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Protection in Animal Models of Brain and Spinal Cord Injury with Mild to Moderate Hypothermia

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Abstract

For the past 20 years, various laboratories throughout the world have shown that mild to moderate levels of hypothermia lead to neuroprotection and improved functional outcome in various models of brain and spinal cord injury (SCI). Although the potential neuroprotective effects of profound hypothermia during and following central nervous system (CNS) injury have long been recognized, more recent studies have described clinically feasible strategies for protecting the brain and spinal cord using hypothermia following a variety of CNS insults. In some cases, only a one or two degree decrease in brain or core temperature can be effective in protecting the CNS from injury. Alternatively, raising brain temperature only a couple of degrees above normothermia levels worsens outcome in a variety of injury models. Based on these data, resurgence has occurred in the potential use of therapeutic hypothermia in experimental and clinical settings. The study of therapeutic hypothermia is now an international area of investigation with scientists and clinicians from every part of the world contributing to this important, promising therapeutic intervention. This paper reviews the experimental data obtained in animal models of brain and SCI demonstrating the benefits of mild to moderate hypothermia. These studies have provided critical data for the translation of this therapy to the clinical arena. The mechanisms underlying the beneficial effects of mild hypothermia are also summarized.

Key words: hypothermia; regeneration; stem cells; traumatic brain injury; traumatic spinal cord injury

Introduction

In the 1950s, moderate hypothermia (30°C) revolutionized the area of cardiac surgery in that lowering body temperature preserved neural function in patients undergoing these surgical procedures. Various pioneers in the area of hypothermia demonstrated in animal models that deep to profound levels of hypothermia (<29°C) appeared to be protective in their experimental studies. Although the benefits of profound hypothermia were certainly witnessed, side effects (including increased infection and cardiac arrhythmias) were also documented as adverse consequences of this therapy. During these investigations, various new drug therapies were advanced that diverted the study of hypothermia except in a few laboratories. The emergence of new neuroprotective strategies in addition to the difficulties in producing hypothermia and maintaining it at critical levels was also a reason why this field was not advanced in the 1970s.

In the 1980s, the observation that small variations in the temperature of the brain during a period of cerebral ischemia significantly affected neuronal vulnerability to that insult certainly helped reignite the field. Various laboratories throughout the world began to study the importance of brain temperature in their experimental animal models, and they reported that indeed relatively mild to moderate levels of hypothermia were neuroprotective. This finding was important because the protection was seen without the significant adverse effects of more profound reductions in systemic temperature.

In addition to the obvious therapeutic considerations of hypothermia, the ability to alter injury outcome by manipulating the temperature of the brain or spinal cord during an insult represented a powerful tool to investigate pathomechanistic issues regarding injury cascades. In the past 20 years, an extensive list of pathomechanisms has been shown to be sensitive to relatively mild variations in temperature. Indeed, many scientists point to the fact that temperature affects many pathological processes when trying to explain the ability of mild hypothermia to protect under a variety of experimental clinical conditions. The purpose of this paper is to review experimental data obtained in various animal models of brain and spinal cord injury (SCI) demonstrating

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the beneficial effects of mild to moderate hypothermia on outcome. In addition, the mechanisms underlying hypothermic protection are also discussed.

Transient Global Ischemia

Models of transient global ischemia have been used for many years to model the histopathological and behavioral consequences of cardiac arrest. This model leads to consistent neuronal vulnerability within the CA1 hippocampus and dorsalateral striatum. In 1987, Busto et al. (1987) first reported that core temperature unreliably reflected brain temperature during periods of global forebrain ischemia induced by four-vessel occlusion. In that study, direct measurements of brain temperature during the ischemic insult showed that, although core temperature could be maintained at normothermia, brain temperature was significantly reduced during the insult unless strategies were put into place to inhibit the intraischemic reduction in brain temperature. Studies subsequently showed that, by artificially maintaining brain temperature at normothermic levels, relatively mild reductions in brain temperature to 34°C significantly protected selectively vulnerable brain regions including the CA1 hippocampus and dorsalateral striatum (Busto et al., 1989a). In other studies using transient global ischemia, scientists reported that a mere decrease of 2°C in body temperature provided 100% protection of neurons in the CA1 region of the hippocampus (Minamisawa et al., 1990; Welsh et al., 1990a). These studies indicated that the biochemical events associated with selective neuronal vulnerability, which were and remain an important topic of research, were extremely temperature sensitive. Although many investigators used whole body cooling to produce the mild hypothermic effect, other studies showed that selective brain cooling during and after a global ischemic insult could also protect the brain from histopathological damage (Kuluz et al., 1992).

In addition to histopathological assessment, mild levels of hypothermia have also been shown to improve functional outcome in transient global ischemia. Green et al. (1992) demonstrated that moderate hypothermia (30°C) during an ischemic period attenuated behavioral deficits including sensorimotor and cognitive functioning. In models of cardiac arrest and cardiopulmonary bypass, Leonov et al. (1990b) examined the effects of mild (34°C) hypothermia in cardiac arrest models in dogs. Both behavioral and histopathological scores at 96 h were improved compared to normothermic animals. Models of cardiac arrest in cats and rats have also showed that moderate hypothermia prevented neuronal degeneration and improved recovery (Horn et al., 1991; Jia et al., 2008). Thus, moderate hypothermia initiated during and after cardiac arrest or cardiopulmonary bypass was shown to be neuroprotective in several animal models.

Postischemic Hypothermia

In clinical situations, the ability of a treatment to protect when initiated after the ischemic insult is a requirement for clinical relevance. In early studies of global ischemia, intraischemic hypothermia was predominantly investigated and subsequent studies began to determine whether delaying the hypothermic event after the ischemic insult would also be beneficial. In a number of studies, significant but partial protection of CA1 hippocampus was documented at relatively short survival periods when a restricted period of hypothermia was initiated after the ischemic insult (Busto et al., 1989a). These early observations were important in that a therapeutic intervention initiated after the primary ischemia period could reduce temperature sensitive pathophysiological events associated with neuronal cell death.

Various investigators began to determine the therapeutic window of hypothermic therapy. Time periods ranging from 30 min to several hours were investigated to determine factors involved in therapeutic protection including window of treatment as well as what duration of ischemia could be tolerated (Colbourne et al., 1998). In 1992, Chen et al. (1992b) failed to demonstrate histopathological protection with postischemic hypothermia after 12 min of global ischemia. However, in other studies including those produced in a dog model of ventricular fibrillation and cardiac arrest, moderate hypothermia (32°C) initiated during recirculation and continued for 3 h reduced overall histopathological damage at 96 h compared to normothermic animals (Leonov et al., 1990a). These studies emphasized that ischemic severity, the duration of hypothermia treatment, the therapeutic window, and the survival period were all important factors in determining whether postischemic hypothermia was protective.

In addition to these factors, the duration of postischemic hypothermia has also been shown to be a critical variable in determining degrees of protection (Buchan and Pulsinelli, 1990; Colbourne and Corbett, 1995; Markarian et al., 1996; Yanamoto et al., 1996). In a study by Carroll and Beek (1992), a 6 h period of immediate postischemic hypothermia was protective, while a relatively short 1 h period was not. Thus, the duration of the postischemic hypothermic period appears to be a significant factor in determining the beneficial effects of postischemic hypothermia. This fact may explain some of the negative effects of hypothermia seen in early studies.

Finally, another factor that appears to be important in the benefits of hypothermia is the duration of survival after the ischemic insult. In early studies, a 3 day survival or less were commonly used as an indicator of protection. Most recently, more clinically relevant survival periods have been examined and have been shown to be a critical factor in determining the long-term effects of hypothermic therapy (Corbett et al., 1997; Dong et al., 2001). In one study, postischemic hypothermia was shown to be protective after a 3 day survival, yet no protection was documented at 2 months after the ischemic insult (Dietrich et al., 1993). These data indicated that brain hypothermia with limited postischemic duration provided only temporary protection from a normothermic global ischemia. These data also emphasized the need to investigate indicators of long-term histopathological and behavioral protection when attempting to translate these preclinical findings to the clinical arena.

Focal Brain Ischemia

Models of focal brain ischemia are generally used to produce pathological outcomes consistent with clinical stroke. In models of focal ischemia, large vessels are occluded by a variety of surgical procedures to produce severe levels of cerebral ischemia in specific arterial territories. Other models of focal ischemia include the injection of particles or clots into cerebral vessels that embolize to small vascular territories to produce single or multiple foci of severe ischemic insults. In

the 1950s, deep hypothermia at levels less than 25°C was shown to protect against focal ischemia. Classic studies by Rosomoff (1959) demonstrated that profound hypothermia protected against middle cerebral artery (MCA) occlusion in various animals, including dogs, monkeys, and rats. In more recent studies, more moderate levels of hypothermia have been investigated in reproducible models of focal cerebral ischemia (Onesti et al., 1991; Pabello et al., 2004). Morikawa et al. (1992) compared normothermia (36°C) and hypothermia (30°C) rats with permanent MCA occlusion. In this early study, a positive correlation between infarct area and temperature was shown. In subsequent studies, milder levels of hypothermia (32–33°C) were shown to reduce volume of neocortical infarction following permanent MCA occlusion (Kader et al., 1992; Scholler et al., 2004). Most recently, mild hypothermia has been shown to be protective in models of MCA occlusion when the hypothermic period is extended for relatively long periods of time (Yanamoto et al., 2001; Clark et al., 2008).

In contrast to permanent MCA occlusion, transient focal ischemia is produced by removing the mechanism of occlusion after the brain has been submitted to a period of focal ischemia. This model therefore leads to a focal ischemic insult, followed by reperfusion injury affecting neuronal populations in specific arterial territories. In contrast to the limited and inconsistent beneficial results seen with permanent MCA occlusion, moderate hypothermia has been shown to be protective in several models of transient MCA occlusion when initiated immediately (Ridenour et al., 1992; Zhang et al., 1993; Huh et al., 2000; Nakano et al., 2007) or in a delayed fashion after occlusion (Karibe et al., 1994; Maier et al., 1998; Corbett et al., 2000). Morikawa et al. (1992) demonstrated that selective brain hypothermia (30°C) during a period of 2 h of reversible MCA occlusion reduced infarct volume. Chen et al. (1992a) reported that whole body hypothermia at 30°C prior to ischemia and maintained for 2 h following MCA occlusion also decreased histopathological damage. More recently, delayed hypothermia has been found to be neuroprotective if given for prolonged durations after transient global ischemia (Colbourne et al., 2000; Kawai et al., 2000; Maier et al., 2001; Kollmar et al., 2002, 2007; Florian et al., 2008). These studies indicate that, following a focal ischemic insult, moderate levels of hypothermia can be initiated during the early reperfusion period and protect against mechanisms of reperfusion injury. These studies have now been conducted in thrombolytic models of stroke with hypothermia improving outcome in these more clinically relevant models of ischemia (Kollmar et al., 2004; Urrea et al., 2004).

Traumatic Brain Injury

Early investigations that made use of profound hypothermia in models of brain trauma yielded inconsistent results. A common problem encountered during aneurysm surgery, for example, was post-operative bleeding during rewarming after profound hypothermia. More recent studies have shown that more moderate levels of hypothermia appear to be neuroprotective in well-characterized rodent models of traumatic brain injury (TBI). Clifton et al. (1991) first investigated the effects of systemic hypothermia (30–36°C) following fluid-percussion brain injury in rats. In that study, hypothermia of 33°C significantly reduced mortality rates as well as attenuated deficits in beam walking, beam balance, and body weight loss compared to normothermia treatment. In a study by Lyeth et al. (1993a), the effects of 1 h post-injury hypothermia (33°C) on behavioral outcome was evaluate. Some evidence for improved behavioral outcome was demonstrated with post-traumatic hypothermia. In 1994, Dietrich et al. (1994) showed that post-traumatic hypothermia (30°C) initiated 5 min after fluid-percussion brain injury reduced overall contusion volume and preserved survival of the overlying cortical neurons. Taken together, these studies demonstrated that cooling after a traumatic insult provided histopathological protection, improved motor deficits and decreased mortality rates (Clark et al., 1996). Subsequent studies have shown that post-traumatic hypothermia also improves cognitive functioning in rats following brain trauma. Bramlett et al. (1995) demonstrated that moderate hypothermia (30°C) initiated 5 min after TBI improved hippocampal-dependent learning and memory using the Morris water maze. The beneficial effects of hypothermia on outcome after TBI have now been replicated in other models of brain injury (Dixon et al., 1998; Yamamoto et al., 1999; Matsushita et al., 2001). Additionally, studies have reported that traumatic axonal pathology, an important predictor of outcome in TBI patients, is also reduced with moderate post-injury hypothermia therapy (Koizumi and Povlishock, 1998; Buki et al., 1999). Thus, post-traumatic hypothermia targets the major pathologies in TBI including contusion, neuronal vulnerability, and traumatic axonal injury.

Traumatic Spinal Cord Injury

Traumatic SCI is another research area that has shown some degree of inconsistent results with hypothermic therapy. In early studies, local cooling was utilized to cool areas of the damaged spinal cord. In those investigations, relatively profound levels of hypothermia were shown in some cases to produce marked neurological and functional recovery after spinal cord trauma (Hansebout et al., 1975). Also, some degree of protection was seen when hypothermia was delayed for 3 h after trauma (Kuchner and Hansebout, 1976). Similar to the studies seen with cerebral ischemia, duration of cooling was also shown to be an important factor in determining whether or not hypothermia improved outcome (Wells and Hansebout, 1978).

In 1992, Martinez and Green showed that regional hypothermia (6–18°C) initiated 1 and 5 h post-injury decreased edema formation and hemorrhage in a cat model of SCI (Martinez-Arizala and Green, 1992). In 2000, Yu et al. (2000) reported for the first time that relatively mild to moderate levels of hypothermia (32–33°C) initiated 30 min after T10 thoracic SCI improved outcome. In that study, hypothermia improved open field motor function as assessed by the Basso, Beattie, and Bresnahan locomotive scale, and also protected against both gray and white matter pathology. Those findings and others showing improved walking as well as reduced overall contusion volume verified that mild hypothermia could be used in the post-injury setting to improve outcome in clinically relevant models of SCI (Ha and Kim, 2008; Morochovic et al., 2008). Most recently, mild hypothermia has also been used to target cervical SCI, a commonly seen clinical problem. In a recent study by Lo et al. (2009), improved forelimb function as well as preservation of motor neurons

and decreased contusion volume was seen in rats cooled after a cervical traumatic insult. Taken together, these studies show that mild to moderate hypothermia improves outcome in models of both thoracic and cervical SCI.

Spinal Cord Ischemia

During various surgical procedures requiring aortic clamp cross-clamping, spinal cord ischemia can result in paralysis. Several studies have investigated the effects of mild cooling on spinal cord ischemia. In 1986, Robertson et al. showed that moderate hypothermia (33°C) increased the duration of ischemia required to produce neurological deficits in rabbits (Robertson et al., 1986). Hypothermia has been found to improve outcome in several animal models of ischemic SCI, including pigs (Colon et al., 1987; Strauch et al., 2004), rabbits (Naslund et al., 1992; Wakamatsu et al., 1999; Tetik et al., 2002), and dogs (Berguer et al., 1992; Tabayashi et al., 1993) Various new methodologies producing local cooling including epidural cooling techniques have also provided positive results (Yoshitake et al., 2007). In a study by Malatova et al. (1995), an epidural cooling technique provided evidence that deep spinal cord hypothermia provided some degree of protection following a regional ischemic insult. Thus, moderate levels of cooling are protective in both traumatic and ischemic SCI models.

Mechanisms Of Hypothermic Protection

Studies from a number of laboratories have reported that subtle variations in temperature have significant effects on a number of pathophysiological mechanisms thought to be important in irreversible neuronal injury and neurological deficits after brain and SCI. The ability of mild hypothermia to target multiple pathomechanisms emphasizes the importance of temperature modifications in effecting the pathophysiology of a central nervous system (CNS) insult. Thus, it has become very common for core temperature to be recorded and rigorously maintained during experiments conducted to investigate pathomechanisms or therapeutic interventions targeting specific injury cascades. Indeed, a reason why mild to moderate hypothermia has been shown to be protective in many models of injury is that it does affect multiple pathomechanisms. The following sections summarize the current thinking regarding basic mechanisms of hypothermic protection.

Metabolic Consequences

In early studies where profound hypothermia was investigated, brain cooling was reported to decrease O_2 consumption and CO_2 production (Bacher et al., 1998). Using 2-deoxyglucose techniques to record local rates of glucose utilization, researchers showed that moderate hypothermia (30°C) reduced glucose utilization compared to normothermia (Tohyama et al., 1998). Metabolic effects of mild hypothermia have also been shown using nuclear magnetic resonance spectroscopy where the metabolic effects of different levels of hypothermia were reported (Chopp et al., 1989; Chen et al., 1992a; Lo and Steinberg, 1992; Kaibara et al., 1999). Thus, hypothermia lowers metabolic and energy demands which can have beneficial effects on cytoplasmic ATP stores and the maintenance of normal transmembrane ion and neurotransmitter gradients. In this regard, some studies have shown that moderate hypothermia reduces the extent of ATP depletion during periods of brief ischemia (Welsh et al., 1990b; Sutton et al., 1991; Ibayashi et al., 2000). The degree of preservation of ATP levels depends on both the hypothermic level as well as the severity of the insult. It is currently felt that, although cerebral hypothermia may not prevent the eventual depletion of ATP or lactate accumulation during a prolonged period of ischemia, hypothermia could certainly slow ATP depletion during a brief ischemia period (Jiang et al., 2004). Thus, an important mechanism for the neuroprotective effects of hypothermia is a reduction or delay in metabolic consumption during the stress of a CNS injury.

Hemodynamic Consequences

The consequences of hypothermia on cerebral blood flow have been somewhat controversial. As previously discussed, the magnitude of hemodynamic consequences with temperature fluctuations depends on various variables including the degree of hypothermia as well as method of inducing the temperature reduction. In 1954, Rosomoff and Holaday demonstrated that systemic hypothermia down to 25°C significantly lowered cerebral blood flow (Rosomoff and Holaday, 1954). However, in a model of selective brain cooling (30.9°C), cortical blood flow measured by laser Doppler flowmetry was shown to increase above control levels (Kuluz et al., 1993). In regards to spinal cord blood flow, Hansebout et al. (1985) reported that local cooling of the spinal cord down to 16°C decreased blood flow compared to normothermic values. In contrast, Zielonka et al. (1974) reported increases in blood flow within a cooled spinal cord segment. The importance of hemodynamic consequences of cooling the brain and spinal cord are important in that reductions in blood flow to critical levels by profound cooling could have adverse effects on tissue preservation and functional outcome.

Excitotoxicity

The effects of moderate hypothermia on glutamate excitotoxicity were first reported using regional microdialysis to assay extracellular levels of various neurotransmitters after global ischemia. In a study by Busto et al. (1989b), intraischemic hypothermia (33°C and 30°C) was reported to attenuate the rise in extracellular levels of striatal glutamate and dopamine after global cerebral ischemia. These studies have been replicated in various models of ischemia indicating that one of the major mechanisms by which temperature affects neuronal vulnerability is through reducing excitotoxicity following cerebral ischemia (Baker et al., 1991, 1995; Mitani and Kataoka, 1991; Globus et al., 1995; Rokkas et al., 1995). Delayed pharmacological treatments that reduce glutamatergic excitoxicity further improve outcome when used in combination with hypothermia therapy (Dietrich et al., 1995; Green et al., 1995; Schmid-Elsaesser et al., 1999; Zausinger et al., 2003; Zhu et al., 2005) and may be a promising avenue for future therapeutic studies. The glutamatergic receptors, AMPA and NMDA, are also modulated by hypothermia. Expression of hippocampal glutamate receptors is decreased after transient global ischemia and this is completely blocked by intraischemic hypothermia (Friedman et al., 2001). The NMDA receptor undergoes phosphorylation at Ser 897 after hypoxic-asphyxic cardiac arrest and this is attenuated by hypothermia (Mueller-Burke et al., 2008). Besides glutamate,

other neurotransmitters are also modulated by hypothermia. For example, Lyeth et al. (1993b) demonstrated that hypothermia (30°C) reduced elevations in cerebrospinal levels of acetylcholine after TBI. Conversely, hypothermia delayed decreases in dopamine, norepinephrine, and serotonin after global cerebral ischemia (Zhang et al., 2008). Nevertheless, other studies have demonstrated that hypothermia (32°C) can improve outcome after CNS injury without attenuating extracellular levels of glutamate and aspartate (Lo et al., 1993; Palmer et al., 1993; Winfree et al., 1996; Koizumi et al., 1997; Huang et al., 1998b). Thus, it is clear that the neurotransmitter response to various types of injury models may be temperature dependent, but that attenuating other injury cascades may be more important in subserving the beneficial effects of hypothermia.

Blood-Brain Barrier

Alterations in BBB permeability after ischemia and trauma are an important vascular consequence that allows for the passage of water, blood-borne exogenous substances and potential neurotoxic agents across the vascular system into the brain parenchyma. Studies have demonstrated the importance of brain and body temperature on the microvascular consequences of cerebral ischemia and trauma. In one study that assessed the effects of intraischemic brain temperature on blood-brain barrier (BBB), mild hypothermia was shown to reduce extravasation of the protein tracer horseradish peroxidase (Dietrich et al., 1990). In models of focal cerebral ischemia, posttraumatic hypothermia has also been shown to reduce BBB permeability (Huang et al., 1998a, 1999). The effects of moderate hypothermia on BBB disruption following TBI have also been investigated (Kinoshita et al., 2002a; Arican et al., 2006). In a study by Jiang et al. (1992), patterns of increased vascular permeability to endogenous serum albumin (IgG) immunoreactivity were greatly reduced compared to normothermic animals. Additionally, extravasation of inflammatory cells is also reduced by post-injury hypothermia after TBI (Whalen et al., 1997a,b; Chatzipanteli et al., 2000a), SCI (Chatzipanteli et al., 2000b), and transient focal cerebral ischemia (Toyoda et al., 1996; Inamasu et al., 2000b, 2001; Wang et al., 2002). Mechanistically, hypothermia may be attenuating BBB permeability by altering matrix metalloproteinases, which are critical extracellular enzymes that can disrupt the BBB (Truettner et al., 2005; Nagel et al., 2008). Because microvascular perturbations including BBB permeability, formation of vasogenic edema and the extravasation of circulating inflammatory cells can adversely affect injury outcome, the effects of hypothermia on the vasculature comprise an important underlying mechanism for the beneficial effects of hypothermia.

Calcium-Dependent Intracellular Signaling

There are pronounced changes in calcium-dependent intracellular signaling pathways after CNS injury. Normal neuronal activity is mediated by signaling through protein kinases and several of these have been documented to be disrupted by TBI and cerebral ischemia. Transient cerebral ischemia inhibits the activity of calcium/calmodulin-dependent protein kinase II (CaMKII), a key protein kinase that mediates synaptic strength, and this is attenuated by hypothermia (Churn et al., 1990; Hu et al., 1995). Protein kinase C (PKC), another calcium-dependent protein kinase, translocates to the membrane after cerebral ischemia and undergoes inhibition; hypothermia rescues the inhibition of PKC activity and its translocation to the membrane (Cardell et al., 1991; Busto et al., 1994; Shimohata et al., 2007a). However, one particular isoform of PKC, PKC delta, is implicated in initiating mitochondrial injury and cytochrome c release after focal cerebral ischemia; conversely hypothermia inhibits increases in PKC delta activity after injury (Shimohata et al., 2007b).

Most recently, various transcription factors that participate in normal neuronal functioning have been shown to be sensitive to temperature manipulations. The immediate early gene c-Fos, which regulates key genetic responses of neurons, is activated by hypothermia after transient global ischemia (Kumar et al., 1996b; Akaji et al., 2003; Pabello et al., 2005). Other cell survival pathways activated by hypothermia after brain injury include extracellular signal-regulated protein kinase (ERK) and c-Jun N-terminal kinase (Hicks et al., 2000). For example, in one study by Atkins et al. (2007), posttraumatic hypothermia (33°C) was shown to potentiate ERK cell signaling pathways associated with cell survival and synaptic plasticity. These studies emphasized that temperature may have profound effects on events associated with irreversible neuronal injury as well as the normal processing of neuronal signals throughout brain circuits.

The neuronal cytoskeleton is highly vulnerable to injury, resulting in beading of dendrites and degeneration of axons. Brain injury results in the breakdown of the cytoskeletal proteins microtubule-associated protein 2 (MAP2) and beta-actin, which is reversed by hypothermia (Miyazawa et al., 1993; Taft et al., 1993; Wu et al., 1995; Haranishi et al., 2005). This effect is most likely mediated by inhibiting calpain activity, a calcium-dependent protease (Liebetrau et al., 2004).

Inflammation and Edema

Attenuation of inflammation is one of the major mechanisms by which hypothermia provides beneficial effects in CNS injury. The inflammatory response after brain and SCI is significantly attenuated by hypothermia. Besides attenuating disruption of the BBB and extravasation of infiltrating inflammatory cells and neurotoxic substances, the endogenous inflammatory response of the CNS is also reduced by hypothermia. Astrocytes and microglia respond rapidly to CNS injury by proliferating around the injury areas and releasing pro-inflammatory molecules as an endogenous repair mechanism. Hypothermia significantly attenuates the activation of both astrocytes and microglia (Chen et al., 1992a; Kumar et al., 1996a; Ha and Kim, 2008). Accordingly, pro-inflammatory cytokines such as interleukin-1β, interleukin-18 and tumor necrosis factor-α are also reduced by hypothermia (Goss et al., 1995; Kinoshita et al., 2002b; Vitarbo et al., 2004; Fukui et al., 2006; Morino et al., 2008). The combinatorial strategy of the anti-inflammatory cytokine interleukin-10 with hypothermia therapy was attempted in both TBI and focal cerebral ischemia (Dietrich et al., 1999; Kline et al., 2002). Synergistic effects of hypothermia and interleukin-10 were only seen in focal cerebral ischemia but not in TBI, suggesting that the mechanisms of inflammation between these two CNS injuries play a role in the effect of hypothermia.

Another major aspect of the inflammatory response to CNS injury is the release of reactive oxygen species by astrocytes and microglia. Hypothermia reduces increases in tissue levels

of superoxide, nitric oxide, and the hydroxyl radical (Globus et al., 1995; Kil et al., 1996; Kumura et al., 1996; Sakamoto et al., 1997; Zhang et al., 2001a; Han et al., 2002; Maier et al., 2002). Additionally, the enzyme responsible for scavenging superoxide, superoxide dismutase, is increased by hypothermia (Lei et al., 1994; DeKosky et al., 2004), and the enzyme responsible for synthesizing nitric oxide, nitric oxide synthase, is attenuated by hypothermia (Kader et al., 1994; Chatzipanteli et al., 1999; Han et al., 2002; Karabiyikoglu et al., 2003; Van Hemelrijck et al., 2005). Hypothermia combined with delayed treatment of a free radical scavenger, N-tert-butyl-a-phenylnitrone (PBN) further improves neuronal survival and hippocampal-dependent memory functioning after transient global ischemia (Pazos et al., 1999), suggesting that hypothermia does not completely attenuate reactive oxygen species production after CNS injury (Nito et al., 2003).

Edema, the swelling of the brain or spinal cord after injury due to water accumulation, is mediated by breakdown of the BBB and the inflammatory response of astrocytes. Brain water content is significantly reduced with hypothermia after focal cerebral ischemia (Park et al., 1998; Kawai et al., 2002). Recent imaging studies have assessed this with magnetic resonance imaging (MRI) and found that reductions in the apparent diffusion coefficient of water (ADC) are also reduced by hypothermia (Mancuso et al., 2000).

Neuronal Cell Death

Evidence for apoptotic cell death has been shown in various models of brain and SCI. Although neuronal necrosis is commonly seen in most injury models, evidence for apoptotic cell death in CNS injury has also been documented using various histochemical and molecular techniques. As with necrosis, apoptotic cell death appears to be sensitive to post-injury hypothermic treatment strategies. Using terminal deoxynucleotidetransferase-mediated dUTP-biotin nick end labeling (TUNEL) staining, DNA fragmentation has been found to be reduced by hypothermia in severe TBI (Brodhun et al., 2001), transient global ischemia (Zhang et al., 2001b), transient focal ischemia (Inamasu et al., 2000a; Phanithi et al., 2000; Prakasa Babu et al., 2000), and SCI (Shibuya et al., 2004). Levels of caspase 3, an important initiator of apoptotic cell death, are highly sensitive to hypothermic treatment (Phanithi et al., 2000; Van Hemelrijck et al., 2005; Lotocki et al., 2006), as is cytochrome c release (Yenari et al., 2002; Zhao et al., 2004, 2005, 2007). Taken together, these studies indicate that apoptotic cell death is another important target by which temperature may affect long-term outcome in various models of CNS injury.

Global Molecular Changes

Recent studies have utilized various genetic markers to evaluate the effects of temperature on molecular events associated with CNS injury. Using a variety of gene arrays, families of genes have been shown to be sensitive to post-injury temperature manipulations in models of ischemia and trauma (Ohta et al., 2007; Gressens et al., 2008; Kobayashi et al., 2008). As previously discussed, families of genes associated with inflammation, apoptosis and other cell signaling cascades are reduced or elevated when brain temperature is lowered. The ability of post-injury temperature to affect the acute and more delayed genetic response to injury is important in that these genes may play a major role in determining the proteomic response that results in secondary injury. Genetic studies are ongoing in many laboratories and represent an exciting research direction for continuing to determine how hypothermia may protect and potentially repair CNS tissues after injury.

Conclusion

Mild to moderate hypothermia has been shown to be protective in various models of brain and SCI. The ability of modest cooling strategies to improve structural and functional outcome in multiple animal models of CNS injury is important as one considers this experimental therapy in the acute treatment of patients with neurological disorders. Indeed, as one reviews the vast literature on neuroprotective strategies targeting cerebral ischemia and trauma, hypothermia appears to be the most consistent treatment in providing significant protection of vulnerable neurons and improved behavioral outcomes. Also, as summarized in this paper, temperature appears to influence a number of pathophysiological mechanisms that are felt to be important in altering cell death and abnormal function. These include cellular, biochemical, and molecular processes affecting a variety of cell types (including neurons, glia, vascular components, and inflammatory cells). The ability of a specific therapy to target multiple cell types and multiple injury cascades is unique, and may explain the positive effects reported throughout the literature by many independent laboratories.

Nevertheless, there remain limitations to the usefulness of mild to moderate hypothermia, and more basic and translational research is required to continue to move this therapy forward. For example, the question of whether there is a single dominant injury mechanism that is responsible for the beneficial effects of hypothermia is commonly considered in study sections and in the experimental literature. Also, when hypothermia does not work, it is not generally clear what went wrong or whether the optimal treatment protocol was administered for the specific situation.

In the following papers in this Special Hypothermia Issue, critical factors and variables, including the level and duration of hypothermia, the rewarming phase, and different injury models, will be discussed in terms of both experimental and clinical situations. Recently, several treatment factors associated with the use of hypothermia have been discussed in terms of negative clinical findings. Thus, additional research should provide critical information regarding how to best utilize hypothermia in specific experimental and clinical conditions and ultimately identify the patient populations that will best benefit from hypothermia therapy. Because combination therapy that includes mild cooling may be advantageous in terms of prolonging the therapeutic window of a drug, thus providing better protection and long-term functional improvements, this research area also requires attention.

Finally, in terms of the clinical use, a number of single institution as well as multicenter trials have been undertaken to test the effectiveness of hypothermic therapy. Both positive and negative findings have been reported with this experimental therapy. Additional trials may be required to confirm findings or alter the treatment protocols to potentially produce more satisfactory results. It is hoped that with continued discussions and experimentation, better approaches for the

use of hypothermic therapy will be clarified and specific patient populations identified that will benefit from this cytoprotective therapy.

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