



## Protective effects of edaravone combined puerarin on inhalation lung injury induced by black gunpowder smog



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### ARTICLE INFO

#### Article history:

Received 9 September 2014

Received in revised form 13 February 2015

Accepted 23 February 2015

Available online 6 March 2015

#### Keywords:

Inhalation lung injury

Edaravone

Puerarin

Black gunpowder smog

### ABSTRACT

**Objective:** The present study aimed to investigate the combined effects of puerarin with edaravone on inhalation lung injury induced by black gunpowder smog.

**Materials and methods:** Male Wistar rats were divided into five groups (control group, edaravone group, puerarin group, edaravone combined with puerarin group and inhalation group). The severity of pulmonary injuries was evaluated after inducing acute lung injury. Arterial blood gas, inflammatory cytokines, biochemical, parameters, cell counting, W/D weight ratio and histopathology were analyzed. Results in lung tissues, either edaravone or puerarin treatment alone showed significant protective effects against neutrophil infiltration and tissue injury, as demonstrated by myeloperoxidase activity and histopathological analysis (all  $p < 0.05$ ). In addition, combined treatment with both edaravone and puerarin demonstrated additive protective effects on smog-induced lung injury, compared with single treatment.

**Conclusions:** Combination of edaravone and puerarin shows promise as a new treatment option for acute lung injury/acute respiratory distress syndrome patients.

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### 1. Introduction

Acute respiratory failure, following smoke inhalation injury, can contribute to significant complications in the presence of severe burn injury [1,2]. In military actions and training, different kinds of smog are used to shield the soldiers or imitate battlefield environments [3]. Lung injury is frequently a component of the polytrauma sustained by military personnel surviving blast on the battlefield [4]. In burn citizens or soldier with inhalation injury, the acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are serious problems, and the most prevalent cause of death [5,6]. ARDS is characterized by a non-cardiogenic pulmonary edema and hypoxia induced pulmonary vasoconstriction. Considering the variable causes of ARDS and the generally and subsequently developing complications of intensive care therapy, a combined interdisciplinary approach today presents a corner stone for successful management of this complex clinical problem. Improvement in local treatment has caused a significant reduction in morbidity and mortality due to infections and local complications. However, only very few data of multi-therapeutical approaches for the first line treatment of ARDS exist [7].

Puerarin (8-beta-D-glucopyranosyl-7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) [8] is extracted from the herbal medicine Radix Puerariae. Previous studies revealed that puerarin might possess many physiological and pharmacological activities and has long been used for cardiovascular diseases, such as coronary artery disease, myocardial damage, and heart failure [9–11]. Puerarin also shows anti-oxidative [12] and anti-allergic activities [13].

As a potent free radical scavenger, edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) has been used clinically in patients with acute ischemic stroke, and improves functional outcomes [14,15]. It was previously demonstrated that edaravone successfully ameliorates lung injury in various animal models of ALI [16–18]. However, it remains to be determined whether Puerarin improves the therapeutic efficacy of edaravone through inhibition of oxidative stress. Thus, the present study aimed to compare the effects of individual versus combined treatment of Puerarin and edaravone on blackpowder smog-induced lung injury in rats.

### 2. Materials and methods

#### 2.1. Chemicals and drugs

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) was produced and supplied by Nanjing Xiansheng Dongyuan Pharmaceutical Co., Ltd. China. Puerarin was produced and supplied by Chengdu Tiantaishan

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Pharmaceutical Co., Ltd. The other chemicals and reagents used were of analytical grade.

## 2.2. Animals

40 male Wistar rats (SPF grade, 4 weeks old) were supplied by the laboratory animal center of the PLA General Hospital, Beijing, China. Filtered tap water and diet were available ad libitum. Rats were housed in pathogen-free cages kept at the temperature of 20–25 °C and 50–70% relative humidity. This study was conducted in accordance with the internationally accepted principles for laboratory animal use and care according to the US guidelines (NIH Publication no. 85-23, revised in 1985), and the study was approved by the Ethics Committee of the Medical College of the People's Liberation Army (PLA).

## 2.3. Induction of acute lung injury and drug administration

The rats were randomly divided into five groups: control group (C group); edaravone group (E group); puerarin group (P group); inhalation group (I group); and edaravone combined with puerarin group (L group), with eight rats in each group. The rat model of inhalation lung injury was reproduced by a self-made smoke generator [19] in L, I, E and P groups.

- (1) The normal group rats were sensitized intraperitoneally (I.P.) with normal saline (NS, NaCl, 0.9%) for seven consecutive days.
- (2) The inhalation group rats were treated with inhalation lung injury followed by daily I.P. NS for seven consecutive days.
- (3) The puerarin group rats were treated with inhalation lung injury followed by daily I.P. puerarin (100 mg/kg) for seven consecutive days.
- (4) The edaravone group rats were treated with inhalation lung injury followed by daily I.P. edaravone (9 mg/kg) for every three days.
- (5) The edaravone combined with puerarin group rats were treated with inhalation lung injury followed by daily I.P. edaravone (9 mg/kg) for every three days and puerarin (100 mg/kg) for seven consecutive days

All rats were sacrificed on day seven.

## 2.4. Surgical procedures

On day 7 after inhalation lung injury, rats were anesthetized by intraperitoneal pentobarbital (50 mg/kg). Then the animals were dissected. And the animals were euthanized by aortic exsanguination. The artery blood was collected in tubes for tests and 0.3 ml of heparinized arterial blood was collected for blood gas analysis using the Roche cobas b blood gas analyzer. After euthanization and opening of chest cavity, rat lungs were thoroughly examined for the morphological lesions. Then the superior lobe of the left lung was kept in 10% neutral formaldehyde for histology. Sections (4 μm thickness) were cut and stained with hematoxylin and eosin (H&E). Tissue lesions and inflammatory cell infiltration were examined using microscope.

## 2.5. Bronchoalveolar lavage fluid (BALF) and cell counting

The right lung, which was isolated and tied off with a micro clamp at the right bronchus, slow intra-tracheal injection of PBS. This procedure was repeated 3 times, with total volume of 2.5 ml. The BALF samples were centrifuged at 1000 g for 10 min at 4 °C to pellet the cells. The cell pellets were resuspended in PBS for total cell counts. We prepared Cytospin for differential cell counts by staining using the Wright-Giemsa staining method.

## 2.6. IL-6 and TNF-α measurements

IL-6 and TNF-α in the BALF and serum were measured by commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's protocol (R&D system, USA). Protein concentration in the BALF, an indicator of vascular permeability, was measured with the bicinchoninic acid method. Other parts of lung tissues were removed and then stored at –80 °C until use.

## 2.7. W/D weight ratio

The left lung lower lobe was weighed immediately after collection and placed into a 55 °C oven to dry for 48 h. The dried tissue was also weighed to determine the W/D weight ratio. The W/D weight ratio was calculated to assess pulmonary vascular permeability.

## 2.8. Measurement of MDA and MPO in lung tissue

The inferior lobe of the left lung was taken for MPO and MDA analyses. MOP and MDA were determined according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering institute, China)

## 2.9. Statistical analysis

All data were expressed as mean ± standard error mean (SEM). All analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). The statistical significance of differences was assessed by one-way ANOVA,  $p < 0.05$  was considered to be significantly different.

## 3. Results

### 3.1. Arterial blood gas analysis

Fig. 1 shows the mean arterial oxygen pressure (PaO<sub>2</sub>). It was found that PaO<sub>2</sub> was 95.11 ± 5.03 mm Hg in the control group. In the inhalation group, the PaO<sub>2</sub> of rats rapidly dropped to 64.6 ± 6.58. E group and P group induced increased PaO<sub>2</sub> and decreased PCO<sub>2</sub> in arterial blood of rats compared with the inhalation group, respectively. The levels of PaO<sub>2</sub> in arterial blood of the L group are higher than the corresponding values in the E group, but they were not significantly different ( $p > 0.05$ ).

### 3.2. Lung W/D ratio and protein concentrations in BALF

The lung W/D ratio (Fig. 2A) and total protein concentrations in the BALF (Fig. 2B), both of which were a measurement of pulmonary vascular permeability, were all markedly increased after smog stimulation. The present study results showed that smog inhalation significantly inhibited the increase of lung W/D ratio and total protein concentrations in BALF.

### 3.3. Lung inflammatory cell accumulation in BALF

As illustrated in Fig. 3, rats exposed to smog showed an increase in the number of total cells, neutrophils (data not shown) and the percentage of neutrophils as compared to the control group ( $p < 0.05$ ). Meanwhile, Edaravone and/or Puerarin treatment led to a significant lowering of the number of total cells, neutrophils (data not shown) and the percentage of neutrophils as compared to the control group ( $p < 0.05$ ). Although the number of macrophages and lymphocytes in I groups (date not shown) significantly increased compared to the control group, the percentage of macrophages and lymphocytes in I groups decreased ( $p < 0.05$ ).

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