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Profound Hypothermia Protects Neurons and Astrocytes, and Preserves Cognitive Functions in a Swine Model of Lethal Hemorrhage[1]

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Background. **Lethal injuries can be repaired under asanguineous hypothermic arrest (suspended animation) with excellent survival. This experiment was designed to test the impact of this strategy on neuronal and astroglial damage in a swine model of lethal hemorrhage. Furthermore, our goal was to correlate the histological changes in the brain with neurological outcome, and the levels of circulating brain specific markers.**

Materials and Methods. **Uncontrolled hemorrhage was induced in 32 female swine (80–120 lbs) by creating an iliac artery and vein injury, followed 30 min later by laceration of the thoracic aorta. Through a thoracotomy approach, organ preservation fluid was infused into the aorta using a roller pump. Experimental groups included normothermic controls (no cooling, NC), and groups where hypothermia was induced at three different rates: 0.5°C/min (slow, SC), 1°C/min (medium, MC), or 2°C/min (fast, FC). Profound hypothermia (core temperature of 10°C) was maintained for 60 min for repair of vascular injuries, after which the animals were re-warmed (0.5°C/min) and resuscitated on cardiopulmonary bypass (CPB). Circulating levels of neuron specific enolase (NSE) and S-100β were serially measured as markers of damage to neurons and astrocytes, respectively. Light microscopy and quantitative immunohistochemical techniques were used to evaluate hippocampal CA1 area and caudate putamen for neuronal injury and astrogliosis (astrocyte hyperplasia/hypertrophy). Surviving animals were observed for 6 weeks and neurological status was documented on an objective scale, and cognitive functions were evaluated using a technique based upon the concept of operant conditioning.**

Results. **Normothermic arrest resulted in clinical brain death in all of the animals. None of the surviving hypothermic animals displayed any neurological deficits or cognitive impairment. On histological examination, normothermic animals were found to have ischemic changes in the neurons and astrocytes (hypertrophy). In contrast, all of the hypothermic animals had histologically normal brains. The circulating levels of brain specific proteins did not correlate with the degree of brain damage. The changes in NSE levels were not statistically significant, whereas S-100β increased in the circulation after CPB, largely independent of the temperature modulation.**

Conclusions. **Profound hypothermia can preserve viability of neurons and astrocytes during prolonged periods of cerebral hypoxia. This approach is associated with excellent cognitive and neurological outcome following severe shock. Circulating markers of central nervous system injury did not correlate with the actual degree of brain damage in this model.**

Key Words: **hypothermia; bypass; thoracotomy; vascular injury; shock; trauma; cognitive functions; CNS injury; astrocytes; neurons; S-100β; neuron specific enolase**

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INTRODUCTION

Severe hemorrhagic shock is associated with extremely high mortality unless the source of bleeding can be controlled promptly [1, 2] Often the underlying injuries are potentially reparable if the viability of key organs (especially the brain) can be maintained during surgical repair and resuscitation. Theoretically, strategies that can safely prolong the ischemia time of the brain (e.g., hypothermia) may improve survival and decrease neurological morbidity. Controlled induction of protective hypothermia is well established in the fields of cardiac, transplant, and neurological surgery [3–6], but not in trauma. However, a number of preclinical studies have shown that induction of hypothermia following severe hemorrhage can dramatically improve outcome [7].

Our team has demonstrated that induction of profound hypothermia (total body preservation) can prevent organ damage and dramatically improve survival in large animal models of lethal hemorrhage [8, 9]. However, clinical examination in animals is not sensitive enough to exclude subtle brain damage. Similarly, an absence of obvious histological abnormalities in the brain many weeks after the insult may represent good healing rather than a lack of injury. Over the last decade assays for the measurement of brain specific proteins in the circulation have become commercially available. If reliable in the setting of traumatic shock, measurement of these biochemical markers would be a very attractive option for the diagnosis and monitoring of brain injury.

Conceptually, any significant insult (e.g., stroke, cardiac arrest, trauma, toxins) to the central nervous system (CNS) can release these proteins into the cerebrospinal fluid, and eventually into the circulation. Although a number of potential markers have been identified, most of the recent research has focused on two proteins: neuron specific enolase (NSE) and S-100β [10]. NSE is a dimeric isoenzyme of gylcolytic enzyme enolase, with a molecular weight of 78 kD and biological half-life of 48 h that is released by cell destruction (not secreted). It is primarily located in the cytoplasm of central and peripheral neurons, and neuroendocrine cells [11]. S100-β is a smaller (10–12 kDa) protein that is primarily located in the cytoplasm of astroglia and Schwann cells, and can either be actively secreted or passively released following cell death [12].

Severe hemorrhage can cause changes that are very similar to those observed in global cerebral ischemia, such as brain edema (cellular swelling), alterations in blood–brain barrier (BBB), apoptosis and/or necrosis [13], and levels of S-100β in the circulation have been correlated with the severity of hemorrhagic shock [14]. In our experimental model cardiopulmonary bypass (CPB) equipment was used to induce and reverse profound hypothermia. The influence of CPB and hypothermia on the degree of cellular damage in the brain, and the levels of circulating biomarkers remains controversial. Increase in the levels of S-100β and NSE has been reported after cardiac surgery, and correlated with development of neurological and cognitive dysfunctions [15, 16]. While induction of mild hypothermia (33°C) has been shown to decrease the levels of NSE (but not S-100β) in patients with cardiac arrest [17], moderate hypothermia (25°C) does not attenuate the levels of NSE or S-100β after repair of congenital cardiac malformations on CPB [18]. It remains untested whether serial measurement of these markers would be useful for diagnosing sub-clinical CNS damage following severe shock and numerous extra-cranial injuries as in our experimental model (uncontrolled hemorrhage, soft tissue trauma, surgical interventions, profound hypothermia, use of CPB, blood transfusions, resuscitation).

In a recent experiment, we evaluated the impact of cooling rates on long-term outcome in a swine model of lethal hemorrhage. The data obtained from this study were divided into two complimentary manuscripts for ease of presentation. The first manuscript described the physiological changes during hypothermic arrest, performance of non-CNS organs, and post-operative complications [19]. The current manuscript provides detailed information about the central nervous system in these animals. By using a battery of assays, our aim was to assess the impact of hypothermia on different cell populations in the brain, and to correlate cellular changes with clinically meaningful parameters (neurological examination and cognitive testing). We also wanted to determine whether the circulating levels of CNS biomarkers accurately reflected the histological extent of brain injury. The hypothesis was that in this lethal model of shock profound hypothermia would: 1) preserve neurons and astrocytes, and 2) protect cognitive functions. Furthermore, we reasoned that the levels of circulating brain specific markers would correlate with the degree of histological brain damage.

METHODS

The Institutional Animal Care and Use Committee approved this study. All of the research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations regarding experiments involving animals. The study adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996 edition. Strict aseptic technique was used for all surgical procedures.

Animal Preparation

There were 32 (n = 8/group) female Yorkshire swine (wt 85–110 lbs., Tom Morris Farms, Reistertown, MD) anesthetized with intramuscular injection of ketamine (10 mg/kg) and inhaled isoflurane. After placement of endotracheal tube, the animals were allowed to breathe spontaneously while light anesthesia was maintained by administering isoflourane (0.5-1%) using a Narkomed M anesthesia machine (North American Dräger, Telford, PA). The right carotid