PERSPECTIVE

The neuroprotective effects of (S)-3,5-dihydroxyphenylglycine preconditioning in middle cerebral artery occluded rats: a perspective as a contrivance for stroke

Stroke is one of the fearsome causes of death that leads to high mortality and morbidity worldwide. Apparently, the management of choice for ischemic stroke is either by thrombolysis or thrombectomy. The paramount challenge in most clinical cases is that those interventions are time-dependent as they need to be administered between 3 to 5 hours after the onset of an ischemic event in order to reduce the number of damaged neurons (Ahmed et al., 2013). In fact that a neuron is incapable of regeneration requires the understanding of the pathophysiology of ischemia which may probe an alternative neuroprotective strategy against an ischemic insult.

The concept of excitotoxicity by Olney and Sharpe (1969) has garnered a great number of basic researches focusing on neuroprotection against ischemia. This paradigm illustrates the fundamental mechanism related to the ischemic cell injury. In general, this concept explains that during an ischemic event, there are excessive releases of presynaptic glutamate neurotransmitter. Upon these, two families of glutamate receptors (GluRs): the ligand-gated ion channels (ionotropic, iGluRs) and the G protein-coupled receptors (metabotropic, mGluRs) are activated by the toxic level of the glutamate. Over-activation of the postsynaptic glutamate receptors results in massive excitations which may lead to the damage then death of the brain cells.

One example of the most studied iGluRs is the N-methyl-D-aspartate (NMDA) receptors (NMDARs) which are endowed with high permeability to calcium ions after being activated by the glutamate. The increase in free calcium ions into the neurons has a major impact on the cellular functions. Obviously, direct inhibition of the NMDARs seems to be the best option in order to arrest the excitotoxic event. However, this option disrupts the physiological roles of NMADRs as the mediators of the synaptic transmission. Throughout the years, the researchers have focused particularly to minimize the side effects resulted from the inhibition of the NMDARs (Xu et al., 2013).

Fascinatingly, in contrast, moderate levels of glutamate agonists protect neurons from damage when subsequently exposed to the toxic levels of glutamate. The term preconditioning was first coined in the ischemic myocardial cells (Murry et al., 1986). Ischemic preconditioning involves a brief exposure of certain level of ischemic insult which results in a fundamental response of protection against injury after subsequent severe ischemic attack. However, due to the fact that neurons are irreversible to damage, ischemic preconditioning is a less favorable approach for ischemic stroke patients. Pharmacological preconditioning poses similar protective effects as ischemic preconditioning without the need of any brief ischemic insults (Balzan et al., 2014). Pharmacological preconditioning appears to be an appealing avenue for the patients who have a higher risk toward suffering of ischemic injury after a brain surgery such as endarterectomy or cerebral aneurysm repair.

One of the Group I mGluRs selective agonists, (S)-3,5-dihydroxyphenylglycine ((S)-3,5-DHPG) has been shown to elicit neuroprotection by preconditioning during an ischemic event *in vitro*. Using hippocampal slice cultures, it has been demonstrated that 2 hours of (S)-3,5-DHPG preconditioning before exposing the cells to the excitotoxic level of NMDA has elicited reduction in the propidium iodide uptakes, prevention of cell nucleus fragmentations and decrement in caspase-3 activities which is associated with the depression of the NMDA induced inward currents (Blaabjerg et al., 2003). Furthermore, those significant observations were evidenced in the 10 and 100 μM as compared to 1 μM (S)-3,5-DHPG preconditioning.

Our present work confirms the neuroprotective effects of (S)-3,5-DHPG preconditioning by using the middle cerebral artery occluded Sprague-Dawley rat as an acute ischemic stroke model (Nik Ramli et al., 2015). In general, we observed that 1 and 10 μM (S)-3,5-DHPG preconditioning significantly reduced the severity of the ischemic injuries after 24 hours of the arterial occlusion. Nevertheless, no significant difference in ischemic injury observed in the 100 μM (S)-3,5-DHPG preconditioning. In fact that the lower dose of (S)-3,5-DHPG preconditioning results in neuroprotection, indicating that the outcomes may facilitate the body systems to keep the constant internal environment *via* nervous, hormonal, vascular, immune and other systems as a whole.

Stroke results in neuronal death which is associated with the major impairment of sensory and motor functions. It is imperative to carry out the sensory and motor assessments in the stroke experimental design in order to monitor the outcomes. Neurological Stroke Scales (NSS) is one of the most common scoring tests used to measure various degrees of neurological impairments such as orientation, motor strength and verbal communication in stroke patients. This test has been modified for assessing neurological impairments in stroke animals, and is named as the modified neurological severity scores (mNSS). mNSS is a general composite test that evaluates several aspects of stroke impairment including motor, sensory, coordination and reflexes. We observed that 1 and 10 μM (S)-3,5-DHPG preconditioning significantly improved the mNSS of the stroke when compared to control ischemic rats (Nik Ramli et al., 2015). On the other hand, 100 μM (S)-3,5-DHPG preconditioning did not show any significant improvement in the mNSS with stroke when compared to control ischemic rats.

Infarct volume is an essential indicator for the level of severity of the ischemic stroke. 2,3,5-Triphenyltetrazolium chloride (TTC) staining is frequently used to determine the degree of infarct induced by focal cerebral ischemia in rats or mice. Using this technique, we observed that the brains of 1 and 10 μM (S)-3,5-DHPG preconditioned rats depicted lesser infarct volume whilst the 100 μM (S)-3,5-DHPG preconditioned rats posed increments in the infarct volume when compared to control ischemic rats. The importance of the significant reduction of the ischemic volumes in 1 and 10 μM (S)-3,5-DHPG preconditioned rats indicates that the penumbra areas have been successfully salvaged and protected by the (S)-3,5-DHPG preconditioning.

One of the main outcomes following ischemic insult is the perturbation of blood brain barrier's permeability which consequently leads to edema of the brain, infiltration of leukocytes and increases risks towards spontaneous hemorrhagic events. To further confirm the effect of (S)-3,5-DHPG in reducing the

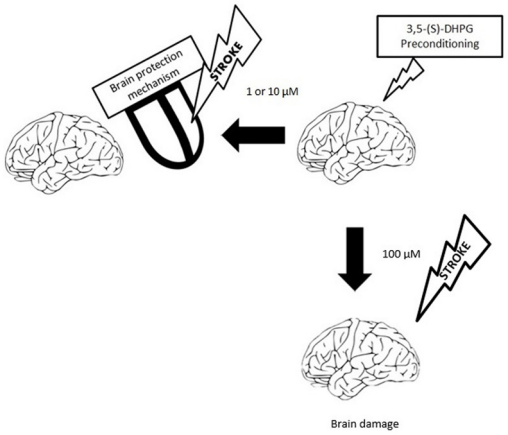

Figure 1 Dose dependent preconditioning effects of (S)-3,5-DHPG against ischemia
Lower doses of (S)-3,5-DHPG (1 or 10 μM) invokes brain endogenous protection mechanism against subsequent ischemic insult while higher dose (100 μM) exacerbates the ischemic brain damage.

ischemic severity, we quantitated the neuron specific enolase (NSE) of the rat's blood serum. It has been reported that NSE is one of the specific biomarkers for prediction of ischemic severity in both experimental and clinical cases (Bharosay et al, 2012). We observed that there were significant reductions in the NSE levels of the 1 and 10 μM (S)-3,5-DHPG as compared to 100 of μM (S)-3,5-DHPG preconditioned rats which implied that the disruption of blood brain barriers were minimal during the ischemic insult when the rats were preconditioned with lower doses.

Many researchers have suggested the potential effects of specific glutamate receptor agonists as the neuroprotective agents which were achieved by direct activations of the protective cellular pathways *via* the pharmacological preconditioning (Pellegrini-Giampietro, 2003). Nevertheless, the fates of the cells depend on the intensity of the stimuli applied which are evidenced by our findings. The medium intensity stimulus such as 1 or 10 μM (S)-3,5-DHPG preconditioning is committed to the cell protection which resulted in reduction of brain damages, while extreme intensity as such 100 μM (S)-3,5-DHPG preconditioning leads to apoptosis and necrosis which were similar in those of ischemic rats without preconditioning (**Figure 1**).

Group I mGluRs represent important sites for interactions between numerous drugs, whereas (S)-3,5-DHPG is known to modulate several pathways *via* various types of neurotransmitters. The stimulation of phospholipase C by group I mGluRs is known to escalate both phosphoinositide turnover and endoplasmic reticulum (ER) stores of Ca^{2+} which relatively effects both neuronal development and necrosis. In addition, group 1 mGluRs activities also lead to diacylglycerol (DAG) formation, a cofactor that remains at cell membrane and further activates protein kinase C. Extended cell culture and animal studies of hypoxia and preconditioning with low concentration of general anesthetic had discussed the effects of moderate release of Ca^{2+} into cytosolic space from ER *via* IP3, resulting in NMDA receptor inhibition and internalization while initiating endogenous neuroprotective mechanism such as the PI3-AKT pathway (Wei and Inan, 2013). On the contrary, application of high dose with prolonged duration of stimulation causes overactivation of IP3 receptors and excessive release of Ca^{2+} from ER, eventually results in apoptosis and protein trafficking.

Due to the nature of neuron which is irreversible to damage, ischemic preconditioning is unlikely to give any benefit to ischemic stroke patients. However, preconditioning is an appealing avenue for surgical procedures which predisposed the patients with higher risk of ischemic brain injury such as endarterectomy and cerebral aneurysm surgery. We demonstrated that preconditioning with lower doses of (S)-3,5-DHPG is protective against subsequent ischemic insult in the acute ischemic stroke rats. Therefore, understanding of mechanisms underlying the preconditioning effect of (S)-3,5-DHPG is a critical point of salvation in this area.

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