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Is There A Place For Cerebral Preconditioning In The Clinic?

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Abstract

Preconditioning (PC) describes a phenomenon whereby a sub-injury inducing stress can protect against a later injurious stress. Great strides have been made in identifying the mechanisms of PC-induced protection in animal models of brain injury. While these may help elucidate potential therapeutic targets, there are questions over the clinical utility of cerebral PC, primarily because of questions over the need to give the PC stimulus prior to the injury, narrow therapeutic windows and safety. The object of this review is to address the question of whether there may indeed be a clinical use for cerebral PC and to discuss the deficiencies in our knowledge of PC that may hamper such clinical translation.

Keywords

translational research; preconditioning; ischemic tolerance; cerebral ischemia

Introduction

Preconditioning (PC) is a phenomenon whereby a sub-injury inducing stress (such as a brief ischemic event; ischemic preconditioning; IPC) can cause protection against a subsequent injurious event (such as a stroke). Ischemic PC occurs in a variety of tissues (1-3), including brain (4-8). A wide range of PC stimuli have been described for brain, varying from transient ischemia, either in the tissue to be protected or in another tissue (so-called remote IPC), to pharmacological agents (5-8). Such PC stimuli can acutely induce a protected state within minutes (classical PC or rapid PC; (6-8)) or the protection may take hours to develop, reflecting a need for altered protein synthesis (delayed PC, second window of protection; (6-8)). In brain, many groups have shown delayed PC, but classical PC can also occur (6-9). Preconditioning stimuli have shown protection against brain injury in animal models that is comparable to the best protection found with pharmacological neuroprotectants delivered after the insult. Thus, for example, in the rat, IPC with a brief ischemic event typically reduces infarct volume after a later permanent or transient middle cerebral artery occlusion (MCAO) by ~40-50% (Table 1).

Understanding how PC exerts protective effects may help identify novel protective pathways suitable for therapeutic modification, but there has also been interest in direct clinical applications of PC. The effects of PC on different human tissues are under investigation by many groups around the world. Thus, there are a number of past and ongoing clinical trials,

primarily focused on cardiac surgery (see the NIH website ClinicalTrials.gov for current trials; (10)). In contrast, in brain, the direct clinical relevance of PC has generally been questioned (8) because of the requirement for PC (rather than post-conditioning; (11)) to occur before the brain injury/disease and the relatively narrow therapeutic window for PC stimuli (either in duration or strength of stimulus).

The purpose of this review is to examine whether there may actually be a potential clinical usefulness of PC in relation to the brain. It addresses the questions of: a) whether there is a clinical utility for PC, b) what are the clinical scenarios in which to test PC and c) what might be the best potential PC stimuli to test in humans? In particular, it will attempt to highlight areas where deficiencies in our knowledge may impede clinical translation. These questions will also be discussed in relation to two similar therapeutic approaches, short-term pretreatment (e.g. giving magnesium sulfate just prior to a surgery; (12)) and long-term prophylactic treatment (e.g. giving antihypertensive agents, anticoagulants or statins to patients at risk of stroke; (13,14)). PC stimuli are a subset of such treatment paradigms and may have advantages and disadvantages compared to other approaches. This review does not discuss the mechanisms underlying PC, unless it is relevant to therapeutic utility. Instead, readers are referred to many excellent recent reviews on that subject (6-9).

Is Preconditioning of Potential Use in the Clinic? Efficacy

There is much preclinical evidence demonstrating the efficacy of PC in reducing brain injury, particularly in relation to cerebral ischemia. There is also some evidence from a naturally occurring event, a transient ischemic attack, that PC may be effective in reducing brain injury in human (15-17) and there is evidence for PC being effective in protecting other tissues in humans, particularly heart (10). The aim of this section is to examine this evidence and highlight some potential deficiencies.

Ischemic stroke - Animal data

By far the largest body of evidence for the efficacy for PC in reducing brain injury is in cerebral ischemia. IPC has been shown to be effective by multiple groups, using different PC stimuli, in both models of global and focal cerebral ischemia and in multiple species (gerbil, rat, mouse, rabbit; Table 2; (4-9)). Brief periods of ischemia can act as both a classical PC paradigm (protection induced within minutes) and a delayed PC paradigm (protection induced within hours), although the latter tends to be more robust. The effects of IPC can be mimicked in vitro with neuronal, glial and endothelial cell cultures (e.g. (18-20)). A potential concern over prior IPC studies in terms of preclinical testing is whether the forms of transient ischemia used as PC stimuli mimics what may occur in the clinic in terms of the depth and area of ischemia.

There are safety concerns over inducing IPC by cerebrovascular occlusion in humans (see below). There has, therefore, been a search for other agents/paradigms that can induce PC. Compared to IPC, these agents/paradigms have generally been less extensively studied in terms of protection against a variety of ischemic insults, the number of species examined and replication between groups of investigators (Table 2). However, a number of these approaches do have potential advantages for clinical development.

A wide range of pharmacological agents act as PC stimuli (8). These include (but are not limited to) inhibitors of metabolism, volatile anesthetics, K_{ATP} channel activators, inflammatory mediators and some naturally occurring dietary compounds. As with IPC, many of these agents have shown protection against permanent and transient ischemia and protection of cultured neural cells as well as in vivo (e.g. (21-24)).

A number of non-pharmacological paradigms can also induce cerebral PC. Thus, repeated exposure to hyperbaric oxygen (but not a single exposure) protects against later ischemic brain damage (25). Similarly, normobaric hyperoxia can also act as a PC agent, reducing later ischemic brain damage in rat (26). Both hypo- and hyperthermia have been reported to induce PC, reducing ischemic brain damage (27,28), as have spreading depression (29,30), acupuncture (31,32) and exercise (33). With these paradigms, most studies have been in rodents with relatively few studies in larger animals (34).

Recently, episodes of ischemia in an organ have been shown to cause PC in other organs, so called remote IPC (35-37). Thus, a prior episode of hindlimb ischemia protects the brain from later transient MCAO (38). This has potentially important clinical implications but it also may impact preclinical studies where often the femoral vasculature is cannulated for blood sampling and drug delivery.

Although there is much evidence on the efficacy of PC, particularly against ischemic stroke, there are deficiencies in the pre-clinical data. Thus, for example, there are concerns over how these results will translate to patients with chronic disease states. It is possible that disease states/co-morbid conditions may prevent the upregulation of defense mechanisms by the PC stimulus. He et al. found that IPC caused less protection against global ischemia in aged compared to young rats (39), Purcell et al. (40) also found that hypertension blunts the protective effects of IPC and Wang et al. found that the microvascular protective effects of IPC (in cremaster muscle) were abolished in the presence of diabetes (41). Alternately, it is possible that there may already be an upregulation in defense mechanisms in disease states so that a further PC stimulus is unable to cause further protection. Thus, Kim et al. found that chronic hypoperfusion can act as a PC stimulus protecting against a later stroke (42) and patients with prior transient ischemic attacks (TIAs) may potentially already be in a preconditioned state (15-17). Similarly, Petcu et al. (43) found that mild systemic inflammation in a rat periodontitis model appears to act as a PC stimulus, protecting against a later transient MCAO.

These relatively few studies indicate that more information on the effects of disease states/co-morbid conditions is needed to inform potential clinical trials (e.g. should diabetic patients be excluded from PC trials). Examination of the effects of aging, hypertension, diabetes, atherosclerosis, inflammation and smoking on PC need to be performed. The effect of gender has also been generally neglected, even though there has been a report that isoflurane-induced PC may have less effect in female rats (44) and that the effects of 3-nitropropionic acid-induced PC varies during the estrus cycle (45).

Cerebral ischemia - Human data

In contrast to the wealth of pre-clinical data, very little is known about the effects of PC on cerebral ischemia in humans. Chan et al. (46) examined whether a prior transient (2 min) artery occlusion 30 min prior to clipping for cerebral aneurysm would affect brain tissue pO₂ and pH during clipping. They found that changes in those two parameters were slower in the preconditioned group suggesting protection. Another approach has been to examine the effect of naturally occurring transient ischemic attacks (TIAs) on later strokes. Two retrospective studies (15,16) and a prospective clinical study (17) have suggested that patients with a prior TIA have reduced ischemic injury compared to patients without a prior TIA. However, two prospective trials found no such effect of a prior TIA (47,48). There are ongoing trials aimed at examining the effects of PC on the brain (see below), but, as yet, there is no clinical data that strongly support the use of PC for brain protection.

Ischemic injury in other tissues - Human data

Compared to brain, the clinical effects of preconditioning on heart, liver and kidney have been more extensively studied. As early as 1993, Yellon et al. (49) found that prior clamping of the aorta to induce IPC could preserve myocardial ATP levels during coronary artery bypass graft (CABG) surgery. Since then, there have been multiple small clinical trials of IPC for cardiac surgery and a meta-analysis by Walsh et al. (50) found evidence that IPC was cardioprotective. However, to alter clinical practice, there is a need for large prospective clinical trials with short- and long-term clinical endpoints comparing outcome to best current clinical practice (10).

Ischemic PC for heart is an invasive procedure (e.g. aortic clamping) and comes with associated risks. There has, therefore, been interest in inducing PC with pharmacological agents. Adenosine, bradykinin, opioids and volatile anesthetics have been examined in clinical studies (10). Anesthetics have been extensively studied because of their current clinical use and ease of administration and meta-analyses of those studies in CABG surgery found evidence of cardioprotection (51,52). Remote IPC has also garnered much interest because of ease of administration (cuff occlusion of upper arm or leg). Takagi et al. (53) published a very limited meta-analysis of remote IPC in cardiovascular surgery and found some evidence of protection.

Many PC trials are ongoing. For example, <http://clinicaltrials.gov> from the NIH currently (September 2009) lists 49 PC trials. Of those 25 are cardiac related (although two of those have cognitive endpoints). It is interesting that 18 of the studies employ remote IPC and eight involve sevoflurane, a volatile anesthetic. This reflects the importance of safety/ease of translation to the clinic (see below) in determining which agents undergo clinical trial.

Non-ischemic brain injury - Animal data

There is some evidence that PC can reduce brain injury in conditions other than cerebral ischemia. For example, brief epileptic seizures in animals may protect against brain injury from later seizures and, interestingly, this protection is also found with IPC (54), although the mechanisms of protection appear to differ (55). This is an example of so-called cross-tolerance, wherein a PC stimulus can protect against a different type of injury (6,56). There have also been studies showing that both IPC and hyperthermic PC can reduce traumatic brain injury (57,58). To our knowledge, the effects of PC on multiple sclerosis and meningitis has not been examined and this may be worth pursuing since PC has a marked effect on neuroinflammation in ischemic stroke (7,8).

Is Preconditioning of Potential Use in the Clinic? Timing

Three major concerns over translating PC to the clinic for brain-related disorders are the fact that the PC stimulus needs to be given prior to the injury, there is a relatively narrow therapeutic window (duration and strength of stimulus) and there are questions over safety. In this section, we examine the first of these issues in relation to the time frame of PC.

Short term PC effects – classical/rapid PC (minutes – hours)

For classical PC, the upregulation in endogenous defense mechanisms and down regulation of potentially harmful pathways occurs within minutes, generally involves protein modification and wanes after 2-3 hours. While extensively studied in heart (59), classical PC has also occurred in brain (6-9), although generally it has been a less robust effect than delayed PC. Because of the rapid onset, this form of PC is best suited to conditions when the onset of a potential injury is known, i.e. a surgery carrying risk of injury. Thus, in heart, there are and have been clinical trials examining the effects of IPC (aortic clamping) and remote PC in CABG surgery (10).

Long-term effects – delayed PC (days)

Delayed PC (second window of protection) involves changes in protein transcription. As changes in protein levels take hours to occur, this form of PC does not induce protection for several hours and protection generally peaks 1-3 days after the stimulus, waning by about 7 days (7,8). Ischemic PC induces very robust delayed protection (7,8). The time frame for this type of PC raises questions over clinical utility. It might be used prior to surgery, but physicians would need access to patients preferably the day before any surgery to administer the stimulus and, dependent upon the risks associated with the stimulus, might need to monitor patients continually until surgery. There are times when patients are at greater risk of neurological events (e.g. patients are at greater risk of stroke within one week after a first stroke (60-65)), but providing protection for one week by PC may be insufficient.

Ultra long-term PC (weeks/years)

As noted above, multiple studies have examined the duration of protection after a PC stimulus and shown that protection peaks one to three days after the stimulus and wanes by about a week (7,8). What has received little or no attention is the question of whether a preconditioned state in the brain can be maintained long-term. This might be possible by giving periodic PC stimuli or by giving an escalating dose of a PC stimulus followed by a maintenance dose. There is some evidence to suggest that this may be possible. Thus, while the protective effects of IPC (a single or repetitive period of transient ischemia) against a subsequent ischemic event wane after about a week, protective effects of chronic hypoperfusion were found after 4 weeks (42). While classical and delayed PC are thought to primarily affect brain injury by protein modification (e.g. phosphorylation) or transcription (6-8), it is possible that ultra long-term PC might also affect brain injury by structural changes within the brain. Thus, exposure to chronic hypoperfusion and hypoxia both can increase cerebral vascular density (42,66).

Whether 'ultra-long-term PC' might be induced by elements in certain diets may be worth examining. Acute PC has been shown with high concentrations of resveratrol, a component of red wine (67), and isothiocyanates, components of cruciferous vegetables, can upregulate brain anti-oxidant defense mechanisms in (68). Whether chronic treatment with such diets might induce a 'pre-conditioned' state and impact later brain injury is a potentially interesting field of study.

Pretreatment vs. preconditioning

Preconditioning is a form of pretreatment. However, whilst most pretreatment therapies rely on the continued presence of the drug during the disease process and pretreatment aids in reducing injury by enabling the drug to be present at the onset of injury, a PC stimulus induces endogenous protective mechanisms and the stimulus can then be removed. Thus, for example, giving a direct free radical scavenger before a stroke would be a pretreatment, but when pretreatment with a compound causes tissues to produce free radical scavengers, we are defining that compound as a PC agent.

Currently, there are many drugs that are given as pretreatments. Thus, a large range of drugs are given to patients at risk of a stroke which either reduce that risk or limit the damage from a stroke should it occur. Examples are: antihypertensive drugs, anticoagulants and, now, statins (13,14). The efficacy of such prophylactic approaches show that there are populations that can benefit from pretreatment and the practicality of giving a pretreatment. Whether such a population exists for PC would depend on the balance of efficacy/safety issues for PC, the time frame of protection and how these compare to current medications.

Drugs have also been given shortly prior to surgery to reduce the risk/severity of potential neurological complications. Thus, magnesium sulfate has shown efficacy when infused from

just prior to carotid endarterectomy (12) and erythropoietin has recently shown a trend towards reducing neurocognitive dysfunction when given the day before and for two days after CABG (69). These results suggest that there are surgeries that can benefit from a pretreatment strategy and that pre-treatment is feasible. Such patients might also benefit from PC (indeed erythropoietin is a suggested PC mediator, (70,71)), but that depends on the balance of efficacy/risk and how that compares to other medications.

While short-duration exposure to a PC agent can induce a protective response, this does not necessarily indicate that the continued presence (administration) of the agent after the onset of injury is without effect. Thus, it may be possible for a PC stimulus to also act as a post-treatment agent. A case in point appears to be deferoxamine. This iron chelator can act as a PC agent (72-74) but, in animal models, it can also reduce brain injury when given after an intracerebral hemorrhage (75,76) or an ischemic stroke (77). Having pre- and post-ictus effects could be a major advantage in a therapeutic.

Is Preconditioning of Potential Use in the Clinic? Safety

Safety is a major concern in translating PC to the clinic (8). Differences in safety profile will likely be (and have been) a major determinant of which PC stimuli proceed to clinic. Some safety issues with specific classes of PC agent are discussed in a later section on the selection of PC agents, but some concerns cover multiple (if not all) PC stimuli and these are discussed here.

The dose range that causes PC is often narrow. For example, for pretreatment with 3-nitropropionic acid (NPA), a suicide inhibitor of succinate dehydrogenase, Hoshi et al. found that 5 mg/kg induced no protective effect against MCAO, 10 mg/kg produced a protective effect, 15 mg/kg produced protection but killed 20% of rats and 20 mg/kg produced no protection and killed 39% of rats (78). While the therapeutic window may differ for different PC agents, a similarly narrow window (in terms of duration of ischemia) is found for IPC. Translating a narrow range to humans is difficult. Particular concerns are that the harmful effects may occur at lower doses in humans or that the safety profile for PC agents may alter with disease state. It is noticeable that Chan et al. (46), examining the effects of prior vascular clipping (IPC) on brain parameters during brain aneurysm surgery, chose to only clip for 2 minutes to avoid potential injury. This raises the question, though, of whether safety concerns may narrow the potential efficacy of any PC effect.

Whether the safety profile for PC agents alter with disease state has received little attention. It may be of particular importance for PC agents that in some way mimic elements of the disease state. For example, those that induce PC by suppressing metabolism under normal conditions might cause injury if given to a patient where metabolism is already suppressed (e.g. under conditions of hypoperfusion). However, the safety profile may be affected in other ways. For example, giving a K_{ATP} channel opener, which regulates insulin secretion in the pancreas, might be contra-indicated in a stroke patient with diabetes. Thus, in addition to the need for efficacy studies in the presence of co-morbid conditions (noted above), there is also great need for pre-clinical studies examining whether the safety window for PC is affected by aging, hypertension, inflammation, diabetes/hyperglycemia or smoking. Currently, there have been very few such studies. However, it is known that vascular occlusions can cause lesions in spontaneously hypertensive stroke prone rats (SHRSP) that cause little or no lesion in the parent normotensive Wistar Kyoto rat strain (79). This difference in susceptibility is because of poor collateral circulation in the SHRSP rats (79).

Another safety concern relates to the observation that a PC may induce neurological symptoms without inflicting detectable tissue damage. In the case of stroke-related studies, the lack of PC-induced injury is normally defined as a lack of a lesion (in vivo) or cell death (in vitro).

However, Hua et al. (80) identified some behavioral deficits after a transient MCAO that has been used as a PC stimulus. The extent and duration of subtle ‘injuries’ that may be induced by PC stimuli in humans will deserve close attention and stresses the importance of using cognitive and functional measures in addition to imaging outputs to assess safety and efficacy.

Which Clinical Scenarios Might Preconditioning Be Used In?

Unless a mechanism can be found to replicate the preconditioned state long-term, it appears that any clinical utility for brain PC will focus on conditions where the timing of potential injury is known. It is envisioned that a preconditioning stimulus would be delivered so that the maximum protection would coincide with the time of highest probability of brain injury. Several potential clinical situations could be considered.

Neurosurgery

All neurosurgical procedures pose a risk for neuronal injury. Vascular neurosurgery often requires temporary and deliberate cessation of regional cerebral blood flow; thus, PC stimuli that work against ischemic injury may be useful in attenuating operative stroke. Although newer surgical techniques have become less invasive and more regionally selective, most surgery involves physical manipulation of normal brain tissue. The mechanism of injury in these operations is likely to be a combination of mechanical injury, inflammatory pathway activation, ischemia, and hemorrhage. Unlike other forms of neuroprotection that selectively target one pathway of injury, most PC stimuli appear to activate common upstream pathways that result in activation of disparate pathways (6-8). Thus, PC may offer a uniquely effective approach to protect tissue that could be susceptible to multiple mechanisms of injury during brain surgery.

Temporary cerebrovascular occlusion is used by many neurosurgeons during surgical manipulations of intracranial aneurysms to ‘soften’ the aneurysm and lower intraoperative rupture (81). The benefit/risk of such occlusions is still debated (81,82). Some neurosurgeons use multiple short periods of temporary occlusion, with barbiturate-induced burst suppression for longer occlusions (81). Given the PC effect of multiple brief periods of ischemia, this type of surgical procedure may be a good avenue for examining the clinical effects of PC on brain.

Vascular surgery

The brain is particularly vulnerable in cardiovascular operations and interventional procedures with arterial catheters. CABG poses a threat of neurological insult due to the bypass procedures and emboli production. The two most common major neurological consequences are ischemic stroke and cognitive dysfunction, both of which are thought to be caused by emboli to the brain. Significant cognitive decline has been reported to occur in ~20-30% of patients at 2-6 months and ~40% of patients by 5 years (83,84), although these might be overestimates (85). PC directed against ischemic damage induced prior to CABG could, in principle, protect against both stroke and cognitive dysfunction.

Another major surgery posing significant risk of stroke is carotid endarterectomy (CEA). In CEA, the carotid artery is interrupted while atheromatous material is cleared and the vessel is repaired. Neurological deficits and stroke can be caused by hemodynamic interruption of flow or by emboli released during or shortly after the operation. It is estimated that about 25% of CEA patients have an early neurocognitive decline after CEA (86,87). Thus, in this relatively short surgery, the time of neurological vulnerability is fairly well demarcated, making CEA a potential target for PC treatments.

Preconditioning could also benefit patients undergoing other cardiovascular procedures, such as carotid artery stenting, which are short procedures that pose relatively short-term risks for

embolic stroke. The number of patients undergoing CABG, CEA, carotid stenting and cerebrovascular surgery (e.g. clipping and coiling of cerebral aneurysms) is high and the rate of neurological events associated with these procedures is significant enough that the risk-benefit of PC could favor general use.

Vasospasm

In patients with subarachnoid hemorrhage (SAH), one of the most feared late complications is vasospasm resulting in ischemic deficits, which may progress to infarction. This condition, generally has an onset of 3-5 days after hemorrhage, with peak vessel narrowing at 5-14 days with resolution by 2-4 weeks (88). A time window of vulnerability to stroke can, therefore, be defined with reasonable certainty. PC triggering anti-ischemic pathways in selected patients at risk of vasospasm might be useful in the prevention of morbidity in the population of SAH patients most vulnerable to further neurological deterioration.

It should be noted that this use of PC would differ greatly from established PC models in that the stimulus would be given after hemorrhage (an injury) but before vasospasm (another injury). How the PC stimulus would interact with the initial hemorrhagic injury is a major question. Some 'sub-injury' inducing PC stimuli may exacerbate brain damage when given to an injured brain. For example, intracerebral injection of a low dose of thrombin which elicits a PC response in normal brain exacerbates brain injury when given after a MCAO (89). There has been a trial of erythropoietin alpha (a potential PC mediator (70,71)) in patients with aneurismal SAH that required clipping (contact E.M. Campoersi, ClinicalTrials.gov Identifier NCT00626574). Patients received erythropoietin one day prior to the clipping and for an additional two days. This trial was terminated as it showed increased mortality in the stroke patients.

Non-stroke related injury

Many injury mechanisms targeted by PC have a role in a variety of brain diseases/injuries and those conditions may be amenable to PC therapy. For example, epilepsy affects ~50 million people worldwide and the recurrent nature of epileptic seizures may make it a plausible target for PC. As noted above, there is preclinical data suggesting that PC can reduce brain injury from seizures (54). PC might also offer a unique prophylactic approach in people at risk of CNS trauma (e.g. battlefield soldiers or athletes in heavy contact sports). In animal models, PC can protect against traumatic brain injury (57,58).

Preconditioning might also have a role in CNS infections and inflammatory conditions. For example, meningitis-induced injury involves inflammatory activation, bacterial toxins and ischemia (90-93). As discussed above, studies on stroke have shown that PC can reduce ischemic brain damage and inflammation (33,94). PC might, therefore, be considered during outbreaks of meningitis. Multiple sclerosis is the most common inflammatory disorder of the brain in the Western world (95). Multiple attacks over time will frequently cause permanent demyelinating damage and functional disability (96). A PC stimulus directed against inflammatory neurological damage could be useful in preventing the integration of damage from MS attacks if the timing can be predicted. Although, for the most part, it is difficult to predict when a relapse of MS will occur, many stressors such as infection (97), are known to trigger an attack. Thus, there could be a role for preventative PC treatment for MS patients who experience a febrile illness.

Limitations to PC in clinical situations

Although preclinical work has established very dramatic efficacy of some PC against brain injury, there are major hurdles to consider in relation to PC trials in any of the above-mentioned situations. Clearly, the risk-benefit ratio must strongly favor treatment. The risks of PC include

the potentially deleterious nature of most PC; for example, inducing hypoxia in a patient who already has vulnerability to ischemic disease could itself result in harm to the patient. Clearly, IPC in a patient with SAH and a preexisting amount of ischemic injury is risky, regardless of the potential benefit in preventing vasospastic injury. Many of the surgical conditions discussed above have increasingly small complication rates due to improved surgical techniques and patient selection. For example, the risk of stroke and death from all causes at 30 days after CEA is commonly less than 3% in good medical centers, but in some centers the risk of intraoperative stroke, which is the main target of PC, is even lower, falling below 1% (98, 99). Thus, the risk of PC must be very low to justify a procedure which will benefit at most 1% of the target population. Another potential limitation that must be overcome is the fairly broad time window of vulnerability to neurological injury. While some targets have strictly defined peaks of neurological injury (e.g. carotid surgery), some disorders have windows of several hours (e.g. bypass surgery) to several days (vasospasm, inflammatory diseases) to several years (neurodegenerative conditions). Finally, as we have stated earlier, the most effective PC stimuli for each disease entity is not known.

To overcome the many hurdles to establishing clinical utility of PC, several avenues of investigation may be instructive. Establishing biomarkers of PC efficacy would be a major advance. The ability to follow a blood marker to establish the window of PC effect would strongly improve the confidence that PC is effective and guide more definitive treatment. Uncovering the basic mechanisms of PC action and matching these to disease mechanisms would allow tailoring of therapy and maximize potential clinical success. Discovering adjunctive agents that attenuate risks involved with PC stimuli would also aid in establishing therapeutic efficacy.

Which Preconditioning Agents Might Be Used?

A wide range of PC agents have been described (6-8). If any of these agents are to be translated to the clinic, the choice of which agent(s) should depend largely on efficacy, safety, ease of translation to the clinic and cost.

Efficacy

A major question is whether there may be inherent differences between different PC agents/paradigms. It is well known that IPC activates a wide range of different protective mechanisms and may inhibit potentially harmful pathways (6-8). There are few studies that have directly compared the degree of protection between different PC agents/paradigms. Pera et al. (100) found that IPC caused a greater reduction in infarct volume (~75%) after transient MCAO than PC with 3-NPA (~51%), but Puisieux et al. (101) found a similar degree of protection with IPC and PC with systemic lipopolysaccharide (LPS; ~31-36%). Table 1 compares the degree of protection (reduced infarct volume) with PC in rat models of permanent and transient MCAO. The average reduction in infarct volume with IPC was 41+/-5% for permanent MCAO, and 51+/-4% for transient MCAO. The average reduction after PC with agents/paradigms other than ischemia was 39+/-6% for permanent MCAO and 45+/-4% for transient MCAO. Thus, in general, it appears that these other agents can mimic almost fully the protective effects of IPC. Certainly, there may be PC agents that partially mimic the effects of IPC, particularly if they act on one of the pathways activated by IPC. An example may be ceramide, a potential downstream mediator involved in LPS-induced PC. Systemic LPS administration causes a marked increase in plasma and brain ceramide levels (102) and, as with LPS, PC with exogenous C8 ceramide causes protection from ischemic brain damage (103). However, the extent of protection (~18% reduction in MCAO-induced infarction) is only half that found with LPS-induced PC (103).

To aid in the successful translation of potential neuroprotectants to the clinic for treatment of ischemic stroke a series of STAIR criteria have been proposed (104). These criteria include determination of the dose response (minimum effective and maximum tolerated dose) and therapeutic time window (for PC this would be the maximum and minimum time the PC stimulus can be given before injury and would depend upon whether the stimulus induces classical or delayed PC or both), use of both histological and behavioral endpoints, replication in more than one laboratory, the use of multiple species, examination of both males and females, testing in the presence of co-morbidities such as hypertension, diabetes and hypercholesterolemia, and examination of whether age affects the protective response. In addition, the studies should meet good scientific standards (e.g. randomization, blinding of observers, appropriate power and sample size criteria, preset inclusion and exclusion criteria and physiological monitoring). Table 2 examines the state of knowledge for some potential PC stimuli with respect to these criteria and cerebral ischemia. The extent to which PC stimuli have met the STAIR criteria varies greatly. While there are reasons to be excited about remote PC (38) and volatile anesthetics (105) as PC agents, the pre-clinical data has many deficiencies that need to be addressed prior to clinical trials where neuroprotection is the main clinical goal (as opposed to being an addition to a heart study or where the use of the PC stimulus is clinically indicated for another reason, i.e. anesthesia).

By far the most studies on the efficacy of PC have used IPC. Those studies have shown that a wide variety of IPC paradigms can induce protection (e.g. transient bilateral common carotid artery occlusion, middle cerebral artery occlusion, cardiac arrest). However, as noted in the previous section, even for IPC, there are still major deficiencies in our knowledge of the effects of aging, gender and co-morbid conditions in IPC induced protection.

In the brain, the preponderance of studies on PC has been for protecting against ischemia. The effects of PC on other disease states have received much less attention. There have been few studies of the effects of PC on cerebral hemorrhage and this is an important caveat if a long-term PC agent/paradigm can be developed. Intracerebral injection of a low dose of thrombin (thrombin PC) protects against brain injury following MCAO and intracerebral hemorrhage in rats (106-108), but it is uncertain whether all PC paradigms would be beneficial in both types of stroke.

Safety profile and ease of clinical translation

Some PC agents have to be given directly into the brain to induce neuroprotection (thrombin, NMDA; (107,109)), probably precluding their clinical use as PC agents. It should be noted, however, some of the effects are receptor mediated and it may be possible to develop blood-brain barrier permeable receptor agonists.

Whilst IPC has extensive pre-clinical efficacy data, questions over how to translate to the clinic and safety issues are major potential hurdles to clinical use. In animal studies, multiple types (duration, frequency) of transient global or focal cerebral ischemia or cardiac arrest have been used as PC stimuli. While showing protection with multiple IPC paradigms helps to demonstrate the robustness of IPC response, many of the paradigms are difficult to directly translate to man. There is, therefore, a need to translate the extent of the 'ischemic insult' induced by the PC stimulus in an animal to that caused by a transient occlusion of a blood vessel in humans in terms of the duration required to elicit a PC response and in relation to the duration that will cause permanent tissue damage. As this will vary between blood vessels (depending upon collateral circulation) and the pre-existing physiological state of the patient, there is a need for surrogate markers to assess the effect of the PC stimulus; e.g. effects on EEG or blood flow.

As noted above, the only clinical study of IPC involving a cerebral artery used a 2 minute artery transient occlusion as the IPC stimulus (46). It is unlikely that the 2 minute occlusion was sufficient to cause tissue damage but there is no information on whether this was sufficient to elicit a full PC response. Intermittent cerebrovascular clipping is used by a number of neurosurgeons for intracranial aneurysm surgery (81). Although the procedure is not done expressly to induce PC, data from such patients may help provide evidence on the safety and efficacy of PC. Data on the safety profile of temporary cerebrovascular occlusions differ. Samson et al. found that patients could tolerate vascular occlusions of less than 14 minutes, but tolerance varied with age and neurological condition (110). Lavine et al. found that all patients who had a transient MCAO for at least 11 minutes had an infarction, whereas none with an occlusion of less than 5 minutes infarcted (111). The safety of the transient occlusion was increased if the patients received an intravenous anesthesia (111). In contrast, Woertgen et al. found that patients undergoing a temporary vascular occlusion for aneurysm surgery were more likely to develop vasospasm (82). In addition, amongst the patients that had a temporary occlusion, the development of both vasospasm and ischemic lesions were associated with longer temporary occlusions (82). Thus, patients that developed vasospasm or an ischemic lesion had an average of 4 minutes of occlusion vs. 2 minutes in patients that did not. This suggests, that in patients with certain neurological conditions, very short transient occlusions can be harmful.

To avoid safety issues associated with inducing IPC by direct cerebrovascular occlusion, a number of groups have proposed using remote IPC (see below). Remote IPC is usually induced by repeat (usually 3 times) transient (5 min) inflation of an arm or leg cuff (37,53,112). The ease of the procedure along with safety is making this a PC stimulus of choice for clinical trials.

Hyperbaric oxygen (HBO) PC (usually 3–5 treatments) can reduce brain injury in animal models of cerebral ischemia and hemorrhage (25,113). HBO has been widely used in humans for a variety of conditions and the safety of this technique has been extensively studied aiding translation to the clinic. Thus, HBO at pressures at or above 4 ATA is associated with side effects and guidelines now recommend that maximum exposures should be 3 ATA (25). A small study (31 control and 33 HBO patients) examined the effect of HBO PC on CABG-associated neurological deficits (114). The HBO PC consisted of three sessions of HBO (2.4 ATA), 24, 12 and 4 hours prior to CABG. That study found reductions in neuropsychometric dysfunction and serum inflammatory markers in HBO PC patients compared to controls. Apart from providing preliminary evidence on HBO PC efficacy, this study shows the feasibility of using the technique in patients. It does require patient access for one day prior to surgery and many institutions do not have access to HBO chambers. There may also be concerns if an acute medical event occurs during HBO exposure, as normally there is a 5-15 minute decompression time.

A range of pharmacological agents induce PC in animals. They may act as a PC stimulus, modulate the signaling cascades involved in PC, enhance effectors that execute the PC response or mimic those effectors (7,8). Whilst it is generally easy to administer such agents, questions over safety and translating pre-clinical data are complex, varying by individual agent. A number of potential agents are already in clinical use for other indications and, therefore, have a known safety profile. For example, deferoxamine PC reduces ischemic damage in heart (72), retina (73) and brain (74) and that iron chelator is already clinically used to treat hemochromatosis. The safety profile of others is still uncertain. This may be of particular concern for agents that act as a 'sub-injury-inducing PC stimulus; e.g. agents that mimic ischemic insults by reducing metabolism.

While it is relatively easy to assess the toxicity of potential pharmacological agents in man, it is more complex to determine whether a sub-toxic dose of that agent is sufficient to induce PC,

particularly if there is a 'U-shaped' dose response. Whilst it might be feasible to examine the effects of more than one dose in phase II or phase III trials, this may be a major impediment to trial development (particularly if there are limited numbers of patients). Alternately, it may be possible to identify systemic markers of the 'preconditioned state'. This is particularly relevant to PC agents that have effects on multiple tissues, including blood cells and serum.

One class of pharmacological PC agents that may be easier to translate to the clinic are volatile anesthetics (105). These agents have a number of advantages: 1) current clinical use; 2) known safety profile; 3) they may already be indicated, as an anesthetic, for the procedure (e.g. as the anesthetic for a neurosurgical operation); 4) some operations are already performed either in awake or anesthetized patients providing potential comparison groups; 5) a surrogate marker, depth of anesthesia, helps to translate the preclinical animal data to human.

Past/current clinical CNS-related PC trials

As mentioned above, there has been one very small trial of IPC in the brain. Chan et al. (46) examined whether a prior transient (2 min) artery occlusion 30 min prior to clipping for cerebral aneurysm. Using brain tissue pO₂ and pH during clipping as an endpoint, they found that a potential beneficial effect of PC. In another small trial, Alex et al. (114) examined the effects of hyperbaric oxygen PC on neuropsychometric dysfunction after CABG and found some evidence of a beneficial effects. Whether the recent erythropoietin trial for CABG by Haljan et al. (69) represents a PC trial is uncertain (the drug was given before and after CABG), but it did also show a trend towards reduced neurocognitive dysfunction.

Currently there are a number of 'CNS-related' clinical trials of PC. Two trials are examining the effects of remote IPC on cognitive outcome after cardiac surgery, one based at the University of Schleswig-Holstein (contact P. Meybohm; ClinicalTrials.gov Identifier NCT00877305) and the other at the Asan Medical Center (contact I.-C. Choi; ClinicalTrials.gov Identifier NCT00953368). Another on the effects of remote IPC on heart and brain damage following carotid endarterectomy has finished enrolling (contact M. Gaunt; ISRCTN register # ISRCTN98544942). Two other trials of remote IPC are underway in Xijing Hospital, one examining the effects of PC on patients undergoing elective neurosurgical procedures (contact H. Dong, ClinicalTrials.gov Identifier NCT00866489), the other examining patients undergoing cervical decompression surgery, ClinicalTrials.gov Identifier NCT00778323). These clinical trials on remote IPC reflect the ease of the treatment and perceived safety. There is, however, very little published pre-clinical data on the effects of this form of PC on brain injury.

Conclusions

In animals, preconditioning is an effective mechanism of protecting the brain from injury. Evidence from heart (and some from brain) suggests that it may also be protective in humans. However, there are major hurdles to surmount before PC can be a viable treatment. In particular, the need for treating the patient before an injury (although this can be done in surgical patients) and questions of safety need to be addressed. In Table 3, we suggest some of the areas of research where further data would help to move PC towards the clinic. Current clinical trials of remote IPC could benefit from greater preclinical data. While examining the effects of PC on ischemia-induced neurological deficits during surgical procedures may be the first step in examining the potential benefit of PC, there may be wider vistas including other forms of brain injury/disease.

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Table 1
Rat: percent reductions in infarct volume after MCAO with different types of preconditioning

Agent	pMCAO	tMCAO	References
ischemic PC	41 +/- 5% (10)	51 +/- 4% (11)	*
chronic hypoperfusion		71%	(42)
3-nitropropionic acid	44 +/- 15% (3)	29 +/- 6% (5)	(100,115-118)
2-deoxyglucose		63%	(119)
hypothermia		40%	(27)
spreading depression	35%	44%	(29,30)
hyperoxia		64%	(26)
hyperthermia		71%	(28)
isoflurane	27 +/- 6% (2)	51%	(21,120,121)
halothane	33		(21)
sevoflurane		26%	(122)
thrombin	63%		(107)
diazoxide/ BMS 191095		30 +/- 2% (2)	(22,123)
C8 ceramide	17%		(103)
NMDA	56%		(109)
LPS		36%	(101)
acupuncture		61 +/- 5% (2)	(31,32)
exercise		79%	(33)

Preconditioning was performed before permanent (pMCAO) or transient middle cerebral artery occlusion with reperfusion (tMCAO; 1-2 hours occlusion). % reductions are for whole brain. Values are means +/- SE with the number of studies in parentheses, or single studies. We have attempted to avoid repeat studies by the same group.

* The following studies were used to calculate the average protection with ischemic PC in pMCAO models (40,124-131) and tMCAO models (71, 94,100,101,120,132-137).

Table 2
Efficacy of multiple types of preconditioning against cerebral ischemia in adults as assessed by potential criteria for progressing to clinical trial

Criteria	Type of Preconditioning				
	Ischemic PC	Remote PC	HBO	Volatile anesthetics *	3-NPA
Classical PC	Yes [#]	Yes (38)		Yes [#]	
Delayed PC	Yes [#]	Yes (38)	Yes [#]	Yes [#]	Yes [#]
Permanent ischemia	Yes [#]	Yes (38)	Yes (138,139)	Yes [#]	Yes (78,116,118)
Transient ischemia	Yes [#]		Yes [#]	Yes [#]	Yes [#]
Multiple laboratories	Yes [#]		Yes [#]	Yes [#]	Yes [#]
Multiple species	r, m, g, rab [#]	r (38)	[^] m, r, g [#]	m, r, d [#]	m, r, g [#]
Females	Yes (140,141)			Yes/No (34,44)	Estrus cycle dependent (45)
Co-morbid conditions	BP [↓] aging [↓] (39,40)				

Ideally, for a PC agent/paradigm to progress to clinical trial for events involving cerebrovascular occlusion, it should have shown efficacy in models of permanent and transient ischemia in multiple laboratories. It should have also worked in multiple species, in both sexes (the majority of studies have been done in males) and irrespective of whether or not major co-morbidities are present. In addition, it may be beneficial if the PC agent/paradigm causes both classical and delayed PC effects, although the importance of this will depend upon the clinical target and treatment strategy.

HBO = hyperbaric oxygen; 3-NPA = 3- nitropropionic acid

[#] References are given in parentheses. Where there are more than three references to list, this is indicated by. For references on those points, readers are referred to reviews covering IPC (5-9), HBO PC (25) and volatile anesthetic induced PC (105), and some of these references for 3-NPA (45,78,100,116,118,142).

[^] For mouse HBO PC, Prass et al. (139) found strain differences with protection in SV129 mice but not C57BL/6.

* combined results for halothane, isoflurane and sevoflurane. m = mouse, r = rat, d = dog, g = gerbil, rab = rabbit, BP= blood pressure

[↓] indicates a reduced effect of PC (with hypertension and aging).

Table 3
Areas of research that could aid the translation of preconditioning to the clinic

➤	Experiments examining whether a preconditioned state can be maintained long term without adverse effects.
➤	Comparison of current outcomes of neurosurgical procedures performed either on awake or anesthetized patients.
➤	Greater pre-clinical data on the effects of remote ischemic PC on brain injury.
➤	Studies examining the effects of co-morbid conditions (e.g. hypertension, diabetes, atherosclerosis, chronic hypoperfusion, inflammation, smoking) on PC efficacy and safety.
➤	Studies examining whether some patients may already have an elevated level of protective mechanisms and are thus less susceptible to PC-induced upregulation.
➤	Determination of surrogate markers that will identify the 'pre-conditioned state' in animals and man.
➤	More studies comparing the effects of PC in aged and young animals, and in male and female animals.
➤	More direct comparisons of the effectiveness and safety of different PC agents
➤	More research into the effects of PC on brain injury/disease other than ischemic stroke (including hemorrhagic stroke and multiple sclerosis).
➤	Having a major neurological component in current heart PC trials.