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Effects of acute hypercapnia with and without acidosis on lung inflammation and apoptosis in experimental acute lung injury

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ABSTRACT

We investigated the effects of acute hypercapnic acidosis and buffered hypercapnia on lung inflammation and apoptosis in experimental acute lung injury (ALI). Twenty-four hours after paraquat injection, 28 Wistar rats were randomized into four groups ($n = 7$/group): (1) normocapnia (NC, $PaCO_2 = 35–45$ mmHg), ventilated with $0.03\%CO_2 + 21\%O_2 +$ balanced N_2; (2) hypercapnic acidosis (HC, $PaCO_2 = 60–70$ mmHg), ventilated with $5\%CO_2 + 21\%O_2 +$ balanced N_2; and (3) buffered hypercapnic acidosis (BHC), ventilated with $5\%CO_2 + 21\%O_2 +$ balanced N_2 and treated with sodium bicarbonate (8.4%). The remaining seven animals were not mechanically ventilated (NV). The mRNA expression of interleukin (IL)-6 ($p = 0.003$), IL-1β ($p < 0.001$), and type III procollagen (PCIII) ($p = 0.001$) in lung tissue was more reduced in the HC group in comparison with NC, with no significant differences between HC and BHC. Lung and kidney cell apoptosis was reduced in HC and BHC in comparison with NC and NV. In conclusion, in this experimental ALI model, hypercapnia, regardless of acidosis, reduced lung inflammation and lung and kidney cell apoptosis.

1. Introduction

Protective ventilation with low tidal volume has been shown to improve survival in patients with acute respiratory distress syndrome (ARDS) (ARDSNetwork, 2000; Ware and Matthay, 2000; Putensen et al., 2009), but may be associated with increased arterial carbon dioxide levels, generating hypercapnic acidosis. Acute hypercapnic acidosis has been suggested to attenuate lung injury in ischemic reperfusion (Laffey et al., 2000a), sepsis (Costello et al., 2009; Higgins et al., 2009), and endotoxin (Norozian et al., 2011) models, raising the possibility of a potential therapeutic application of hypercapnia in acute lung injury (ALI) (therapeutic hypercapnia).

It is still unclear whether acidosis should be buffered in therapeutic hypercapnia to reduce the systemic side effects associated with low arterial pH (pHa) (Bautista and Akca, 2013). Buffering acute hypercapnic acidosis with bicarbonate infusions was permitted in the ARDS Network tidal volume study (Brower et al., 2004). In experimental ALI induced by pulmonary ischemia–reperfusion (Laffey et al., 2000a) or cecal ligation and puncture (Higgins et al., 2009), buffering reduced the protective effects of hypercapnic acidosis, but worsened lung damage in *E. coli*-induced ALI (Nichol et al., 2009).

Hypercapnic acidosis affects not only lung inflammation, but may influence the apoptosis process as well (Shibata et al., 1998). Since CO_2 is highly diffusible through biologic tissues, it may modulate apoptosis not only in the lung but also in peripheral organs. To our knowledge, no study has evaluated, in experimental ALI, whether buffering acute hypercapnic acidosis may dampen cell apoptosis in different organs.

We hypothesized that hypercapnia with acidosis would reduce lung inflammation and apoptosis in lung and distal organs, whereas buffering may decrease these beneficial effects. The aim of this

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study was to investigate the effects of acute hypercapnia with and without acidosis on lung inflammation and lung, kidney, and liver cell apoptosis in paraquat-induced ALI.

2. Methods

2.1. Animal preparation and experimental protocol

This study was approved by the Ethics Committee of the institution where the work was carried out. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the U.S. National Academy of Sciences.

In 28 Wistar rats (weight 250–300 g, age 8 weeks), paraquat was administered (15 mg/kg by intraperitoneal injection). After 24 h, animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and midazolam (5 mg/kg). An intravenous line (Jelco 24G) was placed in the tail vein and anesthesia was maintained intravenously with ketamine (50 mg/kg/h) and midazolam (2.5 mg/kg/h). A comparable amount of sedative and anesthetic drugs was given to all rats. The depth of anesthesia was similar in all animals. Animals were placed and kept in supine position throughout the whole experiment. After median neck incision, a polyethylene catheter (PE 10) was introduced into the right internal carotid artery for blood sampling and mean arterial blood pressure (MAP) measurement (SCIREQ, Montreal, Canada). Under spontaneous breathing of room air (T0), blood (300 μL) was drawn into a heparinized syringe for measurement of arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), pHa, and bicarbonate (HCO_3^-) (i-STAT, Abbott Laboratories, Abbott Park, IL, USA). Animals were then paralyzed (pancuronium bromide 2 mg/kg intravenously) and mechanically ventilated (Servo-i, MAQUET, Sweden) in volume-controlled ventilation with tidal volume (V_T) = 8 ml/kg, respiratory rate (RR) = 80 breaths/min, inspiratory/expiratory ratio = 1:2, fraction of inspired oxygen (FiO_2) = 0.21, and positive end-expiratory pressure (PEEP) = 5 cmH_2O. Animals were randomly allocated to three groups (n = 7 per group): (1) normocapnia (NC) – mechanically ventilated as described above to maintain $PaCO_2$ between 35 and 45 mmHg; (2) hypercapnia (HC) – mechanically ventilated with a gas mixture containing 5% CO_2, 21% O_2, and balanced N_2 (Linde Gas Therapeutics, Lidingö, Sweden) to maintain $PaCO_2$ between 60 and 70 mmHg; and (3) buffered hypercapnia (BHC) – mechanically ventilated with a gas mixture containing 5% CO_2, 21% O_2, and balanced N_2 (Linde Gas Therapeutics, Lidingö, Sweden) to maintain $PaCO_2$ between 60 and 70 mmHg. After 5 min, a bolus of sodium bicarbonate solution (8.4%) was given intravenously equivalent to one-third of the dose calculated according to the following equation: HCO_3^- (mEq) = 0.3 × weight (kg) × base excess (BE) (Sirieix et al., 1997). The remaining seven animals were not mechanically ventilated (NV) and were used for measurement of lung histology and molecular biology analysis (Fig. 1). Arterial blood gases were analyzed at 0 (T0), 10 (T10) and 60 (T60) min after randomization.

After 60 min of mechanical ventilation, animals were euthanized and lungs and distal organs prepared for histological examination and molecular biology analysis.

2.2. Lung histology

At 60 min, a laparotomy was performed and heparin (1000 IU) injected into the vena cava. The trachea was clamped at end-expiration (PEEP = 5 cmH_2O), and the abdominal aorta and vena cava were severed, yielding massive death by exsanguination. The right lung of each animal was quick-frozen by immersion in liquid nitrogen, fixed with Carnoy's solution, and embedded in paraffin (Nagase et al., 1992). Four-micrometer-thick slices were cut and stained with hematoxylin-eosin. Morphometric analysis was performed with an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines of known length coupled to a conventional light microscope (Olympus BX51, Olympus Latin America-Inc., Brazil). The volume fraction of the lung occupied by collapsed alveoli (alveoli with rough or plicate walls) or normal pulmonary areas (those not exhibiting overdistended or plicate walls) were determined by the point-counting technique (Weibel, 1990) at a magnification of ×200 across 10 random, non-coincident microscopic fields (Riva et al., 2008; Saddy et al., 2010). Neutrophils, mononuclear leukocytes, and total cells in the lung parenchyma were evaluated at ×1000 magnification and determined by the point-counting technique. Diffuse alveolar damage (DAD) was quantified using a weighted scoring system (Silva et al., 2013). Briefly, values from 0 to 4 were used to represent the severity of edema, hemorrhage, inflammatory infiltration, and alveolar collapse, with 0 standing for no effect and 4 for maximum severity. Additionally, the extent of each parameter per field of view was scored on a scale of 0 to 4, with 0 standing for no appearance and 4 for complete involvement. Scores were calculated as the product of severity and extent of each feature, and ranged from 0 to 16. The cumulated DAD score was calculated as the sum of single score characteristics, yielding scores from 0 to 64. Two investigators blinded to the origin of the material examined the samples microscopically.

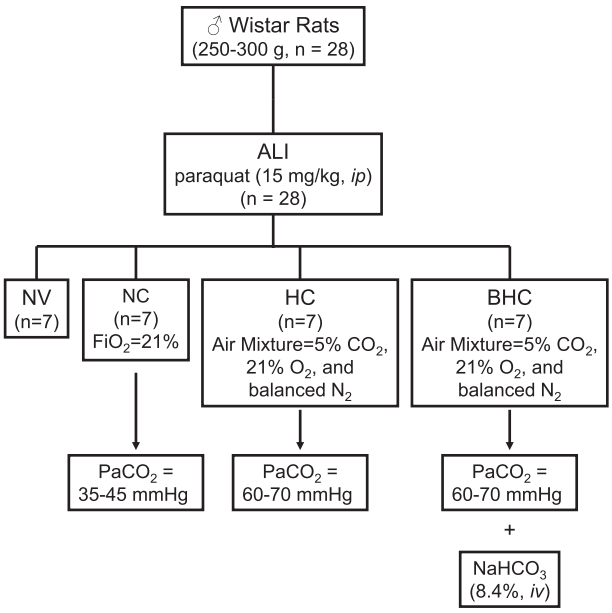

Fig. 1. Schematic flow chart of the study design. Acute lung injury (ALI) groups were randomized as follows: (a) normocapnia (NC), (b) hypercapnia (HC), and (c) buffered hypercapnia (BHC). For lung morphometry and molecular biology analysis, an additional non-ventilated (NV) ALI group was used.

2.3. Apoptosis assay of lungs, kidneys, and liver

Left lungs, kidneys, and liver were removed, fixed in 4% buffered formalin, and embedded in paraffin. To assay cellular apoptosis, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) staining was performed by two pathologists unaware of group allocation. Apoptotic cells were detected using an In Situ Cell Death Detection Kit, Fluorescein (Boehringer, Mannheim, Germany). Nuclei without DNA fragmentation stained blue as a result of counterstaining with hematoxylin (Oliveira et al., 2009;