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Cepharanthine mitigates pro-inflammatory cytokine response in lung injury induced by hemorrhagic shock/resuscitation in rats

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ABSTRACT

Background: Cepharanthine possesses strong anti-inflammation capacity. We sought to clarify whether cepharanthine could mitigate pro-inflammatory cytokine production in acute lung injury induced by hemorrhagic shock/resuscitation (HS/RES). The involvement of heme oxygenase-1 (HO-1) was also investigated.

Methods: Male Sprague Dawley rats were allocated to receive HS/RES, HS/RES plus iv cepharanthine or HS/RES plus cepharanthine plus the HO-1 activity inhibitor tin protoporphyrin (SnPP) and denoted as the HS/RES, HS/RES + CEP, and HS/RES + CEP + SnPP group, respectively. HS/RES was achieved by blood drawing to lower mean arterial pressure (40–45 mmHg for 60 min) followed by shed blood/saline mixtures re-infusion. The rats were monitored for another 5 h before sacrifice.

Results: Arterial blood gas, lung permeability and histologic assays (including histopathology, neutrophil infiltration, and lung water content) confirmed that HS/RES induced significant lung injury. Significant increases in pulmonary levels of tumor necrosis factor-α, interleukin-1β, interleukin-6, prostaglandin E_2 and cyclooxygenase-2 confirmed that HS/RES induced a significant inflammatory response in the lungs. Cepharanthine significantly attenuated the pulmonary pro-inflammatory cytokine production and lung injury induced by HS/RES. However, the protective effects of cepharanthine were blocked by SnPP, the potent HO-1 activity inhibitor.

Conclusion: Cepharanthine significantly mitigates pro-inflammatory cytokine response in acute lung injury induced by HS/RES in rats. The mechanism may involve the HO-1 pathway.

1. Introduction

Hemorrhagic shock followed by resuscitation (HS/RES) are two early pathophysiological phases in the sequela of the clinical reversal of hemorrhage [1]. Hemorrhagic shock induces tissue ischemia and oxidative stress. Reperfusion induced by resuscitation causes the release of toxic mediators from ischemic tissues and the subsequent triggering of inflammation cascades [2], which in turn leads to additional local and distal tissue injury. In the process, the free heme released from damaged erythrocytes catalyzes the formation of reactive oxygen species and greatly increases cell dysfunction [3–5]. Under physiological conditions the enzymes of heme oxygenase (HO) tightly regulate the catabolism of heme to produce metabolites that possess anti-oxidative and anti-inflammatory properties [6,7].

The lung tissues, being a downstream filter that receives the entire cardiac output, are most vulnerable to the toxic metabolites released from ischemic organs after HS/RES [8]. HS/RES readily upregulates the expression of inflammatory molecules in lung tissues, which leads to overt pulmonary inflammation and the progression of lung injury [9–12]. Therapies aimed at attenuating pulmonary inflammation may have beneficial consequences against lung injury induced by HS/RES [8].

Cepharanthine is a biscoclaurine alkaloid derived from *Stephania cepharantha*, a plant of the Menispermaceae family [13]. Cepharanthine possesses potent *in vivo* and *in vitro* anti-neoplastic, immunomodulatory and anti-inflammation effects [14–18] and has been used clinically to successfully treat radiation-induced leukopenia, hair loss, bronchial asthma, and certain types of allergic inflammation [19,20]. Moreover, in animal

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models of sepsis cepharanthine can exert protective effects and mitigate inflammatory responses [21,22].

To date, the question of whether cepharanthine can mitigate pro-inflammatory cytokine response in acute lung injury induced by HS/RES remains unstudied. To examine this hypothesis, we conducted this study using a rodent model of HS/RES. Moreover, it has been shown that induction of heme oxygenase-1 (HO-1) exerts protective effects against HS/RES [23]. We also examined whether HO-1 was involved in mediating the anti-inflammatory effect of cepharanthine.

2. Materials and methods

2.1. Animal preparation

Ninety male Sprague–Dawley rats (BioLASCO Taiwan Co., Ltd, Taipei, Taiwan) weighing 250–300 g were used in the experiments. All animal experiments were approved by Institutional Animal Use and Care Committee, Taipei Tzu Chi Hospital (100-IACUC No. 003). All experiments were done in accordance with the guidelines of the National Institutes of Health. The rats were anesthetized using an intramuscular injection of a ketamine/xylazine mixture (110/10 mg/kg respectively). Additional doses of ketamine/xylazine mixture (30/3 mg/kg respectively) were given hourly until the end of the experiment. The rats were placed supine on a heating pad. A rectal temperature probe was inserted and the body temperature was maintained at 37 °C throughout the experiments using the heating pad and heating lamps. Polyethylene catheters (PE-50, Becton Dickinson, Sparks, MD, USA) were inserted into the right femoral artery for blood pressure monitoring and the left femoral vein for blood withdrawal and intravenous (iv) injection, respectively. A tracheostomy was performed to allow airway clearance.

2.2. HS/RES protocols

Protocols of HS/RES were adapted from our previous study [24]. In brief, hemorrhagic shock was achieved by blood drawing over 10 min to reduce mean arterial pressure (MAP; BIOPAC System, Santa Barbara, CA, USA) from the physiologic level to 40–45 mmHg. The shed blood was stored in a syringe containing 20 units of heparin at room temperature. This lowered MAP was then kept for 60 min by drawing or re-infusing blood as needed. Resuscitation was achieved by re-infusing the shed blood, supplemented with twice the maximum blood volume drawn of normal saline over a 10-min period. All rats were monitored for another 300 min.

2.3. Experimental protocols

The rats were randomized to 5 experimental groups ($n = 18$ in each group). The Sham group received sham operation (i.e., cannulation of vessels and tracheostomy) plus a 30 μL intravenous injection (iv) of dimethylsulfoxide (DMSO) vehicle (Sigma, St Louis, Mo, USA). The Sham + CEP group received sham operation plus cepharanthine (5 mg/kg, iv; LKT Laboratories, Inc. St. Paul, MN, USA). The HS/RES group received HS/RES plus vehicle. The HS/RES + CEP group received HS/RES plus cepharanthine (5 mg/kg, iv). The HS/RES + CEP + SnPP group received HS/RES plus cepharanthine (5 mg/kg, iv) plus the potent HO-1 enzymatic activity inhibitor tin protoporphyrin (SnPP, 30 mg/kg, iv; Enzo Life Sciences, Farmingdale, NY, USA). All rats received intravenous injection of vehicle, cepharanthine, or cepharanthine plus SnPP immediately before resuscitation or at corresponding time points in the Sham groups.

At 300 min following resuscitation, arterial blood (0.5 mL) was drawn from 12 randomly selected rats in each group. Arterial blood gas (ABG) was immediately analyzed with a blood gas analyzer (Gem Premier 3000; Instrumentation Laboratory, Bedford, MA, USA). The lung samples were then harvested immediately after euthanization with pentobarbital (100 mg/kg, iv). The remaining 6 rats of each group were used for lung permeability measurements.

2.4. Lung tissues collection and bronchoalveolar lavage (BAL)

For the above-mentioned 12 rats from each group, the left main bronchus was tied and the left lung was excised. The superior and inferior lobes of the left lung were separated, and the inferior lobe was snap frozen in liquid nitrogen and stored at −80 °C. The left superior lobe was used for wet/dry weight ratio measurement. Then, 6 of these 12 rats from each group received right lung perfusion with 4% formaldehyde and then excised. For the remaining 6 rats, the right lung was lavaged 5 times with 3 mL sterile saline and the BAL fluid (BALF) was collected [24]. An aliquot of BALF was diluted 1:1 with trypan blue dye for counting total cell number. The remaining BALF was collected and centrifuged. The protein concentration of the supernatant was measured using a BCA protein assay kit (Thermo Fischer Scientific Inc., Rockford, IL, USA).

2.5. Histologic analysis

The formalin-fixed and paraffin-embedded lung tissues were serial sectioned and stained with hematoxylin and eosin. Histologic features including alveolar wall edema, vascular congestion, hemorrhage, and polymorphonuclear (PMN) leukocyte infiltration were examined under a light microscope using our previously published protocol [24]. Each histologic feature was scored on a 5-grade scale: 0 (normal) to 5 (severe). The overall lung injury in each rat was classified as normal to minimal when the sum of the scores was 0–5, mild when 6–10, moderate when 11–15 and severe when 16–20.

2.6. Wet/dry weight ratio and myeloperoxidase (MPO) activity assay

Wet/dry weight ratio (i.e., lung water content) and MPO activity (i.e., quantification of tissue PMN accumulation) were analyzed by protocols we have previously described [25]. The freshly harvested left superior lobe was weighed and then dried in the oven at 80 °C for 24 h. The lobe was then weighed again in dry condition. The wet/dry weight ratio was then calculated. For MPO activity assay, the snap-frozen lung tissues were homogenized and centrifuged. The suspension was then sonicated and the supernatant was obtained and incubated in a water bath at 60 °C for 2 h. MPO activity was measured using a MPO fluorometric detection kit (Enzo Life Science) according to the manufacturer's instructions.

2.7. Lung permeability

Lung permeability was determined by the Evans blue dye (EBD) extravasation [26]. In brief, rats received 30 mg/kg (iv) of EBD (Sigma) at 300 min after resuscitation. A blood sample (1 mL) was drawn at 5 min after EBD injection to determine the plasma EBD concentration. The rats were then euthanized at 20 min after EBD injection and BAL was performed, as above-mentioned. The collected BALF was then centrifuged at 1500 rpm at 4 °C for 10 min. The EBD concentrations of the BALF and the plasma were then analyzed by spectrophotometry at 620 nm. The EBD concentration of the BALF was then compared to that of the plasma to determine lung permeability.