditioning on the model of transient focal cerebral ischemia in rats

M. Ozgur Taskapilioglu [a], Tulin Alkan [b,*], Bulent Goren [b], Kudret Tureyen [c], Soner Sahin [a], Ozlem Taskapilioglu [d], Ender Korfali [a]

[a] Department of Neurosurgery, Uludag University School of Medicine, Bursa, Turkey
[b] Department of Physiology, Uludag University School of Medicine, Gorukle, TR-16059 Bursa, Turkey
[c] Department of Neurosurgery, Suleyman Demirel University School of Medicine, Isparta, Turkey
[d] Department of Neurology, Uludag University School of Medicine, Bursa, Turkey

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ABSTRACT

We investigated the neuroprotective effects of pre- and postconditioning on infarct volume in the transient middle cerebral artery occlusion (MCAo) model in rats. Thirty-two male rats were divided into occlusion, preconditioning, postconditioning and both pre- and postconditioning groups. MCAo (120 minutes) was monitored with continuous cerebral tissue oxygen (O_2) pressure ($PtiO_2$). Pre-conditioning comprised 10 minutes of MCAo, 24 hours prior to the 120 minute MCAo. The postconditioning algorithm was 30 seconds of reperfusion followed by 30 seconds of MCAo. This cycle was repeated 3 times at the onset of reperfusion. Comparison of infarct volumes showed a significant difference between the conditioned groups and occlusion group. Although there was better protection in the preconditioning group compared with the other two conditioned groups, the results did not reach statistically significant levels. The results suggest that preconditioning, postconditioning and pre/post conditioning have protective effects on cerebral ischemia.

1. Introduction

Over recent years, neuroscientists have acquired considerable evidence to demonstrate that the mammalian brain can adapt to cerebral ischemia, thus increasing the chances of surviving subsequent injury.[1] Organisms respond with protective mechanisms to recurrent insults. Preconditioning by exposure to sublethal hypoxic stress hours or days before severe hypoxia decreases cell death, and this resistance of the brain to injury is known as tolerance.[1] Ischemia, hypoxia, hypothermia, hyperbaric oxygenation and metabolic inhibitors may be preconditioning stimuli.[1–3] Brain cells, even without preconditioning, try to decrease cellular damage and death by using their own defense systems against ischemia.[4] Any stimulus capable of causing neural trauma may protect the central nervous system by upregulating endogenous pathways that will increase endurance to ischemia or trauma.[5]

Revascularizing the occluded vessels to allow timely reperfusion is one of the strategies being pursued for acute stroke. However, reperfusion itself generates overproduction of free radicals, which leading to reperfusion injury. Reperfusion injury is a complex process involving endothelial and microvascular dysfunction, impaired blood flow, metabolic dysfunction, cellular necrosis and apoptosis.[6] Brief alternating periods of reperfusion-reocclusion at the beginning of reperfusion is defined as postconditioning.[6]

Ischemic pre/postconditioning provides a new insight into the molecular mechanisms responsible for endogenous neuronal protection and this indicates a necessity for new strategies to increase the durability of brain cells to ischemic insult.[1,2,6,7]

Cerebral ischemic pre/postconditioning protects against stroke, but is clinically feasible only when the occurrence of stroke is predictable. Reperfusion plays a critical role in cerebral injury after stroke.[6] The prediction of the possible development of stroke in neurosurgical practice is commonly seen at the following times: during aneurysm surgery while utilizing temporary clip application, during the development of vasospasm after early surgical intervention for ruptured aneurysm, while using temporary clip application on a main arterial trunk, while performing temporary artery occlusion during superficial temporal-middle cerebral artery anastomosis, or during reperfusion with tissue plasminogen activator for stroke.

In this study we investigated the effects of focal ischemic preconditioning and postconditioning alone, and in combination, using the transient focal cerebral ischemia model in rats.

* Corresponding author. Tel.: +90 224 295 4016; fax: +90 224 442 8855.
E-mail address: talkan@uludag.edu.tr (T. Alkan).

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2. Materials and methods

2.1. Animals and care

Thirty-two Sprague-Dawley male rats weighing 275 ± 25 g obtained from Uludag University Experimental Animals Breeding and Research Center were used in this study. Uludag University Animal Care and Use Committee approval was granted for all applications of the experiment (16.05.2006/5).

All the animals were kept in individual cages at constant room temperature under a controlled 12-hour light/dark cycle with free access to water and food. Induction of anesthesia was administered in a glass chamber with 4% isoflurane mixed with 70% nitrous oxide (N_2O) and 30% oxygen (O_2), then maintained with face mask, with 2% isoflurane. The body temperature of the rats was monitored by rectal probe and the rats were placed on a heat controlled pad while under anesthesia to maintain their body temperature at 36.5 °C to 37.5 °C. All experimental procedures were performed using an operation microscope (Opmi 99, Carl Zeiss, Jena, Germany).

2.2. Measurement of rat cerebral tissue O_2 pressure

After the rat was placed in a left-side-up position, a 1 mm diameter burr hole was opened in the left temporal bone, 3.5 mm to the left of the midline and 2.0 mm behind the bregma, using a high speed drill (Aesculap Microtron GD 412, Tuttlingen, Germany). This site was expected to be in the core of the infarct.[8–11] A PE 90 polyethylene tube was fixed to the burr hole with cyanoacrylate. After this procedure the rat was placed in a supine position and a cerebral tissue O_2 probe (LICOX PMO Brain Oxygen Monitoring System, Integra LifeSciences, Plainsboro, NJ, USA) was introduced into the tube, to a depth of 4.5 mm from the surface of the skull. The Clark-type Licox-sensors used in this study have a diameter of 0.45 mm, a pO_2-sensitive sensor length of 5 mm and a surface area for measuring pO_2 mentioned in the literature between 7.1 mm^2 and 15 mm^2.[12] The measurement of continuous cerebral tissue oxygen pressure ($PtiO_2$) was started following 10 minutes of calibration and continued throughout the middle cerebral artery occlusion (MCAo). The rat's $PtiO_2$ values were reduced more than 50% after occlusion, and if they continued at this level throughout the occlusion period, the rats were included the experiment.

2.3. Transient focal cerebral ischemia model

Transient focal cerebral ischemia was performed according to the intraluminal thread model for MCAo as described.[8,9] A midline incision was made on the ventral surface of the neck. The external carotid artery was tied by a silk suture from its distal part and another lose suture was placed on the proximal part of the external carotid artery to fix the occlusion suture. Then the common carotid and internal carotid arteries were occluded using micro aneurysm clips. An arteriotomy was performed on the external carotid artery. A 3-0 nylon monofilament (Ethilon; Ethicon, Somerville, NJ, USA) suture with a silicon-embedded tip was gently introduced into the lumen of the internal carotid artery up to 20 ± 1 mm.

The experimental groups were as follows:

1 Occlusion group ($Group_{occ}$) ($n = 8$) - MCAo was performed for 120 minutes following the placement of the $PtiO_2$ probe;
2 Preconditioning group ($Group_{precon}$) ($n = 8$) - the MCA was occluded for 10 minutes (preconditioning) following the placement of $PtiO_2$ probe. The cerebral $PtiO_2$ was again put in place 24 hours later and the MCA was occluded for another 120 minutes.
3 Postconditioning group ($Group_{postcon}$) ($n = 8$) - MCAo was performed for 120 minutes after placement of the cerebral $PtiO_2$ probe. The postconditioning algorithm was 30 seconds of reperfusion followed by 30 seconds of MCAo and this cycle was repeated 3 times at the onset of reperfusion.
4 Preconditioning and postconditioning group ($Group_{pre/postcon}$) ($n = 8$) - MCA was occluded for 10 minutes (preconditioning) following the placement of the $PtiO_2$ probe. The cerebral $PtiO_2$ probe was again put in place 24 hours later and the MCA was occluded for 120 minutes. The postconditioning algorithm was the same as postconditioning group.

2.4. Assessment of neurological symptoms

All animals were assessed 1 hour after surgery according to Bederson's 4-score scale: Grade 0, no observable deficit; Grade 1, forelimb flexion; Grade 2, decreased resistance to lateral push (and forelimb flexion) without circling; and Grade 3, same behavior as grade 2, with circling.[13] Only rats that scored less than 6 points were used for the subsequent experiments.

In all groups, the rats were sacrificed at the end of the 24-hour reperfusion, after the 120 minutes of the MCAo period. The brains were removed immediately and kept at −80 °C for 5 minutes, then sectioned into 2 mm coronal slices using a pre-chilled commercial rat brain matrix (Zivic Labs, Portersville, PA, USA). These slices were used for the calculation of the infarct area and volume. The experimental procedures for each group are shown in Figure 1.

2.5. Staining and measurement of infarct volume

To show the infarcted area, 1 mg of 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma-Aldrich, St Louis, MO, USA) was dissolved in 100 mL phosphate buffer, pH 8.5. The brain sections were put into this solution in an incubator at 37 °C for 30 minutes.[14]

Coronal sections were scanned by a high resolution scanner (HP Scanjet 4850, Palo Alto, CA, USA). The infarct areas (where the borders were defined by the TTC stains) were calculated by an investigator blind to the study groups, using Image J software (National Institutes of Health, Bethesda, Maryland, USA) as described previously.[15,16] The non-infarcted areas in the left cerebral hemisphere were subtracted from the total area of the right cerebral hemisphere and multiplied by the slice thickness (2 mm) to determine the infarct volume for each section. The volumes of the four sections were added to give the total infarct volume for one rat.

2.6. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 13.0 for Windows (SPSS, Chicago, IL, USA). Continuous and categorical variables were presented on the basis of the average (± standard deviation, S.D.) and the frequency (n and percentage) respectively. The Kruskal-Wallis test and the Mann-Whitney U-test were used to compare the distributions of continuous variables among groups according to normality test. The Wilcoxon rank test was performed for comparison within groups. Two-sided hypothetical tests were used and a value of $p < 0.05$ was considered statistically significant in all statistical analyses.

3. Results

No statistical difference (mean ± SEM) was found between the groups in terms of body weight ($Group_{occ}$ 251.81 ± 10.86 g; $Group_{precon}$ 273.47 ± 17.63 g; $Group_{postcon}$ 260.57 ± 14.52 g;