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PERSPECTIVE

SYSTEMIC AND LOCAL HYPOTHERMIA IN THE CONTEXT OF CELL REGENERATION

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Abstract

Local and systemic cooling is an inducer of cell proliferation. Cell proliferation and transdifferentiation or stem cells differentiation involves microenvironment regulation such as temperature. Mild hypothermia downregulates the production of pro-inflammatory cytokines and reduces the immune response against pathogens. In addition, mild tissue cooling improves endothelial cell function. Endothelial cells are involved in angiogenesis during regeneration strategies; therefore, their death is catastrophic and affects regeneration, but not cell proliferation. The potential mechanism underlying the effects of local or systemic hypothermia on cell regeneration has not yet been elucidated. Hypothermia reduces the production of reactive oxygen species and organelle activity. Hypothermia therapeutic effects depends on the targeted organ, exposure duration, and hypothermia degree. Therefore, determining these factors may enhance the usage of hypothermia more effectively in regenerative medicine. The paper introduces the hypothermia role in paracrine/endocrine cell secretion, reception, and the immune state after local and systemic hypothermia application.

Keywords: cryotherapy; hypothermia; regeneration: immune cells; cytokine; proliferation; differentiation

INTRODUCTION

Local cryogenic application or hypothermia exposes the organism and tissues to a state of hypoactivity and induces the activation of survival mechanisms such as autophagy. Induced hypothermia is a medical procedure aimed to achieve therapeutic effects such as reducing organ dysfunction after cardiac arrest, global brain ischemia, neonatal asphyxia, and even sepsis through either endovascular cooling or surface cooling (1). On a molecular basis, the mitogen-activated protein kinase (MAPK) signaling pathway is activated primarily by hypothermia. This serine/threonine protein kinase is involved in the regulation of extracellular stimuli such as inflammatory stimuli, mitogens, stress, or shock. The overall effect of hypothermia on tissue is various. In many cases, it is protective by activating extracellular signal-regulated kinases-1/2 (ERK1/2) (2), whereas in many other cases it results in activation of apoptosis and suppress inflammation (3,4).

Hypothermia protects ischemic endothelial cells from damage and death by activating Jun N-terminal kinase (JNK) and p53 (5,6). Also, in human umbilical vein endothelial cells, hypothermia reduces the expression of the MCP-

1 and IL-8 as well as inhibiting the expression of E-selectin. However, the expression level of ICAM-1 is not affected (7). Local application of hypothermia is preferred to avoid the systemic adverse effects of hypothermia such as coagulopathy, arrythmias, denervation syndrome and polyneuropathy, infection, pulmonary edema, bleeding diathesis, bladder atony, and hypokalemia.

The application of local hypothermia induces vasoconstriction. Later events involve a decrease in the metabolic activity of the cells that reduces the demand for nutrients (increases glucose level) and oxygen as well as tissue acidity (pH). These environments can be used in case of myocardial infarction, spinal cord injury, and strokes as well as in other hypoxemic organs (8). However, systemic hypothermia not only induces vasoconstriction but also induces cellular anabolic activity to increase heat production.

In this review we introduce the roles of hypothermia in paracrine/endocrine cell secretion, reception, and the immune state after local and systemic hypothermia application.

LOCAL APPLICATION OF HYPOTHERMIA TREATMENT INFLUENCES PARACRINE AND ENDOCRINE SECRETIONS

Hypothermia has been reported to reduce the production of nitric oxide and leukotrienes in several in vitro studies (9,10). Therefore, vasoregulation disorders and endothelial cell dysfunction are induced. Due to endothelial cells over activity, endoplasmic reticulum impairment develops as well as energy deprivation due to energy sources use and mitochondrial impairment as a result of free radicals' formation and mitochondrial DNA damage.

Interesting recent findings have shown that hypothermia induces the expression of the neuroprotective mediator interferon regulatory factor 4 (IRF4) and down-regulates the secretion of pro-inflammatory mediators (11). Moreover, hypothermia-induced IRF4 changes the balance of macrophages to the side of M1. The general effect of hypothermia is known to reduce the immune state of the individual, which is why people generally get some infections during colds (12). Therefore, sterility is required during the application of localized or generalized hypothermia in terms of the regeneration strategy. Whilst hypothermia can trigger damage in the body, mild or moderate hypothermia can induce therapeutic effects.

LOCAL APPLICATION OF HYPOTHERMIA TREATMENT INFLUENCES THE IMMUNE SYSTEM

In a recent in vitro study conducted by He et al., the murine B16f10 model showed that cryothermal application to the model increases the activation of CD4 + CD25 T cells and decreases the activation of myeloid-derived suppressor cells and regulatory T cells. CD4 + CD25 +. Moreover, the effects of cryogenic on the humoral immunity involves induction of the CD8+ T cells activity and their differentiation into memory cells. Also, cryotherapy induces the differentiation of the CD4+ T cells into Th1 and Tfh, CD4-CTL subsets (13). Many of these cells' secret mediators of regeneration including vascular endothelial growth factor, glial cell line–derived neurotrophic factor, brain-derived neurotrophic factor, and angiogenic mediators, such as hepatocyte growth factor and insulin-like growth factor-1. Therefore, promoting differentiation is involved in the regeneration strategy.

In another recent study it has been shown that application of local hypothermia reduces inflammatory mediators in injured spinal cord (MIP1β, IL1β, MIP1α, IL8, MCP) (14). Significant changes in inflammation markers were observed; decrease in MIP1β (from 111.2 pg/mL to 41.144 pg/mL), IL1β (from 13.5 pg/mL to 8.478 pg/mL), MIP1α (from 67.0 pg/mL to 46.766 pg/mL), IL8 (from 2013.9 pg/mL to 1754.107 pg/mL), MCP (from 1821.5 pg/mL to 1069.221 pg/mL). Whereas, non-significant changes were seen in IL10, tissue IP10, IL4, IL1α, and GROα (14). Moreover, cooling of the spinal cord was accompanied by metabolic deteriorations such as increased lactate (from 5.1 mM to 7.0 mM), lactate/pyruvate ratio (from 33.9 to 146.9), glucose (from 2.3 mM to 3.3 mM), glutamate (from 10.9 μM to 17.3 μM); and decreased glycerol (54.5 μM to 41.1 μM) (14). Local hypothermia suggests that it increases anaerobic glycolysis and ATP production, which is required for cell survival despite the quality and quantity of ATP produced. Hypothermia minimizes the progression of cell necrosis and apoptosis during unfavorable conditions by reducing inflammation and inhibiting activation of the apoptosis cascade. However, some

inflammatory mediators pose a protective effect (IL-1) on the injured cells. Therefore, inhibition of the production of these mediators is probably harmful (12). The precise balance and accurate determination of the required degree of hypothermia for each targeted organ is crucial to achieve therapeutic effects from local hypothermia.

Local hypothermia does not affect the level of IL-1, IL-6, and IL-10. Mild hypothermia reduces the level of the proinflammatory cytokines (IL-6) and increases the anti-inflammatory cytokines IL-10 as well as preserving the level of HSP70 (7).

In terms of cell regeneration, up-regulation of pro-inflammatory cytokines (IL-6, IL-8, IL-4) and up-regulation of the required sources of energy combine in positive feedback for the activation of cell regeneration genes. It's of debate whether IL-6 is a proliferation inducer or inhibitor (15). However, IL-6 was found to induce regeneration of hepatocytes, retinal ganglion cells, heart, human mesenchymal stromal cells and kidney, as well as differentiation of bone marrow mesenchymal stem cells into osteoblasts through one of the signaling pathways of IL-6; STAT3, AKT, and ERK1 / 2, as well as inhibition of the intrinsic signaling cascade of the PTEN or SOCS3 (16, 17, 18, 19, 20, 21). Il-6 was found to induce the expression of nonspecific transcription factors according to the type of target cell (22). In osteogenic cell there is upregulation of ALP, ANKH and PIT1 expression, whereas in stem cells there is induced expression of the NANOG, SOX2 and REX1 as well as CD44 and CD105 (22). IL-6 alone is not sufficient for cell induction regeneration. Activation of suitable transcription factors is mandatory, as the well as presence of gp130 and IL-6 receptor on the target cell (23). For example, for induction of heart regeneration, activation of Pim-1 transcription factor is required (24). However, many cells do not express the IL-6 receptor, but the presence of a soluble IL-6 receptor can bind to gp130 of cells that do not express the IL-6 receptor.

INFLUENCES OF SYSTEMIC HYPOTHERMIA ON PARACRINE & ENDOCRINE SECRETIONS

Systemic hypothermia results in over activity of the adrenergic nervous system, and over production of epinephrine from the chromaffin cells of the adrenal gland medulla occurs. The effects of the sympathetic nervous system are achieved by the presence of adrenoceptors in most organs and tissues. Furthermore, systemic hypothermia activates the thermoregulation center in the hypothalamus to cause shivering and heat production.

Local inflammatory cytokines under hypothermia are significantly elevated and local tissue damage continues to result in a systemic inflammatory response. In particular, IL-6 is significantly higher in hypothermic animal models compared to normothermic animal models. Persistent elevation in IL-8 level is seen in both control and treatment groups. However, no significant changes were observed in local IL-10 levels, nor was the rapid reduction in IL-10 observed in hypothermic animals (25).

Moreover, hypothermia is known to increase the release of proinflammatory mediators in endothelial cells (26). Therefore, hypothermia probably induces prolonged elevation of inflammatory mediators' and this has been reported to be involved in cardiac arrest during rewarming (27). Another study showed that hypothermia reduces the release of pro-inflammatory cytokines and protects damaged tissue, particularly the lung alveoli, from hypoxia (28,29).

SYSTEMIC HYPOTHERMIA INFLUENCES THE IMMUNE SYSTEM

According to recent studies in an animal model, hypothermia significantly elevates the systemic level of pro-inflammatory cytokines IL-6, high mobility group box nuclear protein 1 (HMGB1), and IL-8, while no significant differences are observed between normothermic and hypothermic animals in terms of IL-10. Moreover, significant reduction in anti-inflammatory heat shock protein 70 (HSP70) have been observed (25).

In hepatocytes, systemic hypothermia prolongs the apoptosis execution phase (up to 3 days) by inhibiting the activity of caspase enzymes and decreasing excitatory transmitters of intrinsic and extrinsic pathways (7). In addition, hypothermia preserves mitochondrial function by down-regulating FAS-mediated apoptosis and increasing anti-apoptotic Bcl-2/Bax ratio (30). Hypothermia reduces liver IL-8 release during hepatocyte injury (31). Hypothermia reduces the oxidative stress of

hypoxia-affected hepatocytes, especially oxidative stress from highly reactive products such as 4-hydroxynonenal protein adducts (HNE) (32). On murine models of neutrophiles, mild hypothermia (35oc) induces release of TNFα that later activates apoptosis.

Interestingly, a recent study compared the effect of head cooling and whole-body cooling in neonates with hypoxia-ischemia encephalopathy (33). The results were statistically non-significant, such that 77% of the head cooling group died or developed disability and 67 % of the whole-body cooling group died or developed disability. The overall effect of systemic hypothermia on spinal cord injury was protective in comparison with the control group (34).

LOCAL AND SYSTEMIC HYPOTHERMIA TREATMENT IN THE CONTEXT OF CELL REGENERATION

Hypothermia as mentioned increases the release of inflammatory mediators on a local and systemic level, especially IL-6. Therefore, induction of cell regeneration by hypothermia is probably currently impossible. However, modification in the duration and the degree of hypothermia plays a central role in the regulation of secreted paracrine and endocrine molecules that can trigger cell proliferation.

Hypothermia has been seen to induce differentiation of implemented stem cells, particularly neural stem cells (NSCs), through microenvironment modulation (35). Modulation of the microenvironment is involved in the regulation of cell proliferation and transdifferentiation, as well as stem cell differentiation and proliferation. Moreover, in stem cells differentiation, microenvironment regulates the expression of cell linage genes. (Figure 1)

A recent in vitro study investigated the role of hypothermia in the proliferation and differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) into neural cells (36). Cytometry analysis has shown that hypothermia is an unfavorable condition for the proliferation and differentiation of BMSCs into neural cells. Furthermore, the study concluded

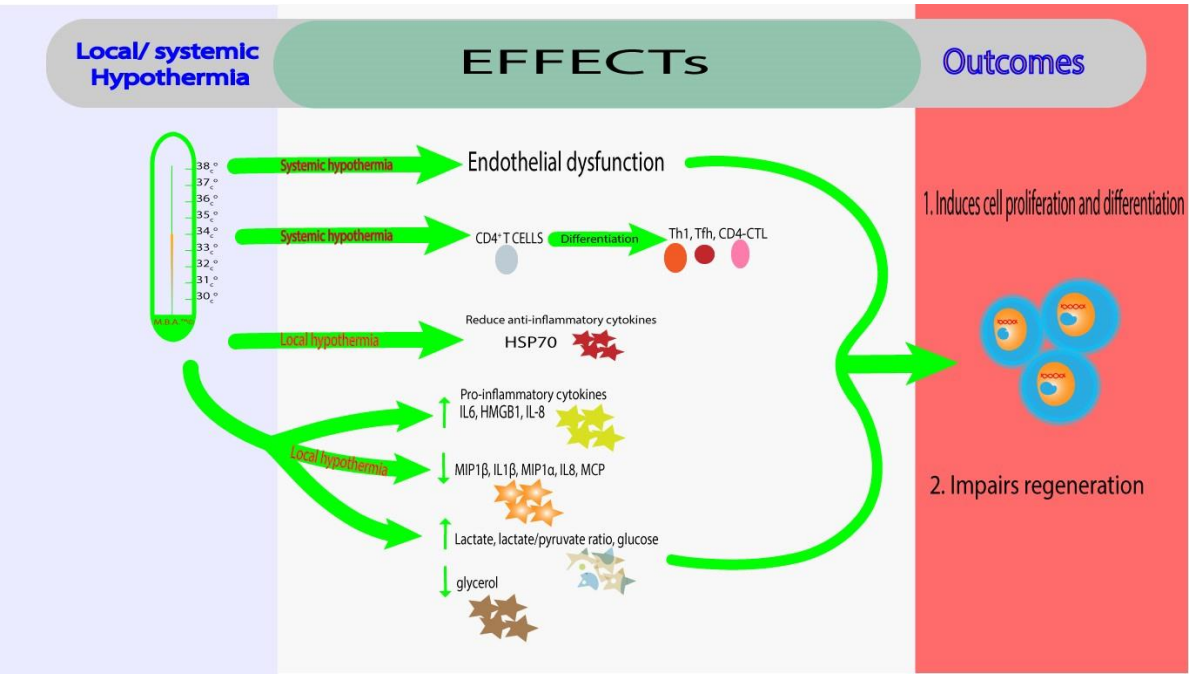

Figure 1. Schematic presentation of local and systemic hypothermia effects on immune system and paracrine/endocrine cells as well as cell metabolism. Local hypothermia increases the secretion of pro-inflammatory mediators and reduces anti-inflammatory mediators. Hypothermia induces endothelial dysfunction that affects angiogenesis, which is crucial for a successful regeneration strategy. Hypothermia improves cell proliferation as well as stem cells differentiation through regulation of the microenvironment. As an outcome, regeneration strategies are not improved under hypothermia.

that small ubiquitin-like modifier (SUMOs) proteins play a crucial role in protecting BMSCs from hypothermia, confirmed by inhibition of the expression of SUMO 1,2,3 proteins that impair the proliferation and differentiation of BMSCs. Furthermore, modification of proteins targeted by SUMO, e.g., antiproliferating cell nuclear antigen, octamer binding transcription factor 4, p53 and hypoxia-inducible factor-1α, are involved in inhibition of proliferation and differentiation (36). SUMOylation is a post-translation modification that is involved in the regulation of apoptosis, nuclear-cytosolic transport, maintaining protein stability, transcriptional regulation, cell cycle progression, and response to stress.

CURRENT APPLICATIONS OF CRYOGENIC AND HYPOTHERMIA IN MEDICINE AND FUTURE PERSPECTIVES

Practically, approved applications of cryotherapy and hypothermia are limited (37). Usually, cryotherapy is used to preserve transplanted organs and cells such as stem cells (38). The use of cryotherapy during ischemic attack on the heart or brain seems to be beneficial by reducing the metabolic state of the cell and the production of reactive oxygen species, as well as induction of survival mechanisms such as autophagy (39, 40, 41). Current approved uses of hypothermia are during subarachnoid hemorrhage, stroke, traumatic brain injury, spinal cord injury, elevated intracranial pressure, neonatal peripartum encephalopathy, and liver encephalopathy (42, 43, 44).

Hypothermia is the only method available to improve the condition of patients with multiple trauma. Several clinical evidences have suggested protective anti-infectious / -inflammatory response of hypothermia in patients with multiple trauma. Furthermore, hypothermia has beneficial effects on hemorrhagic control (31, 45, 46, 47). Hypothermia reduces glucose metabolism and oxygen demand in brain tissue by 2-4 fold for each 10°C reduction in temperature (48). Combining hypothermia and neural stem cells in regeneration of injured brain tissue has improved the differentiation of neural stem cells into newly functional neurons (35).

DISCUSSION

The molecular mechanism of the effects of hypothermia on the organism remains largely uncovered, and additional elucidations are required to rule out the potential underlying mechanism in which hypothermia induces its effects. Targeting the underlying signaling pathway can modify the effect of hypothermia and lead to controllable therapeutic effects of hypothermia on the targeted tissue and even systemic hypothermia.

Currently, only mild hypothermia has a therapeutic effect. In contrast, aggressive hypothermia results in acute complications that are life threatening. Deeper cooling than hypothermia risks the induction of ice formation the avoidance of which will require cryoprotective agents. These are water soluble and need sufficient cell penetration as well as low cell toxicity to protect complex tissues (49).

A more recent study demonstrated that hypothermia reduces the expression of NADPH oxidase subunits (gp91phox, p67phox, p47phox, and p22phox), reduces transmembrane glucose transport, particularly in neural cells (GLUT1,3) (50). This suggests a strong effect of pharmacological hypothermia on the cell vitality.

The effects of hypothermia on cell regeneration depend on the type of tissue and whether or not it is well nourished. Inducing mild hypothermia (body core temperature 32.5 °C for 1 day) of ischemic heart was found to reduce heart rate, blood pressure, and increase lactate level, sublingual microcirculation, as well as inotropic and vasopressin requirement (27, 51, 52, 53, 54, 55, 56, 57, 58).

A recent multicenter study demonstrated that the effect of local hypothermia in the coronary artery during reperfusion of ischemic heart of 22 patients was a reduction in the infarction area by 7.1% in the control but not the hypothermic group of patients (59, 60).

Several medications have been seen to induce the therapeutic protective effects of hypothermia, especially in brain tissue, including excitatory neurotransmitter inhibitors (61). Atorvastatin, phenobarbital, exendin-4, melatonin, cannabidiol, and bumetanide have been shown to enhance the neuroprotective effect of hypothermia (62, 63, 64, 65, 66, 67). When mild hypothermia (33 °C) was applied to the ischemic brain for 4 hours, the infarct tissue was reduced by 22% compared to normothermic animal models. Furthermore, moderate

hypothermia (30°C) preserves 46% of the infarcted area compared to normothermic models. Additionally, mild and moderate hypothermia has been shown to decrease the translocation of cytosolic/nuclear apoptosis inducing factor in the penumbra two days after stroke, and the release of cytosolic cytochrome c from the mitochondrial membrane (68, 69).

CONCLUSIONS

Hypothermia is useful for preserving newly regenerated tissue from apoptosis and death. However, several cellular metabolic processes require a classical optimal temperature to run. Therefore, currently, localized hypothermia is more advantageous in the concept of tissue regeneration, where part of the tissue is targeted. The effect of hypothermia on tissue protection remains unclear (70).

The general effect of hypothermia is known to reduce the immune state of the individual, which is why people generally get some infections during colds (12). Therefore, sterility is required during the application of localized or generalized hypothermia in terms of the regeneration strategy. The body response to hypothermia is potentially damaging. Therefore, mild or moderate hypothermia is required to induce therapeutic effects.

Hypothermia poses promising therapeutic advantages in enhancing cell regeneration in combination with other cell regeneration inducers such as transcription factors, micro RNAs, micro molecules, microenvironment, epigenetic modifications, and DNA methylation/demethylations. The regenerative effects of hypothermia lie in modifying the immune state, endocrine and paracrine secretions, as well as cell reception and metabolism. Each cell can be considered an endocrine and exocrine secretion, since during cell injury all cells start to secret some mediators into the blood and or into the extracellular space. Therefore, these immune mediators and cell metabolism modification can play a central role in promoting regeneration.

List of abbreviations: ERK 1/2; extracellular signal-regulated kinases 1/2, HMGB1; high mobility group box nuclear protein 1, HSP70; heat shock protein 70, JNK; Jun N-terminal kinase, MAPK; mitogen-activated protein kinase, NSCs; neural stem cells

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