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Preconditioning and postconditioning for neuroprotection: The most recent evidence

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Stroke is a leading cause of morbidity and mortality, with perioperative stroke being an important complication in the practice of anaesthesia. Unfortunately, pharmacological treatment options are very limited and often not applicable in the perioperative period. The notion of applying a subtoxic stimulus prior to an otherwise lethal event is termed preconditioning. The main focus of the article is on describing the different concepts of preconditioning, including remote ischaemic preconditioning and anaesthetic preconditioning, as well as postconditioning and summarizing the most recent discoveries in this exciting field.

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Stroke is the third leading cause of death in the United States and a leading cause of disability worldwide.¹ Perioperatively, the stroke risk varies, but is nearly 10% in some types of cardiac surgeries, such as cardiac valve replacements and carotid endarterectomies.² While risk modification is helpful, effective treatments are limited, and can often be used in very few patients, especially perioperatively. One study showed that, of all ischaemic stroke patients presenting for medical care, only 7.5% received tissue plasminogen activator (tPA) treatment.³ Thus, there is great need for further therapies for stroke and other neuroischaemia.

A promising treatment strategy is that of preconditioning. Preconditioning is the notion that a subtoxic stimulus is applied to a tissue incurring a response in that tissue that then protects it from, or limits the damage when a similar, or even worse, an otherwise lethal stimulus would follow. For example, ischaemic preconditioning (also known as “induction of ischaemic tolerance”) in the brain is the idea that exposing the brain to short periods of non-damaging ischaemia affords protection, or tolerance of, a later ischaemic event that would typically be devastating to the brain.

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A comprehensive monograph on the topic of pre-emptive brain conditioning would greatly exceed the space available such that this review rather aims to summarize the most recent advances in the science pertaining to this exciting subfield of neuroprotection research. We will portrait novel findings explaining mechanisms and efficacy of ischaemic preconditioning, remote ischaemic preconditioning, and anaesthetic preconditioning. Additionally, we will discuss the newest discoveries relating to the principle of postconditioning. Finally, we will summarize the most recent discussion about potential gender differences in the sensitivity for or the efficacy of pre- and postconditioning.

Preconditioning: A strategy to induce tolerance against cerebral ischaemia

The phenomenon of ischaemic preconditioning was first identified in 1986 in myocardium,⁴ but has since been observed in multiple organs, including the brain.⁵ Clinically, neuroprotection via ischaemic preconditioning may be occurring when a patient suffers a transient ischaemic attack (TIA) prior to an ischaemic stroke. Multiple studies have shown that those suffering an ischaemic stroke have less severe deficits if they had a TIA prior to the stroke.^{6,7,8}

While ischaemic preconditioning was the first type of preconditioning identified, multiple methods of inducing the endogenous protective mechanisms of cells have now been described. A summary of some types of preconditioning is outlined in Table 1.

Today, there are multiple strategies that are recognized as inducing brain preconditioning, and, depending on which stimulus is used, neuronal tolerance can be observed in at least two temporal profiles: rapid brain preconditioning, in which the stimulus incurs neuroprotection within minutes, and delayed brain preconditioning, in which neuroprotection develops after a delay of hours to days. Understanding the mechanisms of preconditioning and finding appropriate timing and exposure to preconditioning could be an important therapeutic model for prevention of neuroischaemia in high-risk patients, especially those about to undergo a procedure with high perioperative stroke risk.

The mechanism of preconditioning

Multiple molecular pathways and protective mechanisms have been identified and continue to be studied in an effort to induce protection of the ischaemic brain. However, no one pathway has been

Table 1

Types of preconditioning and their definition listed with some of the most recent peer-reviewed publications. LPS-Lipopolysaccharide.

Paradigms for establishing preconditioning	Definition/Principle—All applied prior to a major ischaemic event	Recent Publications	Additional information
Ischaemia	Exposure of brain to subtoxic levels of ischaemia in short bursts	11,17,19,29,50,64,65	Immediate vs. delayed protection
Pharmacological	Exposure to therapeutic agent which triggers endogenous cellular protection.	20,27,31,66,67	Ex. erythropoietin, penicillin
Anaesthetic	Exposure to volatile anesthetics within pharmacological concentration ranges	33,35	Majority of studies done with isoflurane. New anesthetic of interest: Xenon.
Immunologic	Application of inflammatory stimulus that is known to be upregulated with ischaemia.	26,28,30	This overlaps with pharmacological preconditioning, but is more specific. For example, LPS is a proinflammatory stimulus seen to be protective at low doses prior to ischaemia ²⁵ .
Hypoxia	Exposure to hypoxia (O ₂ concentrations <21%) with normobaric pressure	21,22	
Remote ischaemia	Exposure of subtoxic levels of ischaemia in distant limb, such as arm or leg.	36,37	Multiple clinical trials being initiated, see www.clinicaltrials.org

shown to be exclusively protective or necessarily more important than the others. Instead, it appears that many of the pathways work in parallel, or together, to induce preconditioning in the brain. These factors include 1) activation of fundamental cellular defense mechanisms such as antioxidant systems, heat shock proteins, and cell death/survival determinants; 2) responses at tissue level, especially reduced inflammatory responsiveness; and 3) a shift of the neuronal excitatory/inhibitory balance toward inhibition and downregulation⁹ 4) stimulation of numerous growth factors and their receptors, including nerve growth factor and brain-derived neurotrophic factor,¹⁰ leading to angiogenesis and 5) proliferation of endogenous progenitor cells with neuroprotective and neurorestorative properties.¹¹

The all-encompassing idea is that preconditioning appears to upregulate the same protective mechanisms seen in surviving cells after an ischaemic event, but, by inducing these mechanisms prior to ischaemia, a benefit is seen, resulting in decreased damage by an otherwise lethal insult.

Multiple recent reviews provide excellent summaries of these protective pathways and mechanisms.^{9,12,13,14,15} In this review we will give a short summary of some of the most recent additions to our increasing knowledge of the mechanisms involved in ischaemia tolerance in neuronal tissue. [Table 2](#) summarizes most recent exciting discoveries in animal models, and lists the newest evidence as to the mechanisms involved.

Mitochondria as neuroprotectants in preconditioning

Mitochondria comprise nearly half of the cell volume in tissues with high-energy consumption, such as the heart and brain.¹⁶ The mitochondrial respiratory chain generates ATP, the most important intracellular energy source. In high energy consumption tissues, the chain produces more than 80–90% of the ATP.¹⁶ It is no surprise, then, that mitochondria appear to be intrinsically involved in the protective mechanism of preconditioning. Preconditioning prevents mitochondrial swelling and protects mitochondrial energy metabolism during cerebral ischaemia by reducing the rate of ATP consumption.¹⁶ The underlying mechanisms of these changes are not fully understood but further detailed information is excellently outlined in a 2009 review by Dirnagl, et al.¹²

One of the mechanistic pathways most recently studied is that of mitochondrial protection after ischaemic preconditioning by activation of ϵ protein kinase C (ϵ PKC).¹⁷ Several signaling pathways downstream from ϵ PKC have been described, and these pathways have been shown to evoke neuroprotection in ischaemia, but never in the context of ischaemic preconditioning. Dave, et al. showed that ischaemic preconditioning significantly increases the level of hippocampal synaptosomal ϵ PKC at two days after the stimulus in male rats.¹⁷ They also showed that ϵ PKC improves mitochondrial function in terms of rate of respiration and conservation of mitochondrial membrane.¹⁷ These results confirm the importance of the ϵ PKC signaling pathway in the mechanism of neuroprotection after ischaemic preconditioning.

Hypoxia-inducible factor and its target genes are agents in neuroprotection

Hypoxia-inducible factor-1 (HIF-1), the most widely known member of the hypoxia-inducible factor (HIF) family, has certainly been shown to be involved in the molecular cascade leading to preconditioning. HIF-1's nuclear expression is increased during hypoxic preconditioning, and chemical HIF-1 inducers, such as desferrioxamine, induce brain tolerance.¹⁸ HIFs appear to downregulate oxidative metabolism by decreasing mitochondrial biogenesis, augmenting glycolysis, decreasing metabolite entry into the citric acid cycle, and promoting removal of free-radical-generating mitochondria by autophagy.^{12,19}

More specifically, hypoxic preconditioning upregulates HIF-1 target genes, such as vascular endothelial growth factor (VEGF), glucose transporter-1 (GLUT-1), and the lesser-known adrenomedullin (AM).²⁰ AM is a 52-amino acid peptide expressed in various tissues, including heart and brain. It has numerous functions, including control of water intake, vasodilation, apoptosis inhibition, and neuro-modulation functions.²⁰ AM exerts its functions via G-protein coupled receptors in the brain.

Most recent data supports the idea that AM is upregulated after hypoxic preconditioning, and that AM is neuroprotective in neurons. However, use of an AM antagonist during hypoxic preconditioning failed to abolish or decrease this protection, showing that AM is not the primary neuroprotective factor

Table 2

Summary of recent findings in preconditioning reveals protection using anaesthetic, hypoxic, and immunologic preconditioning. OGD-Oxygen-glucose deprivation; min-minutes; h-hours; TLR, Toll-like receptor; MCAO, middle cerebral artery occlusion; ODN, oligodeoxynucleotide; PC, preconditioning; Isch, Ischaemia.

Preconditioning model	Ischaemia model	Animal species	PC duration × frequency	Time interval PC ⇌ Isch	Effects and outcomes	Mechanisms tested /discussed	Reference
3.3% Sevoflurane, 75% Xenon, 75% Nitrous oxide	OGD × 75 min	Mouse neuronal-glia cell co-cultures	2 hrs × 1	24 h	Sevoflurane and Xenon lead to preconditioning of neuronal cells	Sevoflurane protection is independent of K _{ATP} channel activation. Effect of Xenon required the opening of plasmalemmal K _{ATP} channels. This may mean xenon PC may mimic the mechanism of ischaemic PC, while volatile anesthetics may not.	33
Hypoxia (0.1%, 0.5%, 1% or 2% O ₂)	OGD × 1 hr	Mouse neurons	30 min × 1 or 1 hr × 1	24 h	Decreased neuronal cell death after 0.1% or 0.5% hypoxic preconditioning	Adrenomedullin is an effector of hypoxic PC induced neuronal tolerance and a potent autocrine and paracrine neuroprotective factor during ischaemia.	20
Pam3CSK4, a TLR-2 specific ligand systemically	MCAO Permanent	Mouse	Single injection	24 h	Decreased infarct size, Mortality and brain edema at 24 hr of reperfusion. Preserved neurologic function.	PC with a TLR-2 ligand protects brain from ischaemic injury.	26
CpG ODN vs. vehicle	MCAO × 60 min	C57/Bl6, TNF- α KO mice	Single injection	72 h	Decreased infarct size in CpG ODN mice	TLR-9 ligands can induce PC in brain <i>in vivo</i> & <i>in vitro</i> . TNF α is required for this ischaemic protection.	27

in hypoxic preconditioning.²⁰ The same authors also showed that astrocyte neuroprotection in preconditioning is independent of the AM pathway.²⁰

In addition to being a target gene of HIF-1, GLUT-1 and another glucose transporter, GLUT-3, may have their own unique roles in protection of neuroischaemia. Hypoxic preconditioning increases levels of GLUT-1 and GLUT-3 mRNA in neurons and levels of GLUT-1 mRNA in astrocytes, possibly via the HIF-1 pathway.²¹ Additionally, glucose transport activity is upregulated in cultured hippocampal neurons and astrocytes after hypoxic preconditioning.²¹ Glucose transport across the cell membrane is the rate-limiting step in glucose metabolism. There is a low ATP production rate during anoxia; necessitating a cell to make more ATP via glycolysis in order to maintain its ATP levels.²¹ Therefore glucose transport may be vital in anoxic conditions.

For example, erythropoietin (EPO), another hypoxia-inducible factor, has recently been shown also to be associated with the effects of experimental hypoxic preconditioning on brain tissue. Theus et al. showed that hypoxic preconditioning protects stem cells to be implanted into ischaemic rat brain.²² Secretion of EPO was increased in these cells after hypoxic preconditioning, as was expression of EPO receptor. The protection seen after hypoxic preconditioning was diminished when an EPO receptor blocker was added, and adding exogenous EPO mimicked the protective effect in the stem cells.²² A clinical trial attempting to take advantage of the protective mechanisms of EPO was previously initiated. The “Treating patients with aneurismal subarachnoid hemorrhage (SAH) with epoetin alfa (EPO)” trial was listed in clinicaltrials.gov. Unfortunately, this trial was cancelled 2/12/09 due to data from another study that showed increased mortality in ischemic stroke patients with EPO treatment.²³ However, other earlier smaller studies have shown that EPO therapy for stroke is beneficial and without harm.²⁴

Immunologic preconditioning

Like ischaemic preconditioning, inflammatory stimuli prior to ischaemia can induce tolerance. There is an abundance of new research investigating the immune system and toll-like receptors (TLRs) in regards to preconditioning (see Table 2). Ischaemia itself can stimulate the immune system causing inflammation in the brain.²⁵ Immunologic preconditioning aims to upregulate those same pathways prior to the ischemic insult, exploiting those same mechanisms in an effort to upregulate endogenous neuroprotective mechanisms. It has been shown that preconditioning with small intraperitoneal doses of lipopolysaccharide (LPS), a powerful trigger of the immune response, stimulates anti-inflammatory and suppresses proinflammatory pathways, while giving large doses of LPS is toxic to the brain.²⁵ Toll-like receptors are a family of signal transduction molecules that play a role in the induction of innate and adaptive immunity.²⁶ Recently, preconditioning with TLR-2, TLR-4 and TLR-9 ligands has been shown to be protective in neuroischaemia.^{26,27,28} This preconditioning strategy must be well timed and the preconditioning doses of TLR ligands thoroughly tested, as these same molecules have been shown to be proinflammatory if given at the time of ischaemia.^{29,30} Nevertheless, the notion of preconditioning with a TLR-specific ligand is an exciting prospect, as the benefits appear to be similar to those seen in ischaemic preconditioning.³¹

Anaesthetic preconditioning

Especially important to the anaesthesiologist is the principle of anaesthetic preconditioning. Inhalational anaesthetics have been shown to induce neuroprotection, which could be especially helpful in decreasing morbidity and mortality from perioperative stroke. Numerous inhalational anaesthetic agents have been shown to be protective in animal models of preconditioning.^{32,33,34}

The most-studied inhalational anaesthetic in regards to preconditioning is isoflurane, primarily due to its low cost and clinical relevance. Protection has been seen in models of permanent and transient focal cerebral ischaemia, and in global ischaemia, and can be immediate or delayed.³² While it appears that volatile anaesthetics are protective, more research is needed to investigate the best time course to attenuate neuronal injury from perioperative stroke.

Xenon, a chemical element and noble gas that acts as a NMDA antagonist, is one of the most exciting areas of new research. Due to its high cost, limited availability, and lack of a delivery system with gas

recycling capabilities, Xenon is not often used clinically. However, it has been shown to decrease infarction size and improve neurobehavioral function in animal models when used as a preconditioning agent.^{34, 35} Advantages of using Xenon as an inhalational anaesthetic include its wide therapeutic window, minimal effect on mean arterial pressure, cardiac contractility, and heart rate, as well as its low blood/gas partition coefficient, which translates into rapid onset and emergence times.³⁵

The mechanism of anaesthetic preconditioning appears to be as complex as that of ischaemic preconditioning, with much overlap between the two. One of the mechanisms may be that of opening of ATP-sensitive potassium (K_{ATP}) channels, which alters reactive oxygen species (ROS) production, diminishes intraintraischemic mitochondrial calcium accumulation, and enhances postischemic mitochondrial energy protection.³²

Remote ischaemic preconditioning

An alternative to the traditional model of ischaemic preconditioning of the organ of concern is the approach of 'remote ischaemic preconditioning'. Remote ischaemic preconditioning (RIPC) is the idea that transient, nonlethal ischaemic intervals of one organ or anatomic region lead to protection from a lethal ischaemic event in another organ. For example, remote ischaemic preconditioning performed in the hind limb protects against subsequent ischaemia in the brain.³⁶ Like ischaemic preconditioning, RIPC has been most extensively studied initially in its role of cardio protection and myocardial ischaemia; yet, it appears an attractive concept for experimental stroke and neuronal protection as well. The mechanism through which RIPC protects is currently unclear, but studies suggest that the underlying mechanistic pathways and signal transduction cascades are similar to that of ischaemic preconditioning and postconditioning.³⁷

Most recently, Ren et al. showed that hind limb remote preconditioning protected against ischaemic damage after focal cerebral ischemia. Rats were exposed to control or one of four subgroups: (1) isoflurane control, (2) two cycles of 5 min occlusion/reperfusion of the left femoral artery, (3) two cycles of 15 min of occlusion/reperfusion, or (4) three cycles of 15 min occlusion/reperfusion. After preconditioning all groups were immediately exposed to focal cerebral ischaemia consisting of bilateral common carotid artery (CCA) occlusion for 30 min combined with permanent occlusion of the left distal middle cerebral artery (MCA) above the rhinal fissure.³⁶ Infarct size, measured at 48 hours with 2% 2,3,7-triphenyltetrazolium chloride (TTC) staining, showed no protection with 5-minute limb preconditioning, but significantly reduced infarct size with two or three cycles of 15 min occlusion/reperfusion. Three cycles of 15 minutes showed the strongest protection. Ren et al. then used the same hind limb preconditioning model involving three cycles of 15 min occlusion/reperfusion, but delayed the CCA and MCA occlusion for 12 hours after the limb ischaemia. Measurement of infarct size revealed that limb pre-conditioning significantly protects against ischaemic injury. In a third series of experiments, Ren subjected groups to two or three cycles of 15 min occlusion/reperfusion vs. isoflurane control and delayed CCA and MCA occlusion for 48 hours. This delayed limb preconditioning showed that three cycles of occlusion/reperfusion reduced infarct size, but two cycles did not protect against cerebral ischemia.³⁶

The study by Ren and co-workers is especially important because it shows protection from remote preconditioning at multiple time points—rapid, delayed, and most importantly, intermediate time points, which is different from what is seen with ischaemic preconditioning. It also demonstrates the efficacy of remote preconditioning with focal ischaemia and with single limb ischaemia as opposed to both limbs, which had previously not been addressed.

Remote ischaemic preconditioning is an attractive model for clinical trials, as it is possible to apply the preconditioning stimulus to a less vital structure, such as a limb or muscle group, instead of directly to the more risky target organ, namely the brain. A search of clinicaltrials.gov and controlled-trials.com revealed several clinical trials investigating the effect of RIPC, six of which are exploring the effect of RIPC on cerebral ischaemia or cognitive function.^{38, 39, 40, 41, 42} One study in particular is investigating the effect of RIPC as a treatment for acute stroke. The blinded, randomized study is currently in the recruitment phase, and will have two arms: (1) thrombolysis + RIPC and (2) Thrombolysis alone.⁴² The outcome of this study may drastically alter the current stroke treatments. Overall, remote ischaemic preconditioning is a very promising method for neuroprotection.

Strategies of postconditioning

Similar to ischaemic preconditioning, the neuroprotective strategy termed ischaemic postconditioning was initially defined in the field of myocardial research, where repeated cycles of brief reperfusion and re-occlusion were demonstrated to reduce the infarct size after cardiac ischaemia, attenuating the damage thought to result from reactive oxygen species.⁴³ This relatively novel concept has now been shown to be effective in experimental focal and global cerebral ischaemia, as well as in the context of spinal cord ischaemia. Neuronal ischaemic postconditioning was initially described as a repetitive series of brief interruptions of reperfusion applied immediately after ischaemia. This idea has more recently been expanded to include postischaemic exposure to agents previously shown as being protective using preconditioning strategies.

For the purpose of this review, rapid ischaemic postconditioning can be defined as postconditioning initiated at reperfusion or up to 60 minutes after reperfusion. Delayed postconditioning is defined as postconditioning performed more than one hour after the initiation of reperfusion.⁴⁴

When specific pharmacologic agents are used it can be difficult to differentiate between “postconditioning” effects and the direct pharmacologic treatment effects on the extent of neurologic injury observed. This review focuses on those studies that used an ischaemic postconditioning model or those that “postconditioned” with anaesthetics. A summary of recent findings and publications can be seen in Table 3.

Like preconditioning, there are now numerous ways described to confer protection using postconditioning. In the past two years, much of the research has been dedicated to discovering the mechanisms of protection with postconditioning and developing models of protection. Each postconditioning model is characterized by the time at which the stimulus is applied after the initial insult, the cycle length of reperfusion/occlusion, and the number of conditioning cycles used. When using anaesthetic agents or pharmacologic agents, the dose must be considered. For example, rapid postconditioning initiated 10–30 s after reperfusion reduced infarct size at 48 hours, while postconditioning initiated 3 minutes after reperfusion was not beneficial.⁴⁵ In contrast, Ren et al. was able to demonstrate a protective effect from delayed postconditioning when they showed that occlusion/reperfusion cycles initiated 3 and 6 hrs after stroke decreased infarct size in rats.⁴⁴ Protection has also been demonstrated *in vitro* using oxygen-glucose deprivation.⁴⁶ Interestingly, it has also been shown that the protective effects of preconditioning and postconditioning are not additive.^{46, 47}

Of equal importance is the question of whether the protection seen by postconditioning continues throughout the postischaemic period, or if the attenuation of ischaemic damage is only temporary. Recent work has shown that the neuroprotective effect remains present for at least 1–2 months after ischaemia, as seen by lesion size and behavioral score.^{44, 48}

One of the few available clinical therapies for stroke is the use of tissue plasminogen activator (t-PA), which is used as an attempt at reperfusion in applicable candidates. However, a negative side effect of t-PA is its possible neurotoxic properties and the increased risk of haemorrhagic transformation of the injured brain tissue in the post-stroke epoch, which obviously can worsen the brain injury.⁴⁹ Recent *in vivo* work found that delayed postconditioning inhibits t-PA worsening of infarction. It also showed that delayed postconditioning decreases oedema formation and blood-brain barrier leakage, while improving metabolism.⁴⁴

In addition to the traditional models of postconditioning by applying brief episodes of ischaemia, anaesthetic application in the postischaemic time period has been shown to be effective. Lee et al. demonstrated that postconditioning using isoflurane could be protective.⁵⁰ First, rats were anesthetized with 60% O₂ – 40% N₂ containing 2% isoflurane. The middle cerebral artery (MCA) was occluded by advancing a 3–0 monofilament nylon suture with a rounded tip to the right internal carotid artery. Isoflurane anaesthesia was then stopped. At 90 minutes, the rats were reanaesthetized with isoflurane and the nylon suture was removed. The treatment group rats were then maintained on 2% isoflurane for 60 mins, while the control group was allowed to awaken immediately. At 24 hours both groups were evaluated for infarct volume, neurologic deficit scores and motor coordination. The isoflurane postconditioning group showed reduced brain infarction and improved neurologic deficit scores and motor coordination when compared to the control group. *In vitro* studies also revealed evidence of isoflurane postconditioning, and also demonstrated that isoflurane concentrations and timing of application after reperfusion significantly affected cell death.⁵⁰

Table 3

Most recent ischaemic/anesthetic postconditioning studies. MCAo, middle cerebral artery occlusion; CCAo, common carotid artery occlusion; OGD- oxygen-glucose deprivation; DHPG, 3, 5-dihydroxyphenylglycine; N/A, not applicable; bilat, bilateral; global, global ischaemia.

Model (species + ischaemia)	Postcon Onset	Occlusion time	Reperfusion time	Cycles	Outcomes measurement	Behavioral test	Mechanisms evaluated	Reference
Rat, MCAo × 120 min	At reperfusion	Propofol 10, 20, or 35 mg/kg/h × 30 min	N/A	1	10 or 20 mg/kg/h doses significantly decreased infarct volume and attenuated neuron apoptosis @ 22 h reperfusion	Decreased neurological deficit scores in treatment group at 22 h after reperfusion.	Protection by propofol may partly be due to activation of PI3 K/Akt pathway leading to decreased apoptosis.	51
Mouse, MCAo × 60 min	1 or 5 days	Hypoxia × 1 h	NA	3x/wk until post -ischaemic day 43	No significant effect on brain lesion for any treatment on T2-weighted MRI @ 48 h or 43 days. Late hypoxic postconditioning brains showed significant decrease in thalamus atrophy @ 43 days.	No change in blinded deficit grading or adhesive removal task compared to sham.	Hypoxia-inducible factor-1 α and its target genes adrenomedullin and erythropoietin are involved in <i>in vitro</i> hypoxic postconditioning-induced neuroprotection.	58
Rat, Focal ischemia of MCAo × 100 min	Multiple groups	Multiple groups	Multiple groups	Multiple groups	Decreased infarct volume @ 48 h.	none	No additive protection with pre + postconditioning. Postconditioning roughly equivalent to preconditioning. Prosurvival protein kinases (extracellular signal-related kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and Akt) show prolonged phosphorylation in the cortex of postconditioned rats, but only Akt contributes to the protection of postconditioning.	57
Rat, MCAo × 60 min.	30 s.	30 s.	30 s.	6	Decreased infarct volume @ 24, 72 hrs.	Improved at 24 & 72 hrs by blinded observer.	Bcl-2 and heat-shock protein 70 expression upregulated; while cytochrome c release to the cytosol, Bax translocation to mitochondria, and caspase-3 activity were downregulated.	54

Rat, permanent MCAo + 30 min of CCAo	Multiple groups (10 s–3 min.)	Multiple groups (10–30 s)	10 s	3 or 10	Decreased infarct volume @ 48 hrs in some groups.	none	Postconditioning's protection was comparable to that of rapid preconditioning, but not as robust as that of delayed preconditioning.	45
Rat, permanent MCAo + 30 min CCAo	30 s	30 s	10 s	3	Decreased infarct volume @ 30 days	Improved behavioural function @ 30 days	Akt activity, increases in ϵ PKC activity, and reductions in MAPK and δ PKC activity contribute to postconditioning's protection	48
Rat, 30 min. bilat. CCAo + permanent MCAo.	Multiple groups 10 s–6 h	Multiple groups 10 s–15 min.	Multiple groups 10 s–15 min.	3–10	Infarct size and brain edema measurement at 48 h; BBB integrity at 6, 24, 48 h;	Improved behavioural deficits on multiple days.	Delayed postconditioning inhibits t-PA worsening of infarction. Delayed postconditioning improved metabolism and reduced edema and blood brain barrier leakage.	44
Rat, In Vitro slice culture, 30 min OGD	5 min.	3 min OGD or 10 μ M DHPG \times 30 min.	N/A	1	Propidium iodide uptake measurement at 24 h.	N/A	No additive protection with pre + post-conditioning. Activation of mGlu1/mGlu5-PI3 K-Akt signaling pathway plays a crucial role in the mechanisms of postconditioning evoked by DHPG.	46
Rat, global 4-vessel occlusion \times 10 min.	Multiple groups 15–60 s.	Multiple groups 15–30 s.	Multiple groups 15–60 s.	3	Neuron counting of hippocampus and cortex at 7 day & 14 days.	Improved spatial learning and memory test xat 7 days.	First report of postconditioning protection in global ischaemia. Cytochrome c disruption & improvement in cerebral blood flow are protective mechanisms.	51

An exciting recent study exhibited a protective effect of propofol in the postischaemic time period. Immediately following 120 minutes of MCA occlusion, rats were placed on propofol drips at 10, 20, or 35 mg/kg/hr for 30 minutes. When compared to control animals, those treated with doses of 10 or 20 mg/kg/h had significantly decreased infarct volume and better neurological scores at 22 hours after reperfusion.⁵¹

Mechanisms of postconditioning

Mechanistically, ischaemic postconditioning is still a largely unknown area. Unlike ischaemic preconditioning, in which many of the protective mechanisms occur independently from stroke, postconditioning cannot be studied separately from the potentially lethal ischaemic event.⁴³ It is, however, vital that the mechanisms of protection in ischaemic and anaesthetic postconditioning are characterized, as postconditioning has the potential to greatly impact the clinical approach to stroke treatment. For the purpose of this review, a brief summary of the recent discoveries in the mechanism of postconditioning will be discussed.

It was originally hypothesized that the major pathway involved in the protective mechanism of ischaemic postconditioning was attenuation of the deleterious effects of reperfusion injury, among others, the overproduction of reactive oxygen species (ROS) or free radicals in the ischaemic brain.⁵² This overproduction of ROS leads to apoptosis and causes an inflammatory response, which exacerbates ischaemic injury.

Recently it has been shown that rapid postconditioning improves cerebral blood flow (CBF) at 30 minutes after reperfusion in both focal and global ischemia.^{45, 53} Rapid postconditioning also has been shown to decrease the level of malondialdehyde (MDA) and increase superoxide dismutase (SOD) activity, suggesting attenuation of lipid peroxidation via decreased levels of superoxide anions in the brain.⁵⁴ Furthermore, rapid postconditioning has recently been shown to reduce cytochrome c release from the mitochondria to the cytosol, a cascade that is central to induction of apoptosis.⁵³ Thus, postconditioning may reduce ischaemic injury by blocking neuronal apoptosis.

Inhibition of inflammation after ischaemic stroke is another protective pathway seen after ischaemic postconditioning. Rapid postconditioning likely inhibits leukocyte accumulation, attenuates the expression of IL-1 β and TNF- α mRNA, and the ICAM-1 protein expression in the ischaemic cortex 24 hours after ischaemia.⁵⁵

Many recent studies examine the role of protein kinases, especially a family of kinases called 'reperfusion injury salvage kinases'.^{48,56,57} Within this family, Akt contributes to survival after stroke.⁵⁶ During harmful ischaemia, Akt is transiently activated via phosphorylation. This activation is extended after postconditioning stimulation.⁵⁷ Additionally, rapid postconditioning increases Akt phosphorylation and activity, and inhibiting Akt phosphorylation partially eliminates post-conditioning's protection.^{54,57} Propofol postconditioning also appears to decrease apoptosis and is associated with an increase in Akt phosphorylation.⁵¹

Like preconditioning, postconditioning appears to have several simultaneous mechanisms working in parallel, including inhibition of inflammation, inhibition of apoptosis, and increased Akt phosphorylation. More research is needed on this subject to further our understanding of its protective effects. For more detailed descriptions of the multiple pathways involved in ischaemic postconditioning protection, refer to the excellent reviews by Pignataro and Zhao.^{56,57}

Gender and age effects on pre and postconditioning

The issue of gender differences in regards to perioperative stroke risk and overall stroke risk is complicated. Many studies show that women may have a greater perioperative stroke risk than men,^{59,60,61} and gender is known to alter experimental ischaemic brain outcomes.⁶² While some of these experimental differences are attributed to hormonal differences, this does not account for all findings. The majority of studies examining cerebral preconditioning and postconditioning use primarily young male models or, in cell culture, unsexed prenatal pups. However, studies exist showing that neuronal preconditioning response differs between genders.

In 2007 Kitano et al. observed that isoflurane preconditioning in ischaemic mouse brain is only protective in young and middle-aged males. In fact, isoflurane preconditioning increased infarct size of young female mice and had no effect on middle-aged female mice. Mechanistically, this study showed that the sex-specific responses to isoflurane preconditioning are mediated through differences in Akt phosphorylation and activation and that male protection is Akt1-dependent, as evidenced by lack of protection in male Akt1 knockout mice.⁶³

More recently, Limatola, et al. showed that Xenon preconditioning in mice undergoing transient MCA occlusion was protective in both males and females with improved functional outcome on focal deficit scales and reduced infarct volumes.³⁵ There was no significant difference between male and female cohorts. Hypoxia-inducible factor (HIF)-1 α and phospho-Akt were quantitatively elevated in both sexes, as seen by western blotting. HIF-1 α induces multiple pro-survival responses, such as renal transcription of erythropoietin and promotion of angiogenesis and glucose uptake. This study interestingly shows that the mechanism of protection is likely not the same in both isoflurane preconditioning and xenon preconditioning. The authors suggest that the lack of gender specificity could be an important clinical consideration regarding the use of xenon in surgical patients at high risk for perioperative stroke.³⁵

While there are very few studies addressing cerebral pre- or postconditioning that examines gender differences to date, the differences observed molecularly and clinically show the need for age and gender stratification when examining pre- and postconditioning. More studies are needed using models that are outside of the traditional young male animal model.

Practice points

- Perioperative stroke can be as high as 10% or more in patients undergoing some cardiovascular procedures or with multiple risk factors.
- There are very few pharmacological or other therapies for stroke.
- *Pre-* and *post*conditioning may be valuable tools for preventing and/or treating stroke in the future.

Research agenda

- The potential for acute and delayed benefits of anesthetic *pre*conditioning creates opportunities for therapeutic interventions in patients at high risk for stroke, especially those who are to undergo a procedure with high risk of perioperative stroke; the time course for such interventions and the necessary exposure warrants further study.
- The clinical efficacy of all types of *pre-* and *post*conditioning should be evaluated in various genders and age groups prior to being used clinically in a widespread fashion.
- The clinical value of remote ischaemic preconditioning for the protection of the ischaemic brain needs to be more thoroughly assessed.
- Is there a role for remote ischaemic *post*conditioning?

Summary

Stroke is a very serious occurrence, with high morbidity and mortality. Perioperatively, stroke is a complication that occurs frequently and is difficult to treat, due to limited options. Preconditioning and postconditioning are new, potential treatments for neuroischaemia that are promising in animal models that exploit the cells innate protective mechanisms to induce neuroprotection. Perhaps the

most promising of all, remote ischaemic preconditioning, is currently being investigated in clinical trials. Further research is needed, in both translational and clinical trials, in the areas of preconditioning and postconditioning paying special attention to the timecourse of the treatment and when protection is at its peak. In addition, scientists must pay special attention to characteristics of the animal model, which can confound data and results need to be interpreted accordingly.

Conflict of interest statement

None.

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