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Pentoxifylline attenuates leukocyte–endothelial interactions in a two-hit model of shock and sepsis

Naomi Kondo Nakagawa, PhD,[a,c] *Ruy J Cruz Jr., MD, PhD,*[b] *Priscila Aikawa, PhD,*[c] *Cristiano J Correia, BSc,*[a] *José Walber Miranda Costa Cruz, PhD,*[a] *Thais Mauad, MD, PhD,*[c] *Haibo Zhang, MD, PhD,*[d] *Mauricio Rocha-e-Silva, MD, PhD,*[a] *and Paulina Sannomiya, PhD*[a,*]

[a] *Laboratory of Cardiovascular Surgery and Circulation Pathophysiology (LIM 11), Heart Institute (InCor), University of Sao Paulo School of Medicine, Sao Paulo, Brazil*

[b] *Starzl Transplantation Institute, Department of Surgery, University of Pittsburgh School of Medicine, Pittsburg, Pennsylvania*

[c] *Department of Pathology, LIM 5, University of São Paulo School of Medicine, Sao Paulo, Brazil*

[d] *Kennan Research Centre of Li Ka Shing Knowledge Institute at Saint-Michael Hospital, Department of Anesthesia, University of Toronto, Ontario, Canada*

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form
10 July 2014
Accepted 16 July 2014
Available online 24 July 2014

Keywords:
Hemorrhagic shock
Cecal ligation and puncture
Intravital microscopy
Microcirculation
Double-injury
Multiple organ failure

ABSTRACT

Background: This study investigated the effects of pentoxifylline (PTX) combined with resuscitation fluids on microcirculatory dysfunctions in a two-hit model of shock and sepsis.
Materials and methods: Male Wistar rats (250 g) were submitted to hemorrhagic shock and reperfusion followed by sepsis induced by cecal ligation and puncture. For the initial treatment of shock, rats were randomly divided into: sham, no injury, no treatment; hypertonic saline solution (HS) (7.5%, 4 mL/kg); lactated Ringer's solution (LR, 3 × shed blood volume); HS + PTX (4 mL/Kg + 25 mg/kg PTX); and LR + PTX (3 × shed blood volume + 25 mg/kg PTX). After 48 h of being exposed to the double injury, leukocyte–endothelial interactions were assessed by intravital microscopy of the mesentery. Endothelial expression of P-selectin and intercellular adhesion molecule-1 (ICAM-1) was evaluated by immunohistochemistry, as well as lung neutrophil infiltration by histology.
Results: Lactated Ringer's solution induced marked increases ($P < 0.001$) in the number of rolling leukocytes per 10 min (two-fold), adherent leukocytes per 100 μm venule length (six-fold), migrated leukocytes per 5000 μm^2 (eight-fold), P-selectin and ICAM-1 expression (four-fold), and lung neutrophil infiltration (three-fold) compared with sham. In contrast, PTX attenuated leukocyte–endothelial interactions, P-selectin and ICAM-1 expression at the mesentery when associated with either LR ($P < 0.001$) or HS ($P < 0.05$). Neutrophil migration into the lungs was similarly reduced by PTX ($P < 0.05$).
Conclusions: Data presented showed thatpentoxifylline attenuates microcirculatory disturbances at the mesenteric bed with significant minimization of lung inflammation after a double-injury model of hemorrhagicshock and reperfusion followed by sepsis.

* *Corresponding author*. Laboratory of Cardiovascular Surgery and Circulation Pathophysiology (LIM 11), Heart Institute (InCor), University of Sao Paulo School of Medicine, Sao Paulo, Brazil. Tel.: 55-11-30618260; fax: 55-11-306 17178.
E-mail address: exppaulina@incor.usp.br (P. Sannomiya).

0022-4804/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2014.07.034

1. Introduction

Hemorrhagic shock and sepsis are the leading causes of death following trauma. In these events, accumulation and uncontrolled activation of neutrophils play an integral role in tissue damage leading to organ injury and multiple organ dysfunction [1–3]. Despite being essential for organ integrity, fluid resuscitation is associated with abnormal and unbridled leukocyte–endothelial interactions, which increase neutrophil transmigration with further amplification of the inflammatory response. In this regard, several resuscitation solutions have been tested to modulate the organ dysfunction triggered by shock and resuscitation [3,4]. Hypertonic saline (HS) has been applied for early management of hypovolemic shock, with advantages of rapid effectiveness and smaller volume over crystalloid resuscitation. In addition to its hemodynamic and metabolic benefits, the administration of HS attenuates neutrophil migration, and decreases lung injury and bacterial translocation after hypovolemic shock [5]. However, the effects of HS are relatively transient. On the other hand, lactated Ringer's solution (LR) has been shown to improve hemodynamics, but may be implicated with endothelial dysfunction and deregulation of immune responses [4,6–9].

Pentoxifylline (PTX) is a synthetic dimethylxanthine derivative that has shown beneficial effects in the down-regulation of leukocyte-endothelial cell interaction by attenuation of the stimulatory molecule intercellular adhesion molecule-1 (ICAM-1) expression in single animal models of hemorrhagic shock and reperfusion (HSR) or sepsis [10–12]. In the present study, we assessed the effects of PTX in combination with HS or LR in a two-hit model of HSR followed by sepsis induced by cecal ligation and puncture (CLP). We hypothesized that the use of either HS or LR combined with PTX for the initial treatment of shock may improve microcirculatory mesenteric blood flow and modulate the lung inflammatory process as compared with resuscitation with HS or LR alone.

2. Materials and methods

2.1. Animals

Sixty-three male Wistar rats (weighing 200–250 g) were housed on standard rat chow pellet diet and water *ad libitum* under a 12-h light–dark cycle with ambient temperature control for a week before the study. The experimental protocol was approved by the local Ethical Committee.

2.2. Hemorrhagic shock/reperfusion and CLP

We used a two-hit model as described previously [6]. Briefly, male rats were submitted to HSR and 24-h later to CLP, totaling to 48-h of experimental protocol (Fig. 1). We used male rats to minimize potential errors during intravital microscopy reading because studies have shown that endogenous estrogens contribute to the regulation of neutrophil infiltration by reducing the endothelial adhesion molecules ICAM-1 and P-selectin expression in tissues exposed to an inflammatory stimulus. Animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and were intratracheally intubated (14F catheter; Johnson & Johnson, Sao Paulo, Brazil) keeping spontaneous breathing at room air. The right femoral artery and vein were cannulated for several purposes: monitoring of the arterial blood pressure (Acqknowledge System MP100; Biopac, Goleta, CA), bleeding, analysis of blood gases and lactate, and for fluid administration. The animals were randomized into five groups: (1) sham group (only anesthesia, intubation, and catheterization), (2) HS group: HSR with HS 7.5% (4 mL/kg), (3) LR group: HSR with LR (3 × shed blood volume), (4) HSPTX group: HSR with HS and PTX (25 mg/kg), and (5) LRPTX group: HSR with LR and PTX (25 mg/kg). As illustrated in Figure 1, hemorrhagic shock was induced by blood withdrawal over a period of 10 min to reach a mean arterial pressure of 40 ± 5 mm Hg. After 1 h of maintenance of hemorrhagic shock, fluid resuscitation was performed, followed by reinfusion of 25% the extracted blood and, 30 min thereafter, arterial and venous catheters were removed and femoral incision closed. The animals were extubated, kept warmed at 37°C for 1 h, and returned to cages for complete recovery with free access to food and water for an additional 24 h (Fig. 1).

After 24 h of the first hit (HSR), all four groups of animals underwent anesthesia and CLP (the second hit). A 2-cm midline abdominal incision was performed to expose the cecum that was ligated just below the ileocecal valve. The cecum was punctured twice with a 22-gauge needle and squeezed [4,6,13]. The bowel was appropriately returned to the abdomen, and the midline incision was closed in two layers. The animals were kept warmed at 37°C for 60 min and returned to cages for full recovery with free access to food and water.

2.3. Hematocrit, blood gases, and blood lactate

Blood samples were collected from the abdominal aorta for hematocrit, blood gas, and blood lactate analyses (Radiometer ABL 555; Radiometer Medical, Kopenhagen, Denmark) that were performed at the first hit in: baseline, 1 h after hemorrhagic shock, and 30 min after resuscitation (T60 and T90, respectively). They were also analyzed at the second hit: before and after CLP (24 h and 48 h, respectively). The sham group was analyzed at baseline, T60, T90, and 48 h.

2.4. Intravital microscopy of mesenteric leukocyte–endothelial interactions

All five groups of animals, including sham rats and those submitted to the two-hit model, were anesthetized as described previously. The animals were maintained on a warm stage at 37°C as previously described [4,6,12,14]. After an abdominal midline incision, the distal ileum and its accompanying mesentery were exposed. The mesentery was continuously perfused along the study period with a warmed (37°C) Krebs–Henseleit solution (113 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2.2H_2O$, 25 mmol/L $NaHCO_3$, 1.1 mmol/L $MgSO_4$, 1.1 mmol/L KH_2PO_4, 5 mmol/L glucose, and pH