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Effect of hypothermia on apoptosis in traumatic brain injury and hemorrhagic shock model

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ABSTRACT

Introduction: The neuroprotective mechanisms of therapeutic hypothermia against trauma-related injury have not been fully understood yet. In this study, we aimed to investigate the effects of therapeutic hypothermia on biochemical and histopathological markers of apoptosis using Traumatic brain injury (TBI) and hemorrhagic shock (HS) model.

Methods: A total of 50 male albino-wistar rats were divided into five groups: Group isolated TBI, Group NT (HT + HS + normothermia), Group MH (HT + HS + mild hypothermia), Group MoH (HT + HS + moderate hypothermia) and Group C (control). Neurological deficit scores were assessed at baseline and at 24 h. The rats were, then, sacrificed to collect serum and brain tissue samples. Levels of Caspase-3,6,8, proteoglycan-4 (PG-4), malondialdehyde (MDA), and nitric oxide (NO) were measured in serum and brain tissue samples. Histopathological examination was performed in brain tissue.

Results: There were significant differences in the serum levels of Caspase-3 between Group NT and Group C ($p = 0.018$). The serum levels of Caspase-6 in Group NT (0.70 ± 0.58) were lower than Group MH (1.39 ± 0.28), although the difference was not statistically significant ($p = 0.068$). There were significant differences in the brain tissue samples for Caspase-3 levels between Group NT and Group C ($p = 0.049$). A significant difference in the Caspase-8 brain tissue levels was also observed between Group NT and Group C ($p = 0.022$). Group NT had significantly higher scores of all the pathological variables (for edema $p < 0.017$; for gliosis $p < 0.001$; for congestion $p < 0.003$, for hemorrhage $p < 0.011$) than Group C.

Conclusion: Our study results suggest that hypothermia may exert its neuroprotective effects by reducing markers of apoptotic pathway, particularly Caspase-3 on TBI and HS.

Introduction

Traumatic brain injury (TBI) and hemorrhagic shock (HS) secondary to a trauma event are still the leading causes of mortality and morbidity, despite all advancements in treatment methods. TBI due the trauma can occur as a primary injury by direct force, or as a secondary injury due to the further metabolic responses (i.e., hypoxia, ischemia, re-perfusion injury, edema, space-occupying lesion, and free radicals) [1,2]. In these cases which are accompanied by HS, in addition to the well-known damage mechanisms, several different mechanisms also play a role in the damage. Cerebral blood flow and auto regulation mechanisms are disturbed due to hypoperfusion and decreased oxygen delivery. As a result, excitotoxicity and mitochondrial deficiency mechanisms are activated and brain tissue becomes more sensitive against injury [3].

In case of HS, maximum vasoconstriction in splanchnic bed and reduction in blood flow occurs to protect brain tissue. Although these changes in blood flow relatively protect brain tissue in the beginning, they cause ischemia and secretion of pro-inflammatory mediators in other organs. However, secretion of pro-inflammatory mediators leads to secondary injury in brain tissue rather than protection [2]. The underlying mechanism of a possible damage, which can occur in the complex case of TBI and HS, has not been fully understood yet.

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There are several studies which are focused on the beneficial effects of hypothermia on survival, organ functions and hemodynamic changes in as well as on biochemical changes in cellular levels trauma and shock models [4–6]. Neuroprotective effects of therapeutic hypothermia include decreasing cerebral metabolism and production of free oxygen radicals thus preventing the of cytotoxic or excitatory amino acid accumulation, and inhibiting apoptosis [6,7]. Although the neuroprotective effects of therapeutic hypothermia can vary depending on the body temperature, it is recommended that treatment should be started at the earliest time point following brain injury [8,9].

Neuronal apoptosis plays an essential role in hypoxic brain injury and causes neural tissue loss similar to acute hypoxic-ischemic encephalopathy. Neuronal apoptotic process involves two pathways: receptor-mediated extrinsic pathway or mitochondria-mediated intrinsic pathway. In both pathways, cell death occurs via Cysteine Aspartate Specific Proteases (Caspases). Caspases belong to an enzyme family which orchestrates apoptosis, necrosis and inflammation. This enzyme family is subclassified into three types: initiator (Caspase- 2,8,9,10), executioner (Caspase- 3,6,7), and inflammatory (Caspase- 1,4,5,11,12,13,14). In the extrinsic pathway, Caspase-3 is activated by Caspase-8 and plays key role in neuronal apoptosis [10–13]. Several studies using caspases have shown that inhibition of caspases provide a neuroprotective effect [11].

This study was approved by . . . University Animal Ethical Committee (no: 2016/45) and we aimed to investigate the effects of therapeutic hypothermia on biochemical and histopathological markers of apoptosis using TBI and HS model.

Materials and methods

Classification of groups

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals. All animals were caged individually in a room with stable temperature under mild-controlled conditions. Based on our experimental model, a total of 50 male albino-wistar rats weighing 350 to 450 g were included in this study. The rats were randomly assigned to five study groups containing 10 rats in each group:

- **Group TBI:** Isolated traumatic brain injury group
- **Group NT:** Administration of normothermia (36–38 °C) in TBI and HS
- **Group MH:** Administration of mild hypothermia (32–36 °C) in TBI and HS group
- **Group MoH:** Administration of moderate hypothermia (28–32 °C) in TBI and HS group
- **Group C:** Control group

All animals were fasted for eight hours before the experiment and water was also limited. The rats were scored for any neurological deficit and body weights were measured. Animals were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally and all rats, except for the control group, received closed head trauma. Then, all animals were placed in supine position, allowed to breath room air spontaneously, and were cannulated to create HS. At the end of the experiment, core body temperature of all animals was monitored using T_r (rectal temperature) probes (SSGL; Biopac Systems, Santa Barbara, CA).

Traumatic brain injury model

The animals were exposed to repetitive closed head trauma method described by Foda and Marmarou (free-drop of a 450-g blunt weight from a 1-m height to induce closed head trauma and diffuse brain injury model). A nickel plate was positioned on top of the vertex position. By enabling the weight to contact with larger area, diffuse cranial injury model was generated. Furthermore, to prevent a rebound injury and respiratory tract complications, heads of the rats were placed and fixed onto a foam block [14].

Volume controlled hemorrhagic shock model

After sterilization, femoral artery of each rat was cannulated with 22-gauge heparinized cannula. Hemorrhagic shock was induced by volume controlled (2 ml of blood per 100 gr body

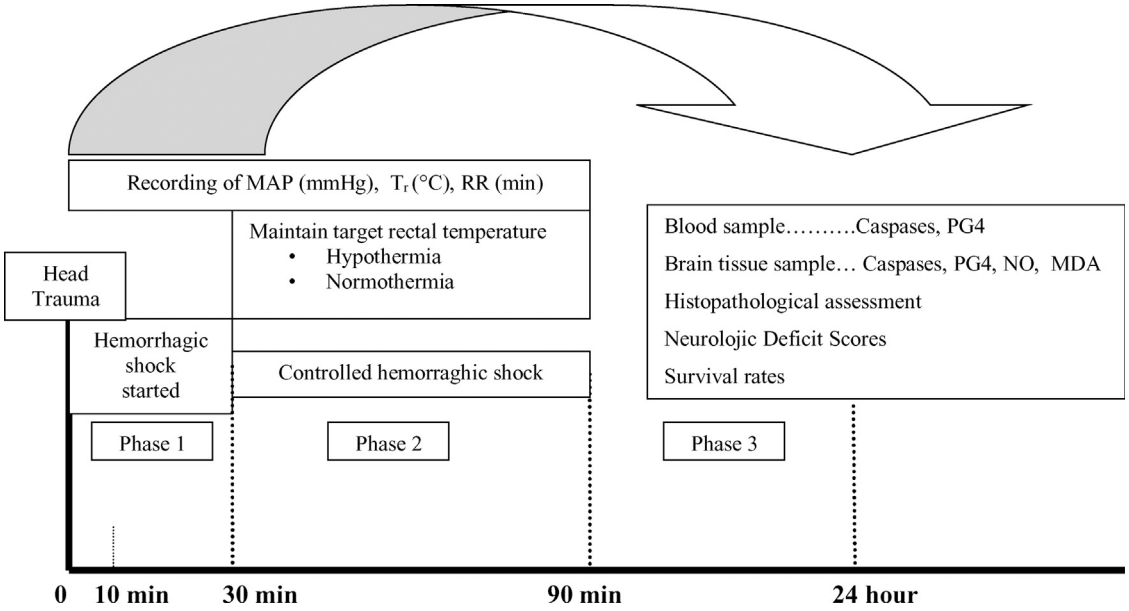

Fig. 1. A time line of the experiment.

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