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Non-pharmaceutical therapies for stroke: Mechanisms and clinical implications

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Abstract

Stroke is deemed a worldwide leading cause of neurological disability and death, however, there is currently no promising pharmacotherapy for acute ischemic stroke aside from intravenous or intra-arterial thrombolysis. Yet because of the narrow therapeutic time window involved, thrombolytic application is very restricted in clinical settings. Accumulating data suggest that non-pharmaceutical therapies for stroke might provide new opportunities for stroke treatment. Here we review recent research progress in the mechanisms and clinical implications of non-pharmaceutical therapies, mainly including neuroprotective approaches such as hypothermia, ischemic/hypoxic conditioning, acupuncture, medical gases, transcranial laser therapy, etc. In addition, we briefly summarize mechanical endovascular recanalization devices and recovery devices for the treatment of the chronic phase of stroke and discuss the relative merits of these devices.

Keywords

non-pharmaceutical therapies; stroke; neuroprotection; acupuncture; hypothermia; brain conditioning; medical gases; transcranial laser therapy; mechanical endovascular recanalization

1. Introduction

Stroke, deemed a worldwide leading cause of death and neurological disability in both immature and adult subjects, continues to wreak physical, psychological and financial havoc

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Conflict of interest

All authors have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning of the submitted work.

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in developing and developed nations alike. Instances of acute ischemic stroke result in heterogeneous changes in CBF (cerebral blood flow) and brain metabolism in the affected region. The human brain and its meshwork of roughly 100 billion interwoven neurons receives close to 15% of the body's resting cardiac output while expending 20% of its oxygen, rendering the organ especially sensitive to such cases of hypoxic or ischemic insult (Clarke and Sokoloff, 1999; Fisher *et al.*, 2009; Chao and Xia, 2010; Arai *et al.*, 2011; Ding *et al.*, 2012; Li *et al.*, 2012a; Albert-Weissenberger *et al.*, 2013; He *et al.*, 2013). As the brain is extremely sensitive to hypoxia or ischemia, protecting brain tissue from injury, simplified as "neuroprotection", has therefore been a long sought after strategy in quelling physiological damage following stroke onset. Decades of research efforts have investigated over 1,000 pharmacological neuroprotectants, including excitatory amino acid antagonists, free radical scavengers, calcium channel blockers, and growth factors that are thought to target and correct different pathophysiological manifestations of ischemic brain injury. Unfortunately, almost all attempts at protecting the brain from ischemic injury have failed at making a successful transition into clinical use. The majority of these failures involved complex pharmaceutical targets and agents. Until now, intravenous rtPA (recombinant tissue plasminogen activator) is the only proven effective treatment in the acute setting. Most neuroprotectants alter only a single step in the broad cascade of biochemical events that lead to ischemic injury, and this over-specification may in part explain failure in the clinical setting (STAIR, 1999). Therefore, it is strongly suggested that stroke be approached with multiple, multifaceted neuroprotective methods capable of ameliorating a broader range of systems gone awry.

Non-pharmaceutical therapies are becoming more common and can be promptly initiated after stroke onset, marking them as ideal to combine with pharmaceutical or thrombolytic therapies which may further salvage affected brain tissue. Indeed, accumulating data suggest that "non-drug" approaches might provide new opportunities for stroke therapy, such as therapeutic hypothermia (Lakhan and Pamplona, 2012; Yenari and Han, 2012), ischemic/hypoxic conditioning (Liu *et al.*, 2009b; Lim and Hausenloy, 2012), acupuncture (Guo *et al.*, 2010; Xia *et al.*, 2010; Xia *et al.*, 2012), certain medical gases (Liu *et al.*, 2011b; Sutherland *et al.*, 2013) and other strategies.

In this review, we focus on the major clinical implications of non-pharmaceutical therapies for acute ischemic stroke and their underlying neuroprotective mechanisms. We also address the recent progress in mechanical endovascular recanalization and recovery devices for the chronic phase of stroke. Together, these non-pharmaceutical therapies present clinically translatable possibilities for both the acute and chronic stages of stroke recovery.

2. Hypothermia

The concept of cooling therapy comes from the phenomenon of hibernation in some mammalian species, which results in lowered body temperature and slowed metabolism. In addition to its use in maintaining organs at low temperatures to prolong transplantation viability, a hypothermic state - even a small, clinically feasible decline in body temperature - was found to prevent neuron death following global ischemia over 25 years ago (Busto *et al.*, 1987). This finding has since prompted intense investigation into hypothermia as a neuroprotective approach, and has been shown in the laboratory to protect against experimental stroke through multiple mechanisms.

2.1. Mechanisms of hypothermia-induced protection against ischemic injury

Although the neuroprotective mechanisms of hypothermia have yet to be fully determined, several consequences of hypothermia seem highly relevant to decreasing the severity of ischemic brain damage, including slowed metabolism and the delay of pathophysiological

progress, anti-inflammatory actions, a reduction of oxygen-based free radical production, decreased excitatory neurotransmitter release, prevention of blood-brain barrier disruption, gene expression alteration under ischemic conditions as well as the regulation of cell death and survival pathways.

2.1.1. Improvements in metabolism—Abnormal cellular metabolism, characterized primarily by the uncoupling of mitochondrial electron transport activity and rapid loss of ATP, is a major cause of brain injury under ischemic/hypoxic conditions (Sims and Muyderman, 2010). Thus, a logical neuroprotective approach would include either limiting or reversing such abnormal metabolic processes without causing irreversible cell death. In some models, hypothermia has been found to decrease metabolic rate and reduce blood flow in the brain (Erecinska *et al.*, 2003). In such cases, low basal temperature was found to decrease brain oxygen consumption and glucose metabolism, and maintain valuable high-energy phosphate compounds such as ATP and pH in ischemic tissues, minimizing the downstream consequences of lactate overload and the development of acidosis (Schaller and Graf, 2003; Gotberg *et al.*, 2010). Furthermore, in a gerbil model of global ischemia, normothermic recovery incurred a significant activity loss from non-synaptic mitochondrial electron transport enzyme complex I and synaptic and non-synaptic complexes II–III and IV (Canevari *et al.*, 1999). Conversely, hypothermic (30°C) recovery led to respiration and mitochondrial enzymatic activity levels indistinguishable from sham-operated controls (Canevari *et al.*, 1999). Although not all studies have indicated that hypothermia prevents ATP loss following ischemia, the recovery rate of ATP in hypothermic animals has been consistently observed in multiple models (Zhao *et al.*, 2007a). The correlation between lowered temperature and the restoration of energetic capacity suggests that the beneficial effects of hypothermia involve, at least in part, mitochondrial function.

2.1.2. Inhibition of inflammatory mediators—Although inflammation is thought to partially contribute to tissue recovery and repair, inflammation can also exacerbate acute cerebral ischemic injury. The ischemic brain triggers innate immune responses that lead to the activation of microglia and circulating leukocytes, generating various molecules including ROS (reactive oxygen species), proteases and pro-inflammatory cytokines. Animal studies have shown that hypothermia may improve neurological outcome by inhibiting various pathological aspects of this immune response following brain ischemia and injury (Deng *et al.*, 2003). Hypothermia suppressed neutrophil extravasation and microglia activation in the affected area and reduced the level of inflammatory mediators, including oxidative stress (Briyal and Gulati, 2010) and adhesion molecules (Choi *et al.*, 2011). Additional studies found that levels of the proinflammatory cytokine IL-1 β were notably reduced by local brain cooling (Fairchild *et al.*, 2004; Wagner *et al.*, 2006), which simultaneously decreased damage inflicted through vasogenic edema. These observations suggest the possibility that hypothermia may protect the blood brain barrier. However, it is important to note that anti-inflammatory cytokines such as IL-10 were also reduced by hypothermia, suggesting that hypothermia may have a more complex role in cytokine modulation (Matsui and Kakeda, 2008). Although much more investigation is required, suppressing inflammatory and downstream responses appears to serve as a neuroprotective mechanism in hypothermia therapy for cerebral ischemia.

2.1.3. Regulation of gene expression—Hypothermia has been found to affect gene expression under ischemic conditions. One study reported that hypothermia inactivated NF- κ B (nuclear factor κ B), a major transcription factor that regulates the expression of many inflammation-related genes. Accordingly, transcription levels of downstream transcriptional targets, including iNOS (inducible nitric oxide synthase) and TNF- α (tumor necrosis factor factor- α) were reduced by lowered temperatures (Han *et al.*, 2003; Yenari and Han, 2006).

In models of focal brain ischemia, hypothermia prevented nuclear NF- κ B translocation and DNA binding by suppressing I κ B phosphorylation and decreasing IKK activity (Han *et al.*, 2003; Yenari and Han, 2006). As NF- κ B also regulates genes involved in cell survival and growth, the comprehensive effect of hypothermia-induced suppression of NF- κ B activity may be complex and is therefore still in the process of elucidation due to the different phases of ischemic injury; even the activation of NF- κ B during these phases is believed to be a multistep process.

Overexpression of HSP70 (heat shock 70kDa protein) effectively reduced NF- κ B activation in mice subjected to ischemic conditions (Zheng *et al.*, 2008). In this study, HSP70 overexpression reduced the phosphorylation of I κ B, thus prohibiting the entry of NF- κ B into the nucleus and suppressed the expression of multiple genes controlled by NF- κ B, including iNOS and TNF- α (Zheng *et al.*, 2008). Endogenous HSP70 expression notably increased with hypothermia (Kaneko and Kibayashi, 2012), and thus could function as the mechanism leading to the suppression of NF- κ B activation.

Excitotoxicity is an important theory that explains the pathophysiology of brain ischemia. The accumulation of excitotoxic amino acids (such as glutamate) is considered to be the base of excitotoxicity. Hypothermia limited calcium influx through AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) channels, one of the major contributors to excitotoxicity under normothermic ischemic conditions. Under ischemic settings, GluR2 (glutamate receptor 2), a subunit of the AMPA receptor that limits calcium influx, is markedly down-regulated and leads to calcium overload, while GluR2 mRNA expression was only modestly reduced upon the addition of hypothermia. (Colbourne *et al.*, 2003).

2.1.4. Balance between cell survival and death pathways—Hypothermia affects cell death processes in acute ischemic stroke models. Cell death pathways can be inhibited on several levels, including modulation of the expression of Bcl-2 (B-cell lymphoma-2) family members, the release of cytochrome c, the activation of caspases, or the activation of the FasL signaling pathway. In transient focal cerebral ischemia models, mild hypothermia significantly attenuated the release of cytochrome c in the brain, but did not alter the expression of Bcl-2 family proteins and caspase activation (Yenari *et al.*, 2002). Hypothermia blocked ischemia-induced translocation of PKC (protein kinase C) δ to the mitochondria and nucleus (Shimohata *et al.*, 2007); inhibition of PKC δ translocation to the mitochondria precludes its formation of reactive oxygen species and initiation of apoptosis. In models of both severe stroke, e.g., MCAO (middle cerebral artery occlusion) for 2 hours or longer, and global ischemia, hypothermia decreased the release of cytochrome c from the mitochondria (Zhao *et al.*, 2007b; Yenari *et al.*, 2002). Consistent with the improvement in stroke outcomes due to genetic or pharmacological disruption of the FAS/FASL pathway, hypothermia prevented FASL cleavage from the cell surface and decreased the amount of soluble FASL in cooled rodent brains (Liu *et al.*, 2008a). The decreased level of soluble FASL was also associated with decreased caspase 8 activation, which occurs downstream of FAS activation (Liu *et al.*, 2008a).

Hypothermia may also affect other molecules involved in apoptosis. The increased expression of HSP70 under hypothermic conditions may lead not only to the alteration of inflammatory gene expression as mentioned above, but may also serve as an important global mediator of cell death and survival pathways (Hu *et al.*, 2013). PTEN (phosphatase and tensin homologue) is a tumor suppressor molecule with pro-apoptotic function when dephosphorylated. Under conditions of early hypothermia in which neuroprotection was observed, phosphorylated (inactive) PTEN levels were preserved, but not under delayed hypothermic conditions that failed to provide a neuroprotective effect (Lee *et al.*, 2009). Although the relationship between phosphorylated PTEN levels and the suppression of

disproportionate amounts of ROS is not well understood, the ROS scavenger S-PBN both reduced infarct volume by 57.2% and maintained levels of phosphorylated PTEN protein. It is possible, then, that hypothermia may act to suppress ROS, leading to preservation of PTEN phosphorylation.

2.1.5. Improved integrity of the blood–brain barrier—The BBB (blood brain barrier) is a physical and metabolic diffusion barrier between cerebral microvessels and the surrounding tissue and is essential for the maintenance of homeostasis and the normal function of the central nervous system (Liebner *et al.*, 2011). Damage to the BBB is mediated by various factors, including free radical production, cytokines, and proteases (Rosenberg, 2012). In addition, structural components of the BBB such as fibronectin, laminin, collagen type IV and tight junction proteins are substrates for a class of extracellular proteinases called MMPs (matrix metalloproteinases) which are responsible for the breakdown of extracellular matrices. (Liu and Rosenberg, 2005). Focus on MMPs in models of cerebral ischemia has intensified due to the observations that activity and expression of MMP-2 and MMP-9 were significantly increased and closely related to BBB disruption, edema formation and intracranial hemorrhage (Yang and Rosenberg, 2011; Chaturvedi and Kaczmarek, 2013), suggesting that MMP activation may target BBB structural components and exacerbate acute brain injury.

Data generated from models of brain ischemia have shown that mild to moderate hypothermia protects the BBB and prevents edema formation (Preston and Webster, 2004; Lee *et al.*, 2005; Kallmunzer *et al.*, 2012). Lending support to the association of MMP activation and BBB damage in ischemic injury, hypothermia, which is protective against BBB damage and ischemia, was associated with a reduction in MMP proteolytic activity and degradation of various components of the extracellular matrix comprising the BBB, including vascular basement membrane proteins, agrin and laminin (Wagner *et al.*, 2003).

Effects on the BBB extend well beyond the time frame of the hypothermic state. Even 5 days following the hypothermic regimen and focal cerebral ischemia, BBB integrity was preserved. On the cellular level, vascular morphology was improved by post-stroke hypothermic treatment, which led to less distortion of endothelial cells and maintained their association with the basement membrane (Duz *et al.*, 2007). Hypothermic cooling also inhibited pericyte migration, which is increasingly recognized to have an important regulatory role in BBB integrity.

The above mentioned mechanisms for hypothermia-induced protection from ischemic brain injury are schematically shown in Figure 1.

2.2. Hypothermia therapy for stroke in clinics

In cardiovascular medicine, the efficiency and safety of induced hypothermia in cardiac arrest has been clearly demonstrated (Zeiner *et al.*, 2000; Bernard *et al.*, 2002; Group, 2002). Indeed, mild hypothermia is recommended by the American Heart Association after ventricular fibrillation. However, the relationship between temperature and ischemic stroke has not yet been completely defined. Hyperthermia has been correlated with increased post-stroke morbidity and mortality in analyses of clinical studies (Hajat *et al.*, 2000; Szczudlik *et al.*, 2003). Conversely, preclinical animal models suggest that mild hypothermia is effective in neuroprotection in both focal transient occlusion models and global ischemia models (described above). Early hypothermia induction in patients with intracranial hematomas and severe traumatic brain injury was correlated with improved outcomes (Clifton *et al.*, 2012), but the effects may have been due to better control of intracranial pressure rather than to hypothermia (Clifton *et al.*, 2011). To date, no clear evidence supports the routine use of mild therapeutic hypothermia in acute stroke patients. Large randomized, controlled and

multicenter clinical trials are still needed to ensure therapeutic effects and demonstrate the safety and feasibility of the therapy and standardize the therapeutic condition.

Despite the disappointing results from clinical trials, replicating the neuroprotection observed in experimental models of hypothermia may be dependent on optimal timing, target temperature, and duration of both hypothermia and occluded vessel reperfusion. Several preclinical studies have shown that hypothermia is effective when induced 1 hour after stroke (Zhang *et al.*, 1993; Colbourne and Corbett, 1995) and that longer cooling increases its neuroprotective effects (Colbourne *et al.*, 2000; Clark *et al.*, 2009), but it is still debated as to how long after stroke hypothermia can be induced and remain neuroprotective. Moreover, the use of rapid cooling techniques may be combined with narrow time window thrombolysis/embolectomy which could further facilitate hypothermia-induced neuroprotection on stroke. However, even the rewarming process may be a sensitive variable to hypothermic neuroprotection, as it has been suggested that, in the presence of ongoing but potentially reversible intracranial hypertension, more prolonged hypothermia may be required and rewarming too quickly may result in temperature overshoot (Varon *et al.*, 2012).

The methods to induce hypothermia can be classified into physical and pharmacological therapies, systemic and local therapies, and surface and endovascular therapies. All methods can be integrated with one another or combined with other neuroprotectants. Many of these methods possess significant caveats or confounds, which will be discussed below.

2.2.1 Surface cooling—Early surface cooling methods included convective air blankets, water mattresses, alcohol bathing, and ice packing. But maintenance of the target temperature using these methods is difficult to control, largely due to skin vasoconstriction and subsequent redistribution of blood flow. However, surface cooling has the considerable advantage of pre-hospital feasibility. The feasibility of surface cooling was addressed by the Copenhagen Stroke Study. In this study, 17 sober stroke patients tolerated mild hypothermia (mean of 35.5°C) for 6 hours with a forced air cooling blanket and were then gradually rewarmed over a period of 6 hours. Although the forced air method was well tolerated and free of confounds (e.g., anesthesia), no significant changes in short or long-term outcomes were found after accounting for factors such as age, gender, and stroke severity compared with 56 non-randomized control patients (Kammersgaard *et al.*, 2000). Still, the well tolerated and anesthesia-free forced air method used, once further studied and refined, may eventually prove to be a highly efficient way of quelling stroke damage.

Localized surface cooling by the use of a cooling helmet is another method of inducing hypothermic conditions quickly and selectively without systemic complications (Wang *et al.*, 2004). However, despite the convenience and portability, shaving is required to improve heat conduction. This delay may be critical when considering the limited therapeutic time for acute stroke patients. In addition, attaining deep brain temperatures are significantly less effective compared to lowering cortical temperatures. The distinction of ischemic injury typically centering in deep brain tissue thus limits the potential for therapeutic benefits of external surface cooling. However, penumbral cortical regions may still attain a therapeutic hypothermic temperature, and may improve functional, if not histological, outcomes.

Krieger *et al* conducted the COOL AID (Cooling for Acute Ischemic Brain Damage) pilot trial, of which was open design and nonrandomized. In total, 19 patients were eligible for the study, and 10 of them were treated with surface cooling (cooling blanket sandwiching) to induce moderate hypothermia ($32\pm1^{\circ}\text{C}$) in combination with intravenous or intra-arterial thrombolysis. No statistical difference in mortality and complications between hypothermia patients and non-hypothermia patients occurred, suggesting that the procedure was well

tolerated, but neurological outcomes were only slightly better in hypothermia patients. However, the lack of statistical significance could be attributed to several limiting factors, such as small sample size and open design (Krieger *et al.*, 2001). Furthermore, the mean time from stroke onset to induction of hypothermia was 6.2 hours, and an additional 3.5 hours was required to achieve the target temperature. The extended timeframe to reach a hypothermic state following ischemia could also negatively impact any potential therapeutic benefit (Krieger *et al.*, 2001).

2.2.2 Nasopharynx cooling—Nasopharynx hypothermia is another method that can be implemented as a prehospital hypothermic procedure. Circulating gases or fluids are either directly applied or contained within balloons by specific devices, and cooling is induced convectively or evaporatively. The cooled medium in the cavity induces direct cooling with close proximity to the brain by cooling the vascular supply in that region. This method cools more quickly than other surface cooling therapies, and is noninvasive and portable. However, similar to the helmet method, the cooling effect is localized, and nasopharyngeal cooling may preferentially cool the regions around the cribriform plate such as the basal frontal lobes, but other regions are only slightly affected at the same time (Mariak *et al.*, 1999).

The nasopharynx hypothermia devices are various. For example, cold saline-filled thin-walled balloons (the QuickCool device, QuickCool AB, Lund, Sweden) or a specially designed nasal catheter that sprays highly evaporative substances (e.g., perfluorohexane (PFC), RhinoChill system (Benechill, Inc., San Diego, CA) can be inserted into the nasopharynx and lead to surface chilling. A randomized trial comparing the feasibility, safety and efficacy of the two methods (Cold Infusions vs. RhinoChill) for rapid cooling of stroke patients has been completed, but the result has not been published (ClinicalTrials.gov, Identifier: NCT01573117).

Another human trial of nasopharyngeal cooling is the Pre-ROSC (Return of Spontaneous Circulation) Intranasal Cooling Effectiveness study. In this clinical study, 83 of 182 patients were randomized to immediate pre-hospital nasopharyngeal cooling (Castren *et al.*, 2010). The outcome data indicated that the nasopharyngeal cooling therapy improved neurological outcomes and decreased mortality. The most common side effect of treatment was reversible nasal discoloration (Castren *et al.*, 2010).

2.2.3 Endovascular cooling—Endovascular cooling induces hypothermia by heat conduction mainly through cerebral blood flow, while local surface cooling does so mainly through brain tissue. Due to low conductivity of brain tissue, surface cooling requires an extended duration to reach the target temperature. Endovascular cooling therapy consists of intra-arterial infusion and intra-venous infusion. Because the cerebral blood flow is dependent upon the perfusion, recanalization therapies are essential. Intra-arterial infusion cooling can easily be combined with other endovascular interventions and has the advantage of cooling the highest risk tissue first. In spite of being capable of reaching the target temperature faster, endovascular cooling is invasive, non-removable and may cause significant complications (Ovesen *et al.*, 2012).

The targeted and rapid localized cooling of high risk tissue can be attained by the intra-arterial infusion of cold fluids (such as recirculated blood and saline) directly into an ischemic arterial territory, using a microcatheter passed distal to an occlusion. In addition to directly targeting at risk tissue, the rapidity by which intra-arterial infusion cools the brain may shorten the onset and therapeutic duration of hypothermia as compared to the slower technique of surface cooling, thus avoiding potential complications that arise with an

extended hypothermic condition. Furthermore, intra-arterial cooling could be easily integrated with other endovascular interventions

As opposed to the localized aspect of intra-arterial infusion, intra-venous infusion therapy induces hypothermia by infusion of cold solution through catheters placed into the inferior vena cava. The COOL AID II trial was a randomized pilot clinical feasibility trial of intra-venous endovascular cooling. Endovascular cooling and rewarming was induced by a device through the inferior vena cava. A core body temperature of 33 °C was targeted for 24 hours. 18 patients received endovascular cooling within 12 hours after a stroke, and 13 of them reached the target temperature of 33°C after an average of 77 minutes. As a control group, 22 patients received standard medical management. Clinical outcomes measured by the NIHSS (National Institutes of Health Stroke Scale) scores were similar in both groups. However, the infarct volume measured by DWI (diffusion-weighted imaging) was lower in hypothermia patients (73%) than in non-hypothermia patients (124%) (De Georgia *et al.*, 2004).

ICTuS-L (Intravenous Thrombolysis Plus Hypothermia for Acute Treatment of Ischemic Stroke) was a randomized, multicenter trial of endovascular cooling and intravenous tissue plasminogen activator in sober patients treated within 6 hours after ischemic stroke. The mean time to reach the target temperature (33°C) was 67 minutes and the duration was roughly 24 hours. Overall, 28 patients were randomized to receive hypothermia and 30 to receive normothermia. NIHSS clinical outcomes were found to be equivalent in both groups at 30 days, and at 3 months there was no statistically significant mRS (modified Rankin Scale) difference. The mortality and the rate of any intracerebral hemorrhage were also similar in both groups. Serious adverse events were more common in the hypothermia groups and the most common serious adverse event was pneumonia. This study demonstrated the feasibility and preliminary safety of combining endovascular hypothermia after stroke with intravenous thrombolysis, but did not confirm the hypothesis that cooling extends the time window for thrombolysis due to the small sample size used (Hemmen *et al.*, 2010).

To circumvent complications (e.g., shivering, or measures to suppress shivering) associated with whole generalized hypothermia, recent research made use of selective ACP (antegrade cerebral perfusion) via the right axillary artery, allowing for hypothermic cooling of the brain only. During hypothermic circulatory arrest in 221 patients, moderate hypothermia induced by ACP during aortic arch surgery was associated with lower mortality and fewer neurologic sequelae than deep hypothermia compared to normothermic patients (Tsai *et al.*, 2013).

2.2.4 Hypothermic combination therapy—Thus far, rtPA is the only proven therapy for ischemic stroke. The effect of hypothermia on thrombolysis is complicated, and may negatively affect each other. For example, recanalization brings increased blood flow and acceleration of heat condition, counteracting hypothermic cooling efforts. Likewise, the thrombolytic activity of rtPA decreased with cooler temperatures in an *in vitro* study (Yenari *et al.*, 1995; Schwarzenberg *et al.*, 1998). Still, there is no conclusive evidence suggesting a direct relationship between hypothermia and the incidence of hemorrhagic complications (Krieger *et al.*, 2001; De Georgia *et al.*, 2004). Several ongoing clinical trials are assessing the efficacy of therapeutic hypothermia in acute ischemic stroke in combination with other therapeutics. The ICTuS II/III study was designed as a prospective, randomized, single-blinded, multicenter phase II–III study in patients presenting within 3 h of stroke symptom onset who are also eligible for intravenous rtPA. Hypothermia is induced during thrombolysis by intravenous insertion of a catheter in the inferior vena cava and perfusion of iced saline to obtain a cooled state of 33 °C (Wu and Grotta, 2013). As an important note,

the protocol calls for the use of anti-shivering interventions. Another ongoing trial assessing the possible synergy between hypothermia and thrombolysis is the EuroHYP-1, a European prospective open, randomized and controlled phase III clinical trial which induces hypothermia using external cooling pads in patients with acute ischemic stroke with or without receiving alteplase administration (Wu and Grotta, 2013). Dr. Grotta and colleagues also carried out several other clinical studies in their SPOTRIAS (Specialized Program of Translational Research in Acute Stroke) application. One of these included combining hypothermia with caffeineol, a neuroprotective combination developed in Dr. Grotta's laboratory and tested in phase I and early phase II clinical trials. These evaluations demonstrated feasibility and safety.

2.2.5 Clinical issues—Despite the clinical feasibility of hypothermic induction, the procedure may lead to several complications or confounds that could affect both outcomes and interpretations. The primary medical complications of hypothermia are composed of cardiovascular complications, pulmonary complications, hematologic complications and immunologic complications. Unlike patients who are intubated and sedated following cardiac arrest or traumatic brain injury, acute ischemic stroke patients are awake, which in turn can impede rapid cooling because of discomfort and shivering. As a result, endotracheal intubation, sedation, pharmacological paralyzation, and anesthesia are induced to prevent shivering. Anti-shivering interventions may play an important role in improving patient tolerance, although pulmonary complications have been related to anti-shivering medications as well as to endotracheal intubation, and surface cooling methods alongside aggressive anti-shivering protocols may cause skin irritation and respiratory suppression (Doufas *et al.*, 2003). Thus, precautionary measures should be implemented to minimize both discomfort and potential complications. Encouragingly, the effects of hypothermia were shown to not be affected by the administration of anti-shivering medication pethidine (Sena *et al.*, 2012), but further studies are needed to develop better techniques to circumvent the use of additional measures used in minimizing complications.

In conclusion, although preclinical results are positive, the outcomes of translational research are still not stable. Appropriate parameters of cooling therapies for stroke still need to be verified. Larger and more definite clinical trials are critical for a better understanding of the full efficacy of hypothermia therapies. Furthermore, investigating the effects of hypothermia when combined with the wide variety of known and theoretical neuroprotectant and revascularization options must also continue. Future directions will push towards increasingly accurate brain-selective cooling and less invasive pharmacological cooling methods, altogether aimed at coming closer to a therapy that yields a balance between comfort, safety, and effective management of hypothermic conditions.

3. Ischemic/hypoxic conditioning

Training the brain to tolerate to ischemia, also called “conditioning”, is a profound neuroprotective strategy for stroke. Conditioning is a process by which previous exposure to a sublethal stress or stimulus confers resilience against a stronger stimulus. The concept of preconditioning was confirmed several decades ago by observing the protective effects of ischemia preconditioning on myocardial ischemia (Murry *et al.*, 1986). The concept was further advanced to cerebral ischemia by the observation that brief bilateral carotid occlusion protected gerbil hippocampal CA1 pyramidal neurons against subsequent prolonged global ischemia (Kitagawa *et al.*, 1991). In addition to these and others' evidence that ischemic preconditioning protects against heart and brain ischemia, the phenomenon of ischemic preconditioning-induced protection extends now to the lung, kidney and liver as well (Fairbanks and Brambrink, 2010). Different methods that are able to develop conditioning have been emerging, including non-drug induced ischemic conditioning and

hypoxic conditioning. Depending on the moment that conditioning is carried out, ischemic conditioning can be classified into preconditioning (before onset of reperfusion) and postconditioning (after ischemia and during reperfusion). However, it is not always feasible, practical, nor safe for stroke patients to receive conventional preconditioning or postconditioning treatment, which requires intra-arterial interventions of occluding and releasing the cerebral artery supplying the brain. RlPostC (Limb remote ischemic postconditioning) is a newly developed postconditioning procedure and is regarded to possess a high potential to become an efficacious protective strategy against brain ischemic injury (Lee *et al.*, 2005). Here we summarize the common mechanism of ischemic/hypoxic conditioning.

3.1. Mechanism of ischemic/hypoxic conditioning

The mechanisms responsible for the induction and maintenance of ischemic/hypoxic tolerance in the brain are still undefined. However, laboratory studies suggest that the mechanisms for ischemic/hypoxic preconditioning involve a wide range of processes, including the improvement of cerebral blood flow, the up-regulation of protective membrane proteins, a reduction of inflammation, increased autophagy function, and the regulation of survival/death signaling, among others.

3.1.1. Improvement in cerebral blood flow and vasculature—Acute ischemic stroke results in heterogeneous changes in CBF in the affected region. There is evidence showing that ischemic/hypoxic conditioning is neuroprotective due to improving CBF in ischemic stroke, likely through reduced reflow vasospasm (Gao *et al.*, 2008a; Hasseldam *et al.*, 2013). Using ^{14}C iodoantipyrine autoradiography to measure CBF at varied occlusion intervals, a striking recovery of CBF during permanent MCAO in preconditioned spontaneously hypertensive rats was observed, presaging reductions in infarct volume (Zhao and Nowak, 2006). Specifically, global CBF detected in the contralateral cortex was better maintained following permanent occlusion in both preconditioned and sham animals, suggesting an effect of surgery (e.g., anesthesia) contributing to global CBF maintenance. In contrast, a progressive reperfusion of penumbra was detected in the ipsilateral cortex of the preconditioned group, independent of global perfusion changes (Zhao and Nowak, 2006).

The mechanisms of vascular alterations are still undetermined. Della-Morte *et al* have demonstrated that PKC ϵ activated by ischemic preconditioning plays a pivotal role in neuroprotection by regulating CBF (Della-Morte *et al.*, 2011). In addition, inhibition of adenosine transporter ENT1 (equilibrative nucleoside transporter 1) with propentofylline increased cerebral blood flow and re-established neuroprotection in hypoxic conditioning (Cui *et al.*, 2013), indicating that maintenance of extracellular adenosine levels may be a mechanism by preconditioning to improve blood flow.

In addition to the observed alterations in blood flow, conditioning appears to directly preserve the vasculature at the molecular and cellular level. HPC (Hypoxic preconditioning) inhibited apoptotic cell death in human brain microvascular endothelial cells through PI3-kinase (phosphatidylinositol 3-kinase)/Akt and the inhibitor-of-apoptosis protein, survivin pathway (Zhang *et al.*, 2007). We recently found that remote post-conditioning protected BBB integrity by diminishing MMP-9 expression and attenuating laminin and fibronectin degradation following experimental ischemic stroke (Ren *et al.*, 2011; Liu *et al.*, 2012c). SphK (Sphingosine kinase)-directed production of sphingosine-1-phosphate, which mediates peripheral vascular integrity via junctional protein regulation, was essential to hypoxic preconditioning-induced stroke protection. In wild-type mice, hypoxic preconditioning significantly strengthened the BBB integrity by increasing triton-insoluble claudin-5 and VE

(vessel endothelium)-cadherin, while SphK2 knockout abolished HPC-induced BBB protection, with loss of VE-cadherin, occludin, and zona occludens-1 (Wacker *et al.*, 2012).

3.1.2. Up-regulation of neuroprotective membrane functional proteins—

Ischemic/hypoxic preconditioning can stimulate some membrane functional proteins to promote endogenous mechanisms of neuroprotection. Among them, the DOR (δ -opioid receptor) may play an important role (Ma *et al.*, 2005; Zhang *et al.*, 2006; Chao *et al.*, 2008; Pang *et al.*, 2009; Chao and Xia, 2010; He *et al.*, 2012). We found that DOR signaling is oxygen-sensitive and up-regulated in both rapid (Zhang *et al.*, 2006) and delayed (Ma *et al.*, 2005) preconditioning. DOR signaling acts at multiple levels, including the regulation of survival and death signals (Ma *et al.*, 2005), inhibition of glutamate toxicity (Zhang *et al.*, 2006), and inhibition of Na^+ influx through the membrane and reducing the increase in intracellular Ca^{2+} , thus decreasing the excessive leakage of intracellular K^+ in the cortex (Chao *et al.*, 2007a). Such protection is dependent on a PKC-dependent and PKA (protein kinase A) -independent signaling pathway (Chao *et al.*, 2008; Chao and Xia, 2010; He *et al.*, 2013). In addition, other mechanisms of neuroprotection include the attenuation of neuronal transmission imbalance, increases in antioxidant capacity, regulation of specific gene and protein expression (Pang *et al.*, 2009), and up-regulation of endogenous opioid release and/or DOR expression (He *et al.*, 2012).

3.1.3. Attenuation of inflammation and the related events—

Several examples of ischemic conditioning attenuate acute inflammatory processes. In the acute phase after stroke, inflammation can be detrimental to the neurological outcome. Ischemic postconditioning decreased myeloperoxidase activity and the expression of interleukin-1 β and intercellular adhesion molecule 1 (Xing *et al.*, 2008), and a long term protective paradigm of limb remote preconditioning inhibited activities of the proinflammatory galectin-9/Tim-3 pathway, iNOS, and nitrotyrosine (Wei *et al.*, 2012). In situ superoxide detection using hydroethidine suggested that postconditioning attenuated superoxide products during early reperfusion after stroke (Zhao *et al.*, 2006). In humans, remote limb conditioning reduced CD11b expression, as measured by flow cytometry and modified leukocyte inflammatory gene expression (Konstantinov *et al.*, 2004). Ischemic conditioning also reduced neutrophil activation and inflammatory gene expression (Shimizu *et al.*, 2010). The promotion of DOR by hypoxic/ischemic conditioning may also lead to suppression of inflammation, as we have recently found that activation of DOR attenuates ischemic increase in TNF- α (unpublished observation). Another mechanism of neuroprotection has been demonstrated by this pathway: a study indicated that ERK (extracellular regulated protein kinases) 1/2 activation induced by PKC ϵ activation may result in NF- κ B translocation to the nucleus followed by COX-2 (cyclooxygenase-2) expression, thus bringing about neuroprotection in mixed neuronal culture (Kim *et al.*, 2010).

3.1.4. Regulation of cell survival/death signaling—

Data derived from morphological, biochemical, and molecular genetic studies indicate that mitochondria constitute a convergence point for neurodegeneration. Mitochondrial dysfunction is also a prominent feature of cerebral ischemic stroke (Perez-Pinzon *et al.*, 2012). Despite the fact that the precise molecular mechanisms underlying preconditioning-induced brain tolerance are still unclear, mitochondrial reactive oxygen species generation and mitochondrial ATP-sensitive potassium channel activation have been implicated in the preconditioning phenomenon. These pathways may be operative not only in brain but also in the brain endothelium. Ischemic/hypoxic preconditioning regulates the expression and translocation of Bcl-2 family proteins to mitochondria (Zhao *et al.*, 2013), attenuating cytochrome c release and caspase-3 activation (Du *et al.*, 2009). Excessive production of ROS and RNS (reactive nitrogen species) induced by ischemia is believed to perturb normal mitochondrial

function and to initiate cellular death pathways. Despite the common association of ROS and toxicity, low levels of ROS production have been found to be protective and may serve as the trigger for activation of IPC-mediated neuroprotective pathways (Thompson *et al.*, 2012). Furthermore, hypoxic preconditioning increased subcortical mitochondrial DNA content and mitochondrial density by a novel mechanism that requires nNOS (neuronal nitric oxide synthase), regulation of PGC-1 α (α -subunit of peroxisome proliferator-activated receptor- γ coactivator-1) and CREB (cyclic adenosine monophosphate) response element binding protein (Gutsaeva *et al.*, 2008). These data indicate that conditioning events can target mitochondria both in terms of modulation of cell death signaling as well as mitochondrial physiology.

Upstream of mitochondrial cell death signaling, multiple pathways are modulated by ischemic conditioning stimuli to counteract the detrimental signaling induced by severe brain injury. Key signal transduction pathways of IPC, such as activation of PKC ϵ , MAPK (mitogen-activated protein kinase) and HIFs (hypoxia inducible factors) are likely involved in IPC-induced mitochondria mediated-neuroprotection (Della-Mortem *et al.*, 2012). Recent findings suggest that signal transducers and activators of transcription, a family of transcription factors involved in many cellular activities, may be intimately involved in IPC-induced ischemic tolerance (Lin *et al.*, 2011). In addition, sirtuins are stress-responsive enzymes which can be activated by IPC and may be linked to modulating protective pathways against oxidative stress, energy depletion, excitotoxicity, inflammation, DNA damage, and apoptosis. Although the functional roles of sirtuins under preconditioning scenarios are still being investigated, they possess unique roles in enhancing cellular function against stress-mediated damage, some of which have been associated with ischemia-reperfusion injury (Morris *et al.*, 2011). The AKT/GSK3 β (Glycogen synthase kinase-3 β) pathway has been recognized as a protective pathway against cerebral ischemic injury. Remote limb conditioning activated and up-regulated the Akt pathway in cerebral ischemia models (Qi *et al.*, 2012; Zhou *et al.*, 2011a), and long term protection afforded by postconditioning was associated with Akt, MAPK (mitogen-activated protein kinase) and PKC (protein kinase C) signaling pathways (Gao *et al.*, 2008b). Protection afforded by hypoxic preconditioning increased ERK and Bcl-2 activity, which counteracted the increased p38 MAPK activity and cytochrome c release observed following severe hypoxia (Ma *et al.*, 2005). The cross-talk between ERK and p38 MAPKs displayed a “yin-yang” antagonism under the control of the DOR-G protein-PKC pathway (Ma *et al.*, 2005). Taken together, the modulation of cell survival and death signaling by hypoxic/ischemic preconditioning appears to be capable of targeting multiple levels of signaling cascades.

3.1.5. Increased autophagy—The degradation of intracellular macromolecules or organelles (such as mitochondria) for subsequent reuse is a process that occurs under physiological conditions in order to maintain intracellular homeostasis. This process, known as autophagy, may also be involved in the protective properties of ischemic preconditioning and postconditioning (Balduino *et al.*, 2012; Sheng *et al.*, 2012a). In PC12 cells, ischemic preconditioning increased the generation and degradation of autophagosomes (Park *et al.*, 2009). Inhibition of autophagy during either the lethal ischemic period or the reperfusion period following the lethal ischemic period significantly decreased the neuroprotective effects of ischemic preconditioning (Park *et al.*, 2009). In a rat model of cerebral ischemic preconditioning, the autophagy inhibitors 3-Methyladenine and bafilomycin A1 suppressed neuroprotection induced by ischemic preconditioning, while rapamycin (an autophagy inducer) reduced infarct volume, brain edema and motor deficits induced by cerebral ischemia (Sheng *et al.*, 2010). We recently reported the protective role of autophagy in rats exposed to limb ischemic postconditioning in the early phase of reperfusion. In this study, autophagy was promoted in an AKT-dependent manner, mainly in neurons in penumbral tissue (Qi *et al.*, 2012). These observations indicate that autophagic activation during

ischemic preconditioning may significantly contribute to tolerance toward a subsequent fatal ischemic insult.

3.1.6. Putative mechanisms underlying remote conditioning—The manner in which remote ischemic-reperfused tissue/organ can transmit protection to the brain or brain vasculature is still unclear. However, three theories have been formed based on the evidence from work in the myocardial and cerebral settings: i) humoral factors acting via the systemic circulation; ii) neurogenic transmission with involvement of muscle afferents and the autonomic nervous system; and iii) effects on leukocytes or circulating immune cells (Hess *et al.*, 2013).

In summary, the mechanisms behind IPC/HPC protection from ischemic brain injury are schematically described in Figure 2.

3.2. Conditioning treatment for stroke in clinical trials

Although a substantial amount of preclinical data strongly indicates that ischemic postconditioning and preconditioning enhances brain and spinal cord tolerance to ischemia and reperfusion, the clinical data on their neuroprotective effects is scant (Koch *et al.*, 2012; Narayanan *et al.*, 2013).

IPC was first described in myocardial ischemia (Zhao *et al.*, 2003), and to date, cardioprotection exerted by ischemic postconditioning has been reported in human trials (Hansen *et al.*, 2010). The first clinical trial of ischemic postconditioning was performed following coronary angioplasty, where postconditioning was induced within 1 minute of reflow by 4 episodes of 1 minute inflation and 1 minute deflation of the angioplasty balloon (Staat *et al.*, 2005). This study confirmed the concept in humans that coronary angioplasty postconditioning could protect the heart during acute myocardial infarction (Staat *et al.*, 2005).

Our recent study correlated repetitive BAIPC (bilateral arm ischemic preconditioning) with reduced stroke recurrence in patients with IAS (intracranial arterial stenosis) (Meng *et al.*, 2012). IAS patients (n=38) received BAIPC treatment along with standard medical treatment, while the control group (n=30) underwent standard medical treatment only. The incidence of recurrent stroke at 90 and 300 days in the BAIPC group was significantly lower compared to the control group, and the frequency of TIA (transient ischemic attack) onset in the duration of 16–30 days after the initiation of treatment was also statistically different. Interestingly, the average time to recovery (modified Rankin Scale score 0–1) was also shortened by BAIPC. Cerebral perfusion status, measured by SPECT and transcranial Doppler sonography, improved remarkably in BAIPC-treated brain than in that of controls (Meng *et al.*, 2012). Further studies regarding the protective effects of remote limb ischemic preconditioning on acute cerebral infarction (ClinicalTrials.gov, Identifier: NCT01672515) are currently underway.

The endogenous protection conferred by conditioning protects the brain itself against future injury by adapting to small doses of harmful stimuli. After being described in myocardial ischemic preconditioning in a canine experimental model (Murry *et al.*, 1986), its neuroprotective effect has also been repeatedly demonstrated in preclinical trials. The ability of the brain to mount an endogenous response to a conditioning stimuli that leads to subsequent protection against future injury has been described in many preclinical animal models, but may have also been indicated in clinical observations. Patients with previous, spontaneously occurring TIAs before cerebral infarction showed a more favorable outcome than those without TIAs. Although TIAs do not necessarily mimic the precise timing and intensity that has been deemed necessary in animal models of preconditioning, the study

indicated that spontaneously occurring transient focal ischemia may induce a tolerance to subsequent permanent focal ischemia (Moncayo *et al.*, 2000).

To date, the translation of preclinical ischemic conditioning protocols to a clinical application has been difficult in terms of both the methodology as well as the establishment of optimal timing. Most preclinical studies of remote ischemic conditioning use vessel clamps to induce hindlimb ischemia, while clinical trials to date have used tourniquets or blood pressure cuffs on the arm as a less invasive means to induce the preconditioning stimulus. In addition, the protection afforded by limb conditioning paradigms appears to be highly sensitive on the establishment of an optimal duration and repetitive ischemic cycles. Typically, preclinical studies have performed unilateral or bilateral femoral ischemia of varying time durations and cycle numbers. Factors such as the total duration of occlusion and the number of cycles need further optimization in clinical settings to determine both feasibility as well as targeted neuroprotective effects.

In designing ischemic postconditioning acute stroke clinical trials, the eligible population should be focused on patients with a high likelihood of reperfusion, either with IV rtPA or with mechanical intra-arterial interventions, since a primary effect of ischemic postconditioning is geared toward the minimization of reperfusion injury. Cardiac ischemia trials indicated that combining daily limb preconditioning with postconditioning may improve survival and left ventricular remodeling in the rat model (Wei *et al.*, 2011), providing support for the application of both preconditioning plus additional postconditioning in the clinical setting. However, a recent clinical trial failed to show the superiority of the combination of limb preconditioning and postconditioning when compared to the control group (Hong *et al.*, 2013). It is worth noting that other non-drug methods of inducing preconditioning, such as physical exercises in a broad sense, have been found to decrease ischemia/reperfusion brain injury in animal models (Li *et al.*, 2004; Ding *et al.*, 2006; Davis *et al.*, 2007; Curry *et al.*, 2010). Indeed, combination therapies with different conditioning may better salvage the ischemic tissue.

Large multicenter clinical studies are still needed to determine the impact of ischemic postconditioning and preconditioning on outcomes after stroke, though both postconditioning and preconditioning may be applied in a safe, economical, noninvasive and convenient fashion with definite neuroprotective potential. The difficulty in predicting the onset of ischemic stroke has limited the neuroprotective potential of ischemic preconditioning. Postconditioning therapy exerts a similar degree of effect compared to preconditioning (Zhao *et al.*, 2006), and its potential feasibility is stronger for the application to both stroke treatment and prevention (Schaller *et al.*, 2003). It is important to better define therapeutic windows by distinguishing different effects of preconditioning on ischemic cascades and CBF at various stages of stroke and the underlying mechanism in future studies. To increase the chance for success of future acute stroke clinical trials, further preclinical testing is needed according to STAIR (Stroke Therapy Academic Industry Roundtable) criteria.

4. Acupuncture

Acupuncture is one of the major modalities in traditional Chinese medicine (TCM). Throughout the long history of China, TCM has advocated for the use of acupuncture in ameliorating a variety of conditions including stroke and other neurological disorders (Xia *et al.*, 2010; Xia *et al.*, 2012). As recorded in the ancient literature, *Huang Di Nei Jing* (The Yellow Emperor's Inner Classic, a product of various unknown authors in the Warring States Period, 475–221 BC), the clinical application of acupuncture on stroke can be traced back over 3000 years. Additional details were described in other historical TCM books such

as *Zhou Hou Fang (Handbook of Prescriptions for Emergencies)* in the year 281 AD and *Qian Jin Yao Fang (Essential Prescriptions Worth a Thousand Gold Pieces)* in 680 AD (Chen *et al.*, 2002; Guo *et al.*, 2010; Zhou *et al.*, 2011b). Since ancient times, the theory and practice of using acupuncture as a stroke treatment has been continually polished and refined throughout China and many other oriental countries. In fact, acupuncture is now recommended by the WHO (World Health Organization, 2003) as one of the alternative and complementary strategies against stroke.

Traditionally, acupuncture was delivered by the insertion of fine needles that were manually manipulated at different acupoints along the body. With the development of acupuncture theory and practice, many alternative techniques have been used to generate acupuncture signals, including acupoint stimulation with electrical currents, magnetic beads, or even by selective drugs. Due to its repeatability and feasibility of standardization, EA (electro-acupuncture) has become increasingly popular in both clinical and experimental studies (Xia *et al.*, 2010; Xia *et al.*, 2012).

The acupuncture modality is relatively convenient and cost-efficient with few side-effects. Acupuncture protocols have the potential to be optimized as an exceptionally useful therapeutic option for protecting the brain against stroke as an early intervention, especially when and where other treatments are not immediately available, such as at the home, during travel, or en route to a hospital. This additionally grants acupuncture therapies a chance of quelling stroke damage in a much more globalized context, extendable to regions with highly limited medical access.

4.1. Potential mechanisms for acupuncture protection against ischemic brain injury

In recent years, a variety of research with modern technologies has made major progress in elucidating the effects of acupuncture on stroke and the underlying mechanisms, especially in the acute ischemic condition. There is substantial evidence showing that either EA, which stimulates specific acupoints for an appropriate period of time with suitable current parameters, or manual acupuncture, can generate beneficial signals to the brain through peripheral nerve pathways (Guo *et al.*, 2010; Xia *et al.*, 2010; Xia *et al.*, 2012). These signals lead to the regulation of cerebral blood flow and modulate the balance between survival and death signals at both cellular and molecular levels (Zhao *et al.*, 2002; Tian *et al.*, 2008; Zhou *et al.*, 2008; Guo *et al.*, 2010; Zhou *et al.*, 2010; Zhou *et al.*, 2011b; Xu *et al.*, 2012; Li *et al.*, 2012; Zhou *et al.*, 2013a; Zhou *et al.*, 2013b). In a side by side comparative study using the MCAO model, EA compared favorably to several pharmacological agents and Chinese herb medicines, such as taurine, “*Salvia miltiorrhizae injection*,” “*Qingkailing injection*,” and “*Xingnaojing injection*” in terms of protective effects against ischemic injury (Zhao *et al.*, 2008).

4.1.1. Increase of cerebral blood flow—The brain is highly susceptible to blood supply insufficiency, due in part to high energetic demands and to limited storage of energetic substrates and oxygen (Sung *et al.*, 2008; Chao and Xia, 2010). The balance between energetic supply and demand can be disrupted by ischemic stress, leading to a disruption of ionic homeostasis and consequential dysfunction of neuronal excitability and synaptic transmission. The failure to maintain ionic homeostasis initiates multiple stress signaling pathways and the triggering of intracellular death signals. Consequently, prolonged ischemic periods lead to brain edema, infarction, neurological deficits, and even death. Therefore, restoration of blood flow to the infarct brain region has an invaluable impact on the treatment of stroke.

After optimizing EA at various acupoints with different current parameters on cerebral ischemia, we found that certain acupoints such as “Baihui” (Du 20) and “Shuigou” (Du 26)

in the head and “Quchi (LI 11)” and “Neiguan” (PC 6) in the forelimb can significantly increase cerebral blood flow and reduce ischemic infarction, neurological deficit, and mortality following ischemic stroke. The effect on the blood flow is greatly dependent on the location stimulated, the stimulation intensity and frequency, and the duration of EA (Zhou *et al.*, 2011b; Zhou *et al.*, 2013a; Zhou *et al.*, 2013b). Interestingly, the increase in blood flow occurred only in the ischemic, but not in the non-ischemic, brain (Zhou *et al.*, 2011b).

The EA-induced increase in blood flow is isochronous to the current impulse and disappeared immediately after the termination of EA, suggesting a rapid-responsive mechanism (e.g., neural reflex) that contributes to the EA-induced increase in cerebral blood flow.

4.1.2. Regulation of neurotransmitters/modulators and membrane proteins—

EA-induced increase in the blood flow likely exerts a major benefit to the ischemic brain. However, recent studies suggest parallel mechanisms contributing to EA-mediated brain protection following ischemic injury (Zhou *et al.*, 2013b). There is substantial evidence demonstrating that acupuncture signals regulate many different neurotransmitters/modulators and membrane functional proteins (Wen, *et al.*, 2010a; Wen, *et al.*, 2010b; Liang and Xia, 2012; Xu, *et al.*, 2012). Among them, DOR appears to play a unique role as an acupuncture-induced neuroprotective protein against cerebral ischemia.

As described in Section 3, DOR exerts neuroprotective effects in the brain (Chao and Xia, 2010; He *et al.*, 2012). Activation of DOR protected against hypoxic and excitotoxic injury in cortical neurons (Zhang *et al.*, 1999; Zhang *et al.*, 2000; Zhang *et al.*, 2002; Zhao *et al.*, 2002; Ding *et al.*, 2012). Pharmacological activation of DOR by the non-specific agonist DADLE ([D-Ala², D-Leu⁵]-enkephalin) reduced glutamate-induced injury in neocortical neurons (Zhang *et al.*, 2000), and was selectively blocked by δ -, but not by μ - or κ -, opioid receptor antagonists. Furthermore, we demonstrated that DOR provided neuroprotection against hypoxic/ischemic insults in various neural models, including neuronal cultures and brain slices subjected to hypoxia or oxygen-glucose deprivation, and *in vivo* brain exposed to cerebral ischemia (Ma *et al.*, 2005; Zhang *et al.*, 2006; Chao *et al.*, 2007a; Chao *et al.*, 2007b; Hong *et al.*, 2007; Chao *et al.*, 2008; Tian *et al.*, 2008; Kang *et al.*, 2009; Yang *et al.*, 2009; Chao and Xia, 2010; He *et al.*, 2012). Intracerebroventricular infusion of the DOR agonist TAN-67 significantly reduced the infarct volume and attenuated neurological deficits, while Naltrindole, a DOR antagonist, aggravated ischemic damage after forebrain ischemia in rats (Tian *et al.*, 2008; Tian *et al.*, 2013a). Similar data generated from other independent laboratories further demonstrated that DOR exerts neuroprotective effects against cerebral ischemic stress in animal models (Lim *et al.*, 2004; Iwata *et al.*, 2007; Su *et al.*, 2007; Govindaswami *et al.*, 2008; Borlongan *et al.*, 2009). In addition, systemic administration of DOR agonist DADLE or Deltorphan-D (variant) reduced infarct volume after transient middle cerebral artery occlusion (MCAO) (Govindaswami *et al.*, 2008; Borlongan *et al.*, 2009).

DOR is highly distributed in the cortex and striatum, major regions targeted by stroke. Since abundant data in acupuncture research have shown that both manual acupuncture and EA can up-regulate endogenous opioid activity (Wen, *et al.*, 2010b; Liang and Xia, 2012), it is very likely that acupuncture signal promotes DOR activity, thus protecting the brain from ischemic injury. In 2002, we showed that intracerebroventricular administration of Naltrindole, a DOR antagonist, largely reversed the EA-induced protection against the ischemic injury, which is the first evidence suggesting the role of DOR in the EA-induced protection against ischemic injury (Zhao, *et al.*, 2002). Furthermore, we observed that EA rescued the level of DOR correlated with the reduction of ischemic infarction in the cortex

(Tian *et al.*, 2008a; 2008b). Intravenous injection of Naltrindole reversed the EA-induced protection (Zhou *et al.*, 2013b). Dr. Xiong and his colleagues also demonstrated that DOR is involved in the EA-induced brain tolerance to cerebral ischemic injury (Xiong, *et al.*, 2007; Li, *et al.*, 2012). Intraperitoneal administration of Naltrindole abolished EA-induced protection against ischemic injury, while *nor*-BNI, a kappa-opioid receptor antagonist, did not significantly affect EA neuroprotection (Xiong, *et al.*, 2007), demonstrating the specific role of DOR in brain protection against ischemic injury. Taken together, these various studies suggest that acupuncture may attenuate ischemic injury in the brain at least in part by the promotion of DOR signaling.

Other neurotransmitters/modulators and membrane functional proteins such as excitatory (e.g., glutamate) and inhibitory (e.g., GABA [gamma-aminobutyric acid]) amino acids, monoamines, neurotrophins such as BDNF (brain derived neurotrophic factor) and their receptors are also involved in acupuncture protection against ischemic brain injury, and have been recently reviewed (Guo, *et al.*, 2010; Xu *et al.*, 2012).

4.1.3. Promoting survival signal transduction—Though acupuncture may influence several neurotransmitters/modulators and accordingly regulate various survival/death signals, the DOR-mediated survival pathway may play a critical role in acupuncture-induced neuroprotection against ischemic brain injury. Recent studies have suggested that DOR activation can stabilize ionic homeostasis, suppress excitatory transmitter release, attenuate disrupted neuronal transmission, increase antioxidant capacity, inhibit apoptotic signals, promote pro-survival signaling and regulate specific gene and protein expression (Ma et al, 2005; Chao et al, 2007a; 2007b; 2008a; 2010a; 2012; He et al, 2013; Tian *et al.*, 2013a; Tian *et al.*, 2013b). For example, Bcl-2 and BDNF-TrkB survival signals are impaired/downregulated in hypoxic/ischemic conditions, while DOR activation rescues/upregulates them (Ma et al, 2005; Tian *et al.*, 2013a; Tian *et al.*, 2013b). Since EA promotes opioid activity in the brain (Wen, *et al.*, 2010a; Wen, *et al.*, 2010b; Liang and Xia, 2012; Xia et al, 2010; 2012) and rescued the level of DOR correlated with reduction of ischemic infarction in the cortex (Tian *et al.*, 2008b), the EA-enhanced DOR activity very likely promotes Bcl-2 and BDNF-TrkB signaling, thereby leading to neuroprotection against ischemic injury.

In addition, the EA-mediated up-regulation of DOR signaling may regulate cellular and intracellular signal transduction at multiple levels, from membrane ionic homeostasis to gene expression and from neuronal integration to glial regulation, which have been largely summarized in recent reviews (Chao and Xia, 2010; He, *et al.*, 2012). The EA-induced specific regulation of intracellular signal transduction has also been summarized recently (Guo, *et al.*, 2010; Xu, *et al.*, 2012). Therefore, the available data strongly suggest that acupuncture can promote survival signaling, while inhibiting death signals, thus rescuing the brain from ischemic injury.

Indeed, such DOR-mediated regulation is important for acupuncture to confer the neuroprotective effect because intracerebroventricular or intravenous injection of Naltrindole, a DOR agonist, largely reverses the EA-induced protection against ischemic brain injury (Zhao et al, 2002; Zhou et al, 2013b). On the other hand, an inappropriate EA stimulation that might not appropriately regulate the survival signaling in the brain does not induce any neuroprotective effect in spite that it still promotes an increase in cerebral blood flow (Zhou et al, 2013b).

4.2. Clinical practice

In past 10 years, many medical institutions, mostly in China and other oriental countries such as Korea, have reported the use of manual- or electro-acupuncture to treat stroke patients.

4.2.1. Controversy in clinical observations—Many of these reports demonstrated that acupuncture has a beneficial effect on patients with ischemic brain injury (e.g., stroke) in acute or chronic phases. After acupuncture therapy, patients showed improved recovery of motor function, reduced spasticity, relief of post-stroke depression, urinary incontinence or even reduction of infarction sizes (Hu *et al.*, 1993; Johansson *et al.*, 1993; Naeser *et al.*, 1994; Si *et al.*, 1998; Wong *et al.*, 1999; Moon *et al.*, 2003; Alexander *et al.*, 2004; Schaechter *et al.*, 2007; Bai *et al.*, 2008; Liu *et al.*, 2008b; Li *et al.*, 2009b; Liu *et al.*, 2009a; Chau *et al.*, 2010).

However, despite these encouraging findings, the lack of strict random controls with appropriate statistical analysis of the data creates uncertainty of the reported efficacy in several of these clinical reports. Furthermore, several of the reports combined acupuncture with other medical treatments, which leads to difficulty in the correct evaluation of effects attributed to acupuncture rather than the adjunct therapy. Also, there is no standard/optimal protocol based on comparative studies in terms of therapeutic windows, acupoints, stimulation parameters, times and durations of treatment, etc. The treatments were mostly based on the personal experiences of the acupuncturists. Some clinical studies combined acupuncture and Chinese herbal medicine for the treatment of post-stroke motor dysfunction. However, most of the primary studies were inadequately designed trials characterized by unknown dropout rates and definitional vagueness in outcome measures. None of the studies approached important end points like death, survival times, rate of dependency, reduction in length of stay in hospital, etc. (Zhang *et al.*, 2009). Combined, these limitations have led to the conclusion that data from the available trials are insufficient to guide continence care of adults after stroke (Thomas *et al.*, 2008).

Although the difficulty of strictly controlling clinical research on patients with such serious disorders is understandable, these fatal issues greatly obstruct the promotion of clinical application for acupuncture in the treatment for stroke. Indeed, there are many reports, both dated and recent, that have indicated the uncertainty of acupuncture treatment for stroke and called for the necessity of more solid evidence in ascertaining the efficacy of acupuncture treatment for stroke (Gosman-Hedstrom *et al.*, 1998; Liu *et al.*, 2006; Hopwood *et al.*, 2008; Kong *et al.*, 2010). Thus, acupuncture, along with the other wide range of interventions pursued in clinical stroke research, require better quality evidence for successful clinical application (Thomas *et al.*, 2008).

4.2.2. Potential solution of the controversy through research—The major controversy surrounding the efficacy of acupuncture in stroke treatment is likely attributed to the differences among various clinical studies in the selection of patients, in the windows of application, and/or in acupuncture methods including acupoints, stimulation parameters (or manner) of acupuncture stimulation and treatment times and durations. Indeed, the selection of proper stimulation approaches is a crucial factor that significantly influences the efficacy of acupuncture. We argue that improved preclinical research will help to establish the basic biology behind neuroprotection afforded by acupuncture as well as delineate therapeutic paradigms to improve standardization into clinical practice.

In the animal model, we observed that EA effectively reduced ischemic injury (Zhou *et al.*, 2011b; Zhou *et al.*, 2013a; Zhou *et al.*, 2013b). When compared side by side to some protective drugs and compounds, EA showed the best effect in the reduction of ischemic infarction. However, we also found that optimized parameters for EA stimulation are extremely important. For example, the specific locations stimulated (acupoints) played a key role in the EA-induced increase in blood flow (Zhou *et al.*, 2013a). Also, the stimulation parameters have a major impact on the EA-induced change in blood flow. No appreciable increase in the blood flow occurred using a stimulus with current intensity less than 0.8 mA,

and the maximal effect was obtained by stimulating at 1 mA. However, the intensity at 1.2 mA failed to boost a further increase in the blood flow (Zhou *et al.*, 2011b). In addition, the EA-induced increase in the blood flow was also dependent on stimulation frequency. EA given at a frequency of up to 40 Hz increased blood flow with a significant effect between 2 and 20 Hz. However, when the frequency was further increased to between 30 and 40 Hz, EA induced significantly less pronounced changes in blood flow. At frequencies at or above 40 Hz, only marginal or no increases in blood flow were observed (Zhou *et al.*, 2011b). These results suggest that besides the locations stimulated (acupoints), the EA-induced increase in the blood flow is very sensitive to electrical frequency. Moreover, over-stimulation even at appropriate locations with optimal stimulation parameters may insult rather than protect the brain in spite that the stimulation still increases CBF (Zhou *et al.*, 2013a). Based on our recent evidence, we hypothesized that non-optimal (e.g., over-stimulation) may trigger some injury factors in the brain, for instance, an anti-DOR mechanism. Therefore, an optimal condition is extremely important for ideal outcome in acupuncture treatment for stroke.

Interestingly, other investigators made similar observations in studies using different models. In a study of EA-induced hypoalgesia in healthy volunteers, subjects were randomized to receive different durations (0 min, 20 min, 30 min, or 40 min) of asynchronous EA stimulations and then subjected to a hypoalgesia test (experimental cold thermal pain threshold model). Thirty minutes of asynchronous EA stimulation yielded the greatest hypoalgesic effect compared with 0, 20, or 40min stimulations (Wang *et al.*, 2009). Optimizing parameters for EA has also been noted to be critical in the cardiovascular system (Li and Longhurst, 2010).

Taken together, more preclinical translational research is needed to better define optimal conditions for maximal efficacy of acupuncture-induced protection from ischemic brain injury. Through it, we may generate valuable insight into applicable guidelines for an improved use of acupuncture for stroke treatment. Furthermore, such studies should establish a consensus on standardized relevant outcome measures in order to design and conduct appropriate randomized controlled trials that adopt those standards for evidence-based practices (Zhang *et al.*, 2009; Asakawa and Xia, 2012).

5. Medical gases

Medical gases, another non-drug approach, are neuroprotective against acute ischemic stroke. These gases range from traditional gases (oxygen and nitrous oxide) to gases such as nitric oxide, carbon monoxide, and hydrogen sulfide (Liu *et al.*, 2011b).

5.1. Oxygen

Hypoxia is a critical component contributing to neuronal death in stroke. Increasing brain tissue oxygenation has long been considered a logical strategy against cerebral ischemia. Oxygen, a commonly used therapeutic agent, has distinct advantages over pharmaceutical drugs (Liu *et al.*, 2011b). The ease of diffusion across the BBB (Hawkins and Egleton, 2008) as well as the relatively safe, well tolerated, and minimal dose-limiting side-effects (Singhal *et al.*, 2005b) position oxygen therapy to likely be effective at improving oxygen supply to brain tissue. Additionally, breakthroughs in chemical engineering have resulted in the creation of oxygen gas-filled microparticles that provide oxygen delivery through direct administration to the blood stream, leading to decreased levels of hypoxemia in rabbits (Kheir *et al.*, 2012). The design of a protocol for testing any potential neuroprotective effects of these microparticles in a stroke model has yet to be developed.

5.1.1. NBO—Although HBO (hyperbaric oxygen therapy) has been extensively studied, NBO (normobaric hyperoxia therapy) has distinct advantages. As HBO requires a chamber or other equipment to create a hyperbaric atmosphere, NBO is simple to administer, inexpensive and can be started by paramedics after stroke onset en route to a hospital. NBO reduced ischemic brain injury and improved functional outcome in several animal models (Singhal *et al.*, 2002a; Liu *et al.*, 2006; Henninger *et al.*, 2007; Yuan *et al.*, 2010; Wu *et al.*, 2012). The neuroprotective effects of NBO may be explained by many possible mechanisms, such as improved metabolism, reduced oxidative stress, and preservation of the BBB. These mechanisms are summarized in Figure 3.

5.1.1.1. Improvement in tissue oxygenation and metabolism: The maintenance of tissue oxygenation is critical for neural cells and is severely affected by stroke. The blockage of blood flow during a cerebral ischemic event induced a rapid decrease of localized interstitial pO_2 as measured by paramagnetic resonance oximetry (30% of pre-ischemic values in penumbra and 4% in the core during cerebral ischemia) (Liu *et al.*, 2004). NBO treatment improved cerebral perfusion and oxygenation in ischemic regions as evidenced by 2-D multispectral reflectance imaging and laser speckle flowmetry, as well as inhibited peri-infarct depolarizations (Shin *et al.*, 2007).

Abnormal metabolism has also been associated with brain injury following ischemic/hypoxic conditions, leading to tissue acidosis and ATP depletion. Ischemic-induced acidosis and ATP depletion in the border zones were attenuated by NBO in a model of focal cerebral ischemia (Sun *et al.*, 2011). In stroke patients treated with inhalation oxygen at a flow rate of 45L/min through a face mask for 8 hours, lactate, a marker for aerobic metabolism, decreased during NBO administration (Singhal *et al.*, 2007). The decrease in lactate suggests that aerobic metabolism can be improved following stroke by NBO. The minimization of energy metabolic dysfunction and the restoration of tissue oxygenation in the acute phase of stroke (i.e., en route to a hospital) might prolong the time window available for reperfusion therapies and the subsequent rescue of the penumbral region (Fisher and Bastan, 2012).

5.1.1.2. Reduction of oxidative stress: Oxidative stress is generated in tissues with abnormal oxygen levels and has been widely considered as one of the primary mechanisms leading to ischemic and/or reperfusion injury (Allen and Bayraktutan, 2009). Although one could hypothesize that oxygen therapy might increase the levels of reactive oxygen species, recent evidence indicates that a short duration of NBO treatment does not increase oxidative stress if started early after stroke onset. NBO treatment during focal cerebral ischemia-reperfusion not only did not increase oxidative stress, as measured by heme oxygenase-1 induction and protein carbonyl formation (Singhal *et al.*, 2002b), but was associated with the reduction of superoxide production and 8-OHdG generation, a biomarker of oxidative DNA damage (Liu *et al.*, 2006). These results indicate that, although NBO supplies oxygen to neural tissue, it appears to reduce oxidative stress. gp91^{phox} is the catalytic subunit of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, and a major source of reactive oxygen species. Pointing to a possible mechanism of how NBO may reduce oxidative stress, NBO inhibited the up-regulation of gp91^{phox} (Liu *et al.*, 2008c). In addition, genomic deletion of gp91^{phox} led to a situation where NBO was no longer able to reduce BBB leakage following cerebral ischemia (Liu *et al.*, 2011a). The effect of NBO on nitric oxide generation has also been examined following stroke. NBO administered during cerebral ischemia delayed and attenuated early nitric oxide generation, possibly through inhibiting nNOS (Yuan *et al.*, 2010). In this study, ischemia caused a rapid production of NO_x^- (nitrite plus nitrate), peaking at 10 min after stroke onset, and then gradually declining to the baseline level at 60 min. NBO delayed the NO_x^- peak to 30 min and attenuated the total amount of NO_x^- . Moreover, NBO showed a similar inhibitory effect on NO_x^- and 3-nitrotyrosine production as that of the specific nNOS inhibitor 7-nitroindazole.

Together, these studies suggest that under appropriate conditions NBO treatment may not cause an observable increase in the production of ROS, but instead may actually decrease ROS generation when penumbral pO₂ is maintained close to the pre-ischemic level.

5.1.1.3. Protection of the blood brain barrier: Induction of MMP-9 is highly associated with the post-ischemic breakdown of the blood brain barrier. NBO treatment inhibited MMP-9 induction in the ischemic brain, and was associated with reduced occludin degradation, Evan's blue extravasation and hemispheric swelling (Liu *et al.*, 2006; Liu *et al.*, 2008c; Liu *et al.*, 2009c), all of which suggesting that NBO treatment protects against BBB damage. The mechanism by which NBO suppresses MMP-9 and attenuates BBB damage appears to involve the above-mentioned inhibition of NADPH oxidase, because i) inhibition of NADPH oxidase with apocynin or knockout of gp91^{phox} resulted in much smaller magnitudes in MMP-9 induction and BBB leakage in the ischemic brain (Liu *et al.*, 2006; Liu *et al.*, 2008c; Liu *et al.*, 2011a), and ii) NBO did not further reduce the induction of MMP-9 when gp91^{phox} was knocked out (Liu *et al.*, 2011a). These results indicate that inhibition of NADPH oxidase-derived ROS production may be an important mechanism underlying NBO-afforded BBB protection. As the tightness of the BBB is crucial to ensure that the ischemic brain safely withstands the return of blood flow, early treatment with NBO may increase the therapeutic time window for thrombolysis and decrease the risk of hemorrhage by attenuating or slowing down the disruption of the BBB.

5.1.1.4. NBO plus rtPA thrombolysis: Both animal and human studies suggest that NBO slows down the process of cell death after stroke. The delay in cell death initiation by NBO may allow for an extension of the therapeutic time window for thrombolysis. The potential for combined NBO treatment with rtPA thrombolysis was recognized several years ago (Singhal *et al.*, 2002a; Henninger and Fisher, 2006). Indeed, several preliminary safety studies have emerged, indicating that the combination of NBO with rtPA did not increase hemorrhage volume at 10 hours or occurrence of confluent petechial hemorrhages at 24 hours in a rat embolic stroke model (Henninger *et al.*, 2009), nor did NBO increase cellular markers of superoxide generation or brain levels of MMP-9 (Kim *et al.*, 2005). These data provide important initial evidence to support the feasibility of combination therapy with NBO and rtPA in ischemic stroke.

However, further studies have yielded inconsistent results as to the proposed synergistic relationship between NBO and rtPA. In one study, combined NBO and rtPA significantly reduced the mortality rate, brain edema, hemorrhage, and MMP-9 augmentation in a filament occlusion rat model with 5-hour ischemia followed by 19-hour reperfusion, as compared with rtPA alone (Liu *et al.*, 2009b). In contrast, another study indicated that NBO itself achieved cerebral blood flow restoration equivalent to rtPA, possibly through direct interaction with endogenous tPA molecules, promoting endogenous fibrinolysis (David *et al.*, 2012b). The combination of NBO and rtPA had no neuroprotective effect on ischemic brain damage in this study, and was concluded to possibly increase the risk of hemorrhage (David *et al.*, 2012b). Further investigation is required to assess the effect of NBO and rtPA combinatorial strategies.

5.1.2. HBO—Although HBO lacks the ready accessibility to treatment compared to NBO, HBO may be even more potent than NBO at conferring ischemic protection (Liu *et al.*, 2011b; Rockswold *et al.*, 2010). Recent studies indicated that the maximal benefits from HBO therapy require early post-stroke administration, short durations, and tissue reperfusion (Veltkamp *et al.*, 2005; Yang *et al.*, 2010). From these studies and others, we have insight into critical issues surrounding HBO therapy, such as the therapeutic time window of HBO and the optimal chamber pressure, and from this, a multicenter HBO trial is currently being planned.

5.1.3. Gas-induced preconditioning—Intermittent hyperoxia has been shown to induce ischemic preconditioning. Compared with hypoxic preconditioning, hyperoxia preconditioning may be safer and more readily available in clinical practice. Consistent with a role for hyperoxia in establishing a preconditioned state, prior exposure to alveolar hyperoxia prevented the hypoxia-induced enhancement of bronchial reactivity to carbachol and histamine (D'Brot and Ahmed, 1991). These studies were carried forward into models of cerebral ischemia, where repeated exposure to HBO (100% oxygen at 2ATA for 1h each) increased the tolerance of the brain against ischemic neuronal damage, and induced HSP-72 expression (Wada *et al.*, 1996). Since then, the protective effects of hyperoxia preconditioning, especially HBO preconditioning, have been investigated in many animal models. Studies have suggested that the protective effects of HBO preconditioning against ischemic injury involves the suppression of inflammatory responses, such as hemeoxygenase-1 (He *et al.*, 2011; Liu *et al.*, 2011c), HIF-1 α (Peng *et al.*, 2008), and COX-2 (cyclooxygenase-2) (Cheng *et al.*, 2011). HBO preconditioning against ODG in cortical neuronal cultures activated the PPAR γ (Peroxisome proliferator-activated receptor γ) pathway, which in turn increased downstream antioxidant enzymatic activities (Zeng *et al.*, 2012). HBO preconditioning attenuated hemorrhagic transformation that correlated with decreased HIF-1 α and MMP-2 and MMP-9 activity in hyperglycemic MCAO rats (Soejima *et al.*, 2012; Soejima *et al.*, 2013). HBO preconditioning also abated mitochondrial-mediated apoptosis in ischemic tissue (Li *et al.*, 2009a), and was associated with autophagy activation (a vital cellular pathway for the degradation of intracellular abnormal mitochondria) following focal cerebral ischemia in rats (Yan *et al.*, 2011).

Intermittent NBO has also been shown to induce ischemic conditioning. When compared to continuous NBO, intermittent NBO treatment (4 short cycles of intermittent NBO/air treatment) administered during ischemia provided similar neuroprotection when assessed at 24 hours after reperfusion, but greater neuroprotection when observed at 72 hours following reperfusion (Liu *et al.*, 2012a). Thus, the extended timeframe to induce neuroprotection suggests that, besides providing oxygen to the ischemic tissue, intermittent NBO likely exerts its neuroprotection through other mechanisms, such as triggering a novel form of postconditioning, such as the attenuation of superoxide generation.

Bigdeli *et al.* investigated the neuroprotective effect of prolonged and intermittent hyperoxia preconditioning. Their results demonstrated that the neuroprotection of intermittent exposure was superior to that of prolonged exposure (24-hour exposure in this study was unacceptable) (Bigdeli *et al.*, 2007).

5.1.4. Clinical trials using therapeutic oxygenation (Hyperoxia preconditioning)—Emerging data suggest that therapeutic oxygenation (normobaric and hyperbaric oxygen therapy) may have positive effects on the neuroprotection of acute ischemic stroke patients, and may even extend the narrow therapeutic time window for stroke thrombolysis as an adjunctive therapy with rtPA (Henninger and Fisher, 2006). To date, the neuroprotective effects of therapeutic oxygenation have been investigated in various models, including global ischemia, forebrain ischemia, surgical brain injury, ischemia-reperfusion injury, neonatal hypoxia-ischemia, intracerebral hemorrhage, and spinal cord ischemia.

It is important to note that supplemental oxygen is not equivalent to therapeutic oxygenation. Supplemental oxygen often requires inhaled oxygen concentrations approaching 100%, and, according to an early observational study, has been associated with a worse one year survival rate in patients with mild-to-moderate stroke (Ronning and Guldvog, 1999). Whether supplemental oxygen is beneficial to patients with severe strokes requires further research. However, supplemental oxygen is not recommended in non-

hypoxic patients with acute ischemic stroke under current stroke guidelines (Jauch *et al.*, 2013).

HBO has failed to show efficiency in three randomized clinical trials, and has been associated with severe limitations, including the possibilities of exacerbating oxygen free radical injury and incurring stroke secondary to air embolization (Anderson *et al.*, 1991; Nighoghossian *et al.*, 1995; Rusyniak *et al.*, 2003). The relationship between HBO in acute ischemic stroke and outcomes of mortality, functional health, and adverse effects was explored by a meta-analysis of clinical studies from four randomized control trials. The study concluded that, based on the current evidence, a beneficial role of HBO for patients with stroke was not supported (Carson *et al.*, 2005). As a result, HBO is not recommended for the treatment of acute stroke in the latest guidelines (Jauch *et al.*, 2013). However, the strength of the conclusions from this meta-analysis was hampered by the determination that one controlled clinical trial and 17 observational studies were deemed to be of “poor quality” and limitations in sample size (Carson *et al.*, 2005).

NBO has been shown to induce vasoconstriction and improve dynamic functional cerebral blood flow in salvageable tissue (Wu *et al.*, 2012), as well as increasing O₂ availability, reducing metabolic disturbances and protecting the blood brain barrier (Shin *et al.*, 2007; Liu *et al.*, 2011a). In addition, it is simple to administer, noninvasive, inexpensive, widely available, and can be started promptly after stroke onset. In 2005, a study at Massachusetts General Hospital investigated the effects of normobaric oxygen therapy in acute ischemic stroke; eight patients that received high-flow oxygen therapy (45 L/min for 8 h) were evaluated by stroke scale scores (NIHSS) and MRI scans (DWI lesion and the DWI-PWI mismatch). High-flow oxygen within 12 hours after onset of ischemic stroke was associated with a transient improvement of neurological impairment (Singhal *et al.*, 2005a). With the addition of three more cases and assessments with multi-voxel magnetic resonance spectroscopic imaging and diffusion/perfusion MRI, NBO therapy decreased lactate levels and preserved N-acetylcysteine levels in the brain, suggesting that NBO improves aerobic metabolism and preserves neuronal integrity in the ischemic brain (Singhal *et al.*, 2007). A subsequent clinical trial using MRI Voxel-based algorithms to evaluate NBO treatment in early phase showed that ischemic lesion growth is attenuated during NBO treatment at 4h (Liu *et al.*, 2012b).

A larger scale trial was initiated in 2007 at Massachusetts General Hospital to compare the safety and therapeutic efficacy of NBO (started within 9 hours of symptom onset) to those of room air (30–45L/min for 8 h). After 85 enrollments, it was terminated because of the imbalance in deaths favoring control arm (ClinicalTrials.gov, Identifier: NCT00414726). In 2010, a randomized pilot study in India evaluated the effect of normobaric high-flow oxygen therapy in patients with acute ischemic stroke by the clinical (NIHSS, mRS) and radiological (MRI) measurements. Twenty patients were randomized to receive NBO. In contrast to the above studies, the result showed that NBO did not improve the clinical scores of stroke outcome in patients with acute ischemic stroke. Furthermore, adverse effects were observed, including cardiac/pulmonary side effects and free radical injury (Padma *et al.*, 2010).

Taken together, the available preclinical and clinical data suggest that NBO may present a promising therapeutic strategy in acute ischemic stroke and TIA (Hadjiev and Mineva, 2009; Hadjiev and Mineva, 2010). Significant benefits may be achieved simply by administering pure oxygen via a face mask, instead of hyperbaric chambers. But its potential risk and curative effect need to be carefully evaluated. Further studies are needed to ensure the safety of NBO and investigate the optimal usage of NBO, including therapeutic time window, duration, flow rates, the combination of thrombolysis and the subtype of the ischemic stroke.

Other associated therapies including the combination of minocycline, cilostazol and edaravone also require more clinical evidence (Nonaka *et al.*, 2008; Nonaka *et al.*, 2009; Jin *et al.*, 2013). Lastly, prospective studies using modern neuroimaging methods are needed to assess the degree and durability of clinical benefits, especially the long-term benefits, from this treatment.

5.2. Other gases

In addition to oxygen, laboratory and clinical evidence has also found that other gases may play a beneficial role in the treatment of stroke. For example, the administration of xenon, argon and helium has resulted in effective neuroprotection in rat models of neonatal hypoxia/ischemia (Zhuang *et al.*, 2012).

Emerging data support the hypothesis that hydrogen, which has the advantage of crossing the BBB easily, might act as a free radical scavenger of hydroxyl radicals, thus promoting neuroprotection. Hydrogen (2%) both reduced the amount of toxic hydroxyl radicals and significantly reduced infarct volume following transient MCAO in rats (Ohsawa *et al.*, 2007). Studies using other rat models confirmed the effects of hydrogen on the improvement of neurological function (Cai *et al.*, 2008; Chen *et al.*, 2010; Matchett *et al.*, 2009). A recent clinical study treated 13 cerebral ischemia patients with hydrogen inhalation and showed that 3–4% hydrogen inhalation over 30 mins was feasible and safe in stroke patients (Ono *et al.*, 2012).

Helium was proposed as a therapeutic gas in 1934, and subsequently heliox (helium/oxygen gas mixture) was proposed as a potential therapy for upper and lower airway obstruction (Barach, 1934). In a side by side comparison of the efficacy of hyperoxia and that of heliox in a rat transient MCAO model, heliox was more effective than hyperoxia in reducing infarct volume and improving neurological deficits if initiated immediately after the onset of ischemia (Pan *et al.*, 2011; Pan *et al.*, 2007). The best protective outcome was observed when heliox (70% helium) was administered immediately after the occlusion (Pan *et al.*, 2011; Pan *et al.*, 2007). Additionally, the protective actions of heliox therapy have been demonstrated in traumatic brain injury, cardiac ischemia and hypoxia ischemia (Coburn *et al.*, 2008; Pagel *et al.*, 2007), but the exact mechanisms behind these protective effects are still unclear.

Xenon is used as an anesthetic gas, and because of its NMDA (N-methyl-D-aspartate) receptor inhibition properties, it has potential neuroprotective roles during instances of brain ischemia. The protective effects of xenon have been identified in various models of rodent hypoxic/ischemia (Natale *et al.*, 2006; Dingley *et al.*, 2008; Ma *et al.*, 2009), but clinical evidence is still limited. In addition to its effects as a single therapeutic, xenon has also been noted to have synergistic/antagonistic neuroprotective effects when used with other therapeutic strategies. The combination of xenon and dexamethasone resulted in neuroprotection (Behrens *et al.*, 1998), and a combination treatment of xenon and hypothermia in both rat and pig models was also neuroprotective (Fries *et al.*, 2012; Sheng *et al.*, 2012b). However, xenon can attenuate rtPA-mediated protection by acting as an inhibitor of rtPA (David *et al.*, 2010), thus any clinical use of xenon would have to occur either in the absence or following rtPA administration.

The neuroprotective effects of argon have been supported by studies both *in vitro* and *in vivo*, and it carries the advantage of being both more affordable and common than xenon, while still capable of protecting against excitotoxic injury (Loetscher *et al.*, 2009; Ryang *et al.*, 2011; David *et al.*, 2012a;). Argon is thought to induce GABA neurotransmitters and thus inhibit NMDA, but, as GABA receptor activation has additional downstream pathway influences, the protective mechanisms at work during argon administration have yet to be

fully determined. Still, promising preclinical results for argon following ischemia suggest that it could be further examined as a potential neuroprotectant for ischemic stroke in a clinical setting.

Taken together, although the neuroprotective effects of these therapeutic gases have been proposed, the definite efficacy and mechanisms should be further validated through in-depth laboratory research and clinical trials.

6. Transcranial laser therapy

TLT (Transcranial laser therapy) has been investigated as a neuroprotective treatment for acute ischemic stroke over 10 years. It may promote functional and behavioral recovery via energy metabolism and by enhancing cerebral blood flow. Two clinical trials, NEST (NeuroThera Effectiveness and Safety Trial)-1 and NEST-2, have evaluated the use of TLT to promote clinical recovery in patients with acute ischemic stroke.

6.1. Mechanisms

Although the mechanism is still incompletely understood, specific wavelengths of near-infrared light can penetrate through the scalp, skull, and the surface of brain tissue, and then convert from light to bioenergy. First, the specific wavelengths (808nm) of light excite cuprous moieties of the mitochondrial cytochrome C oxidase enzyme. This energy absorption within mitochondria increases ATP, leading to significant improvements in energy metabolism and cell viability. Such maintenance of homeostatic cellular functions helps to prevent the spread of ischemia-induced death within the penumbra (Mochizuki-Oda *et al.*, 2002; Desmet *et al.*, 2006; Lapchak and De Taboada, 2010). The biological effects of TLT were shown to be specific to the wavelength and not due to thermal effects of the laser beam (Lapchak, 2010). Other mechanisms may contribute to TLT-induced neuroprotection, such as the promotion of neurogenesis, the production of endogenous neurotrophic factors and the increase of CBF alongside the concentration of cortical NO (Leung *et al.*, 2002; Oron *et al.*, 2006; Uozumi *et al.*, 2010). Taken together, TLT imposes neuroprotective effects by inducing photobiological stimulation to increase mitochondrial energy supply and CBF.

6.2. Clinical trials

Two randomized double-blind clinical trials with TLT induced by the NeuroThera device (Photothera, Carlsbad, CA) using near-infrared light with a wavelength of 808nm have been completed with reproducible and measurable clinical improvement in acute ischemic stroke patients (Lampl *et al.*, 2007; Zivin *et al.*, 2009). A third trial is currently being developed (Zivin *et al.*, 2012).

The NEST-1 was a prospective, double-blinded, randomized, placebo-controlled, multicenter trial involving 120 subjects with strokes who were ineligible for intravenous thrombolysis. In 120 patients, 79 patients received TLT therapy that used low-energy ($10\text{mW}/\text{cm}^2$) lasers at 20 predetermined locations on the scalp with 2 min of irradiation per site, over 20 sites, within 24 hours (mean application at 16 hours) after stroke onset. Significantly better neural function outcome (mRS and NIHSS) in the TLT-treated group were observed compared to the control group. In addition, there was no difference in mortality. All evidence indicated the safety and effectiveness of the treatment for ischemic stroke in humans (Lampl *et al.*, 2007).

A larger follow-up phase III trial, NEST-2, used an identical procedure as described for NEST-1. In 660 patients, 331 patients who were accorded with the specific criterion received TLT within an average of 14.6 h post-stroke, and failed to find the same significant

effectiveness in NEST-1. However, it confirmed the safety of the therapy and the suitable range of stroke patients (Zivin *et al.*, 2009).

The NEST-3 trial (ClinicalTrials.gov, Identifier: NCT01120301), with the purpose of demonstrating the safety and efficacy of TLT in the treatment of acute ischemic stroke by assessing the mRS at 90 days post stroke, is a double-blind, randomized, sham-controlled, parallel group, multicenter, pivotal study and planned to enroll 1000 subjects. (Zivin *et al.*, 2012). But NEST-3 failed to reach significance upon interim analysis in October 2012. The independent Data Monitoring Committee (DMC) found that the trial failed to pass a pre-specified hurdle for efficacy in a futility analysis performed on greater than 550 randomized patients who had completed their Day 90 evaluation. Upon unblinded review of the data, the DMC identified no safety concerns with the therapy. But no efficacy was detected either. So enrollment into the NEST-3 study was suspended and PhotoThera (the sponsoring company) has closed and dissolved.

Lapchak et al used a rabbit large clot embolic stroke model to determine the safety TLT in combination with rtPA, and demonstrated that TLT did not significantly affect hemorrhage rate, hemorrhage volume and the 24-hour survival rate in embolized rabbits (Lapchak *et al.*, 2008). Future clinical trials with an enrollment of patients receiving rtPA should be applied to confirm the neuroprotection of TLT.

Since the efficiency of TLT therapy is still in question, further studies are likely to shed some light on the benefits and shortcomings of TLT therapy. The thermal effects of the laser beam may also affect safety and efficiency. Furthermore, electromagnetic wave energy is mainly concentrated on cortical tissue and cannot reach the deep brain parenchyma. Thus, the application of TLT in acute ischemic stroke is still in its early stages, and larger and more specific clinical trials are required to confirm optimal laser settings, patient population, therapeutic time windows, as well as to develop more advanced devices, all aimed towards creating a new paradigm for the treatment of acute ischemic stroke.

7. Mechanical Endovascular Recanalization

Mechanical endovascular recanalization therapies rehabilitate cerebral blood flow in occluded arteries, including thrombectomy devices which extract, disrupt and aspirate clots, balloon angioplasty or stents which displace clots to the periphery of vessels, and augmenting perfusion devices. These therapies require tailor made devices, qualified organizations, competent medical staff, and above all, timely treatment.

The common devices of recanalization include the Merci Retriever, Penumbra System, Phenox Retriever, Catch Device, Solitaire Device, and the Trevo Device. As indicated by the FDA (Food and Drug Administration), Merci is used for restore neurovascular flow, Penumbra is used for revascularization for acute stroke, and Solitaire and Trevo are approved for intracranial clot removal.

Two small scale trials indicated that the Solitaire Device and Trevo Device show better safety and efficacy than the Merci Retrieval System in endovascular recanalization in acute ischemic stroke (Nogueira *et al.*, 2012; Saver *et al.*, 2012). The latest guidelines recommend that, although the usefulness of the Merci, Penumbra System, Solitaire FR, and Trevo thrombectomy devices is certain, proven efficacy still needs more clinical evidence to improve patient outcomes. The usefulness of other mechanical thrombectomy devices as well as emergent intracranial angioplasty/stenting have not been established (Jauch *et al.*, 2013).

Research on ultrasound enhanced recanalization is also ongoing. Owing to acoustic cavitation, ultrasound devices may enhance thrombolysis and improve recanalization rates compared to IV rtPA alone, thereby having the potential to improve the safety and efficacy of rtPA treatment. Adjuvant ultrasound using microbubbles enhanced rtPA thrombolysis *in vitro* and *in vivo* (Hitchcock and Holland, 2010; Nolte *et al.*, 2013). By using a combination of ultrasound and endovascular techniques, the ultrasound transducer can be placed at the tip of the endovascular catheter can thus circumvent interference by bone (Tachibana *et al.*, 1999). A SPOTRIAS study carried out by Dr. Grotta's group is the development of a hands-free transcranial ultrasound unit, and its safety and efficacy is currently being tested in a phase 3 international prospective randomized study (NIH RePORT Project Number: 5P50NS044227-10). If positive, ultrasound enhanced thrombolysis could become widely applicable wherever IV rtPA is given.

Recently, several studies regarding the use of endovascular treatment for acute ischemic stroke with large artery occlusion yielded negative results, and have presented additional challenges to the creation of effective stroke therapies. The IMS III (interventional management of Stroke III) was a phase III, randomized, multicenter, open label, 656 subject (434 patients to endovascular therapy and 222 to intravenous rtPA alone) clinical trial (2006–2012). This study randomly assigned eligible patients who had received intravenous rtPA within 3 hours after symptom onset to receive additional endovascular therapy (Merci, Penumbra system, Solitarie FR) or intravenous rtPA alone, and compared the mRS and NIHSS scores between the combined therapy group and the intravenous rtPA alone group. Although the trial demonstrated similar safety outcomes, no significant difference in functional independence with endovascular therapy after intravenous rtPA was found, as compared with intravenous rtPA alone (Broderick *et al.*, 2013).

The SYNTHESIS Expansion was a multicenter, open-treatment clinical trial with a blinded end point. 362 patients (181 patients received endovascular therapy and 181 intravenous rtPA) with acute ischemic stroke within 4.5 hours after stroke onset were randomly assigned to study groups (2008–2012). In this study, endovascular therapy did not appear superior to standard treatment with intravenous rtPA in safety and efficacy (Ciccone *et al.*, 2013).

The MR RESCUE (mechanical retrieval and recanalization of stroke clots using embolectomy) was a phase IIb, randomized, controlled, open-label (blinded outcome), and multicenter trial (2004–2011). Randomly assigned patients within eight hours after the onset of large-vessel, anterior-circulation strokes were subjected to mechanical embolectomy (Merci Retriever or Penumbra System) or received standard care. All patients were stratified according to the existence of penumbra as measured by pretreatment computed tomography or magnetic resonance imaging of the brain. The presence of a favorable penumbral pattern on neuroimaging did not lead to a differentiation of patients who might benefit more from endovascular therapy for acute ischemic stroke. (Kidwell *et al.*, 2013). In addition, embolectomy was not found to improve outcomes over the standard care.

Although the conclusions above are consistent and argue against the earlier hypothesis that endovascular therapy may be associated with improved outcomes, there are several limitations of these studies that may affect the strength of the conclusions. First, efficacy is strongly related to the rapidity of initiation of treatment, and the time of interventional therapies in these studies was delayed as compared with rtPA therapy. The minimization of any delays to endovascular therapy initiation could significantly affect the results. Secondly, the time spans of these studies are long, and there were advances in techniques and clinical practices during the study period. Therefore, the efficacy of the new devices and as compared with intravenous rtPA alone remains insufficient. Third, the neuroimaging came from a single time point and may have changed by the time of recanalization in patients

undergoing embolectomy. Follow-up imaging was also not available for all patients. The latest imaging technologies and real-time analysis will be helpful to provide more accurate and credible results. In addition, the lack of a significant benefit of endovascular therapy may be related to the counteraction which is brought about by the operation, and unnecessary damage may be minimized by the accumulation of operational experience. However, facing the currently high rate of recanalization with endovascular treatment, overly accelerated steps should be halted in order to comprehensively theorize and deliberate over which future directions to take with endovascular mechanical recanalization therapy.

8. Recovery devices for chronic phase of stroke

Technology such as robotic devices, repetitive transcranial magnetic stimulation, functional electrical stimulation, vagus nerve stimulation, and transcranial direct current stimulation aims to improve recovery in the chronic phase of stroke. These devices are designed and developed to improve speech, motor and sensory function by facilitating brain function recovery and to preventing disuse myophagism. We divided them into two categories, i.e., the stimulus supporting devices and the exercise recovery devices.

The stimulus supporting devices contain FES (Functional Electrical Stimulation), rTMS (repetitive Transcranial Magnetic Stimulation), VNS (Vagus Nerve Stimulation), and tDCS (transcranial Direct Current Stimulation) (Wilkie *et al.*, 2012; Kondo *et al.*, 2012; Bastani and Jaberzadeh, 2012; Khodaparast *et al.*, 2013). The FES devices facilitate motor ability and decrease activity limitation caused by stroke. The rTMS is a noninvasive method of restoring excitability and voluntary control of down-regulated neurons in the stroke hemisphere. It has been shown to be effective in the setting of low-frequency stimulation. VNS significantly reduces the extent of stroke-induced lesion of brain parenchyma and results in movement during rehabilitation. The tDCS is used to polarize underlying brain regions through the application of weak direct currents by electrodes on the scalp. The current regulates the neuronal excitability to facilitate cortical function.

Exercise recovery devices are deemed as bioengineered devices that focus on extremity impairment reduction after training. Meanwhile, they can measure motor performance objectively and will contribute to a detailed phenotype of stroke recovery. For instance, the AMES (assisted movement with enhanced sensation) device is designed to produce functional cortical changes by assisting the subject as the patient attempts to move the limb (assisted movement) and enhancing movement sensation by vibrating the muscles during movement (enhanced sensation). The training performs more effectively when in combination with EMG Biofeedback (Cordo *et al.*, 2009).

The BCI (Brain Computer Interface) device for stroke rehabilitation has been developed to assist people with motor disability. It works by detecting the motor intent of the user from electroencephalogram signals to drive the robotic rehabilitation via Haptic Knob. The developmental direction of this device is moving towards decreasing electrode interference and improving the accuracy of results (Daly *et al.*, 2009; Tam *et al.*, 2011).

9. Concluding remarks

Stroke is a serious neurological condition involved in complex pathophysiological mechanisms at multiple levels (Clarke and Sokoloff, 1999; Chao and Xia, 2010; Puyal *et al.*, 2013; He *et al.*, 2013). A single pharmaceutical drug may not work properly at all critical levels in ischemic brain injury. This may be one of the key reasons behind the past unsuccessful efforts to achieve therapeutic goals through a pharmacological means. In contrast, non-pharmaceutical treatment such as hypothermia, preconditioning and

acupuncture may induce a comprehensive effect on the ischemic brain at different levels, thus protecting the brain from ischemic injury. Current research has shed a light on such a possibility. In fact, even physical exercise can modulate mitochondrial-mediated pathways and thus protect the brain from injury (Marques-Aleixo *et al.*, 2012). Furthermore, a combination of various treatment strategies may be the most hopeful methodology due to flexibility and multiple targets of action.

However, more in-depth research is required for translatable and effective applications of these non-pharmaceutical approaches in clinical settings. For example, the benefits brought forth by the research and development of new devices alongside the advancement of physical therapies are limited and cannot be reliably quantified. As a result, therapies based on the pathophysiological, cellular and molecular biological mechanisms combined with the multimodal imaging are still in need of higher levels of clinical evidence. Moreover, although many studies show that acupuncture is potentially a useful modality for the treatment of stroke, its optimal conditions and therapeutic time windows for clinical practice need a strong basis supported by solid data from preclinical and translational research.

Translational medicine is the key success factor in developing stroke treatment, as well as improved the efficiency of translation. It is our belief that we may eventually find an efficient and effective way to apply non-pharmaceutical modalities to achieve maximal benefits for ischemic brain by scientifically quantifying therapeutic outcomes with reliable indexes and parameters, elucidating the precise mechanisms underlying the outcomes/effects, and eventually establishing practicable, and personalized therapeutic protocols.

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Abbreviations

ACP	antegrade cerebral perfusion
AMES	assisted movement with enhanced sensation
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
BAIPC	bilateral arm ischemic preconditioning
BBB	blood brain barrier
BCI	Brain Computer Interface
Bcl-2	B-cell lymphoma-2
BDNF	brain derived neurotrophic factor
CBF	cerebral blood flow
CREB	cyclic adenosine monophosphate response element binding protein
COOL AID	Cooling for Acute Ischaemic Brain Damage
COX-2	cyclooxygenase-2
DADLE	(D-Ala ² , D-Leu ⁵)-enkephalin)
DOR	δ -opioid receptor
DWI	diffusion-weighted imaging

EA	electro-acupuncture
eNOS	endothelial nitric oxide synthase
ERK	extracellular regulated protein kinases
ENT1	equilibrative nucleoside transporter 1
FDA	Food and Drug Administration
FES	Functional Electrical Stimulation
GABA	gamma-aminobutyric acid
GluR2	glutamate receptor 2
GSK3β	Glycogen synthase kinase-3 β
HBO	hyperbaric oxygen therapy
HIF	hypoxia inducible factor
HPC	Hypoxic preconditioning
HSP70	heat shock 70 kDa protein
IAS	intracranial arterial stenosis
ICTuS-L	Intravenous Thrombolysis Plus Hypothermia for Acute Treatment of Ischemic Stroke
IMS III	interventional management of Stroke III
IκB	inhibitor of NF- κ B
IKK	inhibitor of NF- κ B kinase
iNOS	inducible nitric oxide synthase
IPC	ischemic postconditioning
MAPK	mitogen-activated protein kinase
MCAO	middle cerebral artery occlusion
MMP	matrix metalloproteinase
MR RESCUE	mechanical retrieval and recanalization of stroke clots using embolectomy
mRS	modified Rankin Scale
NADPH	nicotinamide adenine dinucleotide phosphate
NBO	normobarichyperoxia therapy
NEST	NeuroThera Effectiveness and Safety Trial
nNOS	neuronal nitric oxide synthase
NF-κB	nuclear factor- κ B
NIHSS	National Institutes of Health Stroke Scale
NMDA	N-methyl-D-aspartate
NO$_x^-$	nitrite plus nitrate
PI3-kinase	phosphatidylinositol 3-kinase

PGC-1α	α subunit of peroxisome proliferators-activated receptor- γ coactivator-1
PKA	protein kinase A
PKC	protein kinase C
pO₂	partial oxygen
PPARγ	peroxisome proliferator-activated receptor γ
PTEN	phosphatase and tensin homologue
RIPostC	Limb remote ischemic postconditioning
RNS	reactive nitrogen species
ROS	reactive oxygen species
ROSC	Return of Spontaneous Circulation
rTMS	repetitive Transcranial Magnetic Stimulation
rtPA	recombinant tissue plasminogen activator
SphK	Sphingosine kinase
SPOTRIAS	specialized program in acute stroke
STAIR	Stroke Therapy Academic Industry Roundtable
TCM	Traditional Chinese Medicine
tDCS	transcranial Direct Current Stimulation
TIA	transient ischemic attack
TLT	Transcranial laser therapy
TNF-α	tumor necrosis factor α
VE	vessel endothelium
VNS	Vagus Nerve Stimulation
WHO	World Health Organization

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Highlights

- Recent research in non-pharmaceutical therapies for ischemic stroke and potential mechanisms
- Hypothermia, preconditioning, acupuncture, medical gas and other therapies for acute stroke
- Mechanical endovascular recanalization and recovery devices for the chronic phase of stroke

Fig. 1. Potential mechanisms of hypothermia-induced protection from ischemic brain injury

Note that hypothermia therapy may reduce neurological injury through cellular and molecular regulation by decreasing metabolism and energy consumption, attenuating mitochondrial injury, inhibiting inflammatory mediators; decreasing MMPs proteolytic activity, inhibiting the FasL signaling pathway protecting the blood brain barrier, increasing HSP70 expression, inactivating NF- κ B and downstream transcriptional targets, limiting calcium influx through AMPA, decreasing excitotoxicity, etc..

Fig 2. Mechanisms of IPC/HPC protection from ischemic brain injury

Note that IPC/HPC may block the biochemical cascades that cause brain injury following cerebral ischemia by improving CBF through PKC activation and adenosine transporter inhibition, inhibiting apoptosis through the PI3-kinase/Akt pathway, protecting BBB by diminishing MMP-9 and increasing SphK2, stimulating DOR signaling and thus regulating survival and death signals, inhibiting glutamate toxicity and maintaining ionic homeostasis, attenuating acute inflammatory processes through the PKC/ERK pathway, inhibiting mitochondrial cell death signaling through the AKT/PKC/MAPK/ERK pathway increasing autophagy.

Fig. 3. Possible pathways involved in oxygenation-induced brain protection in ischemia

Note that hyperoxia therapy may induce neuroprotection against acute ischemic stroke by improving tissue oxygenation and metabolism, reducing ischemia-induced acidosis and ATP depletion, up-expressing MMP-9, attenuating BBB leakage, inhibiting NADPH oxidase and decreasing free radical (ROS/RNS), thus reducing oxidative stress.