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Influence of therapeutic hypothermia on regeneration after cerebral ischemia

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Abstract

The protective effect of therapeutic hypothermia in cerebral ischemia is well accepted in experimental models, and some clinical studies show that there is benefit in humans as well. Long-term observations in animal and clinical studies have documented recovery of neurological function following hypothermia treatment. Diminished damage by hypothermic protection should contribute to the recovery in many ways but hypothermia appears to enhance regeneration of brain tissue as well. Since regeneration of the brain after damage initiates within hours and is active days and weeks after stroke, prolonged hypothermia might affect regenerative processes which have been documented to occur in these time frames. While there is a lack of data at the basic and clinical levels, the mechanism of neuroregeneration by hypothermia is unclear. Yet, we speculate that hypothermia enhances regeneration by positively influencing neurogenesis, angiogenesis, gliogenesis and synapse/circuit formation after stroke. In this chapter we will provide up-to-date data from experimental studies and clinical reports on the effect of therapeutic hypothermia on neuroregeneration, with perspectives on future research.

1. Introduction

Recent studies have demonstrated that neurogenesis is evident following injury in the adult brain within the germinal niches in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) as well as neocortex, spinal cord, tegmentum, substantia nigra, amygdala, brain stem and regions adjacent to the injury [1]. Neurogenesis is thought to be stimulated by cytokines, chemokines, neurotransmitters, and reactive oxygen/nitrogen species released by dying neurons and activated macrophages, microglia, and astrocytes [2]. Complete neurogenesis requires multiple steps including proliferation, migration, differentiation, survival, and integration of new neurons into the existing circuitry of the brain. However, while neurogenesis signals appear after injury, successful replacement or regeneration of the damaged brain is scarcely observed. Thus, attempts at successful neurogenesis often fail.

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Abrupt changes after stroke disrupt brain homeostasis and shift the environmental condition from maintenance of mature brain cells to resurrection of dormant stem cells. The initial environment after stroke is considered as pro-regenerative. But with the progression of brain injury, the environment becomes anti-regenerative again. Thus, therapies should be explore that can promote successful regeneration of injured brain by preventing or reducing this anti-regenerative environment. Therapeutic hypothermia is not only known to prevent serious damage by inhibiting cell death and suppressing the damaging effects of inflammation, but it also stimulate stem cells by modulating wide spectrum of biological events [3,4]. We expect that these features of hypothermia lead to an overall net benefit to brain regeneration. Especially when maintained for longer durations, therapeutic hypothermia would be expected to modulate many steps of the regenerative processes. In this chapter we will provide up-to-date data from experimental studies including stroke, traumatic brain injury and other acute brain injury models, and clinical reports on hypothermic neuroregeneration and suggest areas in need of further investigation.

2. Hypothermia and regeneration in experimental models

In contrast to neuroprotection, which targets the salvage of dying cells, neuroregeneration strategies attempt to enhance signaling pathways involved in the regeneration and remodeling of damaged tissue. Neurotrophic factors in the brain control synaptic function and plasticity and sustain neuronal cell survival, morphology, and differentiation. Studies in which hypothermia had neuroprotective effects against ischemic brain insults, brain levels of brain-derived neurotrophic factor (BDNF) [5,6], glial-derived neurotrophic factor (GDNF) [7] and neurotrophin [8] were all increased. The scope of regeneration will be discussed as it pertains to neurogenesis, neuronal connectivity, angiogenesis, and gliogenesis.

2-1. Neurogenesis

While neurogenesis in the uninjured aged brain is markedly reduced, some rodent studies have shown that acute brain insults initiate the proliferation of neural stem cells in the subventricular zone and the hippocampal subgranular zone, [9]. After stroke, ischemic neurons also lose synaptic connectivity and undergo cell death. It is becoming increasingly recognized that endogenous recovery processes are also activated after stroke, leading to neurogenesis and synaptogenesis. However, these regenerative processes are probably not successful or are incomplete, as evidenced by the permanent disability in most stroke patients. Furthermore, rodent studies indicate that neurogenesis is reduced in aged brains, and stroke seen more frequently in the elderly [10].

Strategies to improve regenerative processes should attempt to enhance proliferation of neuronal precursor cells, migration of precursor cells to the injury area, differentiation of these precursor cells into mature neurons and reconnection between neurons. A few experimental studies have demonstrated the beneficial effects of hypothermia on neurogenesis [3,11–13] and therefore hypothermia. Cooling has been shown to differentially affect neurogenesis in uninjured animals. In one study that examined neurogenesis in the developing brain, reduction of brain temperature to 30°C for 21 h decreased the number of proliferating cells in the subgranular zone of the hippocampus, but not the periventricular zone [14]. However, under conditions of hypoxia–ischemia in the developing brain,

hypothermia to 33°C enhanced the maturation of neural progenitor cells in the striatum and inhibited apoptosis of proliferating neural stem cells that were already increased by ischemic stimuli [13].

In addition to it obvious beneficial properties, could also be used as a model to understand the underlying mechanisms of how to promote endogenous recovery of the injured brain. The mechanism of how cooling may enhance regenerative properties could be explain, in part, by its effect on reducing apoptosis. For example, enhanced neural stem cell survival seems to be linked to the cooling-induced upregulation of the anti-apoptotic protein BCL-2 [13]. In a study of cultured neural stem cells, mild hypothermia also inhibited apoptosis, increased the number of nestin positive cells and inhibited stem cell differentiation into astrocytes [15]. Adult rodents exposed to forebrain ischemia and subjected to mild hypothermia had increased numbers of newborn neurons in the dentate gyrus compared to animals exposed to ischemia without cooling [3]. By contrast, another study in adult rats with forebrain ischemia showed that hypothermia had no effect on neurogenesis [16]; however, the duration of hypothermia in this study was rather short (33 °C for 45 min) and occurred relatively early, either during the ischemic period or during the immediate reperfusion phase. Therefore, it is possible that hypothermia may not have any effect on neurogenesis if it is not applied during critical time window(s) that has yet to be clearly defined. More research in this area is needed; in particular to determine the optimal conditions under which cooling might be expected to positively influence neurogenesis and whether cooling may improve neurogenesis in aged brains exposed to ischemia and related insults.

2-2. Neuronal connectivity

In addition to stem cell genesis, repair of neuronal connectivity is crucial to functional recovery after stroke. To repair the loss of neuronal connectivity, neurite outgrowth and formation of new synapses are essential. A few studies have examined the role of hypothermia on neuronal circuit repair. At the morphological level, neurite and axonal outgrowth were enhanced by applying deep hypothermia (17 °C) in organotypic brain slices [17]. A genomic analysis study in a rat model of traumatic brain injury demonstrated that mild hypothermia had a significant effect on gene expression. An analysis of hippocampal gene expression profiles from rats exposed to hypothermia following traumatic brain injury revealed statistically significant differences in 133 transcripts compared to injured normothermic rats. Of these, 57 transcripts were upregulated and 76 were downregulated after injury. Those genes involved in synapse organization and biogenesis were especially upregulated in hypothermic animals compared to normothermic group [18]. Although the scientific literature is still scant, current data suggest that overall, hypothermia supports regenerative processes by enhancing synapse formation and reorganization. The precise mechanisms explaining the hypothermic effects are not clear. We assume inflammation is one of the key players since hypothermia influences the inflammatory response after brain injury and inflammatory cytokines play a major role in modulating neurite outgrowth and regeneration [17,19].

2-3. Angiogenesis

Mild hypothermia has been shown to enhance angiogenesis in focal cerebral ischemia [20], spinal cord injury [21] and traumatic brain injury models [22]. Although these angiogenic effects by hypothermia are presumably beneficial to repair processes, their clinical significance is still uncertain. In fact, a few studies suggest that angiogenesis may actually be detrimental to brain repair. For example, one study of acute stroke patients showed that an early dominance of pro-angiogenic factors, including platelet-derived growth factors (PDGFs), vascular endothelial growth factors (VEGFs) and their receptors, stromal cell-derived factor 1 (SDF1) and hepatocyte growth factor (HGF), was associated with mild short-term neurological deficits, but that an acute anti-angiogenic status (as defined by elevated plasma endostatin levels) also predicted a worse long-term functional outcome [24]. Furthermore, pharmacologic stimulation of angiogenesis using high-dose VEGF impeded recovery of neurological function in a rat model of global cerebral ischemia and caused neuronal damage in uninjured control brains [25]. However, neuroblasts which will further differentiate into fully functional neurons were identified in close proximity around the immature newly created vascular network after stroke [23]. In another study, hypothermia reduced secretion of vascular endothelial growth factor by cultured retinal pigment epithelial cells [26] and suggests effect of hypothermia on angiogenesis might be diverse in depending on the tissue.

2-4. Gliogenesis

Oligodendrocytes succumb to brain insults and undergo cell death with a susceptibility that is similar to neurons, and hypothermia attenuates oligodendrocyte death, demyelination and circuit dysfunction [27]. Hypothermia (32°C) increased the number of oligodendrocyte precursor cells in a primary culture taken from embryonic mouse brains [28]. As a result, greater numbers of oligodendrocyte precursor cells that undergo cell cycle progression were maintained in a less well-differentiated state. However, an *in vivo* study using a hypoxia model in preterm fetal sheep demonstrated that hypothermia (30°C) was associated with an overall reduction in hypoxia-induced loss of immature oligodendrocytes, but did not prevent the hypoxia-induced reduced proliferation of oligodendrocytes within the periventricular white matter [29].

Reports of the effects of hypothermia on endogenous cell genesis in the injured and uninjured brains are somewhat conflicting. Some reports [14,29] indicate that hypothermia suppresses stem cell proliferation, whereas many reports indicate the opposite [3,15,28], and some even suggest that cooling promotes progenitor cell differentiation towards neurogenesis over gliogenesis [13,15]. Hypothermia to temperatures lower than 30°C seems to suppress cell proliferation and phase-specific and nonspecific cell cycle arrest as a result of reduced energy supply [14]. However, small temperature decreases seem to protect against progenitor cell death [15,29]. Thus, we speculate that mild hypothermia enables the differentiation of precursor cells while preventing apoptosis, and that cooling to lower temperatures seems detrimental to cells and blocks their proliferation.

Astrocytes comprise the largest population of cells in the ischemic core during the subacute to chronic period after stroke [30], and reactive astrocytes are the main component of the

glial scar. However, glial scar formation in the brain can obstruct neurite outgrowth and regeneration [31], and blocking astrocyte activation and related reactions can exacerbate inflammation and increase injury responses [31]. Thus, enhancement of gliogenesis may do some harm. How hypothermia affects gliogenesis has not yet been studied in any depth.

3. Conclusion and future perspectives

Although the effect of therapeutic hypothermia in brain regeneration after stroke is far from clear, under specific conditions it seems to have beneficial roles in survival, proliferation, differentiation and migration of stem/progenitor cells, and reconstruction of neural circuitry. Clearly, more research is needed in this area. To date, most of the studies addressing this topic applied cooling relatively early on. Yet, its effects on regeneration were observed days to months later. Thus, identifying the key events linking early cooling and its downstream effects on regenerative processes need to be identified. It is also conceivable that the beneficial effects of therapeutic cooling may not require early intervention, and would have obvious implications at the clinical level where intervention may potentially be initiated days to weeks and even months later.

Although there is no specific treatment to enhance or promote neuroregeneration at present, there is substantial ongoing research in this area, such as small molecules, growth factors and cell base therapies. When such therapies become available, combination therapy with therapeutic cooling and pharmacological interventions should certainly be explored. Therapeutic hypothermia has the potential to enhance the brain's endogenous restorative mechanisms, possibly with the aid of pharmacological or cell-based treatments. In case of drug combination therapy, very little is currently known about the effects of hypothermia on the pharmacokinetics and pharmacodynamics of drugs and this will be another field of future interest. As neuroregeneration strategies develop, there will be increasing needs for neurological or biological markers to predict outcomes in patients as we are facing the need in case of the patients resuscitated after cardiac arrest [32,33]. These prognostic biomarkers will be crucial in informing treatment decisions. Neural regeneration is still a field in its infancy, and there are still many questions that remain as to whether and how therapeutic cooling may play a role.

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