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Research Report

Noninvasive limb remote ischemic preconditioning contributes neuroprotective effects via activation of adenosine A1 receptor and redox status after transient focal cerebral ischemia in rats

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ABSTRACT

Purposes: To investigate whether activation of adenosine A1 receptor (A1R) through limb remote ischemic preconditioning (RIPC) by a noninvasive tourniquet contribute neuroprotective effects against rat focal cerebral ischemic injury induced by transient middle cerebral artery occlusion (MCAO). Methods: One hundred twenty-eight Sprague–Dawley (SD) rats were randomly assigned into eight groups (*n=16 each*): MCAO, Control, 8-cyclopentyl-1,3-dipropulxanthine (DPCPX, Adenosine A1 receptor antagonist), RIPC, DPCPX+RIPC, Vehicle+RIPC, 2-chloro-N^6-cyclopentyladenosine (CCPA, Adenosine A1 receptor agonist) and CCPA+DPCPX groups. All animals underwent right middle cerebral artery occlusion (MCAO) for 2 h. Limb RIPC consisted of three cycles of 5-minute ischemia followed by 5-minute reperfusion in right hind-limb by tourniquet application. Neurological deficit scores were evaluated 24 h after reperfusion, and then the infarct volume was assessed with diffusion weighted imaging (DWI) and 2, 3, 5-triphenyltetrazolium chloride (TTC) staining. Inflammation was assessed by serum tumor necrosis factor α (TNF_α) and NO; oxidative stress was estimated by malondialdehyde (MDA) and 4-hydroxyalkenals (4-HAD), superoxide dismutase (SOD) activity and GSH. Results: Animals in the RIPC, Vehicle+RIPC and CCPA groups developed lower neurological deficit scores and smaller brain infarct volumes than the Control group ($P<0.01$). Animals in the DPCPX, DPCPX+RIPC and CCPA+DPCPX groups developed higher neurological deficit scores and larger brain infarct volumes than the RIPC, Vehicle+RIPC and CCPA groups ($P<0.01$). DPCPX abolished the protective effects of RIPC and CCPA. RIPC or CCPA induced a significant increase in brain MnSOD (manganese SOD) activity and NO generation, and this activity was abolished by DPCPX pretreatment. RIPC or CCPA induced a significant reduction ($P<0.05$) in the GSH and MDA+4HDA concentration and an accumulation in the GSSG concentration in both compartments (serum and tissue) as compared with the MCAO group. Conclusions: The present study demonstrates that limb RIPC induced by noninvasive tourniquet reduces cerebral ischemic injury in rats, and the effect of neuroprotection may depend on the activation of adenosine A1 receptors. CCPA

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pretreatment can induce delayed ischemic tolerance against cerebral ischemia/reperfusion injury. These protective effects are associated with a reduction in oxidative stress, inflammation and endogenous antioxidant preservation.

1. Introduction

Stroke is the leading cause of death and morbidity worldwide. Following an acute cerebral infarction, the prompt restoration of cerebral blood flow in the infarct-related cerebral artery, using either thrombolysis or primary cerebral angioplasty, remains the most effective intervention for limiting cerebral infarct size, and improving clinical outcomes. Obviously, current strategies to stroke are still inadequate and novel therapies are urgently needed (Jaffer et al., 2011).

Limb remote ischemic preconditioning (RIPC) has been studied for more than 18 years in the research field of myocardial ischemia (Przyklenk et al., 1993), and recently it is an emerging concept for experimental stroke. Our previous experiment and some other studies have confirmed the cerebral protective roles of limb RIPC in the animal models of transient focal cerebral ischemia or whole cerebral ischemia (Jin et al., 2006; Zhao et al., 2004, 2007). And limb RIPC has great clinical advantages since the limb is easy to handle and relatively resistant to ischemia/reperfusion injury. To date, most studies such as mentioned above used infrarenal aortic, iliac or femoral artery occlusion which is invasive to induce hind limb ischemia. Limb occlusion by tourniquet or blood pressure (BP) cuff is especially relevant for clinical application because it is a safer noninvasive and comparatively inexpensive procedure. However, whether limb RIPC induced by noninvasive tourniquet or BP cuff protects against focal cerebral ischemic injury is not established.

The molecular mechanism underlying RIPC and its signaling pathways remain largely unclear. One candidate signaling molecule is adenosine, which was shown to be involved in RIPC of the heart by brief renal ischemia and brief mesenteric artery occlusion (Liem et al., 2002; Patel et al., 2002; Tapuria et al., 2008). Transient activation of adenosine A1 receptors by 2-chloro-N6-cyclopentyladenosine (CCPA), 24 h before an ischemic insult, induces delayed myocardial protection in rats and this protection is associated with enhanced manganese superoxide dismutase (MnSOD) and inducible NO synthase (iNOS) expression (Dana et al., 2000; Zhao et al., 2000). Our previous study also found that brief exposure to isoflurane (isoflurane preconditioning) before focal cerebral ischemic injury could mimic the cerebral protective effects of ischemic tolerance which is related to the A1R activation (Liu et al., 2006). The involvement of adenosine in RIPC by brief hind limb ischemia induced by noninvasive tourniquet of the brain has not been studied to date.

According to these considerations, we hypothesized that limb RIPC would induce the ischemic tolerance mediated by the A1R in brain. Therefore, the present study was conducted to explore whether limb RIPC induced by noninvasive tourniquet reduces focal cerebral ischemic injury, and whether antagonism of the A1R with 8-cyclopentyl-1,3-dipropulxanthine (DPCPX) attenuates the rapid cerebral tolerance induced by brief limb remote preconditioning, and whether the transient activation of A1R with CCPA 24 h before MCAO is able to mimic the protective effects of limb RIPC by reducing the oxidative damage associated with MCAO and this neuroprotection effect is related to a mechanism involving cellular redox status in a rat model of focal cerebral ischemia and reperfusion.

2. Results

No differences were found in the rectal temperature, mean arterial blood pressure, arterial pH, $PaCO_2$, PaO_2 and blood glucose values during preconditioning among groups seen in Table 1. Arterial blood gases showed that there was no respiratory depression during anesthesia. After recovery from anesthesia, no animal showed abnormal behavior.

Focal cerebral ischemia was performed on 128 rats; all rats survived until 24 h after reperfusion. Animals in MCAO, Control and DPCPX groups developed severe neurological damage with NDS of 2(1–3), 2(1–3) and 2(1–4) respectively shown in Table 2. Limb RIPC reduced the neurological damage with the NDS of 1(0–3) in RIPC and Vehicle+RIPC groups ($P<0.01$ *vs Control group*). Administration of DPCPX before preconditioning attenuated the neuroprotective effect of RIPC with the NDS of 2 (1–3) in the DPCPX+RIPC group and that of CCPA with the NDS of 2 (1–4) in the CCPA+DPCPX group ($P<0.01$).

Brain infarct volumes determined by DWI scanning and TTC staining 24 h after reperfusion from 120 min MCAO were presented in Fig. 3. Figs. 4 and 5 showed both DWI and TTC infarct volume in RIPC, Vehicle+RIPC and CCPA groups were

Table 1 – Physiological variables during the preconditioning (n=5).

Group	pH	PaO_2 (kPa)	$PaCO_2$ (kPa)	BG (mmol/L)	MAP (kPa)	T (°C)
Control	7.43±0.05	42.83±3.01	5.83±0.32	6.81±0.46	15.39±0.54	37.2±0.28
RIPC	7.42±0.04	44.76±2.86	5.00±0.57	6.34±0.69	16.15±0.60	37.3±0.40
Vehicle+RIPC	7.42±0.05	45.96±2.91	5.92±0.26	6.32±0.73	15.93±1.05	37.3±0.35
DPCPX+RIPC	7.41±0.03	45.88±2.46	5.80±0.57	6.98±0.40	16.45±0.60	36.4±0.15
DPCPX	7.45±0.05	45.66±2.68	5.89±0.21	6.75±0.86	16.48±0.74	37.2±0.45
CCPA+DPCPX	7.39±0.02	45.52±2.41	5.43±0.66	5.89±0.46	14.83±0.76	37.4±0.42