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Estradiol mediates the long-lasting lung inflammation induced by intestinal ischemia and reperfusion

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ABSTRACT

Background: Lung inflammation is one of the main consequences of intestinal ischemia reperfusion (intestinal IR) and, in severe cases, can lead to acute respiratory distress syndrome and death. We have previously demonstrated that estradiol exerts a protective effect on lung edema and cytokine release caused by intestinal IR in male rats.

Materials and methods: We investigated the role of estradiol on the generation of interleukin (IL)-1β, IL-10, vascular endothelial growth factor (VEGF), and cytokine-induced neutrophil chemoattractant 1 (CINC-1) in a female rat model of intestinal IR. Blood and bone marrow leukocytes were also quantified. Seven-days-ovariectomized rats were subjected to intestinal IR by occlusion of the superior mesenteric artery for 45 min. After reperfusion of the tissue for 2 h, the rats were sacrificed. Lung tissue was collected, cultured for 24 h and assayed.

Results: We observed a significant increase in serum levels of IL-10, CINC-1, uric acid and circulating, but not bone marrow, leukocyte numbers. In addition, intestinal IR induced a significant increase in the *ex-vivo* lung levels of IL-1β, IL-10, and VEGF. Treatment with 17β-estradiol before the induction of intestinal IR prevented the systemic release of IL-10, CINC-1, and uric acid, but it did not affect the leukocytosis. In addition, 17β-estradiol significantly prevented the *ex-vivo* release of IL-1β and VEGF from lung tissue.

Conclusions: We demonstrated that intestinal IR interferes with lung homeostasis, priming the tissue to generate proinflammatory mediators for at least 24 h postischemia. Furthermore, our data confirm that the inflammatory responses caused by intestinal IR are estradiol mediated.

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Introduction

Intestinal ischemia and reperfusion (IR) is a life-threatening medical disorder characterized by the systemic release of a wide spectrum of inflammatory mediators and oxygen radicals, thus activating the innate immune response and resulting in local and remote organ dysfunction.[1-3] During ischemia, low tissue oxygenation leads to an energy imbalance whereby the adenosine triphosphate/adenosine monophosphate ratio is shifted to increased levels of adenosine monophosphate that, in turn, are catabolized to hypoxanthine and uric acid.[4] The release of uric acid is elevated after an ischemic event and hence is considered an indicator of low oxygenation of the gut tissue.[5] Uric acid is also a well-recognized stimulus of the innate and adaptive immune response.[6,7]

Acute lung inflammation is a major consequence of intestinal IR and is characterized by leukocyte migration, increased microvascular permeability, and release of proinflammatory mediators. In severe cases, this inflammation can lead to acute respiratory distress syndrome, multiple organ failure, and death. Despite advances in pharmacologic therapies, the mortality rate of acute respiratory distress syndrome is still high (around 40%), indicating a need for a better understanding of its underlying mechanisms.[8,9] We have previously demonstrated that ovariectomized (OVx) rats develop a significantly higher lung microvascular permeability and neutrophil influx after intestinal IR in comparison to female rats with intact ovaries. Interestingly, the replenishment of these OVx rats with 17β-estradiol prevented the increase of lung microvascular permeability by a nitric oxide–mediated mechanism, but it did not affect neutrophil migration into the lung.[10] These results suggest that endogenous estrogens protect female rats against some of the inflammatory repercussions of intestinal IR. Moreover, we have also shown that treatment with 17β-estradiol protects male rats against lung injury induced by intestinal IR, confirming the important role of female sex hormones in this process.[11] Estradiol has also been shown to be protective in experimental models of IR in other organs such as heart,[12,13] brain,[14,15] kidney,[16] and liver.[17,18]

In this study, we sought to investigate the role of estradiol on the systemic release of inflammatory mediators after 2 h of intestinal reperfusion in female rats, and on the *ex-vivo* release of mediators by lung tissue after 24 h of culture. To this purpose, we measured estradiol modulation of the release of serum and tissue cytokines, the number of leukocytes in the bone marrow and blood compartment, and the generation of uric acid in an OVx rat model.

Materials and methods

Animals and study groups

Seven days after ovariectomy, female Wistar rats (180-220 g) from our Department's animal facilities were assigned to three groups: (1) Sham IR: rats surgically manipulated but not submitted to intestinal IR; (2) IR: rats submitted to intestinal IR; and (3) IR + E: rats treated with estradiol and 24 h later submitted to intestinal IR. These studies were approved by the Animal Care Committee of the Institute of Biomedical Sciences, University of Sao Paulo, following the guidelines of the National Council of Animal Experimentation that regulates animal research according to Brazilian Federal Law (Report no. 111/10/03, 2013).

Ovariectomy

Rats were anesthetized with isoflurane (2%) and an incision was made on the lower part of the abdomen; the ovaries were identified and removed free from the adherent tissue. After ovariectomy, the animals received a single dose of Pentantibiotic (570 mg/kg, intramuscularly), Tramadol (5 mg/kg, intraperitoneally), and Paracetamol (530 μg/mL, in the drinking water *ad libitum* for 3 d). The effectiveness of the ovariectomy was verified 7 d later by cell patterns in vaginal smears and only rats with smears compatible with diestrus phase (elevated number of leukocytes) were used in these experiments. In addition, at the end of each experiment, the weight of the uterus was measured and compared with uteri from rats with intact ovaries. Animals with significant loss in weight of their uterus in comparison to controls were considered successfully ovariectomized.

Intestinal IR model

After 7 d of ovariectomy, the rats were anesthetized with ketamine–xylazine (100 and 20 mg/kg, respectively, intraperitoneally) and the superior mesentery artery was occluded for 45 min, after which the clip was removed and sutures performed to close the abdomen. After 2 h of reperfusion, all animals were euthanized by an over dose of ketamine–xylazine.

Ex vivo *culture of lung tissue*

Lungs were perfused through the pulmonary artery with 20 mL of phosphate buffer salt solution (30 mL/min). Samples of lung parenchyma were cut into small pieces and incubated in 24-well plastic microplates (four pieces per well) containing Dulbecco's Modified Eagle Medium (DMEM) at 37°C in a humidified atmosphere with 5% CO_2. Aliquots of the supernatants were collected after 24 h of incubation for cytokine quantification.

Quantification of interleukin-1β, interleukin-10, vascular endothelial growth factor, and cytokine-induced neutrophil chemoattractant 1

The levels of interleukin (IL)-1β, IL-10, vascular endothelial growth factor (VEGF), and of cytokine-induced neutrophil chemoattractant 1 (CINC-1) were determined in serum by Milliplex commercial ELISA kit (Merck Millipore, EUA). The concentration of IL-1β, IL-10, and VEGF were quantified using Duo Set commercial ELISA kit (R&D System, Minneapolis, MN) in aliquots of DMEM collected from lung tissue cultures. All assays were conducted following the specifications of the manufacturer.