Profound Hypothermia Decreases Cardiac Apoptosis Through Akt Survival Pathway

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BACKGROUND: Hypothermia increases the tolerable ischemia time for myocardium in hemorrhagic shock, but precise mechanisms are not clearly established. Here we studied activation of Akt cell survival pathway in a rodent model of emergency preservation and delayed resuscitation.

STUDY DESIGN: Wistar-Kyoto rats underwent 40% blood volume arterial hemorrhage during 10 minutes and were randomized into 2 groups based on core body temperatures (n = 7/group): hypothermia (15°C) and normothermia (37°C). Hypothermia was induced by infusing cold isotonic solution using cardiopulmonary bypass (CPB) setup. After reaching target body temperature, low-flow state (CPB flow rate of 20 mL/kg/min) was maintained for 60 minutes. Hypothermic rats were rewarmed to baseline temperature; all rats were resuscitated on CPB and monitored for 3 hours. The normothermia group underwent identical CPB management. Sham rats (no hemorrhage, no instrumentation) were used as controls (n = 7). Tissues were harvested at the end of experiment.

RESULTS: Induction of hypothermia increased survival rates (100% versus 0% in normothermia group). Western blot analysis of cardiac tissue revealed increased levels of phospho-Akt (active) in hypothermia and sham groups compared with the normothermia group ($p < 0.05$). Among downstream targets of Akt, phospho-GSK-3β (inactive), phospho-Bad (inactive), β-catenin, and Bcl-2 were considerably elevated in the hypothermia group compared with the normothermia group. Hypothermia also showed decreased activity of caspase-3 protein compared with normothermia ($p < 0.05$), suggesting decreased apoptosis.

CONCLUSIONS: Profound hypothermia increases survival in a rodent model of hemorrhagic shock and prolonged low-flow state. Hypothermia preserves Akt signaling pathway in cardiomyocytes with a concurrent decrease in cardiac apoptosis. (J Am Coll Surg 2009;209:89–99. © 2009 by the American College of Surgeons)

Hemorrhage is the leading cause of preventable deaths in military and civilian trauma.[1,2] A large number of these injuries are potentially reparable, and often the limiting factor is the period of normothermic ischemia that can be tolerated by the brain (< 5 minutes)[3,4] and the heart (20 minutes).[5] Typical cardiopulmonary resuscitation strategies, such as cardiac compression and forced ventilation, are futile in the setting of exsanguinating cardiac arrest.[6] Strategies that can maintain organ viability during ischemia and attenuate additional cellular damage during reperfusion can alter dismal outcomes. Induced hypothermia is well known to preserve cerebral and myocardial viability during periods of ischemia.[7] Recently, our group has applied this concept of therapeutic hypothermia in clinically relevant large animal models of trauma and shock.[8] Hypothermia was rapidly induced after life-threatening injuries to achieve "total body preservation." Injuries were repaired during this period of preservation, followed by controlled resuscitation and active rewarming. Implementation of this strategy, termed *emergency preservation and resuscitation* (EPR), has been shown to increase survival and preserve neurologic function after life-threatening hemorrhage, without a substantial increase in postoperative complications, such as bleeding and sepsis.[9-16]

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Abbreviations and Acronyms
CPB = cardiopulmonary bypass
EPR = emergency preservation and resuscitation
PI3K = phosphoinositol 3-kinase

Historically, hypothermia-induced cellular protection has been attributed to a decrease in tissue metabolism and oxygen consumption.[17,18] Recently, there has been increasing evidence that hypothermia also affects several molecular pathways involved in inflammation,[19] stress response,[20] and apoptosis.[21] One particular molecular pathway, shown to be involved in hypothermia, is the phosphoinositol 3-kinase (PI3K) and its downstream target, the serine/threonine protein kinase, Akt pathway (PI3K/Akt). Activated by a variety of extracellular signals, PI3K phosphorylates Akt (phospho-Akt), thereby activating it. Activated Akt promotes cell survival by phosphorylating and inactivating most of its substrates.[22,23] Two such proapoptotic target proteins, GSK-3β (glycogen synthase-3β) and BAD (Bd-xL/Bd-2-associated death promoter) are shown in Figure 1. Recent studies have shown that moderate hypothermia protects against cerebral ischemia by preserving Akt signaling in the neurons.[24,25] Effects of profound hypothermia on Akt signaling in vivo after hemorrhagic shock and prolonged low-flow state remain unknown.

In the current study, we investigated the cellular mechanism of action of therapeutic hypothermia and specifically its involvement in the Akt survival pathway in the heart. We hypothesized that induction of profound hypothermia would preserve Akt signaling in the heart, resulting in decreased apoptosis after hemorrhagic shock and a prolonged low-flow state.

METHODS

The protocol for the study was approved by the Institutional Animal Care and Use Committee. A previously described rodent model of EPR using cardiopulmonary bypass (CPB) in hemorrhagic shock was used in this study.[26] Adult male Wistar-Kyoto rats (Harlan) were anesthetized with 3% isoflurane, and 1% bupivacaine was injected at the operative sites to achieve local anesthesia. Isoflurane (0.7%) was used to maintain anesthesia during the experiment. Animals were orotracheally intubated with a 14-gauge catheter (Braun Medical Inc) and mechanically ventilated (Kent Scientific Corporation) at a rate of 40 breaths/min and a positive end-expiratory pressure of 5 cm H_2O. Tidal volumes were adjusted using a pressure-controlled mode to keep PCO_2 of 35 to 40 mmHg. The fraction of the inspired oxygen was adjusted to maintain pulse oximetry readings > 95%. The left femoral artery was cannulated with polyethylene catheter (PE50; Clay Adams) and used for hemodynamic monitoring, using a Ponemah Physiology Platform (Gould Instrument Systems), and for inducing arterial hemorrhage. The right femoral artery was cannulated with a 20-gauge catheter, which served as an in-flow CPB cannula. The right internal jugular vein was cannulated with a modified 5-hole 14-gauge cannula advanced into the right atrium and used as a venous outflow CPB cannula. A rectal probe was used to monitor body temperature. During surgical preparation, normothermia was maintained using a forced-air blower. After a 5-minute equilibration period, rapid exsanguination (40% blood volume arterial hemorrhage [0.027 mL/g body weight] for 10 minutes) was performed through the left femoral artery catheter.

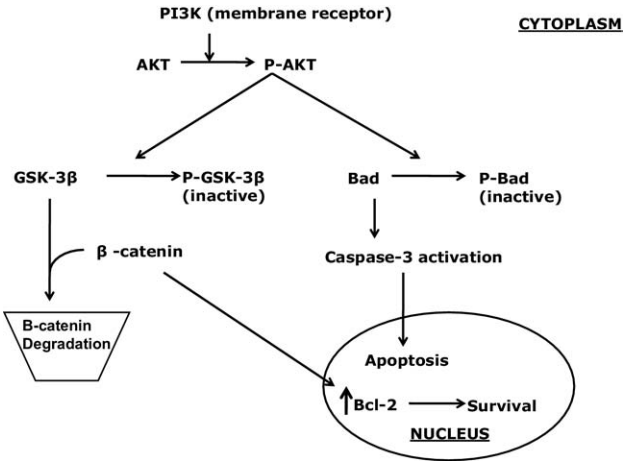

Figure 1. Phosphoinositol 3-kinase (PI3K)/Akt survival pathway.

After the arterial hemorrhage, rats were put on CPB and randomized into 2 groups: hypothermic EPR (0°C flush; n = 7), normothermia (37°C flush; n = 7). Sham animals (no hemorrhage/no instrumentation; n = 7) served as normal controls. CPB was started at a flow rate of 50 mL/kg/min. CPB reservoir was primed with heparinized plasmalyte A. For the H group, animals were cooled to a target temperature of 15°C for 40 minutes with a combination of cold CPB return and surface cooling. Similar volume of flush fluid (at 37°C) was used for the normothermia group for 40 minutes.

CBP setup

The CPB circuit (Fig. 2) consisted of a roller pump (Masterflex pump Model L/S Computerized Drive with an MF Easyload II Pumphead, Model 77201-60; Cole Parmer), tubing, a custom-designed oxygenator, and open reservoir (M Humbs Engineering). The oxygenator contained a 3-layer capillary membrane (prime volume of 4 mL, gas