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Effect of repetitive ischemic preconditioning on spinal cord ischemia in a rabbit model

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Abstract

A completely randomized controlled study based on a rabbit model was designed to study the effect of repetitive ischemic preconditioning (IPC) on a spinal cord ischemic reperfusion injury. Twenty four white adult Japanese rabbits were randomly assigned to one of the 3 groups ($n=8$ per group): Group I: sham-operation group, Group II: ischemic reperfusion group, and, Group III: IPC group. Spinal cord ischemia was induced by infra-renal aortic cross-clamp for 45 min in Group II. Before 45 min ischemia, the rabbits in Group III underwent four cycles of IPC (5 min of ischemia followed by 5 min of reperfusion). Post-operative neurological function, electromyography (EMG) of rear limbs, and spinal cord histopathological changes were measured. The concentrations of calcium, magnesium, copper, and zinc in spinal cord were measured in the 7th day. The neurological function and histopathological changes in Group II were significantly different from those in Group I or Group III ($P<0.05$ or 0.01). There was a more significant change of EMG in Group II than that in Group III ($P<0.05$). The concentrations of calcium and copper in Group II were significantly higher ($P<0.05$ or 0.01), but magnesium and zinc were significantly lower ($P<0.05$) than those in Group I. Calcium and copper in Group II were significantly higher ($P<0.05$), but zinc was significantly lower ($P<0.01$) than those in Group III. In conclusion, repetitive IPC can protect rabbit spinal cord from ischemic reperfusion injury in a timely manner, which is associated with corrections of imbalance of calcium, magnesium, copper, and zinc in the ischemic region.

Keywords: Ischemic preconditioning; Spinal cord; Calcium; Magnesium; Copper; Zinc

Introduction

Paraplegia remains a devastating complication after operations on the descending and thoracoabdominal aorta (Flores et al., 2005). The incidence of this severe complication is unpredictable and has been reported to be as high as 40% in some series (Svensson et al., 1993). The risk of paraplegia during aortic surgery is determined by the interaction of four independent processes: (1) decrease in spinal cord blood flow; (2) rate of neuronal metabolism; (3) post-ischemic reperfusion injury; and (4) post-reperfusion blood flow. Several techniques have been evaluated for efficacy in reducing paraplegia after aortic cross-clamping:cerebral spinal fluid drainage (Crawford et al., 1991; Cina et al., 2004; Safi et al., 1996), distal aortic perfusion (Safi et al., 1996; Verdant et al., 1995), and regional hypothermia of the spinal cord (Black et al., 2003; Cambria et al., 1997). Despite such efforts, no method has totally prevented the development of paraplegia. Here we report the results of experiments involving repetitive ischemic preconditioning (IPC) of the spinal cord as a novel strategy for spinal cord protection.

IPC is an endogenous cellular protective mechanism whereby brief, non-injurious periods of ischemia render a tissue more resistant to a subsequent, more prolonged ischemic insult.

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Munyao et al. (1998) and Matsuyama et al. (1997) had used, respectively, single IPC to prevent the spinal cord from injury in the aorta operations. It had been reported that single IPC had credible protective efficiency on ischemic reperfusion injury of the spinal cord, but the reperfusion interval between the IPC and the subsequent prolonged ischemic event was several hours or days. Although much research in the brain had demonstrated neuroprotection with IPC involving long reperfusion intervals which ranged from 1 day to 5 days (Kitagawa et al., 1991; Glazier et al., 1994; Chen et al., 1996; Chopp et al., 1989), recent evidence suggested that shorter reperfusion intervals of 10 min to 6 h also provided neuroprotection (Perez-Pinzon et al., 1997; Reshef et al., 1996; Abe and Nowak, 2004). Such a widely time window of interval reperfusion suggests that multiple mechanisms are present in IPC protection of the brain. We hypothesize that repetitive IPC of the rabbit spinal cord by aortic occlusion will reduce neurologic deficit and that the mechanisms of repetitive IPC can be acutely invoked by a 5-min reperfusion interval between the last IPC and the subsequent ischemic event.

Materials

The experiment was carried out at the Medical College of Wuhan University between September and December in 2004. Twenty-four white adult Japanese rabbits of either sex, weighing 2.0–2.5 kg, supplied by the Experimental Animal Institute of Wuhan University [certification: SYXK(e)20040027], were used in this study. All animals were fed in standard cages and were randomly assigned to one of the 3 groups ($n=8$ per group): Group I: sham-operation group, Group II: ischemic reperfusion group, and, Group III: IPC group.

Experimental methods

The rabbits were anaesthetized with 3% pentobarbital (0.5 ml/kg), endotracheally intubated, and connected to a small-animal respirator machine (Model DH140B, Zhejiang Medical Instrument Factory of Zhejiang Medical University). Respiration was controlled at 30 times per minute with the rate of expiration/inspiration as 1/1.5. The level of $PaCO_2$ was maintained between 4.67–6 kPa. Ringer's solution (10 ml/kg per hour) was infused through an ear vein during the procedure, and additional dose of 3% pentobarbital (0.5 ml/kg) and recuronium (0.5 mg/kg) were administered at regular intervals throughout the experiment. Following intravenous heparin administration, the right femoral artery was exposed for a catheter with an arterial line which was connected to a pressure/heart transducer (LIFESCOPEE9, Light and Electricity Company, Japan) for continuous monitoring of the aortic pressure, and cardiogram was recorded. Rectal temperature was monitored and maintained at 38 °C under a warming light.

Establishment of injury model of the spinal cord

The injury model of the spinal cord was established according to the Naslund's method (Naslund et al., 1992): The rabbit's skin was incised along the lateral vertical side of erector spinal muscle below the costal verge of left, the abdominal aorta was exposed outside the peritoneum, and small-diameter silicone plastic tubing was placed around the abdominal aorta just distal to the 2 cm distance from the left renal artery. Aortic occlusion was induced by pulling and clamping the surrounding plastic tubing. In Group I, abdominal aorta was not cross-clamped. Spinal cord ischemia was induced by infra-renal aortic cross-clamp for 45 min in Group II (After the occlusion, distal blood pressure decreased immediately, and the pulsatilify disappeared), and reperfusion was lasted for 7 days. Rabbits in Group III underwent four cycles of ischemic preconditioning, i.e. clamping abdominal aorta for 5 min followed by reperfusion for 5 min, then the rabbits were subjected to 45 min ischemia, followed by 7 days reperfusion. An antibiotic (800 000 U penicillin) was administered intramuscularly immediately after the operating procedures. The wounds were then sutured and the rabbits were returned to their home cages for observation.

Hindlimb motor function

During post-operative follow-up, the rabbits were neurologically assessed at 4, 8, 12, 24 and 48 h and every day after 48 h, respectively, by an observer who was unaware of the grouping, using the modified Tarlov criteria: 0: paraplegia with no lower-extremity motor function; 1: poor lower-extremity motor function (flider of movement or weak antigravity movement only); 2: some lower-extremity motor function with good antigravity strength but an inability to draw legs under body and/or hip; 3: the ability to draw legs under body and hip but not normally; 4: normal motor function.

Hindlimb needle electromyography (EMG)

Hindlimb needle electromyographies of quadriceps femoris muscles and biceps muscles in rabbits at the 7th day after operation were recorded with 3202 type EMG apparatus (Nihon Kohden Company, Japan) by a physician who was blinded to the study groups. Parameters observed included spontaneous potential appearance, with 1 spike being defined as rare visible, 2 spikes as sometimes visible, 3 spikes or more as visible in a great quantity, and motor unit potential (MUP). MUP score was assessed using the following criteria: 1: interference pattern; 2: mixed-interference pattern; 3: mixed pattern; 4: mixed-simple pattern; 5: simple pattern.

Histopathology of the spinal cord

After scoring the neurological function for 7 days, all animals were terminated and the tissue of the spinal cord at the level from L3 to L5 was removed and fixed with 10% formalin. Cross-sections of the spinal cord were stained with hematoxylin and eosin. Neurological injury was evaluated at ×400 magnification by an experienced histopathologist under the light microscope, who was unaware of the experimental conditions and the neurological outcome. Histopathologic changes were graded in terms of Naslund's standard (Naslund et al., 1992): Grade I: The neurons were normal, or the vacuole and granule denaturation of cytoplasm in neurons were observed accidentally.