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Neuroprotective mechanism of ischemic postconditioning in mice: a possible relationship between protein kinase C and nitric oxide pathways

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ARTICLE INFO

Article history:
Received 7 January 2014
Received in revised form
6 February 2014
Accepted 14 February 2014
Available online 19 February 2014

Keywords:
Cerebral ischemia
ischemic postconditioning
Neuroprotection
Chelerythrine
L-arginine
Memory

ABSTRACT

Background: The present study was conducted to pharmacologically investigate the role of protein kinase C (PKC) pathway in neuroprotective mechanism of ischemic postconditioning (iPoCo) and determine the influence of nitric oxide (NO) signaling in PKC-mediated effects.

Materials and methods: Bilateral carotid artery occlusion of 12 min followed by reperfusion for 24 h was used to produce ischemia and reperfusion (I/R)–induced cerebral injury in male Swiss mice. Memory was assessed using Morris water maze test. Degree of motor incoordination was evaluated using inclined beam-walk test, rota-rod test, and lateral push test. Cerebral infarct size was measured using triphenyltetrazolium chloride staining. Brain acetylcholinesterase activity, thiobarbituric acid reactive species, nitrite/nitrate, and reduced glutathione levels were also estimated.

Results: Bilateral carotid artery occlusion followed by reperfusion produced significant rise in cerebral infarct size, acetylcholinesterase activity, and thiobarbituric acid reactive species levels along with the fall in nitrite/nitrate and glutathione levels. A significant impairment of memory and motor coordination was also noted. iPoCo consisting of three episodes of 10 s carotid artery occlusion and reperfusion significantly attenuated infarct size, memory impairment, motor incoordination, and altered biochemicals. iPoCo-induced neuroprotective effects were significantly abolished by chelerythrine (a nonselective PKC inhibitor). L-Arginine, an NO precursor significantly attenuated I/R-induced injury and mimicked the neuroprotective effect of postconditioning. Furthermore, this protective effect of L-arginine on I/R injury and iPoCo was abolished when it was coadministered with chelerythrine.

Conclusions: It may be concluded that neuroprotective mechanism of iPoCo involves PKC mediated pathway with NO signaling as an essential step.

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0022-4804/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2014.02.019

1. Introduction

Ischemic stroke, a prominent and an acute manifestation of cerebral ischemia and other cerebrovascular diseases, is a syndrome characterized by rapid onset of neurologic injury due to interruption of blood flow to the brain [1]. Because of limited therapeutic strategies, stroke is the second leading cause of death in the world, which has profound negative social and economic effects.

Reperfusion to ischemic tissue is the only way to salvage ischemic injury but full reperfusion itself results in additional injury known as ischemia-reperfusion (I/R) injury [2–4]. This necessitates the exploration of novel therapeutic approaches, such as ischemic preconditioning and ischemic postconditioning (iPoCo). Preconditioning is a well-established phenomenon and had been used since 1980s to attenuate I/R-induced injury [5]. However, inability to predict the onset of ischemia in clinical settings led to the discovery of a new concept of postconditioning (PoCo), in 2000s whereby brief repetitive cycles of ischemia with intermittent reperfusion followed by prolonged ischemia elicited tissue protection [6]. Protective effect of iPoCo has been documented in different organs like heart [7], brain [8], kidney [9], liver [10], spinal cord [11]. The major advantage of PoCo is its clinical feasibility and it has been observed to produce tissue protective effects in various clinical settings [12,13]. There is an impressive array of molecular mechanisms contributing to (PoCo)-induced tissue protection, which includes triggers, mediators, and end effectors [14]. However, neuroprotective mechanism of iPoCo is poorly understood.

Protein kinase C (PKC) is a family of serine-threonine kinases that participate in numerous biological processes [15,16]. The activation of PKC-linked transduction pathway has been implicated in the cardioprotective mechanism of remote iPoCo of the myocardium [17]. A study conducted by Balafanova *et al.* [18] showed that nitric oxide (NO) donors promote translocation and activation of PKCε in an NO and peroxynitrite-dependent fashion. NO induces peroxynitrite-mediated tyrosine nitration of PKCε in rabbit cardiomyocytes *in vitro*, and nitrotyrosine residues were also detected on PKCε *in vivo* in the rabbit myocardium preconditioned with NO donors [18]. Furthermore, NO donor S-nitroso-*N*-acetyl-DL-penicillamine has shown to induce PKCε activation and cardiac protection *in vivo* [19]. These results implicate NO signaling as an essential step in cardioprotection induced by PKC. However, role of PKC in neuroprotective mechanism of iPoCo remains to be investigated.

Therefore, the present study has been designed to pharmacologically investigate the role of PKC in neuroprotective mechanism of iPoCo using chelerythrine as a PKC inhibitor and its modulation by L-arginine, an NO precursor using mouse model of bilateral carotid artery occlusion (BCAO) cerebral ischemia.

2. Experimental

2.1. Animals

Male Swiss mice weighing 20 ± 5 g, maintained on standard laboratory diet (Kisan Feeds Ltd, Mumbai, India) and having free access to tap water were used in the present study. They were housed in the departmental animal house and were exposed to 12-h light/dark cycle. The animals were randomly divided into separate groups, each group comprised of eight animals ($n = 8$). All the animals were naive to Morris water maze (MWM). The experiments were conducted in a semi-sound proof laboratory. All experiments were carried out between 10:00 and 16:00 h. The animals were acclimatized to the laboratory conditions 5 d before behavioral study. All the behavioral studies were performed in a blinded manner. The experimental protocol was duly approved by institutional animal ethics committee. The care of the animals was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. - 107/1999/CPCSEA).

2.2. Drugs and chemicals

Chelerythrine (MP Biomedicals LLC, Santa Ana, CA), L-Arginine (MP Biomedicals LLC) and chloral hydrate (Riedel-deHaen, Seelze, Germany) were dissolved in distilled water, respectively. All other chemicals used in the present study were of analytical quality. All drug solutions were freshly prepared before use.

2.3. Induction of global cerebral ischemia

Global cerebral ischemia in mice was induced surgically according to the methods of Himori *et al.* [20] and as described by Kaur *et al.* [21]. Mice were anesthetized using chloral hydrate (400 mg/kg, intraperitoneally [i.p.]). After 12 min of global cerebral ischemia, reperfusion was allowed for 24 h. The animals were shifted individually to their home cage and were allowed to recover.

2.4. Induction of iPoCo cycles

For the iPoCo episode, the carotid arteries were reoccluded for a period of 10 s followed by 10 s of reperfusion time. Three such cycles of I/R were instituted immediately after BCAO at the onset of prolonged reperfusion [22].

2.5. Assessment of cerebral infarct size

At the end of 24 h of reperfusion after global cerebral ischemia, animals were killed by spinal dislocation and the brains were removed and placed immediately in ice-cold saline for 10 min. Brain samples were then sliced into uniform coronal sections of about 1 mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride at 37°C in 0.2 M tris buffer (pH 7.4) for 20 min [23]. The infarct size was measured by volume method using NIH image software (NIH Image 1.61) (National Institutes of Health, Bethesda, MD) [24].

2.6. Evaluation of memory using MWM test

Learning and memory of the animals was assessed by using MWM test [25,26]. MWM procedure is based on the principle