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Esmolol attenuates lung injury and inflammation in severe acute pancreatitis rats

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ABSTRACT

Background: Recent studies suggest that beta-adrenergic blockers attenuate systemic inflammation and improve survival in sepsis. We investigated whether esmolol can reduce lung injury and modulate inflammatory response in a rat model of severe acute pancreatitis (SAP).
Methods: A taurocholate-induced SAP was used, with or without continuously intravenous pumping of esmolol (15 mg/kg/h). Heart rate and arterial pressure were monitored. Nine hrs after esmolol administration, blood was drawn for blood gas analyses and cytokine (interleukin(IL)-6, tumor necrosis factor (TNF)-α) detections, lungs and pancreata were isolated for measurements of myeloperoxidase (MPO) activity and histological damage. In an additional 20 animals, rats were randomized into SAP or SAP + esmolol groups to assess effects of esmolol on survival time.
Results: Treatment with esmolol was associated with improved survival time (11.1 ± 1.6 h vs. 9.2 ± 2.0 h, $p = 0.044$) and less severe disease, as assessed by lung and pancreas histology. Blood gas analyses were ameliorated in esmolol group. Arterial PO_2 increased (109.7 ± 12.4 mmHg vs 93.9 ± 4.1 mmHg, $p = 0.008$) while lactate levels (2.1 ± 0.5 vs 3.1 ± 0.7 mmol/L, $p = 0.001$) decreased in SAP + esmolol group as compared with SAP group. Esmolol treatment also abated the increase in bronchoalveolar lavage fluid protein and proinflammatory cytokines. Furthermore, esmolol reduced SAP-induced plasma amylase activity ($p = 0.02$), blunted the expression of TNF-α ($p = 0.003$) and IL-6 ($p < 0.001$), and decreased pancreas/lung MPO activities.
Conclusions: Continuous infusion of esmolol, a selective beta-1 adrenergic blocker, improves outcome, reduces inflammatory responses and also offers lung and pancreas protective effects in SAP rats. This may offer novel therapeutic strategies in treating patients suffering from SAP.

1. Introduction

SAP is a systemic inflammatory disease with high morbidity [1]. In the early phase of SAP, inflammatory cells such as neutrophils and macrophages are recruited and activated. The activation of inflammatory cells results in a significant elevation of various proinflammatory mediators such as TNF-α, IL-1 and IL-6, which contributes to the development of organ dysfunction [2]. Morbidity of SAP in the early phase strongly relates to occurrence and persistence of organ dysfunction. And acute lung injury is the most common complication of pancreatitis and frequently a factor in early death of SAP patients [3]. Although numerous therapeutic strategies have been designed to reduce inflammation and lung injury in SAP, only few of them have proven clinically useful.

Elevated sympathetic activation occurs during several distinct, pro-inflammatory disease states, such as sepsis and burn injury [4,5]. The adrenergic system is a key modulator of organ function and the immune system [6]. And recent studies showed suppression of sympathetic nerve activity by beta-1 adrenergic blockers might possess a new therapeutic capability in sepsis by attenuating excessive inflammatory responses and organ failures [5,7]. In contrast, administration of the nonselective beta-adrenergic receptor antagonist, propranolol, increased mortality when administered before or within 48 h of septic insult [8]. In conclusion, selective beta-1 receptor blocker may have beneficial effects in sepsis. Since SAP and sepsis share a similar early hyperinflammatory phase, we suppose beta-1 adrenergic blocker might play a protective role during SAP. To test this hypothesis, we treated

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rats with beta-1 adrenergic blocker (esmolol) and evaluated effects of this treatment strategy on survival time, lung injury, severity of pancreatitis and systemic inflammation in a SAP model.

2. Methods

2.1. Animals

Male Sprague-Dawley rats weighing 250–350 g were purchased from Experimental Animal Center of Jinling hospital. Animal experiments were conducted in accordance with the standards for the care and use of animal subjects, as stated in the Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of the Jinling hospital. The rats were deprived of food but allowed access to water ad libitum 12 h before the experiments.

2.2. Anesthetics and surgical procedures

Rats were anesthetized with intraperitoneal injection of urethane (1 g/kg) initially. And SAP was induced by retrograde intraductal injection of sodium taurocholate solution [9]. Briefly, laparotomy was performed through a midline incision, and the duodenal loop was exposed. The biliopancreatic duct was closed with a microaneurysm clip placed at the liver hilum to prevent reflux of the infused taurocholate into the liver. A PE-50 catheter was inserted into the biliopancreatic duct through a small incision in the duodenum. After cannulation, 1 mL/kg 3.5% sodium taurocholate (Sigma, St Louis, Mo, USA) was injected into the biliopancreatic duct under steady manual pressure over a period of 60 s. Five minutes after the injection, the microaneurysm clip was removed and the abdomen was closed in 2 layers. After dissection of femoral vessels, the femoral artery was cannulated for measurements of arterial pressure and heart rate.

2.3. Study protocol 1

20 animals were randomized into two groups: (a): SAP group ($n = 10$): rats underwent retrograde intraductal injection of 3.5% sodium taurocholate. After a 30-min period of stabilization, a catheter connected to a micro-infusion pump was inserted into the femoral vein for fluid replacement (normal saline, 10 mL/kg/hr); (b) SAP + esmolol group ($n = 10$): 30-min after SAP modeling, rats received continuously intravenous administration of esmolol (15 mg dilute in 10 mL normal saline) (Qilu pharmaceuticals company, Jinan, China) at the rate of 15 mg/kg/hr via the femoral vein. The chosen dose of esmolol was based on preliminary data demonstrating an approximately 20% decrease in heart rate when administered intravenously to healthy rats under anesthesia. Simultaneously, the survival time (hours) was monitored from the induction of SAP model to the death of the animal.

2.4. Study protocol 2

Rats were randomly assigned to one of the three groups: (d): SAP group ($n = 10$), the same as group a; (e) SAP + esmolol group ($n = 10$), the same as group b; (f) control group ($n = 10$): a laparotomy without insertion of PE-50 catheter into a biliopancreatic duct was performed in control rats.

9 h after normal saline or esmolol injection, arterial blood was drawn from the cannulated femoral artery for blood gas analyses and inflammatory cytokine measurements. Simultaneously, bronchoalveolar lavage fluid of the left lung was sampled for measuring the protein concentration by commercially available BCA protein assay kit (Thermo, IL, USA) and TNF-α/IL-6 concentrations by ELISA method (R&D Systems Inc, Minneapolis, Minn, USA). After that, the right lung was taken out for wet-to-dry weight ratio and MPO measurements and scoring severity of lung damage. In the end, the pancreas was also resected for histological evaluation.

2.5. Wet-to-dry weight ratio

The right lungs were removed and weighed to measure wet lung weight. And then the lungs were dried in an oven (60 °C) for 3 days to obtain dry lung weight. Pulmonary wet-to-dry weight ratios were calculated as follows:

Wet-to-dry weight ratios = (Wet lung weight − Dry lung weight)/Dry lung weight.

2.6. Blood analysis

Plasma levels of IL-6, and TNF-α were determined using commercial enzyme-linked immunosorbent assay kits (R&D Systems Inc, Minneapolis, Minn, USA). Plasma amylase, lipase, blood urea nitrogen, creatinine and cystatin C levels were measured according to standard methods.

2.7. MPO assay

MPO activity, a marker of neutrophil and macrophage parenchymal infiltration, was measured as described previously [10]. Lung/pancreas tissues were homogenized in 5-mM potassium phosphate buffer (pH = 6). Homogenates were centrifuged (30,000 rpm, 30 min at 4 °C); pellets were resuspended in extraction buffer (5 mm potassium phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide) and subjected to 3 cycles of freezing and thawing. The supernatants generated (13,000 rpm, 15 min at 4 °C) were assayed for MPO activity using kinetic readings for 3 min. At last, absorbance was measured at 460 nm and sample protein concentrations were determined (BCA method) with the results presented as MPO units per gram of protein.

2.8. Morphological examination and grading

Lungs and pancreata were collected and fixed in 10% formaldehyde solution, then embedded in paraffin for hematoxylin and eosin staining. The specimens were examined under light microscopy by a pathologist blinded to group assignment. The lung injury and pancreas injury were scored according to the criteria described previously [11,12].

2.9. Statistical analysis

Data are expressed as mean ± SE. The Kaplan–Meier analysis and log-rank test were used for the survival data. The remainder of the data were analyzed with ANOVA followed by LSD test if equal variances assumptions were satisfied and Dunnett's T3 if not. The significance level was accepted at $P < 0.05$.

3. Results

3.1. Effects of esmolol administration on hemodynamic parameters and survival

The mean survival time in SAP + esmolol group was 11.1 ± 1.6 h, which was longer as compared with that in SAP group (9.2 ± 2.0 h, $p = 0.044$) (Fig. 1). SAP modeling resulted in great increases of heart rate and decreases of mean arterial pressure through the whole process. Esmolol treatment decreased heart rate at 3 h, 6 h and 9 h time points and had no effect in mean arterial pressure (see Fig. 2).

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