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Review

Effects of Therapeutic Hypothermia for Neuroprotection from the Viewpoint of Redox Regulation

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Redox regulation has recently been recognized as an important factor in acute illnesses as well as in chronic diseases. It has also become a target for neuroprotection in acute intensive care. Despite its well-known therapeutic effects, therapeutic hypothermia has recently been re-evaluated for its potential use in emergency and critical care medicine. Hypothermia is an undesirable physiological condition that can increase oxidative stress and decrease anti-oxidative potency. However, many studies have shown that under ischemia/reperfusion conditions, therapeutic hypothermia actually suppresses enhanced oxidative stress and maintains or increases anti-oxidative potency. This review provides an overview and outlook for the future of therapeutic hypothermia for neuroprotection from the perspective of redox regulation in patients with post-cardiac arrest syndrome and traumatic brain injury.

Key words: post-cardiac arrest syndrome, traumatic brain injury, therapeutic hypothermia, oxidative stress, intensive care

Stabilization of the systemic oxygen supply is the main goal of emergency and intensive care [1]. Meeting tissue demands by balancing oxygen delivery and consumption is crucial for the management of critically ill patients [2]. However, simultaneous hyperoxia is detrimental [3]. In 2010, Kilgannon *et al.* [4] reported that arterial hyperoxia is independently associated with increased in-hospital mortality among patients admitted to the intensive care unit following resuscitation from cardiac arrest. Redox collapse is considered to be one of the major reasons. Therefore, knowledge and techniques that provide sufficient management of the redox regulation are necessary for intensive care specialists (Fig. 1) [5, 6].

For the management of redox regulation in an intensive care setting, reducing the increase in oxidative stress is as important as controlling the oxygen supply. Within the filed of acute medicine, research focused on redox regulation is newer than that concentrated on chronic diseases such as diabetes mellitus, although many studies focused on oxidative stress in emergency settings have been reported [7]. Indeed, oxidative stress is now one of the major targets of intensive care medicine [8]. Among the various treatments available for oxidative stress, therapeutic hypothermia has been utilized to promote anti-oxidative neuroprotection in the daily management of multiple diseases [9]. In conjunction with adequate intensive care management, therapeutic hypothermia is achieved by a single method or a combination of the following methods (Fig. 2) [10]: 1), wrapping a patient with a blanket (or pad) in which cold liquid (or air) circulates [11]; 2), inserting

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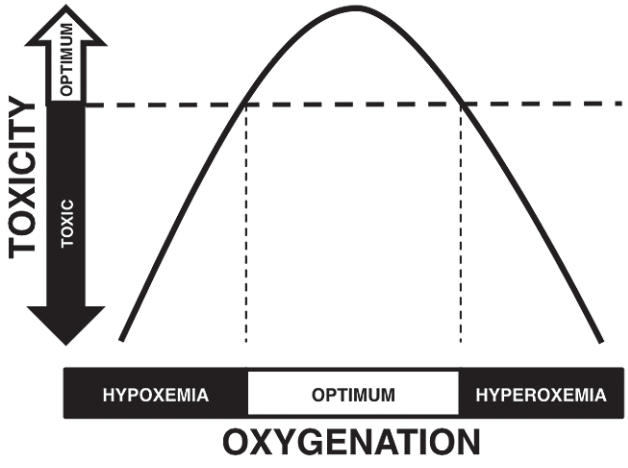

Fig. 1 Dual nature of oxygen. Oxygen has a two-sided character, both benefiting and harming humans and other mammals. The implementation of precisely controlled and balanced oxygenation might be beneficial for avoiding the damage from inadequate oxygenation, which is mainly due to the production of reactive oxygen species. In other words, too much oxygen (hyperoxemia) may be just as harmful as insufficient oxygen (hypoxemia), because of the increased production of reactive oxygen species (oxygen toxicity) under both conditions. ATP: adenosine triphosphate.

a special endovascular cooling catheter intravenously [12]; 3), administering cold Ringer's solution; and 4) introducing an extracorporeal circulation system. Overall, the target temperature is typically set to 33-34°C (*i.e.*, mild hypothermia), which is maintained for 24-48 h, and rewarming is introduced at a steady pace. Mild therapeutic hypothermia has been shown to improve neurological outcome in post-cardiac arrest syndrome (PCAS) [13-15] and in neonatal hypoxic encephalopathy (neonatal asphyxia) [16-19]. In addition to these two disorders, mild therapeutic hypothermia may be a promising therapy to treat traumatic brain injury (TBI) [20, 21] and acute encephalopathy [22].

However, there is still no global consensus on the effectiveness of therapeutic hypothermia. In this report, we will provide an overview of the efficacy of therapeutic hypothermia for neuroprotection from the perspective of redox regulation, based on the findings from PCAS and TBI studies.

Techniques		Advantages	Disadvantages
External cooling system	Cold water / Blanket	Tight thermoregulatory capacity Reducing the risk of over-cooling in induction	Skin reactions
Endovascular cooling system	Cold water / Balloon	Rapid and accurate establishment of the target temperature Stable maintenance	Need special catheter Central venous cannulation with the risk of venous thrombosis and infection
Cold infusion	Ice-cold lactated/ acetated Ringer's solution	Easy and rapid induction Applicable regardless of location	Difficult temperature maintenance
Extracorporeal circulation system (e.g. ECMO, dialysis)	Pump	Rapid induction	Highly invasive Need anticoagulant
Others	• Iced saline gastric lavage • Cooling helmets	• Water immersion system • Trans-nasal cooling devise	

Fig. 2 Cooling techniques. Various techniques are now available to induce and maintain therapeutic hypothermia. Non-invasive external cooling devices have recently made remarkable progress and have been shown to provide a sufficient cooling effect comparable to that of invasive catheter techniques. However, neither technique reliably provides sufficient induction and/or maintenance of therapeutic hypothermia when used alone, and thus these methods must often be supplemented with other approaches. ECMO: extracorporeal membrane oxygenation.

Pathophysiology of PCAS and TBI - Oxidative Stress and Lipid Peroxidation

To determine how oxidative stress affects the pathophysiology of PCAS and TBI, we reviewed the induction of oxidative stress and the mechanisms by which it injures the brain (Fig. 3). Ischemia and reperfusion of tissue have been conclusively implicated in PCAS and TBI pathology [23, 24], which often follow cardiac arrest or brain damage induced by a direct impact injury. During ischemia, the interrupted or decreased oxygen supply is typically balanced by a decrease in metabolism. However, during chronic ischemia, the production of adenosine triphosphate decreases, because the demand for more oxygen exceeds the supply. This event results in the influx of calcium ions followed by the opening of voltage-dependent Ca^{2+} channels induced by malfunctioning Na^{+}/K^{+}-ATPase and the subsequent depolarization of the cell membrane. Calcium influx induces the production of reactive oxygen species (ROS), which is followed by the up-regulation of nitric oxide synthase [25], as well as activation of the xanthine oxidase system, the arachidonate cascade, and the mitochondrial electron transport system [26].

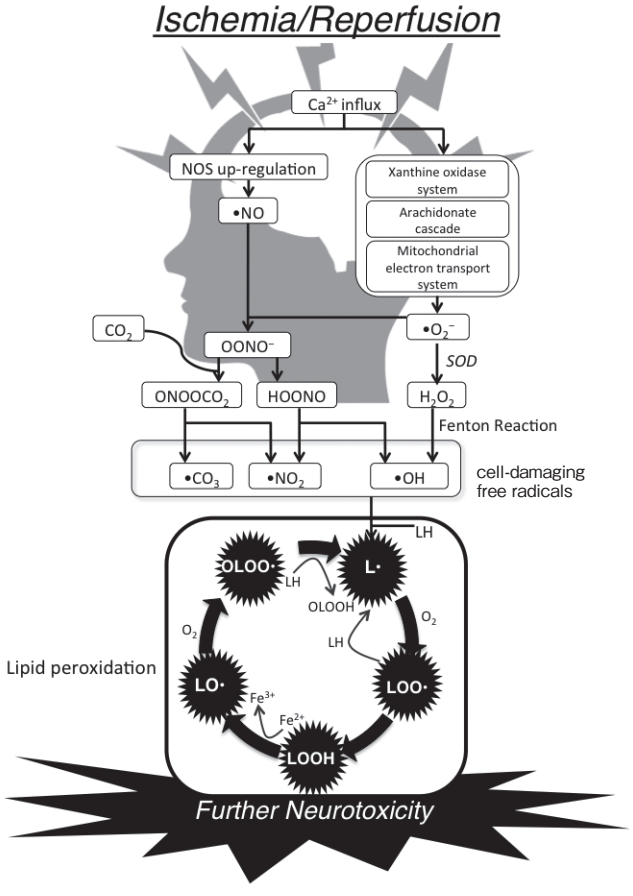

Fig. 3 The oxidative stress cascade in diseases with ischemia/reperfusion. The up-regulation of NOS and the formation of superoxide anion result in increased production of ROS and RNS, which further leads to oxidative stress and enhanced lipid peroxidation. These oxidative molecules further promote oxidative stress through a positive feedback loop, resulting in exacerbation of neuronal damage. H_2O_2, hydrogen peroxide; HOONO, peroxynitrous acid; L•, lipid radical; LO•, lipid alkoxyl radical; LOO•, lipid peroxy radical; LH, polyunsaturated fatty acid; LOOH, lipid hydroperoxide; NOS, nitric oxide synthase; $O_2•^-$, superoxide; •OH, hydroxyl radical; $OONO^-$, peroxynitrite; $ONOOCO_2$, nitrosoperoxocarbonate; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase.

Superoxide anion ($•O_2^-$) is generated after reperfusion resumes the oxygen supply. Superoxide dismutase (SOD) immediately converts superoxide anion to hydrogen peroxide, which is subsequently converted (by the Fenton reaction in the presence of iron) to the strong oxidant hydroxyl radical (•OH). The brain contains the large amount of iron, and thus provides a favorable condition for the generation of hydroxyl radicals [27]. Superoxide anion forms peroxynitrite ($OONO^-$) in the presence of nitric oxide (NO) (produced from activated NOS). Peroxynitrite combines with carbon dioxide to produce nitrosoperoxocarbonate ($ONOOCO_2$), which induces $•NO_2$ and $•CO_3$ [28].

These three by-products (*i.e.* $•OH/•NO_2/•CO_3$) are cell-damaging free radicals with very high reactivity; they target the brain in particular, because it is a rich source of polyunsaturated fatty acids. The interaction with the phospholipid bilayer of the cell membrane triggers peroxidation of lipid, which is relatively stable and hydrophobic, and thus easily diffused into the cytoplasm. This results in irreversible damage for the cell membrane or neuronal proteins.

Oxidative Stress in PCAS and TBI

Findings from animal experiments. Numerous laboratory studies have revealed that oxidant/antioxidant effects play a major role in the pathophysiology of PCAS and TBI. In the brains of cats exposed to a 7.5-minute cardiac arrest, thiobarbituric acid-reacting substances (TBARS) and conjugated dienes were

increased by 339% and 286%, respectively, compared with the levels before cardiac arrest [29]. Exposure of human umbilical vein endothelial cells to plasma from out-of-hospital cardiac arrest (OHCA) patients increases the production of ROS and decreases anti-oxidants such as SOD, glutathione, and glutathione peroxidase [30]. This indicates that plasma from OHCA patients induces acute endothelial cell toxicity leading to cell death. In the cat TBI model (induced by fluid percussion), $\bullet O_2^-$ in the cerebrospinal fluid is significantly increased compared with the control [31]. An increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the nuclear and mitochondrial regions has been shown to occur early after cortical impact injury in rats [32]. Moreover, oxidative injury of the blood-brain barrier (BBB) by shock wave pressure results in vascular edema, BBB leakage, and neurovascular inflammation and degeneration [33].

Findings from clinical studies. Several clinical studies have also shown an involvement of oxidative stress in PCAS and TBI. One of these studies revealed the importance of oxidative stress in patients with cardiac arrest using the oxidative stress index, which is a ratio of the total oxidative status to the total antioxidative status. This study demonstrated that the oxidative stress index was increased in patients with PCAS and TBI, and was found to be an independent predictor of early mortality after cardiopulmonary resuscitation [34]. In addition, reductions of ascorbic acid and glutathione in patients with TBI have been shown to involve a decrease of antioxidative status [35]. Presently, peroxiredoxin VI is a high-profile anti-oxidant marker, as its enzymatic activity is greatly decreased in the cerebrospinal fluid of TBI patients. Recovery of peroxiredoxin VI activity 24 h after patient admission is a favorable prognostic factor, whereas a continuous decrease in activity is a sign of poor prognosis [36].

Hypothermia and Oxidative Stress

Hypothermia causes various adverse effects related to temperature, including electrolyte abnormality, glucose intolerance, arrhythmia, coagulopathy, and immune suppression [37]. Moreover, the rewarming process increases the oxygen demand as a result of metabolic enhancement, and thus rapid rewarming may result in circulatory failure, leading to an exacerbated outcome. Therefore, the ability to transport oxygen decreases during hypothermia and its rewarming process, thereby producing ROS and reactive nitrogen species (RNS), hence oxidative stress [38]. Animal experiments have shown the effects of hypothermia on oxidant/antioxidant levels. Alva *et al.* [38] have demonstrated the effects in rats that were cooled at a mean rate of 0.25°C per minute to reach 22°C and then rewarmed 1 h after hypothermia at a rate of 0.35°C per minute to 37°C. These interventions elevated nitrites/nitrates and lipid peroxidation and decreased antioxidants, such as SOD and catalase, in the plasma [38]. Despite these effects, hypothermia has been shown to confer a neuroprotective effect, and thus it was recently recommended as a treatment for PCAS and neonatal hypoxic encephalopathy.

Therapeutic Hypothermia and Oxidative Stress in Ischemia/Reperfusion

Multiple studies have demonstrated the positive effects of hypothermia against oxidative stress in animal ischemia/reperfusion models (Table 1) [39-43]. Recently, Ostadal *et al.* [44] reported interesting *in vivo* data that could be applied under clinical settings. In their study, mild therapeutic hypothermia (33°C) was found to be superior to normothermia (36.8°C) against oxidative stress in porcine cardiac arrest. After 20 min of cardiac arrest, the circulation was restored by extracorporeal membrane oxygenation. Moreover, the reactive oxygen metabolite (ROM) levels were significantly lower in the hypothermia group than the normothermia group [44]. In a clinical study evaluating ROM production (derivatives of ROM; d-ROM) and the antioxidant capacity in the plasma of PCAS patients, the level of d-ROMs during hypothermia (33°C) was significantly suppressed compared with pre-hypothermia levels. This level increased with rewarming and was correlated with brain temperature [45].

Further studies will be needed to determine why the effects of hypothermia differ according to the presence or absence of ischemia/reperfusion. One possible mechanism for these differential effects may be the presence of GluR2 (the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit). GluR2 in hippocampal CA1 neurons inhibits calcium influx, and during ischemia, this subunit is downregulated. However, hypothermia (32°C) 1 h after forebrain ischemia attenuates this down-regulation, resulting in the inhibition of calcium influx, hence excitotoxicity [46]. In addition, microarray analysis in a rat model of TBI

has shown that hypothermia (33°C) alters miRNA expressions, which may subsequently affect redox regulation [47]. Therefore, differences in GluR2 levels could be responsible for the discrepancy in the hypothermia protocol, in addition to the target temperature, speed of cooling or rewarming, or duration of hypothermia, all of which varied among the studies (Fig. 4).

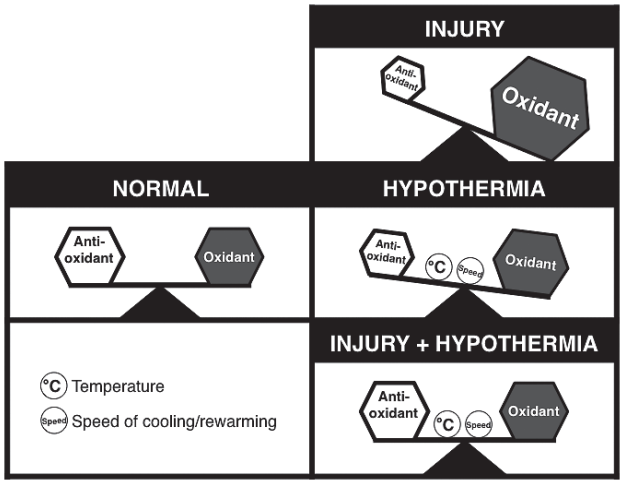

Fig. 4 Therapeutic hypothermia and redox regulation. The oxidant–anti-oxidant status is normally balanced, but injury can induce an oxidative environment. While hypothermia itself also promotes an oxidative environment, therapeutic hypothermia for ischemic/reperfusion damage can suppress oxidative stress and augment anti-oxidative action. The reasons for the discrepant effects of hypothermia between the presence or absence of ischemia/reperfusion remains unknown. However, the oxidant–anti-oxidant balance may be influenced by surrounding factors, including target temperature and speed of cooling and rewarming.

Future Outlook

The mechanism underlying therapeutic hypothermia is not just restricted to redox regulation. Numerous studies have demonstrated that this therapy is neuroprotective in multifactorial ways (Table 2) [10]. However, some clinical studies with large population sizes revealed that mild hypothermia management (compared with a targeted temperature management of 36°C) did not contribute to better outcome for patients with traumatic brain injury or post-cardiac arrest syndrome [48,49]. The discrepancy developed between experimental/animal data and human clinical trials of the effects of hypothermia management [50]. The question at this point is, for whom is mild hypothermia management beneficial? Unfortunately, there is no clear answer. The heterogeneous nature of human injury plays a large role in this knowledge gap. The evaluation of redox environment in a clinical setting may enhance our

Table 2 Mechanisms of neuroprotective action of hypothermia

Decreased cerebral metabolism of glucose and oxygen consumption
Reduced apoptosis and mitochondrial dysfunction
Prolonged cerebral excitatory cascade
Decreased the inflammatory response
Reduced vascular and membrane permeability
Reduced oxygen radical production
Altered expression of “cold shock proteins”
Minimized risk of thrombosis
Reduced risk of epileptic activities through electrical stabilizing properties

Table 1 Effects of therapeutic hypothermia on redox regulation in animal models of ischemia/reperfusion

Authors	Model	TT (°C)	Therapeutic effects of hypothermia
Brooks *et al*., 2002 [40]	MCAO in pigs	33–35	Preserved NAA and GSH in the brain
Karabiyikoglu *et al*., 2003 [41]	MCAO in rats	33	Decreased iNOS expression and preserved nitrotyrosine-positive cells in the brain
Stefanutti *et al*., 2005 [42]	I/R in rats	30–32	Decreased MDA and NOx in the plasma Decreased MDA and preserved GSH/GSSG ratio in the ileum
Grezzana Filho *et al*., 2011 [43]	I/R in rats	26	Decreased TBARS and catalase in the liver
Alva *et al*., 2013 [44]	Hypoxia (10% O_2) in rats	22	Decreased TBARS and NOx in the plasma Decreased TBARS and preserved GSH/GSSG ratio in the liver

GSH, glutathione; GSSG, glutathione disulfide; iNOS, inducible nitric oxide synthase; I/R, ischemia/reperfusion; MCAO, middle cerebral artery occlusion; MDA, malondialhehyde; NAA, N-acetylasparate; NOx, nitric oxide (nitrate plus nitrite); TBARS, thiobarbituric acid reactive substances; TT, target temperature.

understanding of this heterogeneity. Therefore, we propose the following hypotheses to explain this heterogeneity and suggest future research that focuses on redox regulation.

Hypothesis 1: Hypothermia therapy is not effective due to a redox collapse too great to be recovered by clinical management. The indication of hypothermia therapy has mainly been discussed with clinical events that are mechanically unrelated to the formation of brain injury (e.g., out-of-hospital or in-hospital cardiac arrest, schockable or non-shockable rhythm) [50]. Little is known about the indications for hypothermia management for TBI or PCAS based on direct factors that aggravate brain injury, such as redox environment. Therefore, we suggest a clinical research that evaluates the association between various points of a redox environment in a clinical setting and the neurological outcome of patients. These measured values may be useful for identifying patients who would most benefit from mild hypothermia therapy. Serum samples, as well as cerebrospinal fluid (CSF), which should directly reflect the neurological environment, should be used to evaluate the redox environment.

Recently, several automatic analyzers for redox environments have become available [51, 52]. We have published studies evaluating the redox status in humans and animals using a Free Radical Analytical System 4 (Diacron International, Grosseto, Italy) and a Free Radical Elective Evaluator (Diacron International) [53-55], by which both oxidative stress (d-ROMs) and the anti-oxidative status (biological antioxidant potential; BAP) can be measured within a few minutes. These devices are compact. A total serum or CSF sample of 20 μL is all that is required to detect both d-ROMs and BAP. The d-ROMs/BAP ratio and BAP/d-ROMs ratio are now commonly used to evaluate the oxidative or anti-oxidative potential, respectively [51, 52, 56].

Several antioxidants, such as progesterone and N-acetyl cysteine, have been demonstrated in clinical trials to be effective antioxidant treatments for TBI patients [57-59]. Although some of these agents were tested in experimental PCAS animal models [60, 61], to date there has been no clinical study involving the use of these agents in PCAS patients. It is possible that better clinical outcomes could be achieved with therapeutic hypothermia by identifying the optimal dosage and therapeutic window for treatment.

Hypothesis 2: Hypothermia therapy is not effective due to aggravated oxidative stress as a result of the clinical management procedure.

1. Muscle relaxants. Shivering is a defense mechanism for temperature regulation that comprises involuntary, rapid, and oscillating contractions of skeletal muscles [62], and is one of the main concerns with the introduction of hypothermia management. Additionally, shivering leads to increased oxygen consumption in response to increased metabolic demands [63]. Although there has been no clinical study about the direct effect of shivering on redox regulation, it is easily assumed that this vital response also affects the redox environment. Therefore, muscle relaxants could prevent this adverse response, and may contribute to better redox management. Conversely, the use of muscle relaxants has disadvantages in critical care management because of muscle weakness [64] and the association with mechanical ventilation and intensive care over longer periods of use [65]. Thus, the use of this medication is a critical evaluation point in therapeutic hypothermia.

2. Sedative agents. Some sedative agents, such as propofol and melatonin, have been reported to contribute to redox regulation and better outcome [66, 67]. The choice of these agents may have an additional effect in hypothermia management.

3. Methods for inducing hypothermia. Multiple clinical reports have cited the effectiveness and safety of both invasive and non-invasive cooling systems [68-70]. There have also been reports investigating the therapeutic window and speed of cooling. Although experimental animal studies have shown that early and fast cooling is essential for PCAS [71-73], clinical trials have yielded conflicting results on the benefit of fast cooling [74-79]; thus there are no established recommendations with respect to the materials and methods for initiating hypothermia introduction. It would be worthwhile to investigate differences in the association between the redox regulation and clinical outcomes under different methods of hypothermia induction.

4. Rewarming. While rapid rewarming has been reported to be associated with worse outcome [80, 81], one study also showed that prolonged rewarming duration of 28 h or longer might increase complications in PCAS [82]. Further studies are needed to determine the relationship between the redox environment and clinical outcome in the rewarming process in order to optimize the rewarming duration.

Conclusion

Further studies will be needed before reaching any definitive conclusions regarding the clinical effectiveness of therapeutic hypothermia for TBI and PCAS. Redox status may be a key factor to bridge the knowledge gap between experimental and clinical studies on hypothermia management.

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