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Hypothermia and valproic acid activate prosurvival pathways after hemorrhage

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ABSTRACT

Background: Therapeutic hypothermia (hypo) and valproic acid (VPA, a histone deacetylase inhibitor) have independently been shown to be protective in models of trauma and hemorrhagic shock but require logistically challenging doses to be effective. Theoretically, combined treatment may further enhance effectiveness, allowing us to use lower doses of each modality. The aim of this study was to determine whether a combination of mild hypo and VPA treatments would offer better cytoprotection compared with that of individual treatments in a hemorrhage model.

Materials and methods: Male Sprague–Dawley rats were subjected to 40% volume-controlled hemorrhage, kept in shock for 30 min, and assigned to one of the following treatment groups: normothermia (36°C–37°C), hypo (30 ± 2°C), normothermia + VPA (300 mg/kg), and hypo + VPA ($n = 5$ per group). After 3 h of observation, the animals were sacrificed, liver tissue was harvested and subjected to whole cell lysis, and levels of key proteins in the prosurvival Akt pathway were measured using Western blot.

Results: Activation of the proapoptotic protein cleaved caspase-3 was significantly lower in the combined treatment group relative to normothermia ($P < 0.05$). Levels of the prosurvival Bcl-2 was significantly higher in the combined treatment group relative to sham, normothermia, and normothermia + VPA groups ($P < 0.005$). The downstream prosurvival protein phospho-GSK-3β was significantly higher in the sham, hypo, and combined treatment groups compared with that in normothermia groups with or without VPA ($P < 0.05$). Levels of the prosurvival β-catenin were significantly higher in the combined treatment group relative to normothermia ($P < 0.01$).

Conclusions: This is the first *in vivo* study to demonstrate that combined treatment with VPA and hypo offers better cytoprotection than these treatments given independently.

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1. Introduction

Hemorrhage is a leading cause of morbidity and mortality in civilian and combat trauma [1,2]. Conventional treatment strategies for hemorrhagic shock (HS) focus on correcting blood loss by administering fluids or blood components. Although fluid resuscitation restores tissue perfusion, specific anti-inflammatory or prosurvival benefits are highly dependent on the choice of fluids [3–5]. Standard crystalloid resuscitation is perhaps the least effective fluid not only due to rapid extravasation out of the vascular system but also by disrupting endothelial and coagulation functions [6,7]. Recent research has focused on novel strategies to maintain cellular viability during shock. For example, treatment with high doses of valproic acid (VPA, a histone deacetylase inhibitor [HDACI]) have been shown to improve survival by activating innate cellular survival mechanisms [8]. Similarly, hypothermia (hypo) decreases tissue metabolism and oxygen consumption, thereby making the body more resistant to oxygen deprivation during shock. Besides decreasing the metabolic demands of the tissues, other nonmetabolic pathways are likely to be involved as well [8–10]. For example, both hypo and VPA upregulate the prosurvival phosphoinositol 3-kinase (PI3K)/Akt pathway [11], which decreases apoptosis by phosphorylating Akt and glycogen synthase kinase-3ß (GSK-3β), ultimately inhibiting the activation of the proapoptotic enzyme caspase-3 (Fig. 1). Although these strategies have both shown to be protective in models of trauma and HS, profound hypo and high doses of VPA each present unique challenges in the clinical setting. Induction of profound hypo requires specialized instrumentation to reduce core temperatures <15°C. Moreover, in rodent models, VPA must be given in a dose that is six-fold higher than is clinically approved, which may have potential side effects. Effective doses of VPA in patients with HS remain unknown, and we are currently trying to fill this gap through a phase I dose escalation trial (ClinicalTrials.gov Identifier: NCT01951560). In theory, combined therapy with both hypo and VPA may yield better outcomes, which would potentially allow us to use lower doses of each, with a better overall safety profile.

However, there is limited literature available about the interplay between VPA and hypo, and the possible benefits of combined therapy. We recently investigated the effects of combined VPA and mild hypo (32°C) treatment using an *in vitro* model of neuronal cells. We showed that combined treatment improves survival and decreases cell death after chemically induced hypoxia in mouse HT22 hippocampal cells [8]. As an extension of our previous work, the present study aims to obtain proof-of-concept *in vivo*, by testing VPA and hypo in a rodent model of HS. We hypothesize that combined therapy with VPA and mild hypo will upregulate the PI3/Akt prosurvival pathway compared with individual treatments alone.

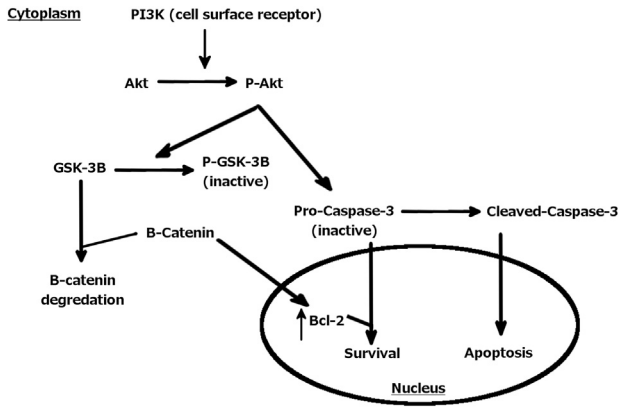

Fig. 1 – Schematic diagram of the PI3K/Akt survival pathway. Adapted from Shuja *et al.* [6].

2. Methods

Guidelines in the Animal Welfare Act and other federal statues were followed for all experiments. The Institutional Animal Care and Use Committee approved this study, and all procedures complied with the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (1996).

2.1. Animal preparation and monitoring

A total of 25 male Sprague–Dawley rats (225–300 g; Charles River Breeding Laboratories, Wilmington, MA) were sedated with 4% isoflurane and maintained at 1%–3% isoflurane for the duration of the experiment. 0.3 mL of 0.25% Sensorcaine (APP Pharmaceuticals, Schaumburg, IL) was administered subcutaneously into the right leg, and the right femoral artery and vein were dissected and cannulated with polyethylene 50 catheters (Clay Adams, Sparks, MD). The arterial catheter was used for hemorrhage and hemodynamic monitoring using the Ponemah Physiology Platform (Gould Instrument Systems, Valley View, OH). The venous cannula was used for VPA administration.

2.2. Treatment groups

Animals were divided into the following treatment groups: normothermic hemorrhage (NH), hypothermic hemorrhage (HH), normothermic hemorrhage with VPA resuscitation (NH + VPA), and hypothermic hemorrhage with VPA resuscitation (HH + VPA). We included normal rats (no hemorrhage or anesthesia) as a control group ($n = 5$ per group). During cannulation, NH and NH + VPA animals were kept at 37°C using a heating pad, and HH and HH + VPA animals were kept at room temperature to induce mild hypo. By the end of cannulation, the hypothermic animals reached a body temperature of around 32°C and after hemorrhage temperature were maintained between 29 and 31°C. Normothermic animals remained at a temperature of 36–38°C for the duration of the experiment. Core temperature was monitored using an indwelling rectal thermometer.

2.3. Hemorrhage and resuscitation protocol

Rats were subjected to a controlled hemorrhage of 40% of total blood volume over 10 min. The 40% hemorrhage volume was