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REVIEW ARTICLE

Cell Physiological Behavior in the Context of Local Hypothermia

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Abstract:

Local hypothermia has protective effects on injured endothelial cells, cardiomyocytes, and neurocytes. Unfortunately, the underlying mechanism of local hypothermia is still unknown. The overall effect of local hypothermia involves changes in cellular and extracellular homeostasis. Reduction in cellular metabolism is the hallmark effect of local hypothermia, resulting in a reduction in energy expenditure already impaired by starvation conditions, such as ischemia. However, on a molecular basis, local hypothermia modifies cell physiology according to the type and the vitality of the cells (brain cells are more important than skin cells; therefore, local hypothermia of the brain tissue is more critical than skin tissue, and the overall reaction of the organism is to prevent the brain from dying). This involves activating survival mechanisms, such as autophagy of brain tissue and apoptosis. The activated signaling pathways are not identical in various tissues. However, the whole machinery signaling axes have not yet been elucidated. Local hypothermia promotes the healing of the injury and improves the proliferation of regenerative tissue, but not differentiation. Hypothermia prevents the transdifferentiation of endothelial cells, neurons, and myocardiocytes. Finally, the therapeutic effects of hypothermia involve activating the nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1).

Keywords: Hypothermia, Neuron, Endothelial cell, Myocardiocyte, Myocardial infarction, Stroke, Angiogenesis, Regeneration, Endothelial dysfunction.

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1. INTRODUCTION

Reduction in the temperature of target tissues is essential as a therapeutic strategy. Local hypothermia applications are limited to preserving transplanted organs, ischemic brain injury, spinal cord injury, pre-operative period in cardiology, and ischemic heart diseases.

Hypothermia affects the cell physiology to adapt to the external environment by reducing temperature. Local hypothermia can be induced in several ways and usually depends on the part of the body. For example, skin hypothermia can be achieved through the application of local ice packs, immersion in water, immersion in ice, ice spray, and ice massage. However, modern devices have been used in cosmetic medicine based on the concept of local hypothermia. Skin cryotherapy is contraindicated in the case of skin anesthesia, peripheral vascular disease, hypersensitivity to cold, urticaria, Raynaud's phenomenon, complex regional pain syndrome, hemoglobinuria, and cryoglobulinemia [1].

Local hypothermia aims to achieve therapeutic advantages by modifying the cellular and extracellular architecture. It is well known that hypothermia reduces anabolic activity, which, in turn, causes the cell to undergo a dormant state with zero energy expenditure [2].

Usually, the major issue is how to preserve partially injured cells from death. Local hypothermia is one of the cornerstones of the strategy. This strategy includes local hypothermia, microenvironment, epigenetics, and transcription factors activation to preserve injured cells and induce local stem cell or fibroblast differentiation or transdifferentiation into the required cell lineage. Each of these factors varies and depends on the type and severity of the injured tissue. Moreover, the treatment plan is determined by the severity of the tissue injury, the type of tissue injured, the duration of the treatment, and the degree of cooling.

Local hypothermia has been used primarily in skin injury, acute liver failure, spinal cord injury, brain injury, and osteoarthritis to preserve partially damaged tissue and prevent the progression of damage [3 - 6]. However, hypothermia increases the rate of infectious complications by suppressing immune cell function and reducing the release of interleukin (IL-2) and antiviral interferons [7].

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2. EFFECTS OF LOCAL HYPOTHERMIA ON NEURONS

Local hypothermia has been used in stroke to reduce infarct progression and maintain the penumbra zone. Furthermore, local hypothermia has therapeutic effects on intracranial pressure, traumatic brain injury, neonatal peripartum encephalopathy, and subarachnoid hemorrhage. A small ubiquitin-like modifier (SUMO) has been shown to translocate from the cytoplasm to the nucleus under hypothermia [8]. SUMOylation modifies intracellular signaling proteins, including responses to stress and cell cycle progression, transcriptional regulation, apoptosis, nuclear-cytosolic transport, and maintaining protein stability. Therefore, hypothermia can modify cell homeostasis to adapt to external and internal changes. Hypothermia can be classified into mild (35- 33°C), moderate (32-28°C), and severe (<28°C). Clinical evidence suggested the protective effects of hypothermia on neuronal function at 33°C but not at 36°C [9].

3. EFFECTS OF LOCAL HYPOTHERMIA ON THE CNS

A recent study on rats aimed to identify the effects of local hypothermia (application of cold on the spinal cord) and general hypothermia on the central nervous system has found that local hypothermia significantly reduces the temperature of the cortex rather than general hypothermia [10]. Monitoring the temperature of the cortex, core, and spinal cord showed that local hypothermia induced a rapid and aggressive drop in temperature in the injured spinal cord of rats rather than in control rats.

During hypothermia, it was found that the permeability of the neuronal cell membrane impairs and results in the overactivation of Na+/K+ ATPase. The critical temperature levels at which the most neuronal activity occurs are between 27 °C to 29 °C. At this temperature range, impairment occurs in M-cholinergic release and receptor activity, as well as K+ efflux from the cytosol, loss of membrane potential, and the ability to generate action potential [11]. Measuring the spike activity of the V brain cortex neuron layer in guinea pigs under hypothermia (21-22°C), it was observed that the cells with high initial neuronal activity (4-8 impulses per second) had either increased firing activity by 34.9% ± 5% (becoming 5.396 -10.792 impulses per second) or decreased firing activity after cooling from 26°C. Whereas neurons with initial low firing activity (zero to 4 impulses per second) dramatically increased their neuronal activity after cooling (24°C) by 231.4% ± 40% (becoming 0-13.256 impulses per second) [11]. However, the effects of hypothermia on neuron signaling and conduction activity remain unclear. These findings suggest that neurons with an initial high-impulse activity can be preserved by hypothermia [12].

Hypothermia following spinal cord injury has been shown to increase levels of interleukin (IL-10), Pentraxin-related protein (PTX-3), and cortisol [13]. These cytokines are mostly anti-inflammatory, with the aim of reducing and localizing inflammation and preventing the development of systemic complications. Therefore, hypothermia preserves the anti-inflammatory effect and reduces inflammation in the target organ.

4. LOCAL HYPOTHERMIA EFFECT ON PNS

Recent studies on experimental animal models have demonstrated that local hypothermia reduced the expression of axon regeneration inhibitors. Results of the Western blot and real-time PCR showed that local hypothermia reduced the expression of RhoA, ROCK-II, NG2, neurocan, brevican, and Nogo-A [14], suggesting that local hypothermia is a good inducer of nerve fiber regeneration. A recent meta-analysis that included studies from the Medline and Embase databases until 2018 showed that local hypothermia (epidural hypothermia) does not significantly improve the locomotor activity of the injured spinal cord compared to whole-body hypothermia (systemic hypothermia) [15]. However, a previous systemic review involving various species of animals found that local hypothermia improves the locomotor activity of the injured spinal cord by 26.2% [16]. The systematic review by the American Association of Neurological Surgeons (AANS)/Congress of Neurological Surgeons (CNS) on hypothermia after spinal cord injury reported that mild hypothermia for acute spinal cord injury is safer and more effective for treatment [17]. Severe and moderate hypothermia provoke spinal cord cell damage by reducing blood supply to the spinal cord, thus resulting in the death of the remaining healthy cells.

5. LOCAL HYPOTHERMIA EFFECT ON CARDIO-MYOCYTES

Hypothermia following cardiac arrest primarily involves when used to mitigate neurological manifestations after acute cardiac arrest [18]. The anti-ischemic effects of local therapeutic hypothermia reduce the mortality rate from acute myocardial infarction by reducing ischemic damage and therefore, reperfusion damage [19 - 22]. Some authors have demonstrated the beneficial effects of local hypothermia on ischemic cardiomyocytes by reducing the size of acutely injured myocardiocytes [21, 23, 24]. Reduction in ischemic cardiomyocyte size has been observed when local mild hypothermia started at the beginning of the controlled ischemia period through the reperfusion period or during the last 20 minutes of ischemia and not during the only reperfusion period [25]. A recent study on farm pigs has shown that hypothermia mitigated ischemia/reperfusion injury by upregulating autophagy, including mitophagy [26]. These findings were consistent with biopsy results from the hearts of patients with therapeutic hypothermia [26]. Autophagy is crucial for the homeostasis and regeneration of cardiomyocytes [27 - 29]. Anti-ischemic effects of hypothermia can be achieved through several strategies, including regional cooling, pericardio perfusion, cold coronary perfusion, topical hypothermia, and possibly whole-body hypothermia [23]. Therapeutic hypothermia for positive cardiac effects ranges from 32-34°C. Local cardiac hypothermia does not reduce reflow and induces microvascular perfusion, and therefore hypothermia reduces acute myocardial injury [30]. However, precise control of the temperature and the microenvironment failed in the reported clinical trials [31]. Hypothermia-induced autophagy is the hallmark of the positive effects of hypothermia on the reduction of acutely injured myocardiocytes, their regeneration, reduction in post-infarction re-modulation, and amelioration of

heart failure [32, 33]. Moreover, the cardioprotective effects of hypothermia realized by the multi-effects on the subcellular level include stabilization of mitochondrial permeability, calcium (Na+/Ca2+) channels homeostasis, production of nitric oxide, and equilibration of reactive oxygen species [34, 35]. The temperature of the body reduces from 36°C to 30°C, which further reduces oxygen consumption by 25%, dysrhythmias, and heart rate, thereby decreasing cardiac output [36, 37]. Bradycardia is manifested by a prolonged QT interval, a QRS complex, and a PR segment. Mild hypothermia preserves cardiac diastolic function and improves systolic function through the positive inotropic effect. However, hypothermia (30 ^{o}C) constricts the coronary arteries and worsens the already occluded artery. However, mild hypothermia (32-34 ^{o}C) is usually accompanied by improved cardiac output and mean arterial blood pressure, as well as perfusion [38]. Hypothermia promotes mitochondrial elongation in cardiac cells *via* inhibition of Drp1 [39]. Furthermore, therapeutic hypothermia inhibits hypoxia-induced cardiomyocyte apoptosis through the MiR-483-3p / CDk9 axis [40]. Delayed mild therapeutic hypothermia reduces oxidative stress on PC12 cells exposed to oxidative stress [41, 42].

6. LOCAL HYPOTHERMIA EFFECT ON ENDOTHELIAL CELLS

Endothelial cell dysfunction plays a central role in the development of most diseases. Therefore, the management strategies for the most common diseases (CVD, CKD, HT, DM, and cancer) involve correcting endothelial cell function. Local hypothermia application as a therapeutic technique for endothelial dysfunction is reliable. Endothelial cell metabolism is reduced by local hypothermia that results in a decrease in demand for energy and excreted toxic and catabolic by-products. Furthermore, the endocrine secretion of endothelial cells is reduced under local hypothermia, such as vascular endothelial growth factor (VEGF) [43]. This was also observed in mesenchymal stem cells; under local hypothermia (30°C), the expression of VEGF-165 was reduced [44]. Therefore, a reduction in the angiogenesis rate results in an impaired regeneration strategy and slow cancer invasion. Endothelial cells are involved in determining the severity of ischemia/reperfusion injury during acute cardiac injury and neuron injury [45]. Moreover, hypothermia was found to ameliorate ischemia / reperfusion-induced endothelial cell apoptosis by modulating JNK signaling and apoptotic pathways [46]. Furthermore, therapeutic hypothermia induces rapid repression of the pro-apoptotic protein Bax (peak anti-apoptotic induced by 6-12 hours) with up-regulation of mitochondrial membrane potential in neuronal ischemia/reperfusion injury [45].

Tumour necrosis factor-a (TNF-α) has been shown to induce endothelial cell dysfunction by increasing cell membrane permeability, actin redistribution, and apoptosis. Recent studies have shown that deep local hypothermia (3°C) significantly reduced TNF- α induced endothelial dysfunction [47]. Hypothermia-induced protective effects were associated with the inhibition of p38 mitogen-activated protein kinase (MAPK)/ heat shock protein 27 (HSP27) and overexpression of MKP-1. Whereas, apoptosis inhibition was mediated by knockdown of p38 MAPK and c-Jun N-terminal kinase (JNK) [47].

Endothelial dysfunction is associated with the release of inflammatory cytokines into the systemic circulation that results in multi-organ signs and symptoms. Classic functions of endothelial cells include barrier function, maintaining haemostasis, immune response, and angiogenesis [48]. Hypothermia reduces the release of pro-inflammatory cytokines and enhances the release of anti-anti-inflammatory mediators from damaged endothelial cells. A recent study showed that hypothermia markedly reduced the level of IL-4, IL-6, and TNF α [49]. Furthermore, hypothermia induced the secretion of vascular endothelial growth factor (VEGF), which is not consistent with the results of other studies [49]. Reduction in lipid peroxidation and release of oxygen species from endothelial cells reduce the overall negative effect of damaged endothelial cells [48]. Furthermore, it has been reported that the effects of hypothermia on endothelial cells are on the organelle level, which includes improving mitochondrial membrane permeability, reducing oxidative phosphorylation rate, decreasing the unfolded protein response of the damaged endoplasmic reticulum, and improving peroxisome lipid metabolism [24, 50].

7. POTENTIAL MECHANISMS OF LOCAL PROTECTIVE HYPOTHERMIA

Regarding the neuroprotective effect of local hypothermia, hypothermia normalizes the neuronal cell membrane permeability, including important electrolytes (Ca^{+2}, Na^{+}, K^{+}, Cl^{-}, Mg^{+2}). Subsequently, neuron edema resolves and improves impulse generation and conduction. In combination with lowering the metabolic activity of neurons, the susceptibility of neuron survival to stroke increases. On the subcellular level, local hypothermia normalizes the mitochondrial membrane permeability and activates mitophagy [51 - 53]. Recent *in vitro* studies have shown that therapeutic hypothermia induced its mitochondrial effects through inhibition of Drp1 and mRNA expression of the capsaicin receptor TRPV1 [39]. Patients with hypothermia have lower levels of circulating mitochondrial DNA, COX3, NADH1, and NADH2 [54]. Furthermore, the reduction in the released free radicals improves cellular antioxidant defense system activity [55]. The presence of a common signaling pathway between apoptosis and autophagy is responsible for the activation of autophagy through mitochondrial stress [53, 56]. Furthermore, local hypothermia modulates endoplasmic reticulum activity. It prevents the accumulation of unfolded protein response through autophagy of the endoplasmic reticulum. The synthesis of lipids and their package in the peroxisomes and Golgi apparatus are preserved and prevent the formation of pathological intracellular lipid vacuoles. Pexophagy plays a central role in the preservation of lipid metabolism and prevents the formation of extra pathological cytoplasmic lipid inclusions. Local hypothermia improves not only neurons but also glial cells, local endothelial cells, and axons. Together, the neuroprotective effect of hypothermia is achieved.

The cardioprotective effects of local hypothermia include stabilization of cell membrane permeability, electrolyte influx

and efflux, and reduction in oxygen demand and its increased supply. At the organelle level, local hypothermia reduces the formation of reactive oxygen species through mitophagy and a reduction in mitochondrial oxidative phosphorylation [39, 57]. Local hypothermia reduces oxidative stress and free radical formation after acute cardiac injury [58]. Moreover, local hypothermia modulates the endoplasmic reticulum and peroxisome activity, and during stress, endoplasmic reticulum autophagy and pexophagy are induced. Local hypothermia induces bradycardia with positive inotropic and bathmotropic effects. Subsequently, oxygen and ATP are preserved for the survival of the myocardiocyte. Similarly, local hypothermia in the heart dilates the coronary arteries and normalises the blood supply. Preventing calcium paradox by normalization of myocardiocytes calcium homeostasis prevents the modulation of post-acute myocardial injury and reduces the risk of additional myocardiocyte complications, such as aneurysm or rupture (Fig. **1**).

The protective effects of local hypothermia on endothelial cells are induced by a similar mechanism, in addition to modifying the endo, exo, and autocrine secretion of endothelial cells. Therapeutic hypothermia (32–34°C) has been found to induce the secretion of endothelin 1, endothelin converting enzyme 1, and endothelin A and B receptors [59]. Furthermore, therapeutic hypothermia upregulates nitric oxide secretion from endothelial cells and neurons [59]. Therefore, endothelial cell function is the basis for the correction of other cell functions (neurons, cardiomyocytes, *etc.*). Local hypothermia was found to reduce the secretion of IL-6 and other pro-inflammatory cytokines, such as IL-8, MCP-1, and COX-2, resulting in the downregulation of cellular inflammatory processes and the reduction of leukocyte recruitment [60]. Local hypothermia induces the expression of Bcl-2, which eventually reduces apoptosis. Mechanically, hypothermia downregulates ERK 1/2 phosphorylation and induces partial IκB-α breakdown that increases NFκB-dependent proinflammatory gene expression [60].

A recent *in vitro* study on Sprague-Dawley rats demonstrated that upregulation of the nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) expression poses protective effects in hypoxic and stressed neurocytes [61]. The results of survival rate, histopathology, hindlimb motor function, immunohistochemistry, and Western blotting after 12, 24, and 48 h of neuronal-induced hypoxia (through administration of 2 mg/kg of vecuronium bromide to induce cardiac arrest) showed that neurons upregulate the Nrf2 and HO-1 gene expression to decrease hypoxia-induced hindlimb paralysis [61]. These effects have been augmented in hypothermia conditions at 33 ±0.5 °C to dramatically reduce the hindlimb paralysis in the Sprague-Dawley rats [61]. Astrocytes play an essential role in the protection of neurocytes from ischemia-induced damage through overexpression of the Nrf2 and HO-1 signaling pathways [62].

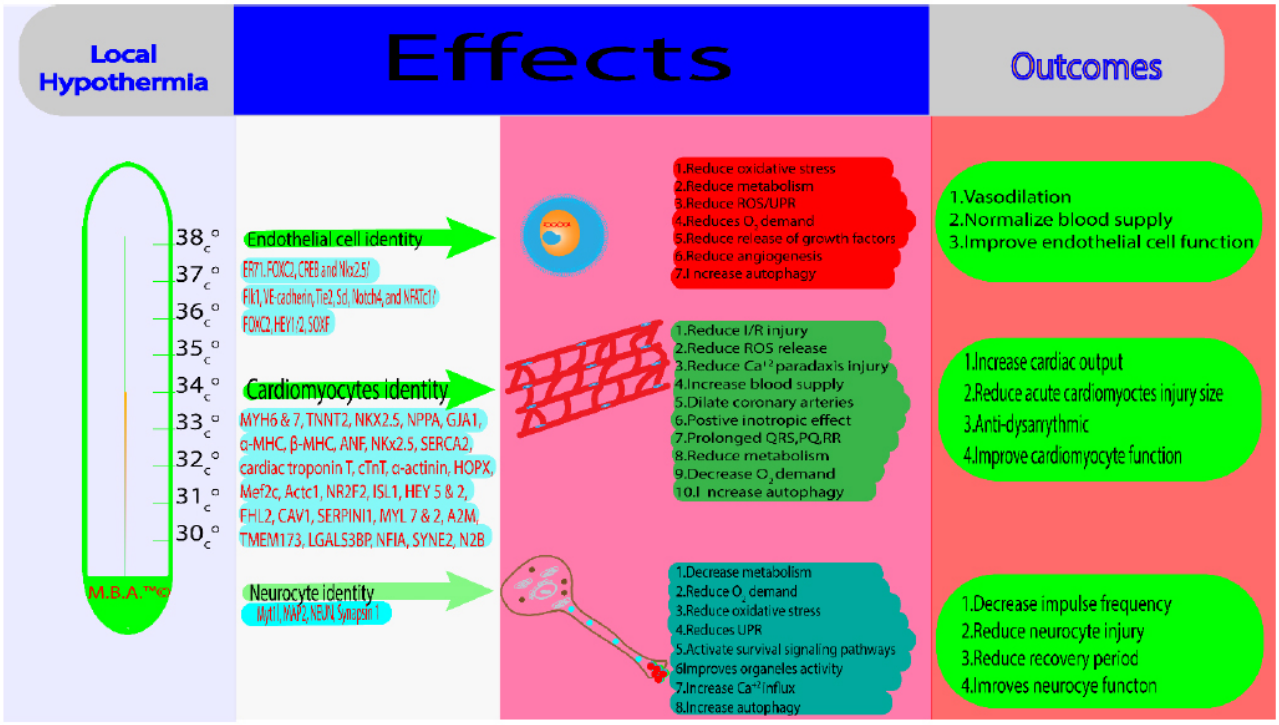

Fig. (1). Schematic presentation of the effects of local hypothermia on endothelial cells, cardiomyocytes, and neurocytes. Local hypothermia modifies the expression of genes through epigenetic modifications, transcription factor activation, small microRNAs, microenvironment, and DNA methylation/demethylation. Also, local hypothermia activates survival signaling pathways in cardiomyocytes, endothelial cells, and neurocytes by activating autophagy and apoptosis. Preferred hypothermia effects are achieved by the combination of endothelial cells modulation and parenchymal cells of the target organ (*e.g.*, endothelial cells and cardiomyocyte protective modification, each of which reduces the size of the post-acute myocardial injury area and slow speed of post-infarction re-modulation).

8. DISCUSSION

During local hypothermia, blood circulation increases in the cooled region, which maintains tissue functionality and integrity. However, prolonged local hypothermia decreases the temperature of the whole body, having systemic effects on the mechanism (signaling pathways), function (physiology), and structure (organic changes) of the body. Reduction in insulin secretion under general hypothermia results in a reduction in glucose uptake by the insulin-dependent tissues (liver, adipose tissue, muscular tissue) [63]. Therefore, hypothermia reduces the metabolic rate and deposits glucose in the brain. Furthermore, low insulin reduces lipogenesis and results in increased fatty acids that are required for cardiomyocytes to function.

Local hypothermia reduces protein metabolism in cells, which is probably due to the downregulation of major transcription factors, such as the global transcription factor Myc and XBP1s [64]. Prolonged hypothermia modifies the expression of genes, transcription factors, epigenetics, and the role of regulatory microRNAs. The effects of hypoxia and hypothermia on cells are quite similar, including activation of survival kinase, serine/threonine kinase, hypoxia-inducible factor 1, oxygen-sensitive transcription factor, MAPK/ERK signaling pathway, and heat shock proteins (HSP27, HSP70, HSP70-1 mRNA) [24, 33]. Therapeutic hypothermia (32°C) induces hypoxia-inducible factor-1 activity by PI3-K and Akt-1 pathways [65]. Therapeutic hypothermia phosphorylates phosphorylation of HSP27 and double phosphorylate Akt. Phosphorylated HSP27-Akt upregulates the production of nitric oxide synthetase-3 [65]. Moreover, nitric oxide synthesis increases, which, in turn, results in mitochondria protein nitrosylation and eventually modifies the production of reactive oxygen species. The general mechanism is involved in all nucleated cells.

One of the critical effects of therapeutic hypothermia on cardiac cells, endothelial cells, and brain cells is preventing the transdifferentiation of these cells into pathological cells, including myofibroblasts [28]. These features make therapeutic hypothermia unique during stressful conditions, including ischemia [66].

CONCLUSION

Local hypothermia is the preferred treatment option for injured neurocytes [10, 67]. The general effect of hypothermia on cardiomyocytes, neurocytes, and endothelial cells depends on the duration of exposure, the degree of hypothermia, and the severity of the injury. Regeneration induction by hypothermia is not sufficient despite that hypothermia induces the proliferation of regenerative tissues [28]. Under hypothermia, cell physiology tends toward dormancy and economic energy expenditure as well as activation of survival mechanisms, such as autophagy. The effects of hypoxia and hypothermia on cells are quite similar, including activation of survival kinase, serine/threonine kinase, hypoxia-inducible factor 1, oxygen-sensitive transcription factor, MAPK/ERK signaling pathway, and heat shock proteins (HSP27, HSP70, HSP70-1 mRNA) [24, 33].

LIST OF ABBREVIATIONS

SUMO	=	Small ubiquitin-like modifier
Nrf2	=	Nuclear factor erythroid 2-related factor 2
VEGF	=	Vascular endothelial growth factor
MAPK	=	Mitogen-activated protein kinase
HSP27	=	Heat shock protein 27
IL	=	Interleukin

AUTHORS' CONTRIBUTION

MB is the writer and researcher and contributed to the collection, analysis of the data, and revision of the manuscript.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The author declared no financial or other conflict of interest.

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