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Therapeutic hypothermia: neuroprotective mechanisms

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1. ABSTRACT

Hypothermia has long been known to be a potent putative neuroprotectant. Experimental evidence and clinical experience show that hypothermia protects the brain from cerebral injury. Recent insights into the mechanisms of cerebral ischemia and reperfusion suggest reasons why hypothermia may be an ideal modality for stroke therapy. Hypothermia protects brain tissue in multiple ways. It retards energy depletion, reduces intracellular acidosis, lessens the ischemia related accumulation of excitotoxic neurotransmitters, and attenuates the influx of intracellular calcium. Additionally, hypothermia suppresses the generation of oxygen free radicals involved in secondary damage associated with reperfusion. It also suppresses the mechanisms related to blood– brain barrier degeneration and postischemic remodeling. The clinical application of therapeutic hypothermia and its limitations will be summarized in this paper. Therapeutic hypothermia is likely to undergo phase III clinical trials in various clinical settings. Novel technologies are being developed to optimize the safety and efficacy of this promising approach.

2. INTRODUCTION

Hypothermia is recognized as perhaps the most robust neuroprotectant studied in the laboratory to date. It has been shown to alter a variety of effects of cerebral injury, including reduction in metabolic and enzymatic activity, glutamate release and re-uptake, inflammation, production of reactive oxygen species, and the expression/downregulation of a host of other genes. Although stroke models vary in methodology, several laboratories have consistently shown that hypothermia reduces the extent of neurologic damage and improves neurologic function.

There is growing evidence that induced hypothermia can have neuroprotective effects in some patients with neurological injury. An association between body temperature, initial stroke severity, infarct volume, and clinical outcome has been recognized (1). Mild to moderate hypothermia has been found to reduce ischemic brain edema in the setting of massive ischemic strokes (2, 3). Several preliminary clinical reports indicate benefits of mild to moderate hypothermia as an adjunct to

thrombolytic therapy. The clinical use of hypothermia is therefore likely to increase in the near future (4).

This review discusses potential mechanisms for hypothermia's neuroprotective effects, followed by potential clinical applications which hypothermia has been shown to be effective, taking into consideration potential limitations and challenges in developing therapeutic hypothermia at the clinical level.

3. HYPOTHERMIC NEUROPROTECTION

3.1. "Dosing" of hypothermia

Factors which need to be taken into consideration when applying hypothermia are the depth and duration of cooling and time of onset. Until now there are no clear therapeutic standards except that cooling should be initiated as soon as possible. Collective laboratory research has suggested that small decreases in temperature are as protective as larger decreases, and prolonging the duration of cooling may also permit more delayed application of hypothermia.

Different levels of hypothermia have been defined, depending on the depth of cooling: mild (> 32 °C), moderate (28 – 32 °C), deep (20 - 28 °C), profound (5 – 20 °C), and ultraprofound (< 5 °C) hypothermia. Deep to ultraprofound hypothermia has been used extensively in the past for resuscitating trauma victims or during high risk surgery such as cardiothoracic surgery requiring cardiac arrest or neurosurgery. Because of the numerous complications of deep to profound hypothermia and the difficulty in achieving and maintaining these temperatures, mild to moderate hypothermia are becoming more attractive alternatives (5). Furthermore, experimental evidence indicates that the extent of neuroprotection is similar whether temperature is reduced to 34 °C or 25 °C (6, 9).

The optimal duration of hypothermia after cerebral ischemic injury is unclear. Some groups have used brief durations of hypothermia (0.5-5 h), whereas others used longer periods (12-48 h), and neuroprotection was observed in nearly all cases (8). In a few studies where the duration of hypothermia was compared directly, durations of 1-3 h appeared effective, whereas 0.5-1 h were not (9, 10). Longer durations may be necessary especially when the initiation of cooling is delayed, and this is corroborated by rodent data indicating robust neuroprotection when hypothermia is delayed by several hours provided cooling is maintained for more than 24 h (11, 12). In clinical stroke, hypothermia may be a more effective neuroprotective strategy if applied for a long duration after the ischemic event, as most patients do not present until hours after the onset of stroke (13, 14). Although a long cooling time seems attractive, this may be offset by an increased risk of complications.

Most experimental data have convincingly shown the robust effects of hypothermia in models where reperfusion occurs. However, whether hypothermia is protective in models of permanent occlusion is less clear, as some groups have documented protection whereas others have not (8,25). The reasons for these discrepancies are not clear, and need further investigation.

3.2. Temporal therapeutic window for hypothermia

From laboratory studies, it has been clear that cooling is remarkably neuroprotective when applied during ischemia. Therefore, hypothermia should be initiated as soon as possible to achieve its optimal beneficial effect. In contrast, the value of postischemic cooling is less clear because of early clinical difficulties and conflicting animal data. However, even with a delay of several hours after the onset of cerebral ischemia, hypothermia was reported to be advantageous compared to control groups maintained at normothermia (14, 15). These observations were important from a treatment perspective and studies were initiated to determine the therapeutic window for postischemic hypothermia. In the rat global cerebral ischemia model, hypothermia to 30 °C commencing 30 min into the start of reperfusion, was reported to be ineffective for protection of the hippocampal neurons(16), but in a gerbil forebrain ischemia model, hypothermia to 30 °C began even 1 h after the start of reperfusion, was reported to be effective if the hypothermia was continued for a long time (14). Yet, studies by Dietrich *et a*l. (17, 18) showed that post-ischemic hypothermia merely delayed the onset of irreversible neuronal injury, unless combined with a second neuroprotectant. However, this latter study only applied hypothermia for 3 h. More recent rodent experiments have shown that a prolonged reduction in temperature (12-48 h) of only a few degrees can provide sustained behavioral and histological neuroprotection as far as 6 months post ischemia onset (11, 12). Thus, the extent of a neuroprotective effect is influenced by the length of the delay and the duration of hypothermia (19).

A few studies concerning the therapeutic time window in the focal cerebral ischemia model have been reported. In a study by Karibe *et a*l. (20), the therapeutic time window for obtaining a brain-protecting effect with mild hypothermia in the case of 2 h of middle cerebral artery occlusion (MCAO) was 10–30 min for the basal ganglia and 30–60 min for the cerebral cortex, and no reduction in the infarct volume was observed when mild hypothermia was begun 1 h after the start of ischemia. Baker *et a*l. (21) also reported that when hypothermia to 24 was begun within 1 h after the start of permanent MCAO, a reduction of the infarct volume was obtained after 24 h, but when mild hypothermia was begun at 2 h after the start of ischemia, no reduction of the infarct volume was observed. Yet other studies in models of temporary MCAO indicate that delays of up to 3 h from the onset of ischemia with hypothermia maintained anywhere from 2-48 h is protective (11, 22-23).

These studies collectively indicate that even delayed post-ischemic hypothermia can reduce the extent of ischemic injury due to focal cerebral ischemia, which is remarkably encouraging for clinicians. What parameters will be effective in humans remains to be seen, but in two clinical studies of therapeutic mild hypothermia in cardiac arrest patients showed neurological efficacy when cooling

began within 2 h of return of circulation and maintained for 12-24 h (7, 24). Studies in ischemic stroke patients are ongoing (24).

4. MECHANISMS OF PROTECTION BY HYPOTHERMIA

Evidence from animal models of ischemic stroke indicates that hypothermia affects a wide range of the processes involved in ischemic brain damage. Hypothermia seems to mitigate neuronal damage at all levels of the ischemic cascade, which suggests a great therapeutic potential for hypothermic therapy to alleviate ischemic brain injury. Hypothermia seems to counteract ischemic brain damage by several mechanisms: lowering of metabolic rate and subsequent energy depletion; reduction of the excitotoxic neurotransmitter release; prevention of blood–brain-barrier disruption and subsequent amelioration of cerebral edema formation; diminishing oxygen free-radical production that results from impaired mitochondrial function and activation of inflammatory cells including microglia; other anti-inflammatory actions; suppression of specific cell death pathways or upregulation of cell survival mechanisms.

4.1. Hypothermic Effect on Metabolism and Cerebral Blood Flow

Hypothermia decreases cellular metabolism by retarding energy depletion and facilitating postischemic glucose utilization (26). For each 1°C decrease in temperature, the cerebral metabolic rate decreases by 5–7%. The Q10 for the cerebral metabolic rate of oxygen (CMRO2), or the ratio of metabolism at 10 °C intervals, has been estimated at 2.5. Since the CMRO2 is the main determinant of cerebral blood flow, hypothermia may provide for a relative improvement in oxygen supply to areas of ischemic brain (see recent reviews) (27,28). During ischemia, cellular metabolism in the penumbra undergoes significant changes. As the neurons continue to fire, potassium ions flood into the extracellular space, calcium ions flow into the neurons leading to cytoskeletal degradation, and ATP concentrations fall as energy depletion continues (29). Hypothermia reduces calcium influx and the subsequent breakdown of intracellular structures (30), improves potassium ion homoeostasis (31), and helps metabolic functions, such as calcium or calmodulin-dependent protein kinase activity to recover (32, 33). However, whether hypothermia's effects on cerebral metabolism will fully explain its protective effect is unlikely. After ischemia, metabolic stores are depleted within minutes, yet neuroprotection has been observed even when hypothermia is delayed by several hours. Furthermore, the extent of protection is not predicted by the Q10 for brain metabolism. That is, the amount of protection when brain temperature is lowered 10C is similar to protection when the brain is lowered 4 °C. Therefore, other factors are likely to play a role. However, this is not to say that preservation of brain metabolic stores does not explain the neuroprotection observed during immediate cooling--in this latter scenario, it likely is.

Under normal conditions, cerebral blood flow (CBF) to the brain is approximately 50 ml/100 g/min. In a feline study, hypothermia proportionately decreased CBF from 48 ml/100 g/min in normothermic animals to 21 and 11 ml/100 g/min at 33 °C and 29 °C, respectively (34). Coupling between CBF and brain metabolism appears, for the most part, preserved at different temperatures. However, under conditions of ischemia, the relationship between temperature and CBF are less clear. Brain ischemia leads to initial decreases in CBF, but upon reperfusion, hyperemia, or increases in CBF above normal is often observed (35). This hyperemia is typically followed by gradual decreases in CBF during the reperfusion phase. Some studies indicate that hypothermia actually increases CBF during the period of ischemia (6, 36), whereas other reports note reduced CBF (34) or no effect (38-40). Mild hypothermia also appears to blunt the early post-reperfusion hyperemia and prevent the gradual decrease in CBF during reperfusion (37)

4.2. Effect of Hypothermia on Excitotoxicity

The amino acid, glutamate acts as a neurotransmitter. Ischemia severe enough to damage the brain causes flooding of excitatory synapses with glutamate due to failure of energy dependent reuptake pumps that normally remove glutamate from the synapse into glia (41-43). The high levels of glutamate, in turn, lead to strong activation of glutamate receptors, especially the N-methyl-D-aspartate (NMDA)-type glutamate receptor. Energy failure also leads to depolarization and voltage-dependent loss of magnesium, which normally blocks this receptor (44). Elevated synaptic levels of glutamate lead to large amounts of calcium entering cells which subsequently activate a variety of death promoting signaling pathways. Various animal experiments have shown that hypothermia improves ion homeostasis and blocks or slows many of these destructive excitatory processes (16, 45-54). Key destructive processes, such as calcium influx, accumulation of glutamate, and release of its coagonist, glycine, are all blocked by hypothermia (46-48).

Mild hypothermia alters neurotransmitter release (55). During focal cerebral ischemia, neurotransmitter release increases within 10-20 min of ischemia onset, peaks within 60 minutes, decreases by 50-90 min and then returns to baseline or decreases substantially by 90–120 min (55-58). Intraischemic mild hypothermia appears to blunt this peak and, in some instances, delays it by 20 minutes (57, 58). Hypothermia between 30 and 33ºC completely inhibits glutamate release (55). By reducing of the glutamate surge, with subsequent reduction of calcium mobilization and ATP expenditure, mild hypothermia is likely to ameliorate cytotoxic edema (59). Even though reduction of presynaptic glutamate release is likely an important mediator of hypothermic neuroprotection, few reports are available regarding the mechanisms for attenuation of ischemia-induced effluxes of neurotransmitters by hypothermia. As it is generally accepted that biosynthesis and uptake of neurotransmitters are temperature-dependent, the degree of glutamate surge is largely depressed and the onset of calcium mobilization is significantly delayed by hypothermia.

The cascade initiated by the disruption in Ca^{2+}

homeostasis continues hours to even days after ischemia. Mild hypothermia has been shown to inhibit the translocation of Ca^{2+}/calmodulin-dependent kinase II and protein kinase C-alpha, beta, gamma isoforms to the synaptosomal fraction. Hypothermia also inhibited fodrin proteolysis caused by ischemia with reperfusion, and subsequent inhibition of the protease, calpain (45). Although Ca^{2+} mediated events continue even days after ischemia, interfering with this process at such late timepoints is not supported by the animal data indicated that the temporal therapeutic window for hypothermia is only a few hours. Therefore, it is likely that these Ca^{2+} triggered events represent irreversible processes no longer amenable to therapeutic hypothermia.

However, a few studies have shown that mild postischemic hypothermia is still effective even when applied after glutamate is released (57, 60). In one study, greater protection was observed at 30 ^{o}C than at 33 ^{o}C (45), even though these two conditions equally reduced excitotoxic neurotransmitter efflux (55). Therefore, hypothermia's effects on ischemic neurotransmitter release per se cannot completely explain its neuroprotective effects.

4.3. Hypothermia and Oxidative Stress and Apoptosis

Following ischemia, and, in particular, ischemia followed by reperfusion, reactive oxygen species (ROS) are formed from injured mitochondria when ischemic brain is exposed to reoxygenated blood. Reperfusion also triggers the generation of ROS through other enzyme pathways such as the glutamate activated xanthine oxidase pathway in neurons and the NADPH oxidase system in inflammatory cells. Several studies have shown that hypothermia attenuates free radical formation and thus provides protection (47, 52, 61, 62). The generation of ROS leads to oxidative stress which leads to a variety of damaging consequences to the ischemic brain. ROS can lead to lipid peroxidation, direct DNA damage and can trigger other cell death pathways such as apoptosis. Hypothermia has been shown to inhibit all of these processes (9, 63-66)

Apoptosis, or programmed cell death, has been documented to occur in the ischemic brain (see reviews (67-69)). In general, there are two major apoptotic pathways, the intrinsic, or mitochondria based pathway, and the extrinsic, or receptor mediated pathway. The intrinsic pathway is triggered by mitochondrial release of cytochrome c followed by activation of the apoptosome (pro-caspase-9 bound to Apaf-1) leading to caspase-9 processing. Activated caspase-9 then activates caspase-3, an effector caspase which leads internucleosomal cleavage of DNA. Mitochondrial cytochrome c release can be modulated by pro- or anti-apoptotic members of the Bcl-2 family. The receptor mediated pathways were first described in immune cells as a means of limiting inflammatory reactions. Binding of a pro-apoptotic ligand to cell surface receptors leads to the recruitment and activation of caspase-8, which eventually leads to activation of caspase-3. Examples of extrinsic apoptotic pathways include Fas/FasL and TNFR/TNF.

Several aspects of apoptosis have been shown to be inhibited by hypothermia (9, 63, 64, 66). Hypothermia has been shown to increase endogenous production of the anti-apoptotic protein Bcl2 (70), reduce DNA fragmentation (71) and inhibit cytochrome c release (72) and caspase activation (64, 73). Less has been studied with regard to receptor mediated pathways and apoptosis in ischemia. However, a few studies have indicated that hypothermia can inhibit Fas (64) and caspase-8 activation (74)

4.4. Effect of Hypothermia on Inflammation

The inflammatory response associated with brain ischemia is thought to contribute to the evolution of tissue injury (see reviews (73,75,76)). Necrotic tissue and ROS stimulate innate immune responses leading to microglial activation, upregulation of endothelial adhesion molecules which leads to infiltration of peripheral leukocytes. Peripheral leukocytes and activated microglia can then secrete a variety of damaging factors such as nitric oxide, inflammatory cytokines, chemokines, superoxide and other proteases which can further exacerbate ischemic injury.

Hypothermia has been shown to have an anti-inflammatory effect by lowering numbers of tissue neutrophils and suppressing microglial activation (78-80,81). Hypothermia appears to reduce tissue leukocytes by preventing their binding to vascular endothelium (81) and downregulating the endothelial adhesion molecule, intercellular adhesion molecule (ICAM-1), thus preventing their ability to infiltrate the brain (76, 82). Furthermore, mild hypothermia also reduces various inflammatory mediators such as nitric oxide and cytokines (83, 84), possibly by inhibiting activation of the inflammatory transcription factor nuclear factor kappa B (NFkB) (85). However, since inflammation is triggered by the presence of necrotic tissue, one could argue that since hypothermia reduces the extent of brain injury, the mechanism of hypothermia may be due to factors upstream of necrosis, and the resulting inflammatory response is proportional to the amount of tissue damage. Interestingly, these anti-inflammatory effects of hypothermia were also observed in models of brain inflammation where cell death did not occur, and would suggest that hypothermia has direct effects on immune responses (85, 86). In humans, hypothermia has been shown to suppress proinflammatory cytokine production in peripheral monocytes and levels of serum IL-6 following brain injury (87, 88).

4.5. Effect of Hypothermia on the blood-brain barrier (BBB): Vascular Permeability and Edema Formation

BBB is composed of endothelial tight junctions, basal lamina, and perivascular astrocytes. The endothelial tight junctions regulate substrate transfer. Cerebral ischemia affects the entire neurovascular unit, and breakdown of the BBB allows blood constituents to move into the interstitial space (vasogenic edema). Hypothermia appears to reduce disruptions in the blood-brain barrier (89, 90) and vascular permeability following ischemia-reperfusion injury (91), thereby decreasing edema formation. Induction of hypothermia also decreases extravasation of hemoglobin following traumatic brain

injury (TBI) (92). This observation supports the concept of a membrane and blood-brain barrier stabilizing effect. The importance of brain edema in the exacerbation of neurological injury in patients with TBI, stroke and anoxic injury following cardiac arrest is well recognized (93-95).

Hypothermia suppresses the breakdown of the blood-brain barrier (40, 96) and decreases intracranial pressure (97). Recent work has focused on the role of matrix metalloproteinases (MMPs) and the destruction of the BBB and extracellular matrix. MMPs are a family of serine proteases which have been detected in ischemic brain (98). They are produced as pro-enzymes cleaved to their active form by various proteases including tissue plasminogen activator (tPA). Activation of MMP-9 is associated with blood-brain barrier breakdown (75) and in at least one study was correlated with areas of hemorrhagic conversion after focal ischemia. MMP-9 rather than MMP-2 has been shown to play a significant role in ischemic injury (99, 100). Inhibiting MMP expression and activation has been shown in laboratory models to reduce the incidence of BBB disruption, edema and hemorrhage, including thrombolytic related hemorrhage (101, 102). A few studies have now shown that therapeutic mild hypothermia not only reduces BBB disruption and edema, but also reduces generation of MMPs and endogenous fibrinolytics, the latter of which are thought to cleave pro-MMPs to their active form (103-105). The mechanism underlying dramatic reductions in intracranial pressure may be linked to the reduction in edema formation as well to decreased intracranial blood volume due to cerebral vasoconstriction.

4.6. Effect of Hypothermia on cell survival mechanisms

While much of the experimental studies examining the mechanisms underlying the protective effect of hypothermia indicate that cooling tends to downregulate or suppress most processes, damaging or otherwise, hypothermia also appears to upregulate several cell survival mechanisms. A few studies have shown that mild hypothermia applied in models of cardiac arrest is associated with increased expression of trophic factors such as glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF) (42, 106) and neurotrophin (107). All of these trophic factors have been shown in various models of cerebral injury to confer a degree of neuroprotection when applied exogenously. Hypothermia also appears to be associated with the upregulation of the anti-apoptotic protein, Bcl-2 (70, 108, 109) and enhances the activity of Akt leading to the prevention of apoptotic cell death (110).

5. INFLUENCE OF HYPOTHERMIA ON COAGULATION AND FIBRINOLYTIC SYSTEMS

Given that the only pharmacological treatment approved for use in acute stroke in the United States is recombinant tPA (rt-PA), it becomes especially relevant to review existing literature on coagulation and fibrinolysis and temperature. In vitro, exogenous application of rt-PA on clot lysis is temperature dependent. Cooling to 30–33 ^{o}C caused a 2–4% decrease in rt-PA activity in a clot lysis assay (111). However, in the absence of added rt-PA, clot lysis paradoxically increased as temperature was lowered. Data available from the manufacturer (Genentech, South San Francisco, CA) indicate that rt-PA activity decreases by 50% when the clot temperature drops from 40 ^{o}C to 30 ^{o}C. This finding was confirmed independently (112); however, these data must be interpreted with caution, because hypothermia suppresses all aspects of the clotting cascade and fibrinolytic pathways, including thrombolytic inhibitors. Hypothermia also suppresses platelet function and may limit platelet aggregation in the microcirculation (113). In one laboratory study of thromboembolic stroke, rats treated with rt-PA and exposed to moderate hypothermia were found to have smaller infarcts compared to normothermic rats given rt-PA (114). However, infarct sizes were no different from rats treated with hypothermia alone, indicating a lack of an additive effect of the two treatments.

A few studies examining the effects of temperature on coagulation and fibrinolytic systems in humans are available. In trauma patients who were hypothermic, the net effect of hypothermia was a slight increase in propensity toward clotting and no net effect on fibrinolysis (115). A small study of moderate hypothermia (32–33 ^{o}C) after head trauma showed no effect on clotting studies or the incidence of delayed intracerebral hemorrhage (116). These patients were hypothermic for 24 h, and rewarmed over 12 h. Coagulation studies were assayed before therapy and 24 h later. In the absence of better data, there is no obvious contraindication to the combination of thrombolysis and hypothermia, but thrombolytic activity may be decreased anywhere from 4% to 20%. No clinical stroke studies are yet available to address the effect of hypothermia on rt-PA efficacy directly, although clinical trials are ongoing (25).

6. POTENTIAL CLINICAL APPLICATIONS OF THERAPEUTIC HYPOTHERMIA

6.1 Clinical applications

To date, the effects of therapeutic hypothermia have been shown in two independent studies to be efficacious for cerebral injury caused by cardiac arrest. After cardiac resuscitation, 80% of patients will remain comatose for more than one hour. Of those remaining comatose hours after resuscitation, only 10 to 30% of them will have a good neurological recovery at one year. While several small studies suggesting a benefit of hypothermia in cardiac arrest on neurological outcome have been reported throughout the decades, results from two prospective randomized trials are now available (24, 81). These clinical trials both showed a substantial benefit of mild hypothermia after cardiac arrest on neurologic outcome. The Australian study was consisted of 77 patients, of which 34 were maintained normothermic and 43 were treated with hypothermia. Cooling began out-of-hospital using surface cooling methods for a target temperature of 33 ^{o}C. The cooling lasted 12 hours and was followed by passive rewarming. More patients in the hypothermic group had favorable outcomes compared to the normothermic group. The European study was a randomized, blinded study conducted at nine centers in five countries. A total of 275

patients were enrolled, 138 in the normothermic group and 137 in the hypothermic group. Cooling began in hospital, following the return of circulation to 32–34 ^{o}C using surface cooling techniques. Duration of treatment was 24 hours with passive rewarming over 8 hours. The primary endpoint was favorable neurological outcome at 6 months, and 55% of the hypothermic group had a favorable outcome, compared 39% of the normothermic group.

Some clinical data suggest that reduction in body temperature may be beneficial to stroke victims as well. In an observational study, it has been suggested that stroke patients presenting with slight reductions in body temperature had better outcomes than those presenting with higher body temperatures (117). Two feasibility trials of therapeutic moderate hypothermia for massive ischemic stroke have been published to date. The first was a report of 25 patients with severe MCA infarction. The mean time from onset of symptoms to initiation of hypothermia was 14 hours, after which patients required 3.5–6.2 hours to achieve a target of 32–33C (2). 56% of patients survived, but herniation related to cerebral edema during rewarming was a prominent complication. The second trial was the Cooling for Acute Ischemic Brain Damage (COOL AID) open pilot study, published in 2001 (4). Hypothermia using surface cooling was induced in 10 patients who suffered massive infarction. It was initiated at a mean of 6.2 hours after stroke onset, and target temperatures were achieved on the average by 3.5 hours after the initiation of cooling. Three-month neurologic outcomes were better in the hypothermia group, although these numbers did not reach statistical significance. A major obstacle in applying hypothermia in humans is rapid attainment of cooling and maintaining target temperatures. Recent technological advances include the use of cooling devices which can more rapidly and stably induce hypothermia. These devices are currently under study for a variety of indications including therapeutic hypothermia for stroke treatment (118).

6.2. Combination therapy

A number of recently published reports support the notion that combination therapy of therapeutic hypothermia with pharmacological agents may be synergistic. In a model of cardiac arrest in dogs, the effect of mild hypothermia may be enhanced by thiopental, as well as the addition of phenytoin and methylprednisolone (119). The fact that mild hypothermia plus lamotrigine together were more effective in inhibiting extracellular glutamate accumulation than hypothermia or lamotrigine alone, suggests the potential for increased neuroprotection by the addition of lamotrigine to mild hypothermia (120). A study of combination of postischemic hypothermia (where protection was previously shown to be only transient) and delayed MK-801 administration indicated that the temporal therapeutic window for attenuating ischemic damage could be considerably prolonged (121). Long term neuroprotection could be observed if hypothermia were combined with IL-10, an anti-inflammatory cytokine, when neither treatment alone was effective (122). Hypothermia combined with N-tert-butyl-alpha-pheylnitrone treatment provided long-term cognitive improvement in a forebrain ischemia model, although the effect of combined treatment in this case did not appear superior to either treatment alone (123). These results collectively support promising strategies for a “cocktail” approach for therapeutic hypothermia. By combining hypothermia with other neuroprotectants, it may be possible to enhance protective effects, reduce side effects and lengthen the maximum time in which such therapies can be initiated.

7. CONCLUSION

Neuroprotection conferred by hypothermia is likely multifactorial, and may explain why many have dubbed it the most robust neuroprotectant studied to date. It is successfully moving from bench to bedside, and has been used in patients undergoing high risk cardiac and neurological surgeries. It could be viewed as the first proven neuroprotectant at the clinical level for improving outcome after cardiac arrest and is increasingly becoming the standard of care in that setting. Preliminary clinical studies of mild to moderate hypothermia in the treatment of acute ischemic stroke are encouraging and studies are ongoing.

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