

Hypoxic tissue damage and the protective effects of therapeutic hypothermia

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Abstract

Several molecules, chemicals and cells are involved in tissue damage during any hypoxic event, such as a cardiac arrest, a respiratory arrest or a cerebrovascular accident. Among them: calcium, protein kinase enzymes, calcium binding proteins, S-100 β protein and adhesion molecules such as intracellular adhesion mol-

ecule-1 (ICAM-1) are frequently cited in the literature. Controversy exists as to whether these “hypoxic aggressors” can be modified favorably by the use of therapeutic hypothermia. This review focus on the role of these different molecules, chemicals and cells and the protective effect of therapeutic hypothermia.

Keywords: Hypoxia, brain injury, CPR, cardiac arrest, hypothermia

Introduction

Therapeutic hypothermia (TH) has been used for over 60 years as an adjunctive therapy in a wide range of critical illnesses. Its use dates back several hundred years, but in modern medicine it was first described in 1940 by Fay [1]. Over the past few years, TH has gained popularity in the therapy of traumatic brain injury, refractory dysrhythmias, during percutaneous coronary intervention for acute myocardial infarction, cardiac arrest, neonatal encephalopathy and asphyxia, near-drowning and in hemorrhagic shock [2-9].

TH can be classified into four different stages: Mild hypothermia has been defined as a core body temperature between 32.2°C – 35°C. Moderate hypothermia is accomplished by decreasing the core temperature between 32.2°C – 28°C. Deep hypothermia is considered below 28°C and higher than 10°C [10]. Temperatures lower than 10°C are considered profound hypothermia [11].

TH has a variety of protective effects in patients with the conditions listed above. The purpose of this article is to briefly review some of the advantages of TH from a basic molecular and cellular standpoint.

The S-100 β Protein and the Effects of TH:

A well characterized role in promoting the survival and growth in specific neuronal populations during development of the nervous system is played by an astrocyte derived protein called S-100 β [12]. This protein has a half-life of approximately two hours [13]. In experimental models it has been documented that this protein is deleterious to the cells if it is over expressed [12]. Significant brain injury is associated to this over expression.

Normally the S-100 β protein is expressed in nanomolar quantities; however at a micromolar concentration, it may trigger significant adverse reactions at the cellular level. High concentrations of this protein have been detected in brains from patients with Down's syndrome, Alzheimer's disease, and brain metastatic carcinoma [14-17]. The rise of serum S-100 β after global or focal interruption of blood flow leads to a release of the protein towards the extracellular space and an efflux of the S-100 β from the cerebrospinal fluid into the blood stream [13]. This release and its quantification has been used by some authors as predictors of neurological outcome after cardiac arrest [18].

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Studies *in vitro* have demonstrated that S-100 β induces apoptosis of neuronal glial cells [13]. The exposure of neuronal cells to a mixture of brain S-100 β causes a sustained elevation of cytosolic free calcium with the concomitant appearance of apoptotic bodies and DNA fragmentation specific to apoptosis [15].

Some clinical and experimental studies have demonstrated that a rise in S-100 β is associated with global edema and a distortion of the astroglial cell membrane integrity [19,20]. Once the S-100 β is released from the cells after the injury, it acts as a positive feedback mechanism to sustain and extend the injury, due to the activation of nitric oxide synthetase, with the generation of nitric oxide and the consequent cellular death [13].

TH may be protective of the neuronal damage that occurs due to S-100 β . Hashimi-Idrisi and coworkers, in two experimental studies demonstrated the mechanism of protection anoxic main injury protection with TH. The alleged mechanism involves the interruption of one or more of the pathways participating in the genesis of anoxic brain injury such as the reduction of neurotransmitter release and the attenuation of the nitric oxide synthetase activity [21,22].

Clinically, serum values of S-100 β that correlate with brain damage are 0.7 micrograms per liter [23,24]. In addition, because of the short half life of the S-100 β protein, a persistently elevated value corresponds to continuous damage to the cells [13]. Patients that undergo mild therapeutic hypothermia, have shown lower levels of S-100 β , however in patients that undergo deep hypothermia, elevated levels of the S-100 β protein, have been shown [13,25].

Reperfusion Injury and the effects of TH

Another mechanism of hypoxic damage is mediated by reperfusion injury due to free radicals, such as superoxide anion and hydrogen peroxide. These oxidative free radicals are produced in the absence of oxygen, and usually after hypo perfusion [26].

Childs and associates, in an *in vivo* hemorrhagic shock model, demonstrated the reduction in microvascular permeability and the production of reactive oxygen (11). In this TH study, measurements of the extravasations of the reactive oxygen species with a solute of fluorescein isothiocyanate-bovine albumin and fluorescent probe of dihydrorhodamine, in rats after severe blood loss. One group of rats was maintained with at a temperature of 37°C, another was kept at 34°C and the last group at 30°C. These authors found that mild and moderate hypothermia, gave a protection to the rat's microvasculature by

decreasing permeability. In addition, the TH groups had less concentration of reactive oxygen species outside the vasculature, as well as less leukocyte migration and an attenuated adherence [11].

In another experimental study, moderate hypothermia initiated after resuscitation, proved to inhibit the accumulation of lipid peroxidase products and the consumption of free radicals scavengers in brain and cardiac tissue [27].

Energy Pathways and TH

In TH, adenosine triphosphate (ATP) storage is preserved and the metabolism kept aerobically [28]. It is also well known that temperature reduction slows ATP generation by a direct effect on rates of synthetic reactions [29]. Moreover, hypothermia also decreases velocities of energy-consuming processes, and the effect on the latter must be greater than the reduction rate of production because levels of high-energy phosphate compounds increase in uncomplicated hypothermia and decrease more slowly during hypoxia ischemia. This indicates that the main targets for TH in the energy pathways are the mitochondria [29].

When the temperature of a cell decreases, intracellular alkalinization will follow. This reduction in temperature stimulates glycolysis and hence increase ATP supply [30]. Takata and associates, in an experimental model, studied the protective effects of mild hypothermia on the neuronal activity, intracellular calcium accumulation and ATP levels during deprivation of oxygen and glucose [31].

Mild hypothermia has minor effects on the maintenance of neuronal activity and ATP levels, but it suppresses calcium accumulation and improves the reversibility of neuronal activity [31]. In addition, mild hypothermia of 12 to 24-h duration after normothermic hypoxic-ischemic insults seems to prevent or ameliorate secondary failures in energy parameters [29].

ICAM-1 and TH

The best well known integrin involved in the tissue damage caused by hypoxia – anoxia is the intercellular adhesion molecule-1 [ICAM-1] (32,33). ICAM-1 is a glycosylated, transmembrane protein, a member of immunoglobulin super family. This protein is involved in cell adhesion by interacting with a number of β 2-integrin families (34). ICAM-1 is expressed on endothelial, epithelial cells and leukocytes. The expression of ICAM-1

produces an accumulation of neutrophils. This, in turn, causes cell damage. ICAM-1 related injury has been well studied in the acute respiratory syndrome, acute lung injury, as well as in ischemic brain injury and myocardial infarction [32].

Kira and coworkers demonstrated that the TH can diminish the expression of ICAM-1, and consequently its effects on adhesion, activation and accumulation of neutrophils, during acute lung injury in rats [32]. In a group of normothermic rats and a group of mild hypothermic rats, a marked increase in expression of ICAM-1 was detected lining the endothelial cells in lungs of the normothermic group, while no increase was seen in the hypothermic rats. Moreover, the neutrophil migration and activity of myeloperoxidase was altered [32]. Wang and associates also showed a reduction in the acute neutrophil tissue infiltration and sub acute monocyte infiltration, in an experimental induced transient cerebral ischemia when TH was utilized [33].

Calcium and TH

The role of calcium ions entering cells upon both reperfusion and previous ischemia has been well studied [35,36]. Sustained levels of free calcium in a reperfusion scenario will produce a massive entry of these ions into

the cells, with the appearance of apoptotic bodies and DNA fragmentation (a herald of cell death). Calcium causes cellular injury by activating lipases, proteases, free radicals, and nitric oxide. These elements damage lipid membranes, mitochondria, and DNA [35].

When cerebral reperfusion occurs there is an increased extra cellular level of glutamate activated channel complexes, which produce an increased calcium levels, free radicals and the consequent activation of degradative enzymes. Hachimi-Idrissi and colleagues have shown that resuscitation combined with mild TH diminishes the elevation in glutamate level, therefore inhibiting its deleterious effects on calcium levels [13,36].

Conclusions

Current data clearly indicates that TH protects the cells against the aggressors in hypoxic or anoxic events. TH can protect the microvasculature, reducing the expression of reactive oxygen species; inhibiting adhesion, activation, and accumulation of neutrophils, preserving the adenosine triphosphate storages and maintaining an aerobic metabolism. TH also reduces serum S-100 β concentrations preventing cellular damage and decreasing edema. TH prevents the adverse effect of high calcium levels when reperfusion occurs.

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