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Pyrrolidine dithiocarbamate improves mortality in a rat model of severe hemorrhage

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ABSTRACT

Background: Hemorrhagic shock is a life threatening condition characterized by diminishing organ function. The aim of this study was to determine whether an effective pyrrolidine dithiocarbamate (PDTC) treatment protocol could be established to decrease organ dysfunction and mortality in a lethal hemorrhagic shock-resuscitation (HSR) model.
Materials and methods: Sprague-Dawley rats were randomized into three experimental groups; HSR alone (HSR), PDTC (100 mg/kg) administered 12 h pre-HSR (PDTC-12), and PDTC administered 1 h post-shock prior to resuscitation (PDTC+1). Hemorrhage was induced by arterial blood withdrawal to a mean arterial pressure (MAP) of 25 ± 5 mmHg for 1 h. Resuscitation was performed until pre-HSR MAP was attained. Blood was collected immediately prior to HSR, 1 h post-shock, and at protocol end. Measurements of base excess, lactate, arterial partial pressure of carbon dioxide ($PaCO_2$) and oxygen (PaO_2), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, blood urea nitrogen (BUN), and lipase were performed.
Results: In PDTC+1 animals, PDTC was ineffective in improving survival. In contrast, survival was significantly increased in the PDTC-12 animals *versus* PDTC+1 and HSR groups. Analysis of physiologic parameters demonstrated that elevations in base deficit and lactate levels following hemorrhage were blunted by PDTC administration in the PDTC-12 group. At time of death, creatinine, ALT, and AST levels were significantly higher in HSR *versus* PDTC-12 animals.
Conclusions: Administration of PDTC 12 h prior to HSR significantly improves survival through preservation of organ function.

1. Introduction

Hemorrhagic shock is a life-threatening condition associated with hypotension leading to inadequate organ perfusion and eventual organ dysfunction. Cellular hypoxia stimulates host-inflammatory responses and oxidative stress [1]. These metabolic stress signals, in turn, induce cell cycle arrest and p53-dependent apoptosis. While the precise molecular mechanisms regulating cell death from hypoxia are not fully understood, previous reports suggest these mechanisms may

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be mediated, at least in part, *via* transcriptional activation of nuclear factor-κB (NFκB) [2]. Under normal conditions the regulatory protein IκBα sequesters NFκB in the cytoplasm. In response to hypoxia, IκBα-targeted phosphorylation, and subsequent degradation, promotes NFκB nuclear translocation. Within the nucleus, NFκB binds to the regulatory regions of numerous genes including those that regulate inflammation, tissue repair and cell fate [3]. Depending on the magnitude and duration of shock, early systemic responses may cause secondary tissue damage and progression of the systemic inflammatory response syndrome [4].

In the United States, hemorrhagic shock ranks among the leading causes of death for patients aged 5-44 [4]. In addition, trauma-related injures account for nearly 500,000 emergency room admission each year and, independent of demographics, hemorrhagic shock is the leading cause of mortality worldwide [4,5]. As adaptive mechanisms act to preserve organ function, cessation of bleeding, and aggressive fluid resuscitation remain the mainstay for managing severe hemorrhage [6]. Following resuscitation, tissue reperfusion restores oxygenation and nutrient supply. However, cellular damage caused by sustained NFκB activation during hypoxia can progress with reperfusion and culminate in multiple organ dysfunction (MOD) and potential organ failure [7].

Disulfiram (Brand name: Antabuse), a drug clinically used for treating alcoholism, acts by irreversibly inhibiting acetaldehyde dehydrogenase resulting in acetaldehyde accumulation (after alcohol consumption), the noxious effects of which deters further alcohol intake [8]. Disulfiram has also been reported to inhibit NFκB activation and down-stream effector molecules [9]. Similarly, pyrrolidine dithiocarbamate (PDTC), a dithiocarbamate analogue of Disulfiram, inhibits NFκB activation and suppresses reactive oxygen species generation and proinflammatory cytokine release [10]. In a sublethal model of HSR, PDTC treatment modulates systemic stress following HSR [11]. To better understand mechanisms that may protect against MOD during hemorrhage, we thus developed a lethal HSR model to evaluate organ function, mortality, and the effect of PDTC on these parameters.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–350 g; Charles River Laboratories; Raleigh, NC) were used for these studies. All experiments were approved by the Institutional Animal Care and Use Committee and conformed to the Federal Care and Use of Laboratory Animals guidelines.

2.2. Surgical procedures

Animals were randomized to sham or hemorrhagic shock and resuscitation (HSR) groups, anesthetized (isoflurane by inhalation), and placed in a supine position as previously described with minor modifications [12]. The neck was shaved, sterilized using aseptic technique, and a cervical incision performed. The right carotid artery and jugular vein were exposed using a minimal dissection technique.

2.3. Sham

The right carotid artery and jugular vein were proximally and distally ligated and transected between ties. The surgical site was flushed with sterile 0.9% saline (37°C) and closed. Isoflurane anesthesia was maintained for 60 min before animals were recovered and returned to a clean cage. A subcutaneous injection of buprenorphine was administered (0.03 mg/kg) prior to surgery, and at 12 h intervals for a maximum of three doses.

2.4. Hemorrhagic shock and resuscitation

The right carotid artery and jugular vein were cannulated with polypropylene tubing (PE50) for blood pressure and heart rate monitoring, blood withdrawal, and resuscitation. Hemorrhagic shock was induced by blood withdrawal from the right carotid artery (≈1 mL/min) to a heparinized syringe (0.02 mL heparin; 1000 U/mL) until a mean arterial pressure (MAP) of 25 ± 5 mmHg was attained and maintained for 60 min by withdrawal-reinfusion of shed blood. A MAP of 25 mmHg was selected as being quantitatively representative of stage IV hemorrhagic shock in humans, as required to achieve cellular injury and MODS [13,14]. Fluid resuscitation was performed using a 1:1 mixture of shed blood and phosphate buffered saline (PBS) (0.9%, 1 mL/min) *via* the jugular vein until pre-hemorrhage MAP was attained. Animals were observed for an additional 10 min to confirm pre-hemorrhage MAP was maintained. Once stability was evident, cannulae were removed, vessels were ligated, and the cervical incision closed.

2.5. Experimental treatment groups

Animals were randomized to one of three experimental groups: (1) PDTC alone (PDTC). An intraperitoneal (i.p.) injection of PDTC was administered (100 mg/kg, dissolved in 0.9% saline), without hemorrhage and animals sacrificed 56 h later. (2) PDTC pre-HSR (PDTC-12). PDTC (100 mg/kg, i.p.) was administered 12 h prior to performing HSR, this time being selected based on the kinetic properties and steady state half-life of dithiocarbamates *in vivo* [15] and previously reported studies [16–18]. PDTC post-HSR (PDTC+1). Animals were subject to hemorrhagic shock and immediately prior to resuscitation PDTC (100 mg/kg) was administered *via* the venous cannula. Animals were resuscitated with shed blood-saline, recovered, and monitored for the remainder of the study period.

2.6. Morbidity and mortality

Animals were closely monitored throughout the experimental period up to 44 h after HSR. Predetermined criteria for humane intervention were established in consultation with the attending veterinarian and animals sacrificed immediately if criteria indicating impending death developed [19].

2.7. Blood collection

Blood samples were collected from HSR groups *via* the arterial cannula immediately prior to initiating hemorrhage (Pre-Hem) and prior to resuscitation (Res). Blood was collected