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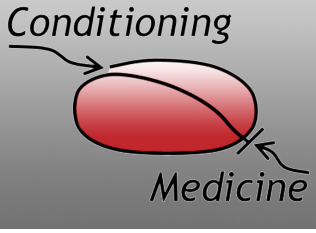

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Potential role of c-Jun N-terminal kinase in ischemic preconditioning and neuroprotection

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Brain preconditioning, a sub-lethal insult that enables resistance to a subsequent lethal injury, has been widely studied as a neuroprotective therapy against several neurodegenerative diseases, various brain-associated injuries including cerebral ischemia, hemorrhage, neonatal hypoxic injury, and trauma. Clinical trials using ischemic preconditioning or remote ischemic preconditioning have investigated the beneficial effects of brain preconditioning in cerebrovascular-related injuries or diseases such as ischemic stroke and subarachnoid hemorrhage.

A wide spectrum of preconditioning stimuli, including transient global/focal cerebral ischemia, remote ischemia, hypoxia, hypothermia, and various pharmacological treatments, have been used to protect the brain in *in vivo* experimental animal studies, and in *in vitro* cell culture systems. The signaling molecules and pathways triggered by preconditioning stimuli, which are involved in cytoprotective mechanisms, have only been recently identified. c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinases (MAPKs) and is activated by various cellular stresses including ischemic injury. JNK signaling is well known as one of the key pathways for regulating neuronal death in many central nervous system injuries. However, JNK signaling has a dual role and is also involved in neuronal survival in brain preconditioning.

In this mini review, we discuss the current clinical trials for ischemic preconditioning and experimental brain ischemic preconditioning, and examine the role of JNK in effective preconditioning against cerebral ischemic stroke.

Keywords: Central nervous system (CNS) injury, Cerebral ischemic stroke, Brain ischemic preconditioning, Neuroprotection, c-Jun N-terminal kinase (JNK)

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Introduction

Several clinical trials have examined the neuroprotective effects of preconditioning against cerebral ischemic vascular- or subarachnoid hemorrhage (SAH)-related brain conditions. Indeed, ischemic preconditioning and remote limb ischemic preconditioning have been employed to attenuate damage induced by ischemic stroke, SAH, multiple sclerosis, subcortical vascular dementia, and brain injury carotid endarterectomy. Many preclinical studies using *in vivo* animal models and *in vitro* cell culture systems have demonstrated the neuroprotective effects of ischemic preconditioning in brain ischemia- or hemorrhage-related injuries, as well as many other central nervous system (CNS) injuries. However, there are still several gaps in our current knowledge that must be addressed to translate the preclinical results into clinical applications for humans.

Preclinical studies aiming to uncover the central molecule or signaling pathway that plays a pivotal role in ischemic preconditioning-mediated neuroprotective effects have successfully identified multiple molecules and signaling pathways of interest. One such pathway is the c-Jun N-terminal kinase (JNK) signaling pathway, which is involved in both neuronal death and survival in various CNS injuries, and thus, has been widely studied as a therapeutic target for brain ischemia and many other brain diseases.

In this mini review, we examine the role of JNK in the neuroprotective effects of preconditioning against cerebral ischemic stroke. A deeper understanding of the molecular mechanisms surrounding brain preconditioning will enable us to develop better therapeutic strategies that more effectively protect the brain against acute or chronic CNS injury.

1. History of the translational approach of ischemic preconditioning to the bedside; clinical trials of preconditioning in cerebrovascular diseases.

Since preclinical studies and human meta-analysis have demonstrated potential neuroprotective and cytoprotective effects of limb ischemic preconditioning (Lehotsky et al., 2009; Ayodele and Koch, 2017), many clinical trials have examined the safety and feasibility of preconditioning against various injuries (Lehotsky et al., 2009; Ayodele and Koch, 2017) (find more information at clinical.org by cross-referencing "Limb preconditioning" as a keyword). So far, several clinical trials examining the efficacy of remote ischemic preconditioning (RIPC) against cerebral ischemic vascular disease or SAH have been completed (**Table 1**). In addition, there are still several ongoing (as of October 6, 2018) clinical trials using ischemic preconditioning or limb RIPC to determine if preconditioning improves stroke rehabilitation or SAH outcomes, and to investigate the beneficial effects or feasibility of using preconditioning to treat ischemic stroke, SAH, multiple sclerosis, subcortical vascular dementia, and brain injury carotid endarterectomy (**Table 2**). Among the cerebrovascular diseases, SAH may be a reasonable model to test whether preconditioning is an effective clinical remedy for reducing SAH-induced brain damage (Koch, 2010; Koch et al., 2011). SAH is caused by an initial primary hemorrhage that is followed by other SAH hallmarks, such as cerebral vasospasm and delayed cerebral ischemia, which are mainly amplified by increased inflammation and activation of various cellular signaling pathways that leads to vasoconstriction (Macdonald et al., 2007). After a SAH, it normally takes 4-10 days until delayed cerebral ischemia is apparent, so limb ischemic pre-, per-, or post-conditioning could be employed during this period (Pluta et al., 2009; Vergouwen et al., 2010).

Koch and colleagues initiated a clinical trial to determine the feasibility and safety of a limb ischemic preconditioning regimen (three instances of the following cycle: 5 min of inflation to 200 mm Hg/5 min of reperfusion using a blood pressure cuff) against SAH (Koch et al., 2011). In a phase 2b study (total of 33 participating patients), the research team reported that limb preconditioning is safe and well tolerated, and that even an increased duration of ischemia (to 10 min) is safe (Koch et al., 2011). The phase 2 study (PreLIMBS) is being conducted with a relatively large patient population (60 patients) to further evaluate the safety and feasibility (as the primary outcome measurement), as well as the clinical outcome (as the secondary outcome measurement) of limb ischemic preconditioning against SAH (ClinicalTrials.gov Identifier: NCT02411266). Other clinical trials have been conducted to explore the feasibility and safety of limb preconditioning in other conditions, such as acute kidney injury in patients

Table 1. Completed clinical trials (stroke- and subarachnoid hemorrhage-related).

Identifier	Title	Aims descripted in the study	Actual Enrollment
NCT01118000	Study on the Cardioprotection and Humoral Mechanism of Limb Ischemia Preconditioning	To assess whether limb ischemic preconditioning protects remote tissue or organs through a humoral mechanism	60
NCT03072914	Effects of Remote Ischemic Preconditioning With Postconditioning on Neurologic Outcome	To evaluate whether RIPC with RIPostC reduce the major neurocomplication in patients undergoing STA-MCA anastomosis	108
NCT01110239	Preconditioning for Aneurismal Subarachnoid Hemorrhage	To determine if RIPC can be safely and effectively instituted in patients with SAH	34
NCT02602977	the Influence of Remote Ischemic Preconditioning on Inflammation During Human Endotoxemia (RISPENDO)	To investigate the effects of (repeated) ischemic preconditioning on inflammation during human endotoxemia	30
NCT01158508	Remote Ischemic Preconditioning in Subarachnoid Hemorrhage (RIPC-SAH)	To study effect of RIPC on cerebral vasospasm following SAH	20
NCT01658306	Clinical Trial on Remote Ischemic Preconditioning and Cerebral Small Vessel Disease (RIPC-SVD)	To test if RIPC might have a beneficial effect on outcomes of cerebral small vessel disease	30
NCT02177981	Impact of Remote Ischemic Preconditioning Preceding Coronary Artery Bypass Grafting on Inducing nEeuroprotection (RIPCAGE)	To determine whether RIPC can reduce the adverse impact of cardiopulmonary bypass on neurological outcome	70
NCT01321749	The Neuroprotective Effect of Remote Ischemic Preconditioning on Ischemic Cerebral Vascular Disease	To observe the effect of RIPC on ischemic cerebral vascular disease	196

Several clinical trials using RIPC to investigate any beneficial effects in cerebral ischemic vascular disease or SAH have been completed (found at *ClinicalTrials.gov*).

Table 2. Current clinical trials.

Identifier	Title	Condition or disease	Aims descripted in the study	Estimated Enrollment (As of Oct. 6th, 2018)
NCT02411266	Preconditioning With Limb Ischemia for Subarachnoid Hemorrhage (PreLIMBS)	Ischemic Preconditioning	A study of limb preconditioning in subjects with SAH who are at high risk of cerebral ischemia in the first 2 weeks after hemorrhage	60
NCT03023150	Ischemic Preconditioning as an Intervention to Improve Stroke Rehabilitation – Froedtert	Ischemic Preconditioning	A study to use ischemic preconditioning as an intervention to improve stroke rehabilitation	50 (active, not recruiting)
NCT02381522	Remote Ischemic Pre-conditioning in Subarachnoid Hemorrhage (RIPC-SAH)	Brain Aneurysms	To investigate the effect of limb ischemia preconditioning on SAH outcome (cerebral vasospasm)	100
NCT02997748	Remote Ischemic Preconditioning After Cardiac Surgery (RIPCRenal)	Cardiac Surgery, Aortocoronary Bypass	To reduce the incidence of AKI by implementing RIPC and to evaluate the dose-response relationship using the biomarkers urinary [TIMP-2] *[IGFBP7] in high risk patients undergoing cardiac surgery	180
NCT02169739	Remote Preconditioning Over Time To Empower Cerebral Tissue (REM-PROTECT)	Ischemic Stroke	To assess feasibility of ischemic preconditioning by preconditioning device	60
NCT03153553	Ischemic Preconditioning, Exercise Tolerance and Multiple Sclerosis	Multiple Sclerosis	To see whether it is feasible to use ischemic preconditioning to improve exercise performance in people with multiple sclerosis	40
NCT03022149	Remote Ischemic Preconditioning for Subcortical Vascular Dementia (RIPSVD)	Subcortical Vascular Dementia	To determine whether the RIPC are effective in the treatment of mild to moderate vascular dementia	52 (active, not recruiting)
NCT03624452	Remote Ischaemic Preconditioning Combined With Exercise Training on Vascular Function.	Cardiovascular Disease Risk	To investigate whether combining 8 weeks of exercise and RIPC is more beneficial to systemic vascular function/ (Cerebro) vascular function than exercise alone	60
NCT02553655	Remote Ischemic Limb Preconditioning In Healthy Volunteers	Cerebrovascular Disease	To determine if remote ischemic leg preconditioning in healthy volunteers improves cerebral vasomotor reactivity	30
NCT03598855	Ischemic Preconditioning and Type 2 Diabetes	Type 2 Diabetes	To determine the impact of 7 days of daily ischemic preconditioning on vascular function and insulin sensitivity in type 2 diabetes mellitus	21 (recruitment completed)
NCT03027011	Remote Ischemic Preconditioning on Brain Injury in Carotid Endarterectomy	Remote Ischemic Preconditioning	To test an intervention (RIPC) in patients undergoing carotid endarterectomy	40
NCT03474952	Effects of Remote Ischemic Preconditioning During Free Flap Reconstruction	Remote ischemic preconditioning (RIPC)	To evaluate the effect of RIPC on tissue oxygen saturation and skin temperature of the flap	50

Several on-going clinical trials (which are "recruiting or completed recruitment") to assess the beneficial effects or feasibility of ischemic preconditioning or RIPC in cerebral ischemia, SAH, multiple sclerosis, subcortical vascular dementia, and brain injury (found at *ClinicalTrials.gov*).

undergoing coronary artery bypass graft surgery, symptomatic intracranial stenosis (Wang et al., 2017), heart and lung injury after abdominal aortic aneurysm repair (Li et al., 2013), carotid artery stenting (Zhao et al., 2017), and other vascular surgeries (find more information at clinical.org).

However, there are still gaps in our current knowledge that need to be filled in order to successfully translate preclinical findings from *in vivo* animal and *in vitro* cell models to patients. For instance, pertinent biomarkers must be found and assessed to determine when the effects of preconditioning will be most beneficial and how long these effects last in the human body. Additionally, it is imperative to optimize the ischemic preconditioning protocols by choosing the appropriate stimulus and duration of the preconditioning stress. Furthermore, age, sex, and comorbidities, which elderly patients may have, should be considered to generate a scientifically proven, well-designed protocol that can be applied in clinical trials. Indeed, comorbidities such as hyperglycemia and hyperlipidemia may interfere with the beneficial effects of preconditioning (Kersten et al., 1998; Kehl et al., 2002; Ungi et al., 2005; Giricz et al., 2006; Yang et al., 2013; Schenning et al., 2015; Ayodele and Koch, 2017). Moreover, older patients (over 65 years of age) do not receive myocardial protection induced by pre-infarction angina, (a form of clinical preconditioning), suggesting that advanced age may nullify the beneficial effects of preconditioning (Abete et al., 1997).

2. Experimental brain ischemic preconditioning *in vivo* or *in vitro*

Cerebral ischemic stroke caused by blocking blood flow and oxygen to the brain is one of the major CNS injuries, leading to severe damage and death of brain tissue. While many studies have attempted to develop a neuroprotective drug against stroke in animal models, only tissue plasminogen activator (tPA) has been applied clinically.

An alternative approach to stroke therapy, ischemic preconditioning that induces tolerance to subsequent severe focal or global ischemia, has been largely studied for many years (Liu et al., 1992; Matsushima and Hakim, 1995; Stagliano et al., 1999; Cardenas et al., 2002; Zhou et al., 2004; Stetler et al., 2014; Chen et al., 2018b). The primary brain ischemia model using rodents is transient or permanent middle cerebral artery occlusion (MCAO) induced by insertion of an intraluminal suture (Stetler et al., 2014; Fluri et al., 2015). After a certain duration of occlusion, the suture is withdrawn, allowing reperfusion to occur (transient focal ischemia) (Stetler

et al., 2014; Fluri et al., 2015). Alternatively, permanent ligation of the MCA without reperfusion (permanent focal ischemia) generates a severe cerebral ischemic injury (Stetler et al., 2014; Fluri et al., 2015). The MCAO model is commonly used in rodent stroke research and generates a reproducible infarction (extent of injury is dependent on occlusion time) in the cortex and striatum, forming an ischemic core and a penumbra surrounding the core (Stetler et al., 2014; Fluri et al., 2015). In addition, the MCAO model is similar to an ischemic stroke in humans because the infarction generated by thrombotic occlusion is localized mainly in the MCA region of the human brain (Fluri et al., 2015; Sommer, 2017). To produce ischemic tolerance against subsequent lethal ischemia, the MCAO method (either transiently or permanently induced) has been commonly used as a preconditioning stimulus (Stagliano et al., 1999; Cardenas et al., 2002; Chen et al., 2018b). One or three 5 min incidents of a brief MCAO before a severe 1h MCAO significantly reduces infarct volume (Stagliano et al., 1999). A short ischemic preconditioning event caused by 10 min of transient MCAO reduces the infarct volume in rat brains subjected to subsequent permanent MCAO (Cardenas et al., 2002). In rats treated with ischemic preconditioning involving 10 min of MCAO prior to 90 min of subsequent severe MCAO, neurological functional deficits, cerebral infarct size, and neuronal death decrease (Chen et al., 2018b).

Cardiac arrest induced by obstructing blood circulation can also cause brain ischemia (Safar, 1986; Petito et al., 1987; Mangus et al., 2014; Stetler et al., 2014). Global ischemia has generally been used in animal stroke models to simulate cerebral ischemic injury caused by cardiac arrest (Petito et al., 1987; Stetler et al., 2014), and this model specifically induces delayed neuronal degeneration in the hippocampal CA1 region. In this model, carotid arteries are occluded transiently, and vertebral arteries are then permanently ligated by electrocoagulation.

Global ischemia has also been used to induce ischemic preconditioning, producing ischemic tolerance to subsequent severe ischemic damage from either focal or global ischemic insults (Liu et al., 1992; Matsushima and Hakim, 1995; Zhou et al., 2004; Stetler et al., 2014). The brief bilateral common carotid artery occlusion (BCCAO) method with vertebral artery ligation or BCCAO with induction of systemic hypotension has also been used to induce global ischemic preconditioning (Liu et al., 1992; Matsushima and Hakim, 1995; Zhou et al., 2004; Stetler et al., 2014). In adult rats, 2 min of global ischemia by BCCAO as an ischemic preconditioning stimulus generates robust neuroprotection in the hippocampal CA1 region following 30 min of reperfusion, 10 min of lethal global ischemia, and 3 days of reperfusion (Perez-Pinzon et al., 1997). Additionally, BCCAO as a preconditioning stimulus 30 min before subsequent MCAO significantly reduces infarct volumes in mice brains, suggesting that the optimal time between the preconditioning stimulus and subsequent lethal focal ischemia is 30 min (Speetzen et al., 2013).

As discussed above, ischemic preconditioning in animal

Table 3. Studies on the role of JNK in brain preconditioning.

Brain Injury Model	Cerebral Ischemia Focal & Global ischemia	(Gu et al., 2000; Gu et al., 2001; Nozaki et al., 2001; Colangelo et al., 2004; Miao et al., 2005; Zhang et al., 2006b; Yang et al., 2008; Du et al., 2009; Zhang et al., 2009; Liang et al., 2011; Maslov and Lishmanov Iu, 2012; Simao et al., 2012; Mirante et al., 2013; Zhang et al., 2013; Wang et al., 2016a; Wang et al., 2016b; Zhang et al., 2017; Zhuang et al., 2017; Shvedova et al., 2018)
	Hemorrhage	(Chang et al., 2015)
	Hypoxia	(Jones and Bergeron, 2004; Gustavsson et al., 2007; Zhang et al., 2007a; Nilsson et al., 2015)
	Trauma	(Lotocki et al., 2006)
	Oxygen Glucose Deprivation	(Meloni et al., 2006; Bhuiyan et al., 2011; Xiang et al., 2014; Pang et al., 2015)
	Pharmacological reagent-induced brain or neuronal injury	(Sugino et al., 2000; Leak et al., 2006; Granziera et al., 2007; Price et al., 2010; Navon et al., 2012; Tripathi et al., 2014; Yang et al., 2015; Fu et al., 2017)
Preconditioning Stimulus	Focal or Global ischemia or Remote ischemia	(Gu et al., 2000; Gu et al., 2001; Nozaki et al., 2001; Colangelo et al., 2004; Miao et al., 2005; Zhang et al., 2006b; Yang et al., 2008; Du et al., 2009; Zhang et al., 2009; Maslov and Lishmanov Iu, 2012; Wang et al., 2016b; Zhang et al., 2017)
	Hypoxia	(Jones and Bergeron, 2004; Gustavsson et al., 2007; Zhang et al., 2007a; Nilsson et al., 2015)
	Oxygen Glucose Deprivation	(Bhuiyan et al., 2011; Pang et al., 2015)
	Hypothermia	(Lotocki et al., 2006)
	Pharmacological Reagent (Melatonin, Sevoflurane, Pitavastatatin, Isoflurane, Riboflavin, Remifentanil, Protease Nexin-1, Resveratrol, Lipopolysaccharide, NMDA, Thrombin, 6-hydroxydopamine, Erythropoietin, 3-nitropropionic acid).	(Sugino et al., 2000; Leak et al., 2006; Meloni et al., 2006; Granziera et al., 2007; Price et al., 2010; Liang et al., 2011; Navon et al., 2012; Simao et al., 2012; Mirante et al., 2013; Zhang et al., 2013; Tripathi et al., 2014; Xiang et al., 2014; Chang et al., 2015; Wang et al., 2016a; Fu et al., 2017)

Among 140 studies (found by searching PubMed with keyword "JNK and preconditioning"), 41 studies involve the connection between JNK and brain preconditioning in various brain diseases or injuries (found by searching PubMed with keyword "JNK and preconditioning and brain"). The table was categorized by brain injury model and preconditioning stimulus.

focal or global stroke models have shown neuroprotective effects. However, in clinical situations, cerebral ischemic preconditioning is difficult to apply due to potential safety and ethical issues in patients (Leape, 2005; Dave et al., 2006; Ren et al., 2008; Hu et al., 2012). Therefore, the use of remote ischemic preconditioning (RIPC) has been considered as an alternative method to ischemic preconditioning (Dave et al., 2006; Ren et al., 2008; Hu et al., 2012). RIPC is minimally invasive and potentially provides a wider therapeutic time window (Dave et al., 2006; Ren et al., 2008; Hu et al., 2012). Non-invasive RIPC of a limb induces neuroprotection against MCAO via changes in the composition of peripheral immune cells (Liu et al., 2016). Four repeated cycles of brief blood flow constriction in the hind-limbs produced an immunomodulatory effect in the spleen during RIPC-mediated neuroprotection against MCAO (Chen et al., 2018a).

Ischemic preconditioning in *in vitro* cell culture models has also been widely studied. The oxygen/glucose deprivation (OGD) model is the most common method for mimicking ischemic preconditioning *in vitro*. Short term OGD preconditioning (0.5 h duration) in cultured rodent cerebral endothelial cells prior to a lethal 2.5 h OGD exposure increased cell viability and blood-brain barrier (BBB) integrity via stabilization of tight junction proteins (An and Xue, 2009). Cultured hippocampal neurons exposed to brief OGD as the ischemic preconditioning stimulus significantly reduced neuronal death after a subsequent lethal OGD injury (Keasey et al., 2016). Additionally, ischemic OGD preconditioning protected cultured rodent astrocytes from a subsequent lethal OGD exposure (Narayanan et al., 2018). Exposure to hypoxic conditions (experimental animals or cells in 8% O_2 chamber) is another method for inducing ischemic preconditioning against ischemic brain injury that has been largely studied in *in vitro* and *in vivo* systems (Gidday et al., 1994; Miller et al., 2001; Bernaudin et al., 2002; Lin et al., 2003; Tang et al., 2006; Zhang et al., 2006a; Zhang et al., 2007b; Zhan et al., 2010; Fan et al., 2011; Yang et al., 2017; Zhan et al., 2017; Zhan et al., 2018). Pre-exposure to hypoxia during the perinatal period protects the neonatal rat brain from hypoxic-ischemic injury (Gidday et al., 1994). Additionally, hypoxic preconditioning is neuroprotective against global cerebral ischemia in adult rats (Zhan et al., 2017; Zhan et al., 2018), and protects rats from cerebral ischemic injury in MCAO models (Wacker et al., 2009; Yang et al., 2017).

3. Ischemic preconditioning for neuroprotection and JNK signaling

Many studies have demonstrated the neuroprotective effects of ischemic preconditioning and other preconditioning treatments against severe subsequent cerebral ischemia, as well as the molecular mechanisms involved in this neuroprotection. C-Jun N-terminal kinase (JNK) is a family of mitogen-activated protein kinases (MAPKs) associated with the cell death accompanying various brain injuries (Ip and Davis, 1998; Irving and Bamford, 2002; Brecht et al., 2005; Kaiser et al., 2005; Kuan and Burke, 2005; Bogoyevitch and Kobe, 2006; Johnson and Nakamura, 2007; Atochin et al., 2016; Kim et al., 2017; Shvedova et al., 2018). This signaling pathway is activated by various cellular stresses such as oxidative stress, inflammation, heat, and osmotic shock (Ip and Davis, 1998; Irving and Bamford, 2002; Brecht et al., 2005; Kaiser et al., 2005; Kuan and Burke, 2005; Bogoyevitch and Kobe, 2006; Johnson and Nakamura, 2007; Atochin et al., 2016; Kim et al., 2017; Shvedova et al., 2018). The JNK signaling pathway plays a central role in cerebral ischemic injury, as well as in myocardial ischemic injury (Kuan et al., 2003; Kaiser et al., 2005; Johnson and Nakamura, 2007; Pei et al., 2008; Shvedova et al., 2018). JNK phosphorylation is induced and its signaling pathways are triggered after cerebral ischemia in rodent models (Hayashi et al., 2000; Irving and Bamford, 2002; Borsello et al., 2003; Ferrer et al., 2003; Okuno et al., 2004; Tian et al., 2005; Kamada et al., 2007; Atochin et al., 2016), and JNK activation exacerbates brain damage following stroke (Davis, 2000; Okuno et al., 2004; Okami et al., 2013). In JNK1 knockout mice subjected to permanent MCAO, brain infarct size significantly increase relative to wild-type mice subjected to MCAO (Brecht et al., 2005). JNK activation caused by an ischemic injury contributes to neuronal cell death through phosphorylation of Bcl-2-associated death promoter (Bad), induction of apoptosis regulator Bim and Fas, activation of caspases -3, -8, and -9, and release of mitochondrial cytochrome *c* (Kuan et al., 2003; Okuno et al., 2004; Carboni et al., 2005; Kamada et al., 2007; Li et al., 2010). However, JNK also plays a dual role in cell death and survival during cerebral ischemia (Waetzig and Herdegen, 2005).

Here, we discuss the dual role of JNK in ischemic preconditioning and various other forms of cerebral preconditioning to subsequent cerebral ischemia. Currently, around 140 studies have reported the relationship between the JNK signaling pathway and preconditioning in various cells, tissues, and organs under many different physiological and pathophysiological conditions (found by searching PubMed with keyword "JNK and preconditioning"). Among these studies, 41 reports involve the connection between JNK and brain preconditioning in various brain diseases and injuries (Table 3) (found by searching PubMed with keyword "JNK and preconditioning and brain"). Pretreatment with 3 min of ischemic preconditioning via four-vessel occlusion prevents the phosphorylation of JNK1 induced by 6 min of lethal ischemia in rat brains (Gu et al., 2000). While 6 min of lethal ischemia significantly reduces neuronal density in the CA1 hippocampal region, 3 min of ischemic preconditioning rescues these CA1 pyramidal cells (Gu et al., 2000). Moreover, in Mongolian gerbils, after ischemic injury by 6 min of bilateral carotid occlusion, JNK expression is highly increased and histological damage occurs in the hippocampal CA1 region. However, animals receiving 2 min of ischemic preconditioning do not experience an increase in JNK expression and lack histological damage in hippocampal and cortical regions (Colangelo et al., 2004). In the rat global ischemia model, 3 min of ischemic preconditioning generated by four-vessel occlusion down-regulates activation of JNK1/2 via N-methyl-D-aspartate (NMDA) receptor-mediated Akt1 activation, and induces neuroprotection in the hippocampal CA1 region (Miao et al., 2005). Similarly, 3 min of ischemic preconditioning with four-vessel occlusion in rat brains subjected to transient global ischemia protects pyramidal neurons in the hippocampal CA1 region and down-regulates activation of c-Jun (Wang et al., 2016b). Additionally, in rat brains, 3 min of ischemic preconditioning by four-vessel occlusion significantly reduces JNK3 activation and apoptotic neuronal death in the CA1 hippocampal region after 8 min of subsequent lethal ischemia (Zhang et al., 2009). This protective effect is abolished by a NMDA receptor antagonist, suggesting that the neuroprotection caused by ischemic preconditioning needs NMDA receptor-mediated JNK3 inhibition (Zhang et al., 2009). In *in vitro* models of NMDA preconditioning in primary rat cultured neurons, NMDA preconditioning-induced neuroprotection against glutamate toxicity is mediated through JNK inactivation (Navon et al., 2012). Furthermore, in *in vitro* models of OGD-induced cell death in cultured microglia, isoflurane preconditioning inhibits activation of TLR4 and JNK, attenuates OGD-induced cell death, and significantly reduces the level of pro-inflammatory cytokines from cultured microglia (Xiang et al., 2014).

However, there are several contradicting studies regarding

the role of JNK in ischemic preconditioning-mediated neuroprotection. In Mongolian gerbil brains, 2.5 min of ischemic preconditioning via bilateral common carotid artery occlusion, followed by 5 min of lethal ischemia, induces c-Jun expression in the hippocampal CA1 region and prevents delayed neuronal cell death (Sommer et al., 1995). Fifteen minutes of ischemic preconditioning via OGD in cultured astrocytes significantly reduces cell death generated by 8 hrs of ischemic injury. Of note, this ischemic preconditioning also promotes JNK activation, and the neuroprotective effects are eliminated upon JNK inhibition (Pang et al., 2015). Moreover, ischemic preconditioning protects primary astrocytes against ischemic injury by upregulating the protective protein 14-3-3γ. However, inhibiting JNK with SP600125, a specific inhibitor of JNK, diminishes the ischemic preconditioning-associated up-regulation of 14-3-3γ (Pang et al., 2015). Thrombin-induced ischemic tolerance protects neurons against subsequent OGD-induced ischemia *in vitro*, and protects the brain from MCAO-related damage *in vivo*, through JNK activation (Granziera et al., 2007). For example, pre-treatment with a low dose of thrombin (0.01U/ml) for 1 h prior to 24 hrs of OGD prevents neuronal cell death in rat hippocampal organotypic slice cultures, and JNK inhibition prevents this thrombin preconditioning-induced neuroprotective effect (Granziera et al., 2007). In addition, intracerebroventricular injection of thrombin 1 day before MCAO significantly reduces the lesion size in mice MCAO brains, and improves functional recovery (Granziera et al., 2007). Again, JNK inhibition blocks this thrombin preconditioning-induced neuroprotective effect and prevents the reduction in infarct volume observed in the MCAO mice brains pretreated with thrombin, thus, indicating that JNK is involved in thrombin preconditioning-induced neuroprotection against transient cerebral ischemia (Granziera et al., 2007). In Mongolian gerbil brains, preconditioning via intraperitoneal administration of a low dose (3 mg/kg) of 3-nitropionic acid induces JNK activation in the hippocampal CA1 region 2 days after injection and prevents delayed neuronal cell death against subsequent ischemia in the hippocampus (Sugino et al., 2000). Preconditioning with 30 min of hypothermia (33 °C) also generates neuroprotective effects in the cerebral cortex of rats following traumatic brain injury through early activation of JNK (Lotocki et al., 2006). In addition, hypothermic preconditioning decreases tumor necrosis factor (TNF) ligand-receptor-1 expression via early JNK activation, triggering neuroprotective signal cascades and suppressing both caspase-3 activation and cleavage of X-linked inhibitor of apoptosis protein (XIAP) during later time points (Lotocki et al., 2006). However, it is also possible that JNK signaling has no role in cerebral ischemic preconditioning-induced ischemic tolerance in the brain. Cerebral ischemic preconditioning in the rat brain up-regulates glial glutamate transporter 1 (GLT-1), which is important for brain ischemic tolerance, and significantly increases the phosphorylation of p-38 MAPK, but does not induce any changes in p-ERK1/2 or p-JNK in the hippocampal CA1 region (Zhang et al., 2017). Moreover, inhibition of p-p38 MAPK suppresses ischemic tolerance in the brain, suggesting that p-p38 MAPK is involved in GLT-1-mediated ischemic tolerance following cerebral ischemic preconditioning (Zhang et al., 2017). When MAPKs and PI3K/Akt were examined in cultured rat cerebral cortical neurons preconditioned with 1 h of OGD prior to 3 hrs of lethal OGD, no relative changes in the phosphorylation or expression of JNK, ERK1/2, and p38 were observed (Bhuiyan et al., 2011). Only Akt showed significant changes in its phosphorylation after ischemic preconditioning with OGD, and Akt inhibition abolished these ischemic preconditioning-induced neuroprotective effects (Bhuiyan et al., 2011).

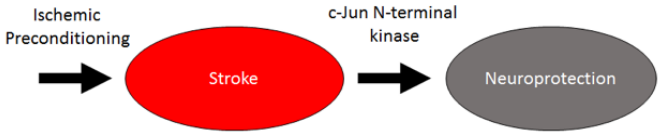

Figure 1. Signaling pathway of ischemic preconditioning. There is scientific evidence to suggest that JNK mediates the neuroprotective effects of ischemic preconditioning in stroke.

Conclusion

Many clinical and pre-clinical studies have been conducted to develop therapeutic treatments for cerebral ischemia and various other CNS injuries. Ischemic preconditioning, including RIPC, is one of main approaches to protect brain or neuronal cells against cerebrovascular ischemic-related injury. However, in order to translate the findings to the clinic, many factors need to be elucidated and identified, such as age/gender-dependency, comorbidities, patient medication history, and standard biomarkers. Essentially, clinical trials or pre-clinical studies should be more "personalized and categorized" in relation to these factors. In addition, experimental studies aimed to identify the key molecule that plays a central role in ischemic preconditioning-mediated neuroprotection against cerebral ischemia should also consider factors such as the extent of the injury, time point, brain tissue area, and cell type. JNK has a dual role in regulating neuronal cell death and survival following a brain injury. Therefore, a therapeutic approach that targets JNK activity in ischemic preconditioning should be carefully developed. A detailed understanding about JNK-mediated molecular mechanisms involved in ischemic preconditioning-induced neuroprotection will lead to the development of improved therapeutic strategies for cerebral ischemia and other CNS injuries.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

Abete P, Ferrara N, Cacciatore F, Madrid A, Bianco S, Calabrese C, Napoli C, Scognamiglio P, Bollella O, Cioppa A, Longobardi G, Rengo F (1997) Angina-induced protection against myocardial infarction in adult and elderly patients: a loss of preconditioning mechanism in the aging heart? J Am Coll Cardiol 30:947-954.

An P, Xue YX (2009) Effects of preconditioning on tight junction and cell adhesion of cerebral endothelial cells. Brain Res 1272:81-88.

Atochin DN, Schepetkin IA, Khlebnikov AI, Seledtsov VI, Swanson H, Quinn MT, Huang PL (2016) A novel dual NO-donating oxime and c-Jun N-terminal kinase inhibitor protects against cerebral ischemia-reperfusion injury in mice. Neurosci Lett 618:45-49.

Ayodele M, Koch S (2017) Ischemic Preconditioning in the Intensive Care Unit. Curr Treat Options Neurol 19:24.

Bernaudin M, Nedelec AS, Divoux D, MacKenzie ET, Petit E, Schumann-Bard P (2002) Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain. J Cereb Blood Flow Metab 22:393-403.

Bhuiyan MI, Jung SY, Kim HJ, Lee YS, Jin C (2011) Major role of the PI3K/Akt pathway in ischemic tolerance induced by sublethal oxygen-glucose deprivation in cortical neurons *in vitro*. Arch Pharm Res 34:1023-1034.

Bogoyevitch MA, Kobe B (2006) Uses for JNK: the many

and varied substrates of the c-Jun N-terminal kinases. Microbiol Mol Biol Rev 70:1061-1095.
Borsello T, Clarke PG, Hirt L, Vercelli A, Repici M, Schorderet DF, Bogousslavsky J, Bonny C (2003) A peptide inhibitor of c-Jun N-terminal kinase protects against excitotoxicity and cerebral ischemia. Nat Med 9:1180-1186.
Brecht S, Kirchhof R, Chromik A, Willesen M, Nicolaus T, Raivich G, Wessig J, Waetzig V, Goetz M, Claussen M, Pearse D, Kuan CY, Vaudano E, Behrens A, Wagner E, Flavell RA, Davis RJ, Herdegen T (2005) Specific pathophysiological functions of JNK isoforms in the brain. Eur J Neurosci 21:363-377.
Carboni S, Antonsson B, Gaillard P, Gotteland JP, Gillon JY, Vitte PA (2005) Control of death receptor and mitochondrial-dependent apoptosis by c-Jun N-terminal kinase in hippocampal CA1 neurones following global transient ischaemia. J Neurochem 92:1054-1060.
Cardenas A, Moro MA, Leza JC, O'Shea E, Davalos A, Castillo J, Lorenzo P, Lizasoain I (2002) Upregulation of TACE/ADAM17 after ischemic preconditioning is involved in brain tolerance. J Cereb Blood Flow Metab 22:1297-1302.
Chang CZ, Wu SC, Kwan AL, Lin CL (2015) Preconditioning with pitavastatin, an HMG-CoA reductase inhibitor, attenuates C-Jun N-terminal kinase activation in experimental subarachnoid hemorrhage-induced apoptosis. Acta Neurochir (Wien) 157:1031-1041.
Chen C, Jiang W, Liu Z, Li F, Yang J, Zhao Y, Ran Y, Meng Y, Ji X, Geng X, Du H, Hu X (2018a) Splenic responses play an important role in remote ischemic preconditioning-mediated neuroprotection against stroke. J Neuroinflammation 15:167.
Chen L, Huang K, Wang R, Jiang Q, Wu Z, Liang W, Guo R, Wang L (2018b) Neuroprotective Effects of Cerebral Ischemic Preconditioning in a Rat Middle Cerebral Artery Occlusion Model: The Role of the Notch Signaling Pathway. Biomed Res Int 2018:8168720.
Colangelo V, Gordon WC, Mukherjee PK, Trivedi P, Ottino P (2004) Downregulation of COX-2 and JNK expression after induction of ischemic tolerance in the gerbil brain. Brain Res 1016:195-200.
Dave KR, Saul I, Prado R, Busto R, Perez-Pinzon MA (2006) Remote organ ischemic preconditioning protect brain from ischemic damage following asphyxial cardiac arrest. Neurosci Lett 404:170-175.
Davis RJ (2000) Signal transduction by the JNK group of MAP kinases. Cell 103:239-252.
Du Y, Li C, Hu WW, Song YJ, Zhang GY (2009) Neuroprotection of preconditioning against ischemic brain injury in rat hippocampus through inhibition of the assembly of GluR6-PSD95-mixed lineage kinase 3 signaling module via nuclear and non-nuclear pathways. Neuroscience 161:370-380.
Fan YY, Hu WW, Dai HB, Zhang JX, Zhang LY, He P, Shen Y, Ohtsu H, Wei EQ, Chen Z (2011) Activation of the central histaminergic system is involved in hypoxia-induced stroke tolerance in adult mice. J Cereb Blood Flow Metab 31:305-314.
Ferrer I, Friguls B, Dalfo E, Planas AM (2003) Early modifications in the expression of mitogen-activated protein kinase (MAPK/ERK), stress-activated kinases SAPK/JNK and p38, and their phosphorylated substrates following focal cerebral ischemia. Acta Neuropathol 105:425-437.
Fluri F, Schuhmann MK, Kleinschnitz C (2015) Animal models of ischemic stroke and their application in clinical research. Drug Des Devel Ther 9:3445-3454.
Fu J, Xia X, Liu Z, Wang Y, Wang Y, Shi Q, Song X, Song E, Song Y (2017) The acute exposure of tetrachloro-p-benzoquinone (a.k.a. chloranil) triggers inflammation and neurological dysfunction via Toll-like receptor 4 signaling: The protective role of melatonin preconditioning. Toxicology 381:39-50.
Gidday JM, Fitzgibbons JC, Shah AR, Park TS (1994) Neuroprotection from ischemic brain injury by hypoxic preconditioning in the neonatal rat. Neurosci Lett 168:221-224.
Giricz Z, Lalu MM, Csonka C, Bencsik P, Schulz R, Ferdinandy P (2006) Hyperlipidemia attenuates the infarct size-limiting effect of ischemic preconditioning: role of matrix metalloproteinase-2 inhibition. J Pharmacol Exp Ther 316:154-161.
Granziera C, Thevenet J, Price M, Wiegler K, Magistretti PJ, Badaut J, Hirt L (2007) Thrombin-induced ischemic tolerance is prevented by inhibiting c-jun N-terminal kinase. Brain Res 1148:217-225.
Gu Z, Jiang Q, Zhang G (2001) Extracellular signal-regulated kinase and c-Jun N-terminal protein kinase in ischemic tolerance. Neuroreport 12:3487-3491.
Gu Z, Jiang Q, Zhang G, Cui Z, Zhu Z (2000) Diphosphorylation of extracellular signal-regulated kinases and c-Jun N-terminal protein kinases in brain ischemic tolerance in rat. Brain Res 860:157-160.
Gustavsson M, Wilson MA, Mallard C, Rousset C, Johnston MV, Hagberg H (2007) Global gene expression in the developing rat brain after hypoxic preconditioning: involvement of apoptotic mechanisms? Pediatr Res 61:444-450.
Hayashi T, Sakai K, Sasaki C, Zhang WR, Warita H, Abe K (2000) c-Jun N-terminal kinase (JNK) and JNK interacting protein response in rat brain after transient middle cerebral artery occlusion. Neurosci Lett 284:195-199.
Hu S, Dong H, Zhang H, Wang S, Hou L, Chen S, Zhang J, Xiong L (2012) Noninvasive limb remote ischemic preconditioning contributes neuroprotective effects via activation of adenosine A1 receptor and redox status after transient focal cerebral ischemia in rats. Brain Res 1459:81-90.
Ip YT, Davis RJ (1998) Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. Curr Opin Cell Biol 10:205-219.
Irving EA, Bamford M (2002) Role of mitogen- and stress-activated kinases in ischemic injury. J Cereb Blood Flow Metab 22:631-647.
Johnson GL, Nakamura K (2007) The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. Biochim Biophys Acta 1773:1341-1348.
Jones NM, Bergeron M (2004) Hypoxia-induced ischemic tolerance in neonatal rat brain involves enhanced ERK1/2 signaling. J Neurochem 89:157-167.
Kaiser RA, Liang Q, Bueno O, Huang Y, Lackey T, Klevitsky R, Hewett TE, Molkentin JD (2005) Genetic inhibition or activation of JNK1/2 protects the myocardium from ischemia-reperfusion-induced cell death *in vivo*. J Biol Chem 280:32602-32608.
Kamada H, Nito C, Endo H, Chan PH (2007) Bad as a converging signaling molecule between survival PI3-K/Akt and death JNK in neurons after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 27:521-533.
Keasey MP, Scott HL, Bantounas I, Uney JB, Kelly S (2016) MiR-132 Is Upregulated by Ischemic Preconditioning of Cultured Hippocampal Neurons and Protects them from Subsequent OGD Toxicity. J Mol Neurosci 59:404-410.
Kehl F, Krolikowski JG, Mraovic B, Pagel PS, Warltier DC, Kersten JR (2002) Hyperglycemia prevents isoflurane-

induced preconditioning against myocardial infarction. Anesthesiology 96:183-188.
Kersten JR, Schmeling TJ, Orth KG, Pagel PS, Warltier DC (1998) Acute hyperglycemia abolishes ischemic preconditioning *in vivo*. Am J Physiol 275:H721-725.
Kim S, Jeong J, Jung HS, Kim B, Kim YE, Lim DS, Kim SD, Song YS (2017) Anti-inflammatory Effect of Glucagon Like Peptide-1 Receptor Agonist, Exendin-4, through Modulation of IB1/JIP1 Expression and JNK Signaling in Stroke. Exp Neurobiol 26:227-239.
Koch S (2010) Preconditioning the human brain: practical considerations for proving cerebral protection. Transl Stroke Res 1:161-169.
Koch S, Katsnelson M, Dong C, Perez-Pinzon M (2011) Remote ischemic limb preconditioning after subarachnoid hemorrhage: a phase Ib study of safety and feasibility. Stroke 42:1387-1391.
Kuan CY, Burke RE (2005) Targeting the JNK signaling pathway for stroke and Parkinson's diseases therapy. Curr Drug Targets CNS Neurol Disord 4:63-67.
Kuan CY, Whitmarsh AJ, Yang DD, Liao G, Schloemer AJ, Dong C, Bao J, Banasiak KJ, Haddad GG, Flavell RA, Davis RJ, Rakic P (2003) A critical role of neural-specific JNK3 for ischemic apoptosis. Proc Natl Acad Sci USA 100:15184-15189.
Leak RK, Liou AK, Zigmond MJ (2006) Effect of sublethal 6-hydroxydopamine on the response to subsequent oxidative stress in dopaminergic cells: evidence for preconditioning. J Neurochem 99:1151-1163.
Leape LL (2005) Ethical issues in patient safety. Thorac Surg Clin 15:493-501.
Lehotsky J, Burda J, Danielisova V, Gottlieb M, Kaplan P, Saniova B (2009) Ischemic tolerance: the mechanisms of neuroprotective strategy. Anat Rec (Hoboken) 292:2002-2012.
Li C, Li YS, Xu M, Wen SH, Yao X, Wu Y, Huang CY, Huang WQ, Liu KX (2013) Limb remote ischemic preconditioning for intestinal and pulmonary protection during elective open infrarenal abdominal aortic aneurysm repair: a randomized controlled trial. Anesthesiology 118:842-852.
Li J, Li Y, Ogle M, Zhou X, Song M, Yu SP, Wei L (2010) DL-3-n-butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway. Brain Res 1359:216-226.
Liang J, Wang J, Saad Y, Warble L, Becerra E, Kolattukudy PE (2011) Participation of MCP-induced protein 1 in lipopolysaccharide preconditioning-induced ischemic stroke tolerance by regulating the expression of proinflammatory cytokines. J Neuroinflammation 8:182.
Lin AM, Dung SW, Chen CF, Chen WH, Ho LT (2003) Hypoxic preconditioning prevents cortical infarction by transient focal ischemia-reperfusion. Ann NY Acad Sci 993:168-178; discussion 195-166.
Liu Y, Kato H, Nakata N, Kogure K (1992) Protection of rat hippocampus against ischemic neuronal damage by pretreatment with sublethal ischemia. Brain Res 586:121-124.
Liu ZJ, Chen C, Li XR, Ran YY, Xu T, Zhang Y, Geng XK, Zhang Y, Du HS, Leak RK, Ji XM, Hu XM (2016) Remote Ischemic Preconditioning-Mediated Neuroprotection against Stroke is Associated with Significant Alterations in Peripheral Immune Responses. CNS Neurosci Ther 22:43-52.
Lotocki G, de Rivero Vaccari JP, Perez ER, Alonso OF, Curbelo K, Keane RW, Dietrich WD (2006) Therapeutic hypothermia modulates TNFR1 signaling in the traumatized brain via early transient activation of the JNK pathway and suppression of XIAP cleavage. Eur J Neurosci 24:2283-2290.
Macdonald RL, Pluta RM, Zhang JH (2007) Cerebral vasospasm after subarachnoid hemorrhage: the emerging revolution. Nat Clin Pract Neurol 3:256-263.
Mangus DB, Huang L, Applegate PM, Gatling JW, Zhang J, Applegate RL, 2nd (2014) A systematic review of neuroprotective strategies after cardiac arrest: from bench to bedside (Part I - Protection via specific pathways). Med Gas Res 4:9.
Maslov LN, Lishmanov Iu B (2012) [Neuroprotective effect of ischemic postconditioning and remote preconditioning. Prospective of clinical use]. Angiol Sosud Khir 18:27-34.
Matsushima K, Hakim AM (1995) Transient forebrain ischemia protects against subsequent focal cerebral ischemia without changing cerebral perfusion. Stroke 26:1047-1052.
Meloni BP, Tilbrook PA, Boulos S, Arthur PG, Knuckey NW (2006) Erythropoietin preconditioning in neuronal cultures: signaling, protection from *in vitro* ischemia, and proteomic analysis. J Neurosci Res 83:584-593.
Miao B, Yin XH, Pei DS, Zhang QG, Zhang GY (2005) Neuroprotective effects of preconditioning ischemia on ischemic brain injury through down-regulating activation of JNK1/2 via N-methyl-D-aspartate receptor-mediated Akt1 activation. J Biol Chem 280:21693-21699.
Miller BA, Perez RS, Shah AR, Gonzales ER, Park TS, Gidday JM (2001) Cerebral protection by hypoxic preconditioning in a murine model of focal ischemia-reperfusion. Neuroreport 12:1663-1669.
Mirante O, Price M, Puentes W, Castillo X, Benakis C, Thevenet J, Monard D, Hirt L (2013) Endogenous protease nexin-1 protects against cerebral ischemia. Int J Mol Sci 14:16719-16731.
Narayanan SV, Dave KR, Perez-Pinzon MA (2018) Ischemic Preconditioning Protects Astrocytes against Oxygen Glucose Deprivation Via the Nuclear Erythroid 2-Related Factor 2 Pathway. Transl Stroke Res 9:99-109.
Navon H, Bromberg Y, Sperling O, Shani E (2012) Neuroprotection by NMDA preconditioning against glutamate cytotoxicity is mediated through activation of ERK 1/2, inactivation of JNK, and by prevention of glutamate-induced CREB inactivation. J Mol Neurosci 46:100-108.
Nilsson GE, Vaage J, Stenslokken KO (2015) Oxygen- and temperature-dependent expression of survival protein kinases in crucian carp (Carassius carassius) heart and brain. Am J Physiol Regul Integr Comp Physiol 308:R50-61.
Nozaki K, Nishimura M, Hashimoto N (2001) Mitogen-activated protein kinases and cerebral ischemia. Mol Neurobiol 23:1-19.
Okami N, Narasimhan P, Yoshioka H, Sakata H, Kim GS, Jung JE, Maier CM, Chan PH (2013) Prevention of JNK phosphorylation as a mechanism for rosiglitazone in neuroprotection after transient cerebral ischemia: activation of dual specificity phosphatase. J Cereb Blood Flow Metab 33:106-114.
Okuno S, Saito A, Hayashi T, Chan PH (2004) The c-Jun N-terminal protein kinase signaling pathway mediates Bax activation and subsequent neuronal apoptosis through interaction with Bim after transient focal cerebral ischemia. J Neurosci 24:7879-7887.
Pang Y, Chai CR, Gao K, Jia XH, Kong JG, Chen XQ, Vatcher G, Chen JG, Yu AC (2015) Ischemia preconditioning protects astrocytes from ischemic injury through 14-3-3gamma. J Neurosci Res 93:1507-1518.
Pei DS, Song YJ, Yu HM, Hu WW, Du Y, Zhang GY (2008)

Exogenous nitric oxide negatively regulates c-Jun N-terminal kinase activation via inhibiting endogenous NO-induced S-nitrosylation during cerebral ischemia and reperfusion in rat hippocampus. J Neurochem 106:1952-1963.

Perez-Pinzon MA, Xu GP, Dietrich WD, Rosenthal M, Sick TJ (1997) Rapid preconditioning protects rats against ischemic neuronal damage after 3 but not 7 days of reperfusion following global cerebral ischemia. J Cereb Blood Flow Metab 17:175-182.

Petito CK, Feldmann E, Pulsinelli WA, Plum F (1987) Delayed hippocampal damage in humans following cardiorespiratory arrest. Neurology 37:1281-1286.

Pluta RM, Hansen-Schwartz J, Dreier J, Vajkoczy P, Macdonald RL, Nishizawa S, Kasuya H, Wellman G, Keller E, Zauner A, Dorsch N, Clark J, Ono S, Kiris T, Leroux P, Zhang JH (2009) Cerebral vasospasm following subarachnoid hemorrhage: time for a new world of thought. Neurol Res 31:151-158.

Price M, Badaut J, Thevenet J, Hirt L (2010) Activation of c-Jun in the nuclei of neurons of the CA-1 in thrombin preconditioning occurs via PAR-1. J Neurosci Res 88:1338-1347.

Ren C, Gao X, Steinberg GK, Zhao H (2008) Limb remote-preconditioning protects against focal ischemia in rats and contradicts the dogma of therapeutic time windows for preconditioning. Neuroscience 151:1099-1103.

Safar P (1986) Cerebral resuscitation after cardiac arrest: a review. Circulation 74:IV138-153.

Schenning KJ, Anderson S, Alkayed NJ, Hutchens MP (2015) Hyperglycemia abolishes the protective effect of ischemic preconditioning in glomerular endothelial cells *in vitro*. Physiol Rep 3.

Shvedova M, Anfinogenova Y, Atochina-Vasserman EN, Schepetkin IA, Atochin DN (2018) c-Jun N-Terminal Kinases (JNKs) in Myocardial and Cerebral Ischemia/Reperfusion Injury. Front Pharmacol 9:715.

Simao F, Matte A, Pagnussat AS, Netto CA, Salbego CG (2012) Resveratrol preconditioning modulates inflammatory response in the rat hippocampus following global cerebral ischemia. Neurochem Int 61:659-665.

Sommer C, Gass P, Kiessling M (1995) Selective c-JUN expression in CA1 neurons of the gerbil hippocampus during and after acquisition of an ischemia-tolerant state. Brain Pathol 5:135-144.

Sommer CJ (2017) Ischemic stroke: experimental models and reality. Acta Neuropathol 133:245-261.

Speetzen LJ, Endres M, Kunz A (2013) Bilateral common carotid artery occlusion as an adequate preconditioning stimulus to induce early ischemic tolerance to focal cerebral ischemia. J Vis Exp:e4387.

Stagliano NE, Perez-Pinzon MA, Moskowitz MA, Huang PL (1999) Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. J Cereb Blood Flow Metab 19:757-761.

Stetler RA, Leak RK, Gan Y, Li P, Zhang F, Hu X, Jing Z, Chen J, Zigmond MJ, Gao Y (2014) Preconditioning provides neuroprotection in models of CNS disease: paradigms and clinical significance. Prog Neurobiol 114:58-83.

Sugino T, Nozaki K, Hashimoto N (2000) Activation of mitogen-activated protein kinases in gerbil hippocampus with ischemic tolerance induced by 3-nitropropionic acid. Neurosci Lett 278:101-104.

Tang Y, Pacary E, Freret T, Divoux D, Petit E, Schumann-Bard P, Bernaudin M (2006) Effect of hypoxic preconditioning on brain genomic response before and following ischemia in the adult mouse: identification of potential neuroprotective candidates for stroke. Neurobiol Dis 21:18-28.

Tian H, Zhang QG, Zhu GX, Pei DS, Guan QH, Zhang GY (2005) Activation of c-Jun NH2-terminal kinase 3 is mediated by the GluR6.PSD-95.MLK3 signaling module following cerebral ischemia in rat hippocampus. Brain Res 1061:57-66.

Tripathi AK, Dwivedi A, Pal MK, Rastogi N, Gupta P, Ali S, Prabhu MB, Kushwaha HN, Ray RS, Singh SK, Duggal S, Narayan B, Mishra DP (2014) Attenuated neuroprotective effect of riboflavin under UV-B irradiation via miR-203/c-Jun signaling pathway *in vivo* and *in vitro*. J Biomed Sci 21:39.

Ungi I, Ungi T, Ruzsa Z, Nagy E, Zimmermann Z, Csont T, Ferdinandy P (2005) Hypercholesterolemia attenuates the anti-ischemic effect of preconditioning during coronary angioplasty. Chest 128:1623-1628.

Vergouwen MD, Vermeulen M, van Gijn J, Rinkel GJ, Wijdicks EF, Muizelaar JP, Mendelow AD, Juvela S, Yonas H, Terbrugge KG, Macdonald RL, Diringer MN, Broderick JP, Dreier JP, Roos YB (2010) Definition of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage as an outcome event in clinical trials and observational studies: proposal of a multidisciplinary research group. Stroke 41:2391-2395.

Wacker BK, Park TS, Gidday JM (2009) Hypoxic preconditioning-induced cerebral ischemic tolerance: role of microvascular sphingosine kinase 2. Stroke 40:3342-3348.

Waetzig V, Herdegen T (2005) Context-specific inhibition of JNKs: overcoming the dilemma of protection and damage. Trends Pharmacol Sci 26:455-461.

Wang H, Shi H, Yu Q, Chen J, Zhang F, Gao Y (2016a) Sevoflurane Preconditioning Confers Neuroprotection via Anti-apoptosis Effects. Acta Neurochir Suppl 121:55-61.

Wang M, Qi DS, Zhou C, Han D, Li PP, Zhang F, Zhou XY, Han M, Di JH, Ye JS, Yu HM, Song YJ, Zhang GY (2016b) Ischemic preconditioning protects the brain against injury via inhibiting CaMKII-nNOS signaling pathway. Brain Res 1634:140-149.

Wang Y, Meng R, Song H, Liu G, Hua Y, Cui D, Zheng L, Feng W, Liebeskind DS, Fisher M, Ji X (2017) Remote Ischemic Conditioning May Improve Outcomes of Patients With Cerebral Small-Vessel Disease. Stroke 48:3064-3072.

Xiang HF, Cao DH, Yang YQ, Wang HQ, Zhu LJ, Ruan BH, Du J, Wang MC (2014) Isoflurane protects against injury caused by deprivation of oxygen and glucose in microglia through regulation of the Toll-like receptor 4 pathway. J Mol Neurosci 54:664-670.

Yang C, Ren Y, Liu F, Cai W, Zhang N, Nagel DJ, Yin G (2008) Ischemic preconditioning suppresses apoptosis of rabbit spinal neurocytes by inhibiting ASK1-14-3-3 dissociation. Neurosci Lett 441:267-271.

Yang Y, Lu F, Zhuang L, Yang S, Kong Y, Tan W, Gong Z, Zhan S (2017) Combined preconditioning with hypoxia and GYKI-52466 protects rats from cerebral ischemic injury by HIF-1alpha/eNOS pathway. Am J Transl Res 9:5308-5319.

Yang Z, Tian Y, Liu Y, Hennessy S, Kron IL, French BA (2013) Acute hyperglycemia abolishes ischemic preconditioning by inhibiting Akt phosphorylation: normalizing blood glucose before ischemia restores ischemic preconditioning. Oxid Med Cell Longev 2013:329183.

Yang ZJ, Wang YW, Li CL, Ma LQ, Zhao X (2015) Pre-treatment with a Xingnaojing preparation ameliorates sevoflurane-induced neuroapoptosis in the infant rat striatum. Mol Med Rep 11:1615-1622.

Zhan L, Wang T, Li W, Xu ZC, Sun W, Xu E (2010) Activation of Akt/FoxO signaling pathway contributes to induction of

neuroprotection against transient global cerebral ischemia by hypoxic pre-conditioning in adult rats. J Neurochem 114:897-908.

Zhan L, Chen S, Li K, Liang D, Zhu X, Liu L, Lu Z, Sun W, Xu E (2017) Autophagosome maturation mediated by Rab7 contributes to neuroprotection of hypoxic preconditioning against global cerebral ischemia in rats. Cell Death Dis 8:e2949.

Zhan L, Lu Z, Zhu X, Xu W, Li L, Li X, Chen S, Sun W, Xu E (2018) Hypoxic preconditioning attenuates necroptotic neuronal death induced by global cerebral ischemia via Drp1-dependent signaling pathway mediated by CaMKIIalpha inactivation in adult rats. FASEB J:fj201800111RR.

Zhang J, Qian H, Zhao P, Hong SS, Xia Y (2006a) Rapid hypoxia preconditioning protects cortical neurons from glutamate toxicity through delta-opioid receptor. Stroke 37:1094-1099.

Zhang M, Gong JX, Wang JL, Jiang MY, Li L, Hu YY, Qi J, Zhang LY, Zhao H, Cui X, Xian XH, Li WB (2017) p38 MAPK Participates in the Mediation of GLT-1 Up-regulation During the Induction of Brain Ischemic Tolerance by Cerebral Ischemic Preconditioning. Mol Neurobiol 54:58-71.

Zhang N, Gao G, Bu X, Han S, Fang L, Li J (2007a) Neuron-specific phosphorylation of c-Jun N-terminal kinase increased in the brain of hypoxic preconditioned mice. Neurosci Lett 423:219-224.

Zhang QG, Wang RM, Han D, Yang LC, Li J, Brann DW (2009) Preconditioning neuroprotection in global cerebral ischemia involves NMDA receptor-mediated ERK-JNK3 crosstalk. Neurosci Res 63:205-212.

Zhang QG, Han D, Xu J, Lv Q, Wang R, Yin XH, Xu TL, Zhang GY (2006b) Ischemic preconditioning negatively regulates plenty of SH3s-mixed lineage kinase 3-Rac1 complex and c-Jun N-terminal kinase 3 signaling via activation of Akt. Neuroscience 143:431-444.

Zhang Y, Park TS, Gidday JM (2007b) Hypoxic preconditioning protects human brain endothelium from ischemic apoptosis by Akt-dependent survivin activation. Am J Physiol Heart Circ Physiol 292:H2573-2581.

Zhang Y, Li YW, Wang YX, Zhang HT, Zhang XM, Liang Y, Zhang XS, Wang WS, Liu HG, Zhang Y, Zhang L, Zheng YH (2013) Remifentanil preconditioning alleviating brain damage of cerebral ischemia reperfusion rats by regulating the JNK signal pathway and TNF-alpha/TNFR1 signal pathway. Mol Biol Rep 40:6997-7006.

Zhao W, Meng R, Ma C, Hou B, Jiao L, Zhu F, Wu W, Shi J, Duan Y, Zhang R, Zhang J, Sun Y, Zhang H, Ling F, Wang Y, Feng W, Ding Y, Ovbiagele B, Ji X (2017) Safety and Efficacy of Remote Ischemic Preconditioning in Patients With Severe Carotid Artery Stenosis Before Carotid Artery Stenting: A Proof-of-Concept, Randomized Controlled Trial. Circulation 135:1325-1335.

Zhou AM, Li WB, Li QJ, Liu HQ, Feng RF, Zhao HG (2004) A short cerebral ischemic preconditioning up-regulates adenosine receptors in the hippocampal CA1 region of rats. Neurosci Res 48:397-404.

Zhuang Q, Dai C, Yang L, Wen H, Wang H, Jiang X, Zhang Y (2017) Stimulated CB1 Cannabinoid Receptor Inducing Ischemic Tolerance and Protecting Neuron from Cerebral Ischemia. Cent Nerv Syst Agents Med Chem 17:141-150.