Resuscitation 80 (2009) 1204–1210

Contents lists available at ScienceDirect

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Dexmedetomidine–ketamine combination mitigates acute lung injury in haemorrhagic shock rats☆

Chen-Hsien Yang[a], Pei-Shan Tsai[c], Tao-Yeuan Wang[b], Chun-Jen Huang[d,e,f,*]

[a] Department of Anaesthesiology, Mackay Memorial Hospital, Taipei, Taiwan
[b] Department of Pathology, Mackay Memorial Hospital, Taipei, Taiwan
[c] Graduate Institute of Nursing, College of Nursing, Taipei Medical University, Taipei, Taiwan
[d] Department of Anaesthesiology, Buddhist Tzu Chi General Hospital, Taipei Branch, Taipei, Taiwan
[e] School of Medicine, Tzu Chi University, Hualien, Taiwan
[f] Department of Pharmacology, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 9 March 2009
Received in revised form 17 May 2009
Accepted 13 June 2009

Keywords:
Inflammation
Cytokine
Inducible nitric oxide synthase
Cyclooxygenase-2
Resuscitation

ABSTRACT

Aim of the study: Upregulation of pulmonary inflammatory molecules is crucial in mediating the development of acute lung injury induced by haemorrhagic shock. Dexmedetomidine and ketamine possess potent anti-inflammatory capacity. We sought to elucidate whether dexmedetomidine, ketamine, or dexmedetomidine–ketamine combination could mitigate acute lung injury in haemorrhagic shock rats.
Methods: Fifty adult male Sprague–Dawley rats were randomized to the sham-instrumented, haemorrhagic shock (HS), HS plus dexmedetomidine (HS-D), HS plus ketamine (HS-K), or HS plus dexmedetomidine–ketamine (HS-D + K) group ($n = 10$ in each group). Haemorrhagic shock was induced by blood withdrawing and the mean blood pressure was maintained at 40–45 mmHg for 120 min. Resuscitation was then performed by infusion of shed blood/saline mixtures. After monitoring for another 8 h, rats were sacrificed.
Results: Histology findings and lung injury score analysis revealed moderate lung injury in rats of the HS, HS-D, and HS-K groups, whereas those of the HS-D + K group revealed mild lung injury. The effects of haemorrhagic shock on increasing cell number and protein concentration in bronchoalveolar lavage fluid as well as water content, leukocyte infiltration, and myeloperoxidase activity of lung tissues were significantly attenuated by dexmedetomidine–ketamine combination but not by dexmedetomidine or ketamine alone. Dexmedetomidine–ketamine combination, but not dexmedetomidine or ketamine alone, also significantly inhibited haemorrhagic shock-induced upregulation of pulmonary inflammatory molecules, including nitric oxide, prostaglandin E_2, chemokine (e.g., macrophage inflammatory protein-2), and cytokines [e.g., interleukin (IL)-1β, and IL-6].
Conclusions: Dexmedetomidine–ketamine combination mitigates acute lung injury in haemorrhagic shock rats.

1. Introduction

Haemorrhagic shock readily causes acute lung injury.[1] Upregulation of pulmonary inflammatory molecules, including nitric oxide (NO), prostaglandin E_2 (PGE_2), chemokine, and cytokines, has been shown to play a crucial role in mediating the development of acute lung injury induced by haemorrhagic shock.[2–5] Therapies aimed at attenuating the upregulation of pulmonary inflammatory molecules may be beneficial against acute lung injury in haemorrhagic shock animals.[6]

Ketamine, an antagonist of the N-methyl-D-aspartate receptor, is a potent anaesthetic and analgesic agent.[7] Ketamine was reported to cause undesired side effects (e.g., emergence agitation, increases in intracranial pressure, etc.) in a dose-dependent manner.[7] To prevent such side effects, the use of a sub-anaesthetic dosage of ketamine in combination with the other anaesthetic agents (e.g., dexmedetomidine) was recommended.[8] Dexmedetomidine is a selective agonist of the α2-adrenergic receptors and a novel sedative and analgesic agent.[9] It was reported that a combination of dexmedetomidine and a sub-anaesthetic dosage of ketamine (i.e., dexmedetomidine–ketamine combination) provides effective sedation and analgesia in clinical practice.[8]

☆ A Spanish translated version of the abstract of this article appears as Appendix in the final online version at doi:10.1016/j.resuscitation.2009.06.017.

* Corresponding author at: Department of Anaesthesiology, Buddhist Tzu Chi General Hospital, Taipei Branch, 289 Jianguo Rd., Sindian City, Taipei County 231, Taiwan. Tel.: +886 2 66289779x2639; fax: +886 2 66289009.
E-mail address: huangcj1112@gmail.com (C.-J. Huang).

0300-9572/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.resuscitation.2009.06.017

Both dexmedetomidine and ketamine possess potent anti-inflammatory capacity.[10,11] For instance, dexmedetomidine reduced endotoxin-induced inflammatory responses in septic rats.[10] Ketamine was found to exert anti-inflammatory effects in septic animals.[11] However, evidence regarding the anti-inflammatory effects of dexmedetomidine–ketamine combination is still lacking. To elucidate further, we thus performed this animal study with the hypothesis that dexmedetomidine, ketamine, and dexmedetomidine–ketamine combination could attenuate acute lung injury in haemorrhagic shock rats. Bronchoalveolar lavage and histology were used to determine lung injury. In addition, changes in arterial blood gas and upregulation of pulmonary inflammatory molecules induced by haemorrhagic shock were also evaluated to further confirm the protective effects of dexmedetomidine, ketamine, and dexmedetomidine–ketamine combination on acute lung injury.

2. Methods

2.1. Animal preparation

All animal studies were approved by the Animal Use and Care Committee of Mackay Memorial Hospital. The care and handling of the animals were in accordance with National Institutes of Health guidelines. Under halothane anaesthesia, two polyethylene catheters (PE-50, Becton Dickinson, Sparks, MD, USA) were placed in right femoral artery and left femoral vein, respectively. After tracheostomy, a 14-gauge intravenous (i.v.) catheter was inserted as a tracheostomy tube. Rats were mechanically ventilated (tidal volume: 4 mL of room air; rate: 35 breaths/min) with a small animal ventilator (Harvard Apparatus, South Natick, MA, USA). Mean arterial pressure (MAP) and heart rate (HR) were continuously monitored (BIOPAC System, Santa Barbara, CA, USA).

2.2. Haemorrhagic shock–resuscitation protocols

Haemorrhagic shock was induced by withdrawing blood over 10 min to lower MAP from the physiologic level (approximately 100–120 mmHg) to hypotension (i.e., 40–45 mmHg), as we previously reported.[12] Withdrawn blood was kept in a heparin-rinsed glass syringe. MAP was maintained constantly by further blood withdrawing or re-infusion as needed for 120 min. Then, resuscitation was performed by re-infusing the remaining shed blood supplemented with normal saline (2 times of the maximum blood volume withdrawn). All rats were monitored for another 480 min and then sacrificed by high dose pentobarbital injection.

2.3. Experimental protocols

Fifty adult male Sprague–Dawley rats (200–250 g; BioLASCO Taiwan Co., Ltd., Taipei, Taiwan) were randomly allocated to one of the 5 groups ($n = 10$ in each group): the sham-instrumented (Sham), haemorrhagic shock (HS), HS plus dexmedetomidine (HS-D), HS plus ketamine (HS-K), or HS plus dexmedetomidine–ketamine (HS-D + K) group. All rats were anaesthetized with halothane (0.75%) throughout the experiment. In addition, rats of the HS-D group received a loading dose of dexmedetomidine (1 μg/kg, i.v. infusion over 10 min; Hospira Inc., Lakeforest, IL, USA) followed by dexmedetomidine infusion (0.5 μg/kg/h, i.v.) until the end of experiment. Rats of the HS-K group received ketamine infusion (1 mg/kg/h, i.v.; Pfizer Pharmaceutical Co., Taipei, Taiwan) throughout the experiment. Rats of the HS-D + K group received a loading dose of dexmedetomidine (1 μg/kg, i.v. infusion over 10 min) followed by infusion of dexmedetomidine (0.5 μg/kg/h, i.v.) and ketamine (1 mg/kg/h, i.v.) until the end of experiment.

2.4. Arterial blood gas (ABG), lung tissues collection, and bronchoalveolar lavage (BAL)

At the end of each experiment, arterial blood (0.5 mL) was drawn. ABG levels were measured with an analyzer (Bayer Healthcare LLC, East Walpole, MA, USA). Then, the lungs were inflated and the left main bronchus was tied and left lung from all rats was removed. The upper and lower lobes of left lung were divided and the left lower lobe lung tissues were snap frozen in liquid nitrogen and stored at −80 °C. The right lung tissues from 5 rats of each group were perfused with 4% formaldehyde and then removed. For the other 5 rats of each group, the right lung was lavaged with 6-mL aliquots of sterile saline for 5 times and the BAL fluid (BALF) was collected. Total cell number and protein concentration of the BALF were then determined, according to a previous report.[13]

2.5. Histology, PMN/alveoli ratio, and wet/dry weight ratio

The formaldehyde-perfused right lungs were fixed, sectioned, and stained with haematoxylin and eosin. Histological characteristics, including alveolar wall oedema, haemorrhage, vascular congestion, and polymorphonuclear leukocyte (PMN) infiltration, were evaluated under a light microscope. Then, each characteristic was scored (0: normal to 3: severe) by a pathologist who was blinded to the experiment. Lung injury was categorized according to the sum of the score (0–3: normal to minimal; 4–6: mild; 7–9: moderate; 10–12: severe inflammation), as we previously reported.[14] The PMN/alveoli ratio was used to assess the degree of PMN infiltration.[15] We counted PMNs and alveoli per high-power field (HPF, 400×) in ten randomly selected areas of each sample and the PMN/alveoli ratio was calculated. In addition, freshly harvested left upper lobe lung samples were weighed and then placed in an oven for 24 h at 60 °C and weighed when they were dry to determine the wet/dry weight ratio (i.e., index of lung water), as we previously reported.[14]

2.6. Myeloperoxidase (MPO) activity, NO, PGE2, chemokine, and cytokines

Snap frozen left lower lobe lung samples were homogenized, re-suspended, sonicated, and centrifuged. The supernatant was collected and MPO activity was then measured by H_2O_2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine, according to a previous report.[16] Concentrations of stable NO metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), of the supernatants were measured using a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA). Concentrations of PGE_2, chemokine (e.g., macrophage inflammatory protein-2, MIP-2), and cytokines [including tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-10] of the supernatants were also measured using enzyme-linked immunosorbent assay (ELISA) (ELISA kits for PGE_2, TNF-α, IL-1β, IL-6, and IL-10, Pierce Biotechnology, Inc., Rockfold, IL, USA; MIP-2 ELISA kit; R&D Systems, Inc., Minneapolis, MN, USA). Previous data suggested a correlation between the ratio of pro-inflammatory cytokine IL-6 to anti-inflammatory cytokine IL-10 and the level of inflammation.[17] We thus also calculated the IL-6/IL-10 ratio of each sample.

2.7. Reverse transcription and polymerase chain reaction (RT-PCR)

Production of NO and PGE_2 is regulated by inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively.[18,19] Transcriptional expression of iNOS and COX-2 of the harvested lung