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Acute lung injury induced by intestinal ischemia and reperfusion is altered in obese female mice

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ARTICLE INFO

This work is dedicated to the memory of Dr. Domenico Spina. He passed away on the 5th December 2016. Dr Spina was a Reader in Pharmacology, and Head of Pharmacology and Therapeutics Research group in the Institute of Pharmaceutical Science at King's College London. His death was premature and he will be greatly missed by his many colleagues, students and friends around the world.

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Obesity
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ABSTRACT

Rational: Acute lung injury (ALI) is a common complication after intestinal ischemia and reperfusion (I/R) injury that can lead to acute respiratory distress syndrome (ARDS). We have previously demonstrated that females are protected against lung damage induced by intestinal I/R through an estrogen mediated mechanism.
Objectives: To investigate the effect of obesity on ALI induced by intestinal I/R in female mice.
Methods: C57Bl/6 female mice were fed with a standard low-fat diet (SD) or a high-fat diet (HFD) for 9 weeks. Intestinal I/R injury was induced by a 45 min occlusion of the mesenteric artery followed by 2 and 24 h of reperfusion.
Results: Significant increase in lung myeloperoxidase expression (MPO) and neutrophil numbers of SD and HFD mice occurred at 2 h and 24 h of reperfusion. Furthermore, HFD mice presented a significant increase in lung eosinophil peroxidase (EPO) expression and eosinophil numbers compared to SD mice. Lung wet/dry weight ratio was significantly greater in HFD mice at 2 and 24 h of reperfusion, accompanied by a significant increase in the expression of inducible NO in the lung tissue and a significant decrease in arterial oxygen saturation at 24 h of reperfusion relative to SD mice.
Conclusion: Obesity predisposes female mice to increased pulmonary oedema and deterioration in gas exchange, which is accompanied by an increase in iNOS expression in the lung.

1. Introduction

The absence of oxygen supply and nutrients caused by ischemia creates a condition in which the restoration of blood circulation (reperfusion) results in oxidative damage of the tissue, release of inflammatory mediators and influx of inflammatory cells to local and remote organs [1–4]. In this scenario, acute lung injury (ALI) is a common complication after intestinal ischemia (I/R) that, when severe, can lead to acute respiratory distress syndrome (ARDS) and death [5]. We have previously demonstrated a significant increase in myeloperoxidase activity (MPO) in the lung and intestines, an increase in lung vascular permeability and the release of significant amounts of serum IL-6, IL-1β and IL-10 following experimental induction of I/R in normal weight rodents [3,6,7].

It is well established that obesity is an important co-morbidity in inflammatory diseases such as asthma, COPD, and diabetes [8,9]. However, the effect of obesity in ALI induced by ischemic trauma is controversial. Early epidemiological data show that obesity is associated with worse outcomes in acute lung injury [10]. It has also been suggested that chronic low grade inflammation observed in obesity primes the lung for injury, increasing the risk of developing ARDS following an ischemic episode [11,12]. In contrast, other authors have reported that increasing body mass index (BMI) is associated with both decreasing plasma inflammatory biomarkers and increasing white blood cells count, but not with increasing mortality, in patients who are critically ill with ALI [13]. More recent studies have confirmed these observations, suggesting that obesity is associated with higher morbidity, but not with an increased risk of mortality in critically ill patients [14].

Interestingly, whilst clinical findings suggest that obesity is an

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important factor in the severity of lung injury following ischemic trauma there is also evidence suggesting that this interaction may be gender influenced. Sex differences in ALI/ARDS have been documented with reports of higher incidence and higher mortality in male patients when compared to females [15–17]. Similarly, another study has demonstrated that male patients are more likely to receive mechanical ventilation than females when in intensive care units [18]. In addition, our group and others have demonstrated that estrogen has a protective effect in experimental models of acute lung injury induced by ischemic trauma [19–21].

In this study, we have investigated whether obesity alters the protective state conferred to female mice in ischemic events. To this end, we have measured inflammatory responses and arterial oxygen levels following intestinal I/R in mice fed either a standard or a high-fat diet.

2. Material and methods

2.1. Animals

Female C57/Bl6 between 20 and 25 g, bred in the animal facility of the Institute of Biomedical Sciences-University of São Paulo. This study was approved by the Ethic Committee of Animals Experimentation of the Institute of Biomedical Sciences-University of São 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).

2.2. Diet

Mice were fed for 9 weeks with a 30% fat diet (Pragsoluções Biociências, Brazil) or with a standard low-fat diet (Nuvilab CR-1, Brazil). All animals were weighed daily and their food consumption in grams recorded.

2.3. Cholesterol and triglycerides levels

Levels of circulating serum cholesterol and plasma triglycerides were determined by a specific enzymatic assay, following the instructions of the manufacturer (Colesterol Liquiform, Triglicérides Liquiform both from Labtest, São Paulo, Brazil).

2.4. Induction of intestinal ischemia and reperfusion (I/R)

Mice were anesthetized with ketamine/xylazine (100/20 mg/kg, i.p, respectively.) The superior mesenteric artery was obstructed for 45 min using a surgical clamp. After clamp removal, the abdomen was sutured and mice were humanely killed 2 or 24 h later with an over dose of anesthetic.

2.5. Wet/dry weight ratio

Wet weight of the lung tissue was recorded immediately after collection and dry weight after 24 h incubation in a dry oven at 60 °C. Edema was assessed calculating the ratio between wet and dry weights of the samples.

2.6. Immunohistochemistry

Expression of neutrophil myeloperoxidase (MPO), eosinophil peroxidase (EPO) and inducible nitric oxide synthase (iNOS) was measured using specific primary antibody against mouse MPO (1:200 MPO bs-4943R, Bioss Antibodies, US), mouse EPO (1:250 EPX bs-3881R Bioss Antibodies, US) and mouse iNOS (1:500 iNOS Novus Biologicals, US). Appropriate anti-rabbit biotinilated secondary antibody was used (1:200 or 1:500, Sigma, UK) and expression determined using 3,3′-Diaminobenzidine (DAB, Sigma, UK). Quantification was made using Image Pro Plus software, v5.5.0.29. Data represent average of percentage of positive DAB staining areas in the lung tissue, measured as 5 fields per sample, 4-5 animals per group.

2.7. Cytokine levels

Serum cytokine levels were measured using a 96-well magnetic beads multiplex plate, following manufacturer's instructions (R&D, Abingdon, UK).

2.8. Neutrophil and eosinophil number in lung tissue

Formulin-fixed lung tissue was processed and embedded in paraffin blocks (Chandon tissue processor, Thermo-Ficher, UK). The tissue blocks were cut into 5 μm slices and stained using conventional hematoxylin and eosin staining. Tissue neutrophils were enumerated manually using a 50 μm^2 grid and the Image J cell counter plug-in software (v. 1.45S). Data represent the average number obtained from 3 random fields per image, 5 images per sample, and 5 animals per group. Eosinophils were identified using the eosinophil-specific stain Luna, which confers a bright red color to the cells [22]. Quantification of eosinophils was made automatically using the Image J plug-in particle quantification software (v. 1.45S). Data represent average number of 5 images per sample, 5 animals per group.

2.9. Oximetry

Before each time point of reperfusion, mice were anesthetized with ketamine/xylazine (100/20 mg/kg, i.p, respectively.) and linked to an oximeter by an infra-red collar sensor (MouseOx, Starr Life Science, USA). Arterial oxygen saturation was measured every 10 s for the period of 1 min, totaling 6 recordings per mouse.

2.10. Statistic analysis

Data are represented as means ± SEM. Comparisons between groups were made by two-way ANOVA followed by Bonferroni post-test or Student's t test. (GraphPad InStat software ver.5.0). Values of $P < .05$ were considered statistically significant.

3. Results

3.1. Characterization of the obesity model

Mice maintained on high-fat diet for 9 weeks were characterized by a significant increase in body weight and abdominal fat deposition in comparison to mice fed with a standard low-fat diet (SD) for the same period of time (Fig. 1A and B). The high-fat diet (HFD) also induced a significant increase in circulating triglycerides (Fig. 1C) and cholesterol levels (Fig. 1D) when compared to mice fed a standard diet. There was no significant difference in basal plasma levels of estrogen between diets (0 h) (Fig. 1E).

3.2. Lung inflammation

Intestinal I/R caused a significant increase in lung tissue neutrophil number after 2 h of reperfusion that remained significant at 24 h in both groups when compared to the 0 h control (Fig. 2A). Immunohistochemistry measurements of MPO expression shows that HFD animals presented higher expression of MPO in the lung tissue in comparison to normal weight mice at 2 h of reperfusion. At 24 h of reperfusion, levels of MPO expression were similar between diets (Fig. 2C). HFD mice presented significantly higher number of eosinophils relative to SD mice at 2 and 24 h of reperfusion (Fig. 2B). This difference was confirmed by measurements of EPO expression in lung tissue (Fig. 2D).