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Valproic acid for the treatment of hemorrhagic shock: a dose-optimization study

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ABSTRACT

Background: Valproic acid (VPA) has been shown to improve survival in animal models of hemorrhagic shock at a dose of 300 mg/kg. Our aim was to identify the ideal dose through dose-escalation, split-dosing, and dose de-escalation regimens.

Materials and methods: Rats were subjected to sublethal 40% hemorrhage and treated with vehicle or VPA (dose of 300, 400, or 450 mg/kg) after 30 min of shock. Acetylated histones and activated proteins from the PI3K–Akt–GSK-3β survival pathway at different time points were quantified by Western blot analysis. In a similar model, a VPA dose of 200 mg/kg followed 2 h later by another dose of 100 mg/kg was administered. Finally, animals were subjected to a lethal 50% hemorrhage and VPA was administered in a dose de-escalation manner (starting at dose of 300 mg/kg) until a significant drop in percent survival was observed.

Results: Larger doses of VPA resulted in greater acetylation of histone 3 and increased activation of PI3K pathway proteins. Dose-dependent differences were significant in histone acetylation but not in the activation of the survival pathway proteins. Split-dose administration of VPA resulted in similar results to a single full dose. Survival was as follows: 87.5% with 300 and 250 mg/kg of VPA, 50% with 200 mg/kg of VPA, and 14% with vehicle-treated animals.

Conclusions: Although higher doses of VPA result in greater histone acetylation and activation of prosurvival protein signaling, doses as low as 250 mg/kg of VPA confer the same survival advantage in lethal hemorrhagic shock. Also, VPA can be given in a split-dose fashion without a reduction in its cytoprotective effectiveness.

1. Introduction

Hemorrhage remains a leading cause of preventable death after civilian and combat trauma. Conventional treatment involves prompt surgical control of bleeding and aggressive fluid resuscitation to reverse hypotension [1]. However, although fluid resuscitation restores circulatory volume, it also leads to hemodilution, increased coagulopathy, edema, and upregulation of proinflammatory mediators and their receptors, which in turn can exacerbate the lethal triad of

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acidosis, coagulopathy, and hypothermia [2,3]. Damage control resuscitation, which involves permissive hypotension and use of blood products instead of crystalloids, is a better alternative but is limited by the availability of these products in remote and austere locations such as the battlefield. Moreover, these resuscitation methods lack any specific properties that can correct the shock-induced changes at a cellular level. For these reasons, we are investigating pharmacologic alternatives to fluid resuscitation that are portable, protect against cellular damage induced by hemorrhagic shock, and are capable of maintaining survival until the injured patients can be transferred to a higher level facility (bridge to definitive care).

One promising pharmacologic alternative is valproic acid (VPA), which is widely prescribed as an antiepilepsy drug [4] but in higher doses can act as a histone deacetylase inhibitor (HDACI) [5]. Although hemorrhagic shock can disrupt cellular "acetylation homeostasis" [6] by suppressing histone acetyltransferase activity, leading to excessive histone deacetylation and suppressed gene transcription, the administration of VPA reverses these changes [7]. Our group has shown that VPA treatment prolongs survival in highly lethal models of hemorrhagic shock in swine and rats [8,9]. Additionally, VPA administration reduces cellular levels of proapoptotic caspase 3 in liver [10]; deactivates inflammatory extracellular signal-regulated kinase, JNK, and p38 mitogen–activated protein kinase pathway [11]; stabilizes intestinal tight junctions [12]; and attenuates systemic effects of ischemia–reperfusion injury in small and large animal models [13,14]. These data strongly suggest that treatment with HDACI can create a prosurvival phenotype in a wide variety of lethal insults, such as hemorrhagic shock, sepsis, and traumatic brain injury [15–17].

The dose of VPA (250–300 mg/kg) shown to cause histone deacetylase inhibition and survival improvement is 6- to 8-fold higher than what is currently approved by the Food and Drug Administration (FDA) for the treatment of seizures and mood disorders (20–60 mg/kg). When given as an HDACI in cancer patients, total VPA doses of >300 mg/kg have been administered in clinical trials but in five to six divided doses. Typically, the single maximum intravenous dose is kept <75 mg/kg to avoid side effects [18]. As massive blood loss is rapidly lethal, splitting the total dose over many days is not a practical option; therefore, we have typically used a single administration of VPA (300 mg/kg) to treat controlled hemorrhagic shock in animal models and have even used a dose as high as 400 mg/kg in a model of ongoing blood loss and massive resuscitation (large volume of distribution) [8]. Single administration of VPA in this large dose (300 mg/kg) has consistently been shown to sufficiently correct the histone acetylation profile and activate numerous prosurvival mechanisms [17]. However, it remains unknown whether 300 mg/kg of VPA is the optimal dose, if larger doses would increase efficacy, or if smaller doses would suffice. High-dose VPA has many potential side effects, such as pancreatitis, hepatic injury, and central nervous system depression. It is therefore logical to use the lowest possible dose that can produce the desired effect; therefore, we designed a three-part dose study to identify the optimal dose. Experiment 1, a dose-escalation study, examines whether VPA doses of >300 mg/kg result in any additional activation of key prosurvival pathways. We measured alterations in the PI3–Akt pathway as the end point because it is a well-known survival pathway that is affected by VPA treatment. Experiment 2 examines whether a given dose of VPA administered in a split fashion (two doses over a period of 2 h) has the same effect on protein activation as a single bolus dose. Experiment 3, a dose de-escalation study, determines whether VPA doses of <300 mg/kg produce survival advantage in the setting of lethal hemorrhagic shock.

2. Materials and methods

Experiments were designed and performed in accordance with the statutes from *The Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996 edition). This study complies with the Animal Welfare Act and other Federal regulations and was approved by our Institutional Animal Care and Use Committee.

2.1. Surgical procedure

Rats had access to food and water *ad libitum* before and after surgery. Male Sprague–Dawley rats (200–333 g; Charles River Laboratories, Wilmington, MA) were anesthetized with 5% isoflurane. Bupivacaine (1%) was injected at the operative site for local anesthesia. A veterinary multichannel anesthesia delivery system (Kent Scientific Corporation, Torrington, CT) was used to administer isoflurane at 0.7%–2% inspiratory fraction. Both femoral vessels were then isolated and aseptically cannulated with PE50 polyethylene catheters (Clay Adams, Sparks, MD) primed with heparinized saline (100 USP U/mL). The venous line was used for sampling, hemorrhage, and administration of drug treatment. The arterial catheter was attached to a pressure transducer for continuous blood pressure monitoring (Ponemah Physiology Platform; Gould Instrument Systems, Valley View, OH).

2.2. Sublethal hemorrhagic shock protocol for experiments 1 (dose-escalation study) and 2 (split-dose study)

Rats were subjected to a sublethal hemorrhage of 40% total blood volume over 10 min. The hemorrhage volume was calculated using the formula: total blood volume (mL) = rat's weight (g) × 0.006 (mL/g) + 0.77 [19]. Blood was withdrawn from the venous catheter using adjustable syringe pumps (Kent Scientific Corporation, Torrington, CT). A baseline (BL) arterial blood gas measurement was taken before hemorrhage and 30 min after hemorrhage for a post-shock (PS) measurement. Blood samples were analyzed using a Stat Profile Critical Care Xpress machine (Nova Biomedical, Waltham, MA). Rats were then randomly assigned to receive either saline vehicle or 300, 400, or 450 mg/kg dose of VPA (Calbiochem, San Diego, CA) given over a span of 10 min. Cannulas were removed, vessels ligated, skin closed, and animals returned to their cage to recuperate before sacrifice at 1, 6, or 16 h ($n = 3$ per time point, resulting in $n = 9$ per group for each of the four groups) after the end of VPA treatment (therefore), and organ tissues were harvested and flash frozen. Sham (instrumentation and no hemorrhage) rats served as controls.