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Therapeutic hypothermia for ischemic stroke; pathophysiology and future promise

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Abstract

Therapeutic hypothermia, or cooling of the body or brain for the purposes of preserving organ viability, is one of the most robust neuroprotectants at both the preclinical and clinical levels. Although therapeutic hypothermia has been shown to improve outcome from related clinical conditions, the significance in ischemic stroke is still under investigation. Numerous pre-clinical studies of therapeutic hypothermia has suggested optimal cooling conditions, such as depth, duration, and temporal therapeutic window for effective neuroprotection. Several studies have also explored mechanisms underlying the mechanisms of neuroprotection by therapeutic hypothermia. As such, it appears that cooling affects multiple aspects of brain pathophysiology, and regulates almost every pathway involved in the evolution of ischemic stroke. This multifaceted mechanism is thought to contribute to its strong neuroprotective effect. In order to carry out this therapy in optimal clinical settings, methodological and pathophysiological understanding is crucial. However, more investigation is still needed to better understand the underlying mechanisms of this intervention, and to overcome clinical barriers which seem to preclude the routine use therapeutic hypothermia in stroke.

Keywords

Hypothermia; Cooling; Stroke; Ischemia; Cerebral Infarct; Infarction

1. Introduction

Since Busto and colleagues (Busto et al., 1987) demonstrated that lowering of brain temperature by only a few degrees could ameliorate neuronal death in 1987, there has been continuous renewed interested in this robust neuroprotective effect. Therapeutic hypothermia has been widely to be one of the most reliable neuroprotective therapies for several cerebral disorders and injuries (van der Worp et al., 2007; Yenari and Han, 2012), including stroke, traumatic injury, global ischemia after cardiac arrest, and hypoxic-ischemic encephalopathy.

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Conflict of interest
There are no conflicts of interest to declare.

Stroke is one of the leading causes of death and disability in this industrialized world. Of these, approximately 87% of all strokes are ischemic (Liu et al., 2016). Despite such high prevalence, there is no proven effective therapy, other than the revascularization within a limited time window. However, due to its restricted time window, patients who can receive such intervention is limited. Based on numerous reports demonstrating that therapeutic hypothermia can provide robust neuroprotection, this therapy has the potential to be one of the most attractive therapies for ischemic stroke (van der Worp et al., 2007; Yenari and Han, 2012). Prior clinical trials showed that hypothermia improved clinical outcomes in comatose survivors of out-of-hospital cardiac arrest (Bernard et al., 2002) and neonatal hypoxic ischemic encephalopathy (Perrone et al., 2010; Tsuda et al., 2017).

To date, numerous pre-clinical studies have shown that cooling affects multiple pathways at various stages of ischemic stroke (Yenari and Han, 2012). During the acute stage of ischemia, decreases in cerebral blood flow disrupts ionic homeostasis, leading to increased intracellular calcium and release of excitatory neurotransmitters. Increased intracellular calcium also causes downstream effects such as mitochondrial dysfunction, leading to increased reactive oxygen species (ROS) generation.(Gonzalez-Ibarra et al., 2011). In the sub-acute stage, apoptotic and inflammatory pathways are initiated hours to days later (Ceulemans et al., 2010). Once initiated, these pathways lead to neuronal cell death through a combination of apoptosis, inflammation, oxidative stress, and excitotoxicity pathways. Therapeutic hypothermia is thought to affect almost every one of these pathways (Yenari and Han, 2012). This multifaceted mechanism is thought to explain its strong therapeutic effect (Yenari and Han, 2012). Thus, it is likely that no single factor can explain its underlying protective effect (Yenari and Han, 2012).

Since therapeutic hypothermia is a promising and attractive therapy for ischemic stroke, a precise understanding of what is known and what is unknown is important. This will allow the optimization and standardization of any future clinical trials, and will first discuss the optimal cooling conditions which might be effective both in experimental models and human stroke. We will also discuss cellular and molecular pathways affected by cooling. Cumulative knowledge may prove helpful to the effective translation of therapeutic hypothermia to clinical settings.

2. Methodological aspects of therapeutic hypothermia

Most pre-clinical studies of hypothermia use small rodent models. In rodents, cooling is performed by applying a cooling blanket or by spraying water or alcohol on the animal’s fur. Unlike humans, target temperatures can be reached within minutes and maintained with reasonable control in the anesthetized animal. Rewarming requires placing the animal on a warming blanket with a heat lamp suspended over the animal. Rewarming takes a similar amount of time as cooling, and no obvious detrimental effects have been reported when this approach is used in small animal models. This is in contrast to what is seen at the clinical level where cooling requires several hours as does rewarming. Further, too rapid rewarming runs the risk of increased brain edema and intracranial pressure (ICP). Cooling for longer durations (that is, for more than 1day) in awake and freely moving animals can be

accomplished by using automated misting systems and overhead fans and appear to be well tolerated to temperatures of 32°C (Colbourne et al., 1996).

A novel method to induce hypothermia in rodents was reported recently (Lamb et al., 2016), where hypothermia was attained by cooling circulating blood at the inferior vena. This approach may complement traditional surface cooling methods, because of its more rapid induction and tighter regulation of temperature.

2.1. Optimal cooling conditions

In animal studies of hypothermia in ischemic stroke, a wide range of depths, durations, and delays to initiation of cooling has been studied (van der Worp et al., 2007). Because optimization of these methodological parameters is crucial for clinical application, several studies had been carried out in various stroke model rodents. Previous work and meta-analyses (Krieger and Yenari, 2004; van der Worp et al., 2007) revealed that relatively small decreases in brain temperature are as protective as lower temperatures. In fact, brain temperatures in the range of 30–34°C (decreased from normal body temperatures of 36–38°C) seem to provide protection that is as robust as temperatures below 25°C. Timing and duration are also important factors involved in achieving neuroprotection induced by therapeutic hypothermia. Early initiation and long duration increase the probability of a good outcome (Kurisu et al., 2016a; Yenari and Han, 2012). Previous meta-analyses showed that the therapeutic efficacy induced by hypothermia was best with lower temperatures, and when treatment was started before or at the onset of ischemia (van der Worp et al., 2007). Furthermore, there appeared to be a small correlation between longer durations of cooling and decreased infarct size (van der Worp et al., 2007). Neuroprotection can also be attained even when cooling is delayed, provided decreased temperature is maintained for long durations (24–48h) (Yenari et al., 2008). This remarkable effect suggests the promise of hypothermic therapy for clinical ischemic stroke. However, at present, the long-term effect of therapeutic hypothermia is not fully clarified yet. Only a few preclinical studies (Colbourne et al., 1999; Maier et al., 2001) have demonstrated the durability of the effect to be at least a few months.

Although cooling can be easily achieved in small rodents, it brings substantial challenges in humans. Considering the issues mentioned above regarding larger body mass and co-morbidities, the adverse effects induced by hypothermia therapy may abrogate any beneficial effect (Esposito et al., 2014). To date, several novel therapeutic concepts were proposed and had been tested pre-clinically, which might be an attractive alternative to traditional hypothermia therapy (Dumitrascu et al., 2016; Esposito et al., 2014). These include selective brain cooling and pharmacological induction of hypothermia.

In humans, established cooling methods for therapeutic hypothermia largely include surface and endovascular cooling (Wu and Grotta, 2013). Surface cooling is attained through the use of ice packs, cooling blankets, or cooling pads. Advantages of surface cooling are its ease of use and its safety. Endovascular cooling is somewhat more invasive, as it involves the insertion of catheters much like that of central lines. The catheter allows chilled saline and/or an endovascular cooling device to circulate via the catheter tip, and temperature is controlled tightly by an external cooling and temperature monitoring module. Although this

method includes invasive technique for stroke patients, it seems that this method seems to cool patients faster and more precisely than the surface cooling (Wu and Grotta, 2013). However, both of these approaches are still met with the challenge of shivering in typically awake stroke patients.

2.2 Clinical challenges

Despite the beneficial effects of therapeutic hypothermia being well-documented in animal studies, its full translation into human trials of stroke patients presents many challenges (Liu et al., 2016). Although the therapeutic effectiveness of hypothermia therapy has long been shown by numerous pre-clinical studies and meta-analysis (van der Worp et al., 2010; van der Worp et al., 2007), the benefit in humans has not established yet (Lyden et al., 2014; van der Worp et al., 2014). Several clinical issues not represented in animal models will need to be overcome. Among these issues is that cooling in humans takes longer, because human have a larger body mass compared to smaller animal used in preclinical studies. Further, typical stroke patients, unlike comatose survivors of cardiac arrest or neonatal populations, may not tolerate cooling due to older age and the many co-morbidities such as diabetes and cardiovascular disease typical of this population (Yenari et al., 2008). In addition, although lower temperatures will surely promote neuroprotection, cooling may also induce unfavorable systemic effects such as shivering in awake stroke patients (Wu and Grotta, 2013), immune suppression (Liu et al., 2016), pneumonia (Lyden et al., 2016) and cardiovascular events (De Georgia et al., 2004). Thus, the design of any clinical studies will need to take into consideration these issues. Further, since thrombolysis is now standard of care in acute ischemic stroke, it is less clear how this intervention should be incorporated into any cooling protocol. Yet, some preclinical studies indicate that mild to moderate cooling should not worsen safety concerns of thrombolytics (Tang et al., 2013). However, there is no standard consensus as to how to deal with these concerns. Furthermore, some clinical trials (Hong et al., 2014; Hwang et al., 2017) showed a promising effect of hypothermia in ischemic stroke patients. These results may provide useful direction in the design of future clinical trials.

2.3 Selective brain cooling

Selective brain cooling is a novel therapeutic approach which aims to induce cooling to only the brain or head, thus circumventing any adverse systemic effects which may result from total body cooling. Since it had been reported that neuroprotection induced by hypothermia depends on lowered brain temperature and does not require whole body cooling (Busto et al., 1987), selective brain cooling would be an attractive alternative to systemic cooling (Esposito et al., 2014). To date, several methods for selective brain cooling for neuroprotection have been reported in animal experiments, including the placement of a cooling coil under the temporal muscle (Clark and Colbourne, 2007), trans-ventricular cooling (Bell et al., 2006), and others. Of these, trans-arterial brain cooling, which is simply performed by perfusion of cold perfusate via the ipsilateral carotid artery, is thought to be one of the most attractive method of attaining selective brain hypothermia in clinical settings (Esposito et al., 2014; Kurisu et al., 2016a; Kurisu et al., 2016b). This approach could be performed endovascularly, and could be readily implemented considering the role of endovascular therapies already in clinical use for acute ischemic stroke treatment.

Furthermore, it seems that this approach can lead to neuroprotection with shorter duration of cooling, and lowering temperature by only 2–3C (Kurisu et al., 2016b). The directly cooling of the neurovascular unit may explain this difference, compared to total body cooling. Recently, a pilot clinical study of this novel approach was published (Chen et al., 2016), but its therapeutic effect is still unclear. Thus, further research is still needed.

2.4. Pharmacologically inducing hypothermia

There has been a recent surge of interest in the investigation of drug-induced hypothermia as a treatment option for ischemic stroke (Liu et al., 2016; Zhang et al., 2013). This therapeutic concept is proposed for the aim of maximizing the beneficial effect of hypothermia in clinical setting with the potential of developing fewer adverse events. Currently, there are eight classes of pharmacological agents/agonists which can achieve hypothermia. These compounds affect a multitude of systems including cannabinoid, opioid, transient receptor potential vanilloid 1 (TRPV1), neurotensin, and thyroxine derivatives, dopamine, gases such as xenon and helium, and adenosine derivatives. Interestingly, some of these drugs, such as those of the cannabinoid families, may not only provide neuroprotection through hypothermia induction but may provide neuroprotection through other pharmacological mechanisms provided by the drug itself (Liu et al., 2016). This may lead to the possibility that pharmacologically induced hypothermia might be more effective than physical cooling through synergistic effects of the drug.

Furthermore, another advantage of pharmacologically induced hypothermia is its effect on central thermoregulation at the level of the hypothalamus (Liu et al., 2016). Several drugs, such as TPRV1 receptor, neurotensin and thyroxine families, have been shown to have effects on thermoregulatory control by decreasing the compensatory hypothermic response during cooling. This effect on thermoregulation could also reduce shivering and vasoconstriction, which often interferes with the therapeutic effect of hypothermia in the typical awake stroke patient. Therefore, these agents might be efficacious in combination with physical hypothermia in reducing discomfort, hastening the cooling process, and prolonging tolerable cooling durations (Liu et al., 2016).

3. Underlying mechanisms of therapeutic hypothermia

The precise mechanism involved in therapeutic hypothermia is not yet fully understood. However, it seems that cooling affects nearly every investigated cell death pathway to date, including excitotoxicity, apoptosis, inflammation, and free radical production. It is likely that no single factor can explain its strong neurorotective effect. Herein, we review the many molecular pathways which have been shown to be affected by hypothermia.

3.1 Effects on the acute stage of stroke

Pathways affected by therapeutic hypothermia during the acute stage of stroke (minutes to hours) are summarized in figure 1. Almost every molecular and cellular pathway known to lead to cell death are affected by therapeutic hypothermia.

3.1.1 Metabolism, cerebral blood flow, and excitotoxicity—The effect of brain cooling on cerebral metabolism and blood flow have been the most widely cited mechanism of therapeutic cooling. It is well known that lowered temperatures prevent loss of essential metabolic substrates and alter cerebral blood flow. Cerebral metabolism, reflected in estimates of oxygen consumption, glucose utilization, and lactate levels, is temperature dependent, and hypothermia decreases each of these parameters. Cooling decreases brain oxygen consumption and glucose metabolism by about 5% per degree Celsius (Yenari et al., 2008). Hypothermia also preserves high-energy phosphate compounds, such as ATP, and maintains tissue pH. Preserving the brain's metabolic stores can prevent downstream consequences of increased lactate production (which is dependent on anaerobic metabolism) and the development of acidosis.

The effect of hypothermia on cerebral blood flow is complex. Under non injury conditions cerebral blood flow decreases linearly between temperatures of 18 to 37°C, and coupling between blood flow and brain metabolism is preserved (Yenari et al., 2008). However, under ischemic conditions, cerebral blood flow is markedly reduced as a result of vessel occlusion. However, when blood flow is restored (reperfusion), there is an overshoot of blood flow (hyperaemia) followed by a temporary reduction in cerebral blood flow. A recent report (Ito et al., 2011; Kurisu et al., 2016b) showed that this post-reperfusion blood flow reduction is a result of microvascular narrowing. Therapeutic hypothermia attenuates this morphological microvascular change, and improves microcirculation in acute stage (Kurisu et al., 2016b)

Therapeutic hypothermia has also been shown to prevent the accumulation or release of excitotoxic amino acids such as glutamate (Yenari et al., 2008; Yenari and Han, 2012). This may be attributable to the effect of hypothermia on metabolism, which preserves tissue ATP levels. ATP is needed to maintain ion gradients. Once ATP loss is triggered by ischemia, efflux of potassium and influx of sodium and calcium down their concentration gradients ensues. Calcium influxes induce direct neurotoxicity as does extracellular accumulation of glutamate, both of which lead to brain cell damage. It seems that hypothermia may also prevent the consequences of excitotoxcity by limiting calcium influx through AMPA channels(Colbourne et al., 2003). Glutamate receptor 2 (GluR2), a subunit of the AMPA receptor is thought to limit calcium influx and its down regulation by ischemia may lead to the entry of excess calcium. A previous report demonstrated that hypothermia attenuates ischemia-induced downregulation of GluR2(Colbourne et al., 2003), and would thus expect to reduce toxic calcium influx through this channel.

3.1.2 Other early molecular events—Hypothermia has also been shown to affect other acute processes associated with ischemia, including the induction of immediate early gene expression (Kamme et al., 1995) and the cellular stress response (Yenari et al., 2005). Some reports showed that one of the stress related proteins, the 70kDa inducible heat shock protein (HSP70), is increased under hypothermic conditions (Terao et al., 2009a), and this might be consistent with HSP70's neuroprotective properties (Yenari et al., 2005). However, it is still unclear whether hypothermic neuroprotection is necessarily mediated through alterations in immediate early gene expression and the cellular stress response (Yenari and Han, 2012).

Micro RNAs (miRNAs), a subset of non-coding RNAs, have been a topic of recent investigation in brain injury models, including stroke, where their expression has been observed to increase as early as 2hour after ischemia onset. It is conceivable that they have an important role in stroke pathogenesis, and the roles of specific miRNAs are currently under investigation. A report (Truettner et al., 2011) in traumatic brain injury models showed that therapeutic hypothermia alters the expression of several miRNAs, as represented by miR-874 and miR-451.

Cold-inducible (or 'cold shock') proteins are also of relevance to hypothermic neuroprotection (Yenari and Han, 2012). Two of these genes, cold-inducible RNA-binding protein (CIRBP) and RNA binding motif protein 3 (RBM3), have been reported to be specifically induced by therapeutic hypothermia (Yenari and Han, 2012). A few studies have begun to explore their significance in neuroprotection and potential mechanisms of protection by hypothermia. For instance, CIRBP mRNA was increased in rodent ischemic brain, with even higher increases in ischemic brains exposed to hypothermia (Liu et al., 2010). RBM3 was also upregulated by hypothermia in neuronal cells, and hypothermia's neuroprotective effect was eliminated when RBM3 expression was inhibited. (Chip et al., 2011)

However, these acute mechanisms might be not enough to explain the full beneficial effect provided by therapeutic hypothermia. The extent of neuroprotection does not proportionately increase with temperature decrease, and therapeutic hypothermia is still effective even when cooling is initiated well after these acute events have occurred (Kurisu et al., 2016a; Yenari et al., 2008). This suggests that hypothermia likely has additional effects on injury, and may even affect pathways in the subacute (hours to days), and even chronic phases (weeks to months) of ischemic stroke.

3.2 Effects on the sub-acute stage of stroke

The subacute stage of stroke is thought to be the period during which secondary injury occurs, and this is generally considered to be anywhere from 1 to 7days post-ischemia (Yenari and Han, 2012). During this period, reperfusion related pathways are activated as a result of the increased generation of reactive oxygen species (ROS) by injured cells. In addition, inflammatory responses are activated, along with other cell death pathways, including those leading to apoptosis. Due to the activation of these pathways, the extent of ischemic injury can worsen during this period, and secondary injury such as blood-brain barrier (BBB) disruption, edema formation and hemorrhagic transformation can also occur (Yenari and Han, 2012). Several studies have demonstrated that hypothermia affects multiple cell death and cell survival pathways (Fig. 2) (Yenari and Han, 2012).

3.2.1 Apoptosis—Therapeutic hypothermia has been shown to affect several aspects of apoptotic cell death. There are two main pathways that lead to apoptosis, and they include the intrinsic pathway and extrinsic pathways. The intrinsic pathway is thought to originate within the cell at the level of the mitochondria (Green and Reed, 1998), whereas the extrinsic pathway is triggered via cell surface receptors (Ashkenazi and Dixit, 1998). Hypothermia has been shown to affect both pathways (Yenari and Han, 2012).

Cooling can suppress the intrinsic (mitochondrial) pathway by regulating the expression of BCL-2 family members, reducing cytochrome *c* release and decreasing caspase activation (Liu and Yenari, 2007). Therapeutic hypothermia is reported to reduce pro-apoptotic BCL-2 family members such as BCL-2-associated X (BAX) and to increase anti-apoptotic members, such as BCL-2. Acting downstream of BCL-2 family proteins, protein kinase C (PKC) family members can also be either pro- (PKCδ) or anti-apoptotic (PKCε). PKCδ translocates from the cytosol to the mitochondria to initiate apoptosis. By contrast, PKCε is anti-apoptotic, and is degraded by caspases. Interestingly, cooling does not seem to alter overall levels of PKCδ, but it blocks its translocation to the mitochondria and nucleus, and stimulates the action of PKCε after ischemia (Shimohata et al., 2007).

The extrinsic apoptotic pathways are also activated by ischemic injury, and are also suppressed by hypothermia. The most widely studied apoptosis-inducing death receptor is FAS, and its ligand FASL. Hypothermia seems to suppress the expression of both proteins (Liu et al., 2008). Although it is known that binding of FAS and FASL is essential to initiate the extrinsic apoptotic pathway via caspase 8 activation, it is still unclear how FASL binds FAS (Yenari and Han, 2012). Some reports indicated that cleavage and solubilization of FASL by activated by matrix metalloproteinases (MMPs) is important to this binding (Powell et al., 1999). In support of this, therapeutic hypothermia was shown to prevent this cleavage, while decreasing levels of soluble FASL and several MMPs (Lee et al., 2005; Truettner et al., 2005). A recently reported mechanism includes the role of dynamin-1, which is a protein described in exocytosis and synaptic transmission, but has been described to also have chaperone functions where it can transport Fas from the endoplasmic reticulum to the cell surface, thus making it available for FASL ligation (Ivanov et al., 2006). Dynamin is also upregulated in the brain in stroke models and its deficiency or inhibition is protective (Kim et al., 2016). Similarly, therapeutic hypothermia also reduces dynamin expression while inhibiting surface expression of Fas (Kim et al., 2017).

Other more recently studied molecules involved in apoptosis are also affected by therapeutic hypothermia as well. Phosphatase and tensin homologue (PTEN) is a tumor suppressor molecule with pro-apoptotic functions. Cerebral ischemia seems to suppress the PTEN phosphorylation, which leads to its deactivation, and promote cell apoptosis(Zhao et al., 2007). While, under cooling conditions, phosphorylated PTEN levels were preserved (Lee et al., 2009). The activated form of this pro-apoptotic protein seems to be associated with hypothermic neuroprotection.

3.2.2 Cell survival—Several neurotrophic factors in the brain have been studied with respect to their therapeutic potential in various acute neurological insults. These proteins control synaptic function and plasticity and sustain neuronal cell survival, morphology and differentiation. In studies of therapeutic hypothermia, levels of brain-derived neurotrophic factor (BDNF) (D'Cruz et al., 2002), glial-derived neurotrophic factor (GDNF) (Schmidt et al., 2004) and neurotrophin (Boris-Moller et al., 1998) were all increased in the ischemic brain.

Furthermore, hypothermia also upregulates other survival factors. As described above, hypothermia upregulates the anti-apoptotic protein BCL-2 and also promotes activation of

AKT (Zhao et al., 2005), a serine/threonine protein kinase that has multiple roles in glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. It seems that activated AKT phosphorylates (and thus inactivates) pro-apoptotic proteins such as glycogen synthase 3β (GSK3 β) and BCL-2 antagonist of cell death (BAD). Cooling seems to preserve AKT activity, and may suggest a specific mechanism of hypothermic protection (Zhao et al., 2005). Thus, although therapeutic hypothermia has generally been documented to suppress or decrease metabolism and protein expression, it can also upregulate or maintain proteins involved in cell survival and growth (Yenari and Han, 2012).

3.2.3 Inflammation—The injured brain stimulates innate immune responses leading to activation of microglia and circulating leukocytes, and these immune cells can then release various molecules, including ROS, proteases and pro-inflammatory cytokines. These molecules can activate more inflammatory cells, leading to a vicious cycle of death and inflammatory activation (Yenari and Han, 2012).

Several animal studies have shown that hypothermia can inhibit these deleterious effects induced by post-ischemic inflammations, and can provide robust beneficial effects on neurological outcomes (Ceulemans et al., 2010). Indeed, therapeutic hypothermia lowers numbers of infiltrating neutrophils and activated microglia in the ischemic area and reduces levels of many inflammatory mediators including ROS (Perrone et al., 2010), adhesion molecules (Deng et al., 2003), proinflammatory cytokines (Wang et al., 2007) (such as interleukin-1β (IL-1β), tumor necrosis factor α (TNFα) and IL-6) and the chemokines CC-chemokine ligand 2 (CCL2; also known as MCP1) and C-C motif chemokine 20 (also known as MIP3α) (Terao et al., 2009b).

Therapeutic hypothermia also affects some transcription factors which are involved in the inflammatory responses. Nuclear Factor κB (NF-κB) is a classic transcription factor involved in the activation of inflammatory responses (Kim et al., 2014). In animal stroke models, therapeutic hypothermia suppresses NF-κB activation through the prevention of nuclear NF-κB translocation and DNA binding by inhibiting the activity of inhibitor of NF-κB kinase (IKK). IKK is required for the phosphorylation and degradation of NF-κB inhibitor (IκB), thereby allowing NF-κB to enter the nucleus, where it can upregulate target genes (Yenari and Han, 2006).

Mitogen-activated protein kinase (MAPK) pathway, also involved in regulating inflammation, is affected by therapeutic hypothermia. In ischemic stroke models, cooling activates ERK in brain endothelial cells. This leads to decreased levels of intercellular adhesion molecule 1(ICAM1), one of many inflammatory factors regulated by MAPK signaling pathway and an adhesion molecule involved in the binding of circulating immune cells onto the brain's vasculature prior to entry into the brain parenchyma (Choi et al., 2011).

3.2.4 Blood-brain barrier—Blood brain barrier (BBB) disruption is caused by structural and functional impairment of components of the neurovascular unit, including tight-junction proteins, transport proteins, basement membrane, endothelial cells, astrocytes, pericytes and neurons. Mild to moderate cooling protects the BBB from ischemic injury. Specifically, therapeutic hypothermia prevents the activation of proteases responsible for

degrading the extracellular matrix, such as the matrix metalloproteinases (MMPs) ((Lee et al., 2005). Activated MMPs have been shown to degrade several tight-junction proteins that make up the BBB, leading to edema formation, influx of leukocytes and serum proteins, and brain hemorrhage. Therapeutic hypothermia suppresses the proteolytic activity of MMPs and subsequent degradation of vascular basement membrane proteins and extracellular matrix proteins agrin and laminin (Baumann et al., 2009).

3.3 Effects on the chronic stage of stroke

Although it has been shown that therapeutic hypothermia can affect various pathways involved in the acute and sub-acute phases of stroke, less has been studied for the chronic phase (Yenari and Han, 2012). Recent work has focused on whether therapeutic hypothermia can provide lasting effects, and whether hypothermia might affect recovery and repair mechanisms that occur in brain long after the acute and subacute stages of injury. It should also be pointed out that particularly in this setting, cooling was applied during the acute and sometimes subacute stages of stroke, yet the effects of cooling appear to impact stroke pathophysiology long after normothermia has been restored.

3.3.1 Neurogenesis—After ischemia, injured neurons lose synaptic connectivity and undergo cell death. In the chronic stage, it is increasingly recognized that endogenous restorative processes are activated, leading to neurogenesis and synaptogenesis (Kernie and Parent, 2010). However, the influence of therapeutic hypothermia on this process is still unclear. There are some reports (Xiong et al., 2011) showing that therapeutic hypothermia can enhance both maturation of neural progenitor cells and proliferation of neural stem cells, and promote post-ischemic neurogenesis and synaptogenesis. In contrast, another study (Lasarzik et al., 2009) showed that hypothermia had no effect on neurogenesis. These discrepancies might suggest that cooling might need to be applied during critical time windows to provide significant effects on post-ischemic neurogenesis, although these time windows have yet to be defined (Yenari and Han, 2012).

3.3.2 Astrogliogenesis and angiogenesis—Astrogenesis and angiogenesis are thought to contribute to brain regeneration following brain injury. Astrocytes comprise the largest population of cells in the ischemic core during the subacute to chronic stage after ischemic stroke (Li et al., 2010), and reactive astrocytes comprise the main component of the glial scar. This reactive gliosis and scar formation are thought to obstruct neurite outgrowth and regeneration, particularly after spinal cord injury, but it is yet unclear whether this applies following stroke.(Gao et al., 2013). Gliosis may also exacerbate inflammation and increase injury responses (Trendelenburg and Dirnagl, 2005). Thus, gliogenesis may actually induce some harm. How hypothermia affects gliogenesis has not yet been completely clarified, although there is one report (Kurisu et al., 2016a) showing that hypothermia prevents post-ischemic reactive gliosis and glial scar formation.

Mild hypothermia has been shown to enhance angiogenesis in several brain injury models (Kuo et al., 2010; Xie et al., 2007). Although this effect of enhanced angiogenesis by hypothermia is presumably beneficial to the repair processes (Yenari and Han, 2012), its significance is still unclear. In fact, a few contradictory studies suggest that angiogenesis

may be detrimental to brain repair, as these newly formed vessels may not function normally (Manoonkitiwongsa et al., 2004), and some have reported vessel ‘leakiness’, which could only serve to exacerbate brain edema and hemorrhage (Abumiya et al., 2005; Kanazawa et al., 2011). To clarify these uncertain but presumably beneficial effects of hypothermia, further research is clearly needed.

4. Conclusions and future directions

Therapeutic hypothermia is perhaps the most robust neuroprotectant studied in the laboratories. This promising therapy affects nearly every metabolic, molecular and cellular event in cell death, ultimately leading to the promotion tissue preservation and repair. This multi-faceted aspect of the therapy is likely what is responsible for its strong neuroprotective effect, and there is likely no single unifying mechanism to neatly explain it beneficial effect. Numerous laboratory studies have collectively contributed important knowledge to understand this remarkable intervention. However, there are obstacles that hamper the translation of the therapy to the clinical setting. These challenges are largely unique to the clinical setting, as many are not reproduced in small animal stroke models.

From a research standpoint, many mechanisms involved in therapeutic hypothermia have been investigated and have been clarified from pre-clinical studies. Understanding how hypothermia affects previously unstudied proteins and pathways may help to reveal previously unrecognized therapeutic targets. However, there are still many areas related to cooling that have yet to be investigated. In particular, how hypothermia influences the recovery and repair processes even weeks to months after cooling has stopped may prove to be some of the most important scientific findings in this area.

In summary, therapeutic hypothermia has its place in the laboratory, translational and clinical realms as a model of neuroprotection and now as a treatment for some ischemic brain injuries. However, more research is still needed to understand its biological significance as well as how and whether it can be effectively applied in clinical settings.

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Highlights

- Therapeutic hypothermia is one of the most promising therapy for ischemic stroke.
- Numerous studies clarified its mechanisms and proved its strong neuroprotection.
- Multifaceted mechanism is thought to contribute the strong therapeutic effect.
- However, several unclear issues are remained to be overcome yet.
- Further investigation is warranted to apply this therapy in optimal manner.

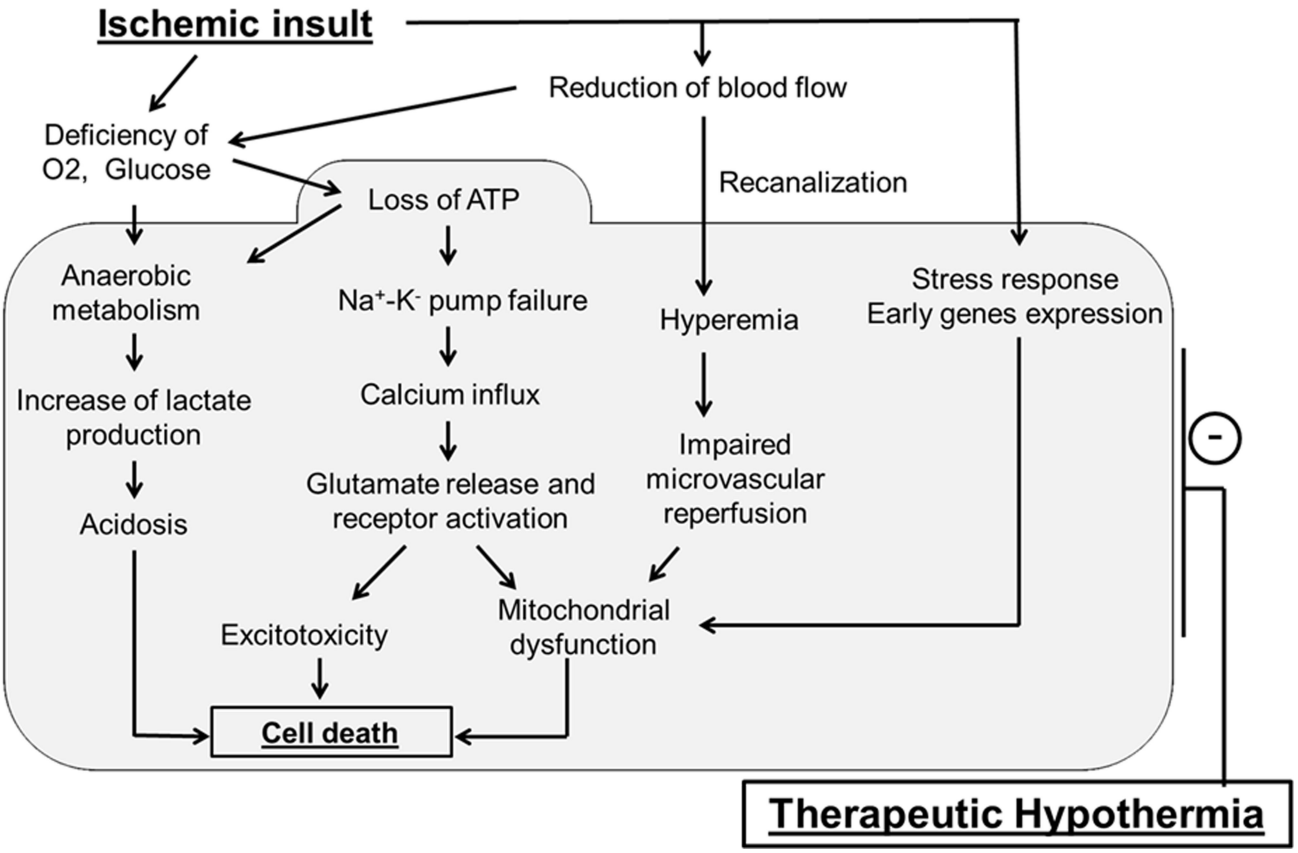

Fig 1.

Mechanisms affected by therapeutic hypothermia during the acute stage of ischemic stroke. Following ischemia, acute events such as loss of oxygen and glucose lead to energy loss (loss of ATP) and ion pump failure. The resulting loss of concentration gradients allows ions to flow down their concentration gradients, leading to cell swelling (cytotoxic edema) and the release of excitatory amino acids (EAAs). All of these events will induce acute cell death. Therapeutic hypothermia has been reported to affect almost every cellular event that leads to cell death.

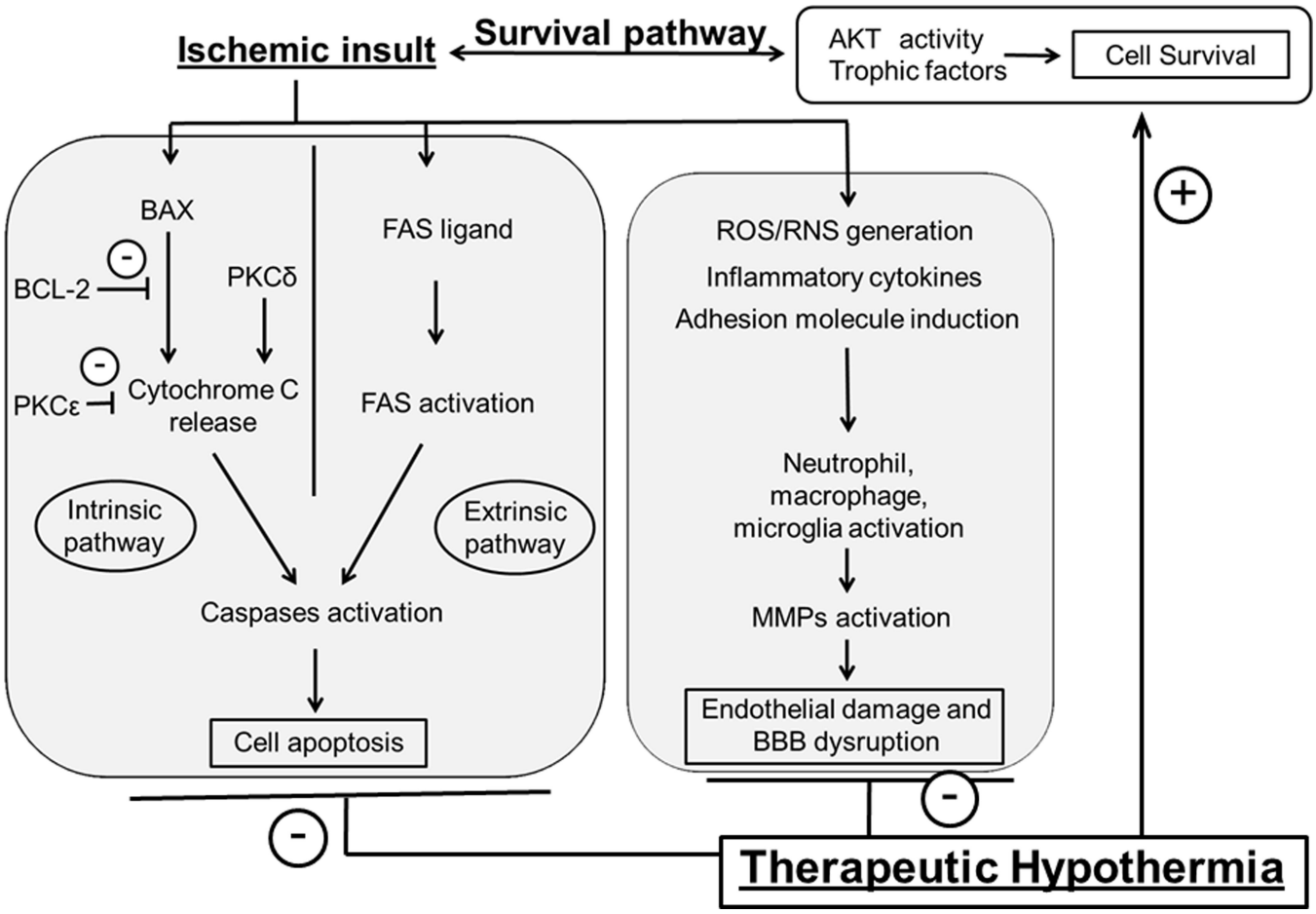

Fig 2.

Mechanisms affected by therapeutic hypothermia on the sub-acute stage of ischemic stroke. Ischemia activates both intrinsic and extrinsic pathway which will lead cell apoptosis. Ischemia also triggers ROS production and inflammatory reaction. These promote endothelial cell damage and BBB disruption. Therapeutic hypothermia has been associated with suppression of both apoptotic and inflammatory pathways, while upregulating cell survival pathways.

BCL-2: b-cell leukemia/lymphoma 2 protein; BAX: Bcl-2-associated X protein; PKC: phosphokinase C; ROS: reactive oxygen species; RNS: reactive nitrogen species; MMP: matrix metalloproteinase; BBB: Blood brain barrier.