

● INVITED REVIEW

Neuroprotective mechanisms and translational potential of therapeutic hypothermia in the treatment of ischemic stroke

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Abstract

Stroke is a leading cause of disability and death, yet effective treatments for acute stroke has been very limited. Thus far, tissue plasminogen activator has been the only FDA-approved drug for thrombolytic treatment of ischemic stroke patients, yet its application is only applicable to less than 4–5% of stroke patients due to the narrow therapeutic window (< 4.5 hours after the onset of stroke) and the high risk of hemorrhagic transformation. Emerging evidence from basic and clinical studies has shown that therapeutic hypothermia, also known as targeted temperature management, can be a promising therapy for patients with different types of stroke. Moreover, the success in animal models using pharmacologically induced hypothermia (PIH) has gained increasing momentum for clinical translation of hypothermic therapy. This review provides an updated overview of the mechanisms and protective effects of therapeutic hypothermia, as well as the recent development and findings behind PIH treatment. It is expected that a safe and effective hypothermic therapy has a high translational potential for clinical treatment of patients with stroke and other CNS injuries.

Key Words: stroke; therapeutic hypothermia; drug-induced hypothermia; ischemia; cell death; inflammation

Introduction

Although recently stroke fell from the fourth to the fifth leading cause of human death in the United States, each year there are still approximately 750,000 individuals who suffer a new or recurrent stroke (ischemic or hemorrhagic) (Writing Group et al., 2016). About 610,000 of them encounter a primary stroke attack, and 185,000 have recurrent stroke events. A stroke attack occurs every 40 seconds, resulting in stroke-related deaths every 4 minutes in the United States. Due to the increasing aged population, the American Heart Association (AHA) has projected that healthcare costs associated with stroke would increase dramatically in the next 20 years according to the increased incidence and prevalence of stroke.

Despite tremendous advancements in understanding the pathogenesis and cellular/molecular mechanisms of stroke over the last few decades, thrombolytic therapy using tissue plasminogen activator (tPA) has been the only drug approved by the Food and Drug Administration (FDA) for treating acute ischemic stroke (Group, 1995). Although recent data show that the rates of tPA application has been increased between 2005 and 2009, still only < 4% of all stroke patients can benefit from the thrombolytic treatment. This is mainly due to the required early treatment and a high risk

of hemorrhagic transformation associated with tPA applications (Adeoye et al., 2011). Thus, there is an urgent need to develop new therapies that have wider therapeutic windows and are more effective for more stroke patients (Shobha et al., 2011).

Over the past two decades, many neuroprotective drugs and treatments have failed the clinical translation from animal models to clinical practice due to the lack of efficacy and, in some cases, intolerable side-effects (Cheng et al., 2004). With the increasing understanding of the cellular and molecular injurious pathways and their interplays in ischemic cascades, it is now increasingly recognized that the conventional strategy of targeting a specific inhibitory or excitatory neuronal receptor or ion channel or a single signaling pathway/gene is far from enough to battle the overwhelming pathophysiological cascades that occur acutely, sub-acutely, and even chronically after a stroke attack. Thus, a global protection paradigm that covers different cell types including neuronal and non-neuronal cells and multiple signaling pathways is necessary to achieve clinically meaningful benefits for stroke patients.

Compelling evidence from pre-clinical research in animal models demonstrated marked protective effects of mild to moderate hypothermia (therapeutic hypothermia) against

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ischemic and hemorrhagic brain damage (Darwazeh and Yan, 2013; Chamorro et al., 2016). Therapeutic hypothermia ameliorates brain damage through inhibition of multiple pathways such as oxidative stress, inflammatory responses, metabolic disruption, and cell death signals (Katz et al., 2004; Choi et al., 2012). Furthermore, therapeutic hypothermia therapy improves functional outcomes in animal models of stroke and traumatic brain injury (TBI) (Polderman et al., 2002; Choi et al., 2012; Lee et al., 2014). In humans, multiple clinical trials involving both surface cooling and endovascular hypothermia have been effective in decreasing certain quantitative metrics that correspond with functional outcomes after TBI, including intracerebral pressure (ICP) and the mean diffusion-weighted imaging (DWI) lesion growth (Schwab et al., 1998a, b, 2001; De Georgia et al., 2004). In clinical practice, mild to moderate hypothermia (3–5°C reduction) is safe and has been used for the treatments of cardiac arrest and hypoxic-ischemic encephalopathy (Dae et al., 2003; Xiao et al., 2013). In fact, therapeutic hypothermia has been incorporated in the American Heart Association (AHA) guidelines for post-resuscitation care for more than 10 years (Sugerman and Abella, 2009). Thus, both pre-clinical and clinical evidence supports that therapeutic hypothermia has a promising potential to be an effective treatment for acute brain injury such as stroke and TBI (Schwab et al., 1998b, 2001; De Georgia et al., 2004; van der Worp et al., 2007; Torok et al., 2009; Kim et al., 2011; Yenari and Han, 2012; Lee et al., 2016a). Currently, there is no other experimental stroke therapy that has demonstrated such strong potential in both basic and clinical research, although challenges on the efficacy and the mechanism of action call for future and more specific investigations (Tahir and Pabaney, 2016). This review will focus on emerging concepts in the protective mechanisms of therapeutic hypothermia for treating patients with stroke, as well as highlighting pharmacologically induced hypothermia (PIH), or drug-induced cooling treatments, that can be applied as an acute hypothermic treatment with some unique advantages.

Mechanisms of Therapeutic Hypothermia against Ischemia-induced Brain Damage

Excitotoxicity

Glutamate mediates excitatory synaptic transmission through the activation of ionotropic glutamate receptors that are sensitive to N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or kainite in the nervous system. The excitatory transmission mediates normal information processing and neuronal plasticity (Won et al., 2002). It has been well established that excessive activation of glutamate receptors mediates the initial step in excitotoxicity. Upon an ischemic insult, the interruption of blood supply in the brain causes the deprivation of oxygen and glucose, leading to impaired energy metabolism (Choi, 1992; Dugan and Choi, 1994). Consequently, it increases glutamate release through membrane depolarization and the subsequent activation of the voltage gated Ca^{2+} channels (Choi, 1992; Dugan and Choi, 1994).

Due to impaired energy synthesis, reuptake of glutamate is also interfered, which results in the excess accumulation of synaptic glutamate. The sustained over-activation of the ionotropic glutamate receptors leads to neuronal death, mostly due to necrotic cell death mechanism.

Recent reports have shown that therapeutic hypothermia prevents the accumulation or release of glutamate (Zhao et al., 2007a; Yenari and Han, 2012). Also, body temperature influences glutamate excitotoxicity during the acute phase of stroke, indicating that the release of these neurotransmitters is temperature dependent (Campos et al., 2012). Hypothermia can directly affect excitotoxicity through regulating the glutamate receptor 2 (GluR2) subunit of AMPA receptors (Colbourne et al., 2003). Hypothermia recovers the downregulation of GluR2 in hippocampal CA1 neurons after global cerebral ischemia in gerbils. After stroke, hypothermia significantly decreases brain glycine levels, which is needed to activate NMDA receptors and accelerate the function of NMDA receptors (Johnson and Ascher, 1987; Kvrivishvili, 2002). Additionally, hypothermia reduces the number of AMPA and NMDA receptors expressed on hippocampal neurons after stroke, which is associated with decreased infarct volume (Friedman et al., 2001; Li et al., 2011). Hypothermia also abates spreading depolarization that occurs after ischemic stroke by reducing the release of excitatory amino acids (EAA) (Nakashima and Todd, 1996).

Oxidative stress

Neurons are exposed to a minimum level of free radicals from both exogenous and endogenous sources in the normal condition (Dugan and Choi, 1994; Won et al., 2002). However, excess accumulation of reactive oxygen species leads to the damage of basic components for cell function and survival. Because the storage capacity of oxygen is limited in the brain, as well as there being a high probability of lipid peroxidation, neurons can be vulnerable to the change of free radical levels when oxygen supply is interrupted. Following stroke, increases in arachidonic acid, nitric oxide, glutamate, and over-activation of glutamate receptors rapidly develop in the ischemic tissue, which coincides with the production of free oxygen radicals such as superoxide (O_2^-), peroxynitrite (NO_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), resulting in neuronal death (Dugan and Choi, 1994; Globus et al., 1995b; Yenari and Han, 2012). The production of free radicals after stroke is temperature-dependent, and the suppression of free radical production is linearly proportional to the decreased temperature (Globus et al., 1995a; Hall, 1997). As a result, hypothermia can significantly reduce the production of free radicals and maintain the endogenous antioxidant activity in injured cells (Globus et al., 1995a).

Apoptosis

Necrosis and apoptosis are two major forms of neuronal cell death after ischemic stroke. Necrosis is a form of cell injury where edema and cellular inflammatory responses occur,

leading to excitotoxic death. Apoptosis is the main type of programmed cell death caused by activation of a cascade of intracellular pathways. This cell death mechanism is regulated by the culmination of interactions between pro-apoptotic and anti-apoptotic signaling genes (Mattson, 2000). Hypoxia and ATP depletion trigger neurons to activate the apoptotic regulatory proteins and several cellular processes, which include mitochondria dysfunction, activation of caspase enzymes, acidosis, calcium imbalance, and other cellular energy metabolism disorders (Won et al., 2002; Xu et al., 2002). These apoptotic events are mostly responsible for some delayed and secondary brain injuries.

Experimental models of ischemia have shown that therapeutic hypothermia prevents neuronal apoptosis through decreasing p53 protein, a transcription factor which activates apoptosis and pro-apoptotic proteins, including Bak, Bax, and NAD depletion (Bargonetti and Manfredi, 2002; Choi et al., 2012; Lee et al., 2014). Hypothermia regulates the levels of apoptotic related genes such as B-cell lymphoma-2 (BCL-2), cytochrome C, and tumor necrosis factor (TNF) pathway genes (Bossenmeyer-Pourie et al., 2000; Zhao et al., 2004, 2007b; Liu et al., 2008). After stroke, hypothermia stimulates anti-apoptotic proteins in the Bcl-2 family, causes the reduction of cytochrome c release into the cytosol, inhibits caspase activation, and thus enhances cell survival (Prakasa Babu et al., 2000; Zhao et al., 2004). Experimental animal models have shown that hypothermia has a beneficial effect on the dysfunction of ATP-dependent Na⁺/K⁺ pumps and Na⁺, K⁺, and Ca⁺ channels and reduces the influx of calcium into the cells, abating neuronal damage (Siesjo et al., 1989; Hall, 1997).

Autophagy

Autophagy is normally a physiological catabolic process for nutrient recycling, involving degradation of damaged organelles and proteins. The process is tightly regulated by autophagy signaling pathways, and alterations in this process may lead to diseases or exacerbate damage under pathological conditions. In recent years, increased autophagy has been identified as one of the pathophysiological mechanism after ischemic stroke (Chen et al., 2014). We were one of the first groups to demonstrate that a suppressing effect of therapeutic hypothermia on autophagy contributes to the neuroprotection after ischemic stroke (Choi et al., 2012). In our investigation, autophagic activity was examined by the formation of microtubule-associated protein light chain 3 (LC3-II) and degradation levels of sequestosome 1/p62 in the penumbra region. Both autophagic factors were decreased by hypothermic treatment using the neurotensin receptor agonist ABS-201. LC3-labeled autophagosome formation and TUNEL/LC3/NeuN triple-labeled cells were also decreased by the treatment. At about the same time, a different group reported that ischemia and reperfusion stimulate cell autophagy and cause cell death, which can be attenuated by mild hypothermia (Cheng et al., 2013). More recent papers demonstrated that hypothermia can inhibit autophagic cell death after

TBI and spinal cord injury (Jin et al., 2015, 2016; Seo et al., 2015). The mechanism underlying the anti-autophagy action of therapeutic hypothermia is an active area of current research.

Inflammation

Inflammatory mechanisms are activated after brain ischemia and act as important mediators in the pathogenesis of stroke-induced primary and secondary injuries (Vila et al., 2000; Gelderblom et al., 2009). Although a certain level of inflammation has beneficial effects required for tissue recovery and repairing, many reports have shown that inflammation is a major pathological mechanism underlying ischemic brain injury (Lakhan et al., 2009; Jin et al., 2010). During ischemia, inflammation is characterized by the production of pro-inflammatory cytokines such as TNF- α , interleukin-1 β (IL-1 β), IL-6, and anti-inflammatory cytokines such as IL-10, as well as the accumulation of neutrophils and the activation of microglia in the injured brain (Huang et al., 2006). Also, ischemia-mediated neuronal damage induces the synthesis and release of chemokines, such as monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), and IP-10 (interferon-inducible protein), which can recruit microglia, monocytes, and neutrophils into the ischemic region (Rappert et al., 2004; Wang et al., 2008; Jiang et al., 2016).

Hypothermia reduces the expressions of this pro-inflammatory immune response such as TNF- α and IL-1 β , but it also regulates the expression of some anti-inflammatory cytokines such as IL-10 and transforming growth factor- β (TGF- β) (Matsui and Kakeda, 2008; Jiang et al., 2016; Lee et al., 2016b). Accumulated data suggest that MCP-1 and MIP-1 α play crucial roles in ischemia-mediated cellular damage. Upregulation of MCP-1 and MIP-1 α were observed after ischemia; while MCP-1 knockout mice show reduced infarct volume after ischemia (Che et al., 2001; Hughes et al., 2002; Wang et al., 2008; Strecker et al., 2013). In addition, MIP-1 α injection exacerbated brain infarction but a broad-spectrum chemokine receptor antagonist using viral macrophage inflammatory protein-2 (vMIP-2), prevented neuronal damage from ischemic insults (Takami et al., 2001; Wang et al., 2008). Hypothermia attenuated the expression levels of chemokines such as MCP-1 and MIP-1 α (Lee et al., 2016b). Recent reports have demonstrated that hypothermia prevented inflammation-mediated cellular damage through regulating both activation of NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and Janus kinase (JAK) and signal transducer/activator of transcription pathway (STAT) signaling.

Microglia/macrophages are highly flexible cells with diverse phenotypes that are involved in the generation of distinct effector cells and functions (Murray and Wynn, 2011; Hu et al., 2012). Various cytokines by diverse stimuli lead to the development of M1 or M2 subtypes, which can express different levels of cell surface markers and secrete mediators such as scavenger receptors, chemokines, and cytokines. Recently, we showed that hypothermia shows

neuroprotective effects partly due to a shift from M1 to M2 type microglia cells. M1 is the classic activated state that is more associated with pro-inflammation, while M2 is more protective and associated with anti-inflammation (Cherry et al., 2014). This is especially important after brain injury, in which the transition to the M2 state not only clears out inflammation, but also initiates brain repair, and a suppressed M2 response results in greater lesion sizes following both stroke and TBI (Xiong et al., 2011; Kumar et al., 2013; Pérez-de Puig et al., 2013). In the hypothermia-treated stroke brain, there were decreased M1 type reactive factors including TNF- α , IL-1 β , IL-12, IL-23, and inducible nitric oxide synthase (iNOS) and increased M2 markers such as IL-10, Fizz1, Ym1, and arginase-1 (Lee et al., 2016b). Thus, hypothermia-related regulation of microglia can ameliorate the detrimental effects of a persistent M1 type microglia, including retrograde delayed degeneration, axonal degeneration, and white matter tract injury (Wilson et al., 2004; Maxwell et al., 2006).

Others

Blood-brain barrier (BBB)

Studies have shown that cerebral ischemia-reperfusion injury causes structural and functional breakdown of the BBB, resulting in increased BBB permeability, and the extent of disruption is directly correlated with the severity and duration of the insult (Latour et al., 2004; Chen et al., 2009). BBB breakdown not only causes brain edema and hemorrhage, but also increases various cytokines and chemokines, predisposing the brain to a secondary cascade of ischemic injury. BBB disruption after stroke, TBI, or other brain injuries is caused by structural and functional impairment of components of the neurovascular unit, including tight-junction proteins, transport proteins, endothelial cells, astrocytes, and pericytes.

Hypothermia prevents the activation of proteases responsible for degrading the extracellular matrix, such as the matrix metalloproteinases (MMPs) which are known to degrade tight-junction proteins (Lakhan et al., 2013). The activity of MMPs and the consequent degradation of vascular basement membrane proteins and the extracellular matrix proteins were reduced by hypothermia (Nagel et al., 2008; Baumann et al., 2009). In addition, hypothermia increases the expression of metalloproteinase inhibitor 2 (also known as TIMP2), an endogenous MMP inhibitor.

Neurogenesis and angiogenesis

Recent reports have shown that hypothermia can affect regenerative activities after stroke. Moderate low temperature (32°C) preserved the stemness of neural stem cells (NSCs) and prevented cell apoptosis. It is suggested that the protective effect of moderate hypothermia is partially associated with preservation of neural stem cells (Saito et al., 2010). Prolonged hypothermia positively interacts with post-ischemic repair processes, such as neurogenesis, resulting in improved functional outcome (Silasi and Colbourne, 2011). Additionally, a few studies have reported on the effect of

hypothermia on angiogenesis (Xie et al., 2007). Hypothermia reduced total infarct volume and increased endogenous brain-derived neurotrophin factor (BDNF) level. The microvessel diameter, the number of vascular branches and the vessel surface area were significantly increased in the hypothermia group, suggesting that mild hypothermia enhances angiogenesis in the ischemic brain. Despite the heightened proliferation of NSCs, other studies have suggested that endogenous neurogenesis may not contribute significantly to neuronal repairs. For example, NSCs from pools such as the subventricular zone (SVZ) may be region-specific and committed to become distinct subtypes and thus will be limited in regenerative versatility (Merkle et al., 2007). However, the increased activity of NSCs may play other roles, such as promoting plasticity following injury (Quadrato et al., 2014; Obernier et al., 2015).

Growth factors

Neurotrophic factors in the brain can regulate neuronal synaptic function and plasticity, cellular survival, differentiation and promote neural regeneration/repair (Wang et al., 2012; Bowling et al., 2016; Wurzelmann et al., 2017). There have been conflicting reports about the growth factor response to hypothermic treatment. Hypothermia showed strong neuroprotective effects following stroke *via* regulating BDNF, glial-derived neurotrophic factor (GDNF), and other neurotrophins (D'Cruz et al., 2002; Schmidt et al., 2004; Vosler et al., 2005). However, in a sheep model, hypothermia shortens the activity of insulin-like growth factor 1 (IGF-1) after hypoxia (Roelfsema et al., 2005). Furthermore, studies have reported that hypothermia may suppress the release of other growth factors, including vascular endothelial growth factor (VEGF) following *in vitro* hypoxia and nerve growth factor (NGF) in a mouse model of TBI (Goss et al., 1995; Coassin et al., 2010). These findings may be attributed to a decrease in overall metabolism of cells in response to hypothermia.

Other putative mechanisms

Given the global coverage of the temperature reductions during therapeutic hypothermia, as well as the vast array of interconnected molecular pathways, there are various other putative mechanisms that contribute towards hypothermia's neuroprotective effects. Other mechanisms include the impact of hypothermia on cerebral metabolism and consequently cerebral perfusion (Rosomoff and Holaday, 1954). The reduction in temperatures may improve brain glucose consumption and reduce the lactate-glucose and lactate-pyruvate ratios after TBI as compared to normothermia controls (Wang et al., 2007). Another pathophysiology that arises after brain injuries like ischemic stroke is cerebral thermo-pooling, in which localized foci of the brain become hyperthermic after injury, thus making it an ideal target for hypothermia (Schwab et al., 1998b). Indeed, therapeutic hypothermia is effective at reducing thermo-pooling after ischemic stroke and in other pathological cases, such as influenza encephalitis (Hayashi et al., 1997; Hayashi, 2000; Faulds and Meekings, 2013).

Table 1 Major classes of pharmacological agents as putative candidates for pharmacologically induced hypothermia (PIH)

Pharmacological class	Mechanisms of action	Efficacy for hypothermia-related neuroprotection	Side effects/ limitations	References
Neurotensins	Activation of neurotensin receptor 1 (NTR1)	Rapid induction of hypothermia (~13 minutes to reach 33°C) that can last for multiple hours (6–8 hours); effectively produce neuroprotection against both ischemic stroke and traumatic brain injury (TBI); decrease lesion volume and improve functional outcomes; HPI201 and HPI363 abolish shivering response	Hyperglycemia, hypoinsulinemia, and hyperglucagonemia at high doses	Bissette et al., 1976; Torup et al., 2003; Katz et al., 2004; Choi et al., 2012; Wei et al., 2013; Lee et al., 2014, 2016b
Transient receptor potential vanilloid 1 (TRPV1) receptor agonists	Activation of TRPV1 to regulate temperature	Can achieve appropriate hypothermic temperatures (33°C); Capsaicin produced neuroprotective effects; DHC induces hypothermia and confers neuroprotection after stroke; Rinvanil conferred neuroprotective effects specific to hypothermia after ischemic stroke; reduces shivering possibly by raising the thermoregulatory set point	Transient hypotension; Rinvanil is ineffective at high doses	Adler et al., 1988; Xu et al., 2011; Muzzi et al., 2012; Cao et al., 2014
Cannabinoids	Activation of cognate receptors CB1 and CB2	Cannabinoids confer neuroprotection both dependent and independent of hypothermia; possibly also ameliorates excitotoxicity and modulation of inflammation; achieves appropriate hypothermic temperatures for the most part (33–35°C)	Hypotension and bradycardia	Gerdeman and Lovinger, 2001; Rawls et al., 2002; Leker et al., 2003; Bonfils et al., 2006; Fernández-López et al., 2012; Suzuki et al., 2012
Opioids	Lowering the set point for cold response by depression of sympathetic tone	K-opioid and δ -opioid agonists are able to induce hypothermia and confer neuroprotective effects	δ -Opioid agonists – hypotension and arrhythmia	Benamar et al., 2002; Sigg et al., 2002; Zhang et al., 2003; Drabek et al., 2008
Thyroxine derivatives	Decarboxylated thyroid hormone derivatives – partially mediated by TAARI activation	Rapid induction to target temperature (31°C) for 6–10 hours, leading to neuroprotective effects; notably eliminate cold response side effects, such as shivering and piloerection	Minimal	Doyle et al., 2007; Ianculescu and Scanlan, 2010; Zhang et al., 2013b
Dopamine receptor agonists	Activation of the 4 subtypes of dopamine receptors possibly modulates body's thermoregulatory set point	Modest decreases in temperature (~1–2°C), but still resulted in neuroprotective effects; may be able to dampen the homeostatic cold response	Hypokalemia	Colboc and Costentin, 1980; Johansen et al., 2003, 2013
Gaseous (xenon, H ₂ S)	Xenon – anesthesia via NMDA inhibition H ₂ S – inhibition of cytochrome c oxidase	Xenon – neuroprotective, but may be independent of hypothermia (mild temperature reduction – 1.3°C) H ₂ S – neuroprotective effect may be due to a variety of secondary effects, including hypothermia (reduced to 31°C)	Xenon – limited availability and high cost, inhibits tPA H ₂ S – low therapeutic index, and foul odor	David et al., 2009; Nicholson and Calvert, 2010; Joseph et al., 2012; Sheng et al., 2012
Adenosine derivatives	Inconclusive	Despite its ability to decrease temperatures (~33.2°C), ATP and AMP both failed to provide neuroprotection (although possibly the chosen doses were inappropriate for the brain, although cardioprotection was observed)	Exacerbation of ischemic damage, severe hypotension, hyperglycemia, metabolic acidosis, hypocalcemia	Tao et al., 2011; Rittiner et al., 2012; Muzzi et al., 2013; Zhang et al., 2013a

This outline describes the currently known mechanisms of action for each of the major pharmacological classes of PIH, as well as their primary central nervous system (CNS) benefits and side effects.

Pharmacologically Induced Hypothermia: a Potential Therapeutic Strategy and Implication in Clinical Stage

Limitations of physical cooling

Therapeutic hypothermia has been proposed as a treatment for stroke and shown to be effective in preclinical and clinical studies. However, despite the obvious beneficial effects of therapeutic hypothermia, its clinical application has been limited due to multiple shortcomings. For patients, current hypothermia protocols, including ice cooling or cooling pads, are generally slow (2–8 hours) and cumbersome, or

invasive in the case of endovascular cooling. The long time-course of surface cooling often requires the concomitant use of anesthetics and paralytics in order to curb shivering, which are performed with endotracheal intubation and ventilation, which carry side effects, such as pulmonary infections (Hemmen and Lyden, 2007). The invasive nature of endovascular cooling can result in increased risk of infections and bleeding, and is not as universally applicable, due to the need for a skilled medical personnel to perform the catheter placement (Glushakova et al., 2016). In addition, physical cooling (PC) evokes shivering responses, which is a defensive metabolic adaptation to cold, as well as peripheral vasocon-

striction. This systemic response makes effective and accurate cooling difficult, and most often the cooling procedure has to be performed under general anesthesia or sedation reagents (Schwab et al., 1998a; Sessler, 2009). Another possible limitation of hypothermia is its association with systemic infections, including pneumonia and sepsis, although this side effect can be treatable (Hemmen et al., 2010; Geurts et al., 2014). Other potential side effects of hypothermic treatment includes hypothermia-induced diuresis, resulting in hypovolemia and electrolyte depletion, thrombocytopenia, and bradycardia (Schwab et al., 2001). Furthermore, while clinical trials have shown hypothermia to be a feasible treatment, there is conflicting data as to whether hypothermia can effectively improve functional outcomes (Krieger et al., 2001; Schwab et al., 2001; Polderman et al., 2002; De Georgia et al., 2004; Nielsen et al., 2013; Lyden et al., 2016). This is largely due to the plethora of parameters associated with TTM, aside from simply the temperature reduction. Even if patients undergo physical cooling to optimal temperatures of 33–35°C, other factors that could affect the outcomes include the severity, location and subtypes of the injury, the time of intervention, the speed/duration of the cooling treatment, and finally other pharmacological agents (e.g., anesthetics or paralytics) administered (Wan et al., 2014; Subramaniam et al., 2015). All of these factors contribute to the inconsistent findings, but they also provide tremendous potential for optimization of therapeutic hypothermia in order to ensure its efficacy.

Recent early pilot studies with clinical trials have provided modest evidence suggesting both the feasibility and efficacy of therapeutic hypothermia, especially when performed in conjunction with thrombolysis (Hemmen et al., 2010; Piironen et al., 2014). These studies have parlayed into a phase III multi-center randomized controlled trial, EuroHYP-1, and the results will have significant ramifications on the implementation of this clinical strategy (Worp et al., 2014). Nevertheless, considering the modest success associated with therapeutic hypothermia thus far, as well as the current ESO guidelines that discourage the use of current physical means of hypothermia to treat ischemic stroke, it is important to continue the exploration of other avenues that can induce hypothermia in safer and more efficient ways (Ntaios et al., 2015). This is important because it may be possible that the aforementioned limitations may be associated more or less with physical cooling, either with surface cooling or endovascular methods. Thus, increasing research attention has recently been drawn to pharmacological reagents that have shown cooling effects in animals and/or humans.

Pharmacologically induced hypothermia

Pharmacologically induced hypothermia (PIH), which targets the peripheral or central thermoregulatory mechanisms, has emerged as a more efficient and safer treatment for brain disorders (Choi et al., 2012; Wei et al., 2013; Cao et al., 2014; Lee et al., 2014). PIH allows for greater control

of temperature changes necessary for TTM. More importantly, we have shown that a PIH therapy targeting the central thermoregulator may suppress external cooling-induced shivering and tachycardia and achieve more rapid and effective hypothermia (Feketa et al., 2013; Lee et al., 2016a). It is well known that shivering, as a physiological defense mechanism against body cooling, is a strong and troublesome reaction that is invariably coupled with physical cooling. This reaction often prevents effective and accurate control of hypothermic treatment. Normally, general anesthesia and/or sedation have to be applied to battle with shivering during physical cooling. PIH using hypothermic drugs acting at the central or peripheral thermoregulatory receptors/channels provides a mechanism-based strategy to eliminate cooling-associated shivering and other responses. This unique effect creates multimodal benefits, including the administration of hypothermic treatment in awake patients without the need for general anesthesia and the avoidance of a number of side effects seen with conventional physical cooling methods.

Currently, there are several major classes of pharmacological agents that are categorized based on their mechanisms of action and thermoregulation target: neurotensin, transient receptor potential vanilloid 1 (TRPV1), cannabinoid, opioid, thyroxine derivatives, dopamine, gas, and adenosine derivatives (Table 1). Other drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) that show mild TTM action have also been investigated in the context of therapeutic hypothermia but are not as effective for inducing and maintaining the minimum temperature reductions (Feigin et al., 2002).

Our group has developed the second generation of hypothermic compounds acting as a selective neurotensin receptor 1 (NTR1) agonist that can pass through the BBB and efficiently reduce the body and brain temperature in a dose-dependent manner (Choi et al., 2012). For example, HPI-201 and HPI-363 (also known as ABS-201, ABS-363) are NTR1 agonists acting at the hypothalamic thermoregulatory set point. It possesses a high affinity for human NTR1, exhibits BBB permeability, and effectively induces regulated hypothermia in rodents, resulting in protective effects and improved functional recovery after ischemic or hemorrhagic stroke or TBI in mice (Hadden et al., 2005; Choi et al., 2012; Wei et al., 2013; Lee et al., 2014, 2016a, b). Other groups showed that an agonist at transient receptor potential cation channel subfamily vanilloid member 1 (TRPV1) can decrease temperature *via* regulation of the peripheral temperature sensitive channel and confer neuroprotective effects after stroke in animals (Feketa et al., 2013; Cao et al., 2014).

Overall, PIH confers several significant advantages over physical cooling that allow for more feasible clinical implementation. These include the targeting of the thermoregulatory center in the hypothalamus in order to decrease the cold set point to reduce compensatory physiological responses to hypothermia. These effects, such as shivering, piloerection, and vasoconstriction, cause discomfort for the patients

and reduces the efficacy of hypothermia (Díaz and Becker, 2010). Importantly, PIH and physical cooling are not mutually exclusive, and the best paradigm may ultimately be a combination therapy of the two in order to create a reliable regimen that uses a lower minimum pharmacological dose (to mitigate potential side effects) to induce hypothermia, but that also dulls the cold response (Muzzi et al., 2013; Liu et al., 2016). Preliminary data has already suggested that combination therapy can provide synergistic effects that augment the benefits of either treatment alone (Lee et al., 2016a).

Conclusion

Therapeutic hypothermia is one of the most promising therapies for neuroprotection against brain injuries such as stroke. The mechanism of action is multifaceted. Therapeutic hypothermia provides a global brain protection rather than targeting a single pathway or a single gene. Therapeutic hypothermia can regulate multiple pathways including excitotoxicity, oxidative stress, apoptosis, autophagy and promote regenerative activities. We expect that these actions can be utilized to show synergistic effects with other neuroprotective treatments such as tPA and help to develop combinatory stroke therapy for clinical treatments. To this end, PIH has been demonstrated to be an effective and more efficient hypothermic therapy that shows high feasibility and translational potential for clinical applications. Further study will be needed to have an in-depth understanding of the multiple mechanisms underlying therapeutic hypothermia and reinforced efforts are necessary to verify the efficacy of PIH compounds in large animals such as non-human primates. It is expected that safer and more effective hypothermia therapies may help develop more clinical treatments for stroke and other intractable brain disorders.

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