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Original Article

Expression of IL-6 and TNF-α in serum after treated with methylprednisolone combined with azithromycin in children with bronchopneumonia

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Abstract: Objective: To observe the changes of the levels of IL-6 and TNF-α in serum of young bacterial bronchopneumonia model mice after treated with methylprednisolone combined with azithromycin. Methods: 36 BALB/c established bronchopneumonia model mice were divided into model group, blank control group and treatment group. Mice in the model group were not treated, the blank control group was given normal saline, and the treatment group was administrated with methylprednisolone combined with azithromycin. 12 mice with the same age (male and female) were selected as normal group. The pathological morphology of lung was observed by HE staining, and the levels of IL-6 and TNF-α in serum were detected by ELISA on the 1st day and the 7th day. Results: The inflammatory response and secretion of the respiratory epithelium in the left main bronchus of the model mice were increased with the swelling of the ciliated epithelial cells of the airway surface. The 7th day's observation after treatment showed that the inflammatory response of the left main bronchus was more obvious in model group, and the epithelial cells were disarranged with the cilia and cell necrosis. The levels of IL-6 and TNF-α in serum of the treated group mice were significantly higher than those of normal mice (P=0.000). On the 7th day after treatment, the levels of serum IL-6 and TNF-α in the model group were decreased. Conclusion: Methylprednisolone with azithromycin can significantly improve the inflammatory response and can effectively reduce lung injury in pneumonia mice.

Keywords: Methylprednisolone, azithromycin, bronchial pneumonia in children, interleukin-6, tumor necrosis factor-alpha

Introduction

Staphylococcus, pneumococcus, Streptococcus, copper green Pseudomonas, Klebsiellapneumoniae, Haemophilusinfluenzae and so on are common pathogenic bacteria that lead to the occurrence of bronchopneumonia in children [1, 2]. Antibiotics are often used as a routine treatment for children with bronchial pneumonia, but resistant strains are increasing and accelerating due to the irregular use of antibiotics [3, 4]. Therefore, to a certain extent, the treatment course is prolonged and the disease is easy to repeat [5, 6]. At the same time, the poor treatment may lead to the development of the disease into the bronchusasthma and infection that can even cause death of children [7-9]. In this study, Klebsiella pneumoniae was used to establish a model of bacterial bronchopneumonia in mice with immunosuppression [10]. The changes of lung tissue and the serum inflammatory factors were observed after methylprednisolone combined with azithromycin treatment.

Materials and methods

Animals

48 BALB/c mice at 2 week-age from SPF were bought from the animal experiment center of Huazhong University of Science and Technology, with female and male by half, the weight of 21-25 g, and the average weight of 23.1+1.9 g. Then the mice were divided into normal group, model group, blank control group and treated group, with 12 rats in each group, half male and half female.

Reagents and instruments

Hydrocortisone injection was purchased from Tianjin Jin Yao Pharmaceutical Co., Ltd (Approval Number: H12020885. Specifications: 2 mL: 10 mg × 10).

Cyclophosphamide for injection was purchased from Jiangsu HengRui pharmaceutical Limited by Share Ltd (Approval Number: Chinese medicine quasi character H32020856. Specification: 0.1 g).

Bacterial fluid: Inoculating Klebsiellapneumoniae on the blood plate at 37°C overnight. Then 4-5 typical colonies were selected to be incubated in meat soup. After 6 hours, the turbidity was adjusted to the same as that of the 0.5 McFarland.

Methylprednisolone tablets were purchased from Pfizer Italia S.r.l., Italy (Approval Number: H20150245. Specification: 4 mg × 30 s).

Azithromycin dispersible tablets were purchased from Zhuhai homology Pharmaceutical Co., Ltd (Approval Number: H20067564. Specifications: 0.25 g × 12 tablets/boxes).

Establishment of bacterial bronchopneumonia mouse model

Cyclophosphamide for injection was dissolved in saline and diluted to 2.5 g/L. 36 mice were intraperitoneally injected with hydrocortisone 0.1 mL every morning and with 0.2 mL 2.5 g/L cyclophosphamide every afternoon. Therefore, the mice were repeatedly administered for 3 days in an immunosuppressed state. Immunosuppressed mice were given cyclophosphamide. After 1-2 hours, the upper right part of the right upper back was sterilized 1 cm from the root of the right ear, and 0.1 mL of bacterial solution was injected with the baby's scalp needle. Survival at 5 hours post injection was considered a successful model.

Dosage regimen: (1) The normal group (12 rats) was given no treatment at all. (2) The model group (12 rats) did not undergo any treatment after the models were successfully established. (3) The blank control group (12 rats) was treated with saline after the models were successfully established. (4) The treated group was gavaged with the Methylprednisolone tablets (40 mg/kg/d) and Azithromycin dispersible tablets (10 mg/kg/d) dissolved in the saline.

Content and method of observation

Half number of the mice was killed on the 1st day and 7th day after models were successfully established. HE staining was used to observe the pathological changes of lung tissue and the levels of serum IL-6 and TNF-α.

HE staining

The mice were killed and the lungs were removed from the chest. 5-7 mL 10% formaldehyde phosphate buffer was injected into the right lung and fixed in 10% formaldehyde solution. One of the largest circumferential diameter of the right sagittal plane or the largest circumferential diameter of the right middle lobe was selected under the conditions of conventional dehydration, paraffin embedding, sectioning and HE staining. Then the pathological changes of the lung tissue were observed.

The detection of IL-6 and TNF-alpha in serum

Mice were killed after anesthesia, then 0.8 mL the serum was added to the test tube with heparin, after 30 min at room temperature, centrifuged by 1.174 g 5 min, and the supernatants were tested. The level of IL-6 and TNF-α were tested with the enzyme linked immunosorbent assay. The operation procedure was carried out in strict accordance with the instructions, and the kit was bought from Wuhan Doctorate Biotechnology Co., Ltd.

Statistical analysis

The data were analyzed with SPSS20.0, and the measurement data were compared with t test, and the counting data were compared with the chi square test. $P<0.05$ was considered to be statistically significant.

Results

Modeling result

36 mice models were successfully constructed and applied to the study.

HE staining results

Bronchial HE staining pathological results: As is shown in **Figure 1**, on the 1st day after models were successfully established, the left main bronchus of the model mice was exudative and edematous, with increased secretion and de-

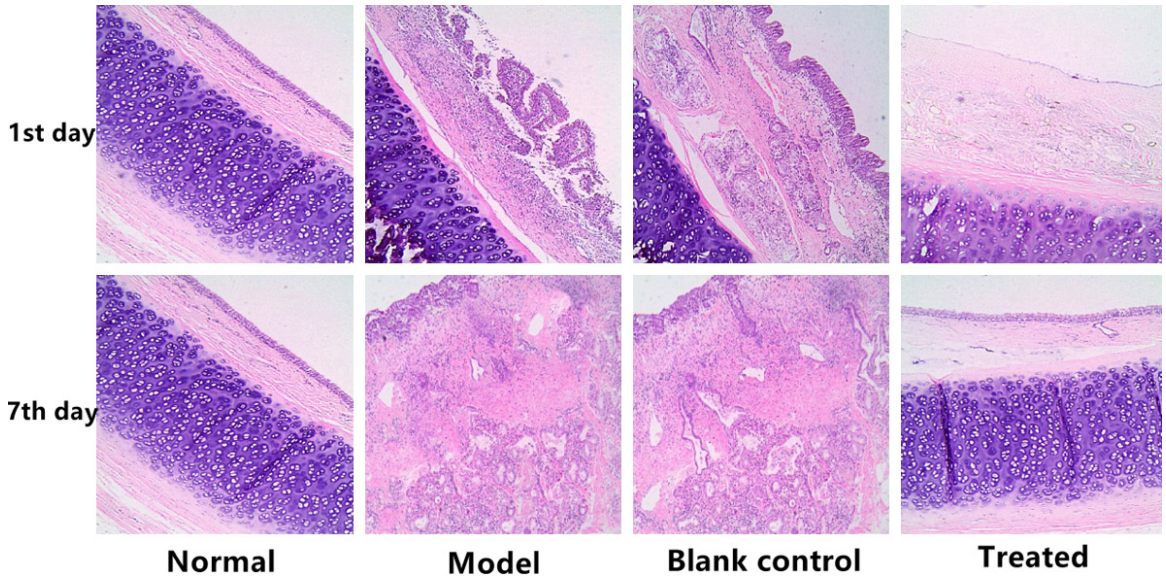

Figure 1. HE staining of the epithelium of bronchial airway in each group on the 1st day and the 7th day (400×).

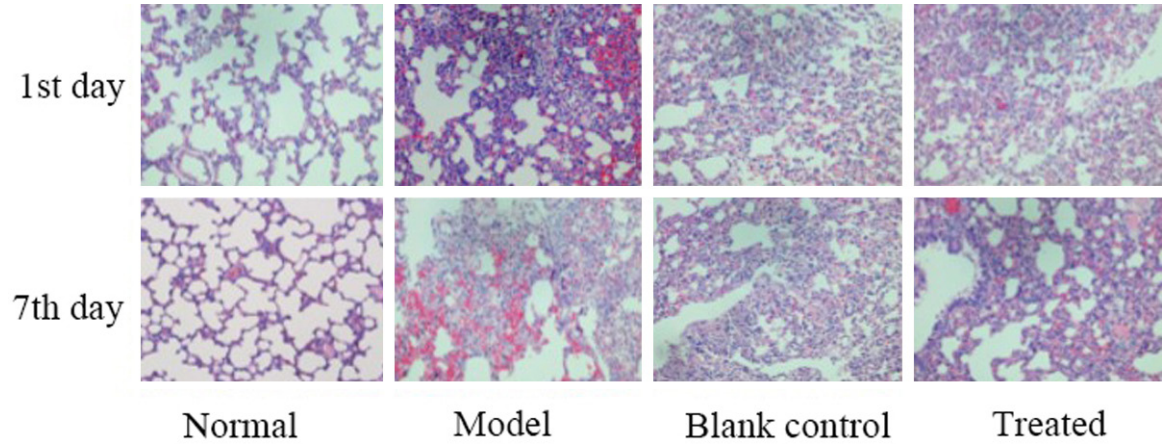

Figure 2. The pulmonary alveolar structure changes with HE staining in each group on the 1st day and the 7th day (100×).

ranged mucociliary epithelial cells on the surface of the airway, while the left main bronchus of the normal mice were smooth, without exudation, edema, increased secretion and deranged mucociliary epithelial cells on the surface of the airway secretion. On the 7th day after treatment, the inflammatory reaction in the left main bronchus of the model group was more obvious, the epithelial cells were in disorder with the necrosis and shedding of cilium and cells, and exudation in the tracheal tissue and interstitial was obvious. In the blank control group, the left main bronchial epithelial tissue exudation and edema were alleviated, and there was no significant difference compared with the model group. However, in the treatment group, the left bronchial airway tissue exudation and edema were significantly reduced, and the surface secretion was significantly reduced. The airway was significantly reduced.

Pathological results of HE staining of alveolar structure

As is shown in **Figure 2**, on the 1st day and the 7th day, the alveolar tissue in normal group mice maintained well the structural integrity, and no inflammatory factors were released. The alveolar structure in the model group was severely

Table 1. Serum IL-6 and TNF-α content at different time points ($\bar{x}$ ± sd)

Group	IL-6 (pg/mL)		TNF-α (nmol/L)	
	The 1st day	The 7th day	The 1st day	The 7th day
The normal group	3507±208	3489±199	4.1±0.2	4.9±0.3
The model group	11533±397*	9915±261	14.7±1.5*	11.8±0.6
The blank control group	11839±457	9728±219	14.3±1.1	12.3±1.1
The treated group	11677±319	6539±203#	14.6±0.9	6.0±0.6#

Compared with normal group on the 1st day, *P=0.000; Compared with respective treated group on the 1st day, #P=0.000.

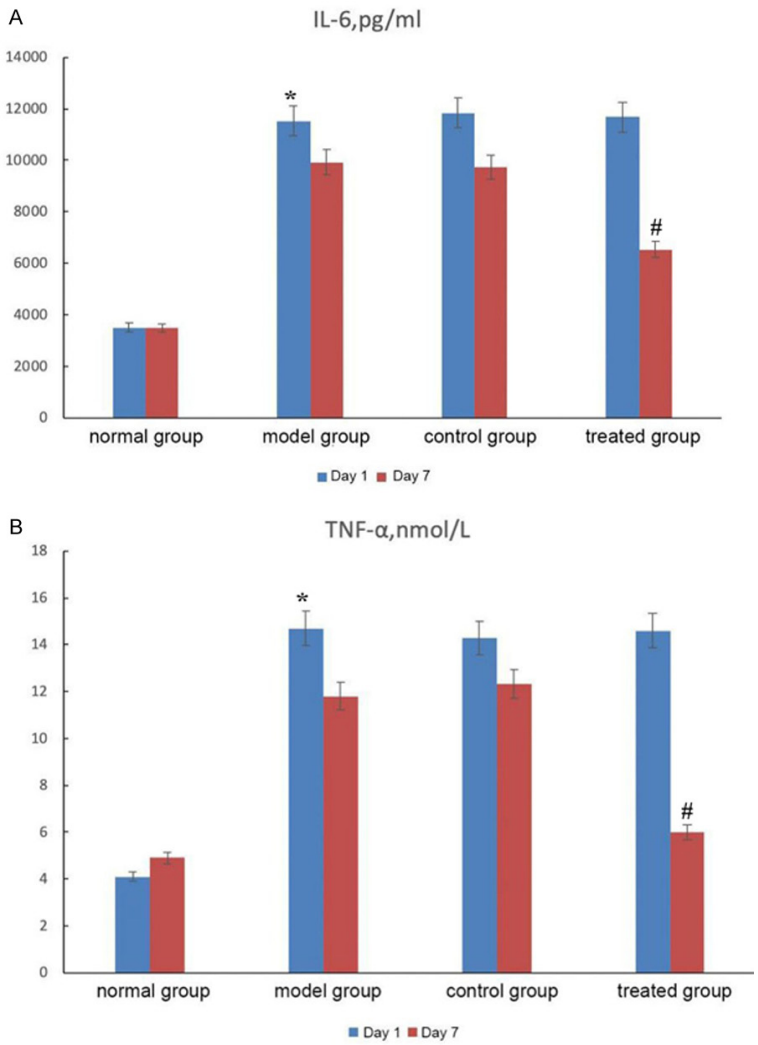

Figure 3. Serum IL-6 (A) and TNF-α (B) in each group. Compared with normal group on the 1st day, *P=0.000; Compared with respective treated group on the 1st day, #P=0.000.

damaged, no intact alveoli existed, and a large number of inflammatory factors were released. The alveolar structure of the blank control group was severely damaged, releasing a large number of inflammatory factors, but there was no significant difference from the model group. There was no significant change in the alveolar tissue of the model group on the first day of the treatment group, but after 7 days of treatment, the alveolar structure damage was significantly improved and the inflammatory response was reduced.

The detection of IL-6 and TNF-alpha

On the 1st day after model was successfully established, the levels of IL-6 and TNF-α in serum of mice with bronchopneumonia were significantly higher than those of normal mice (t=13.902, P=0.000). On the 7th day after treatment, the level of serum IL-6 and TNF-α in the model group and the blank control group decreased, but there was no significant difference compared with that on the 1st day (P>0.05). The levels of serum IL-6 (6539±203 vs 11677±319) and TNF-α (6±0.6 vs 14.6±0.9) in the treated group on the 7th day were significantly decreased (t= 13.895, P=0.000; t=14.083, P=0.000) compared with those on the 1st day, and were significantly lower than those in the model group and the blank control group. However, compared with the normal group, serum serum IL-6 and TNF-α levels in the treated group were still significantly higher (t=9.275, P=0.001; t=8.913, P=0.001). The results were as shown in **Table 1**; **Figure 3A** and **3B**.

Discussion

Bacterial bronchopneumonia in children is mainly caused by bacterial infection such as Streptococcus pneumoniae and Klebsiellapneumoniae and is commonly treated with antibiotics [11]. However, there is no further clinical study. Animal experiments are an important means to study the effect of drug treatment and pharmacodynamics. However, due to the lack of an ideal model of early lung infection pathogen invasion, animal studies of bacterial bronchial pneumonia are rare.

The study suggests that an ideal mouse model of bacterial pneumonia can be obtained by using Klebsiella pneumoniae to inhibit the immune system of mice [10]. White blood cell infiltration and inflammatory reaction in the bronchi and alveoli are mainly pathological changes in this model [12, 13]. However, the performance of pulmonary edema is not obvious.

Clinically, methylprednisolone and azithromycin are commonly used in the treatment of children with bronchopneumonia [14, 15]. The clinical efficacy has been approved by doctors, but there are few reports on their application against bacterial bronchopneumonia. In this study, Klebsiella pneumonia was used to establish a model of bacterial bronchopneumonia in mice under immunosuppressive condition. After treatment with methylprednisolone combined with azithromycin, pathological changes and serum inflammation of lung tissue were detected in mice.

In this study, a successful model of bacterial bronchopneumonia was established in mice with Klebsiella attacking the mice in the immunosuppressive state. All 36 mice were successfully modeled which indicated the feasibility and practicability of our method for modeling, and suggested a new choice for studying disease and drug research in clinic.

In this study, the pathological changes in the lungs of all the mice were observed by HE staining. The results showed that the left main bronchus of the model mice was exudative and edematous, with increased secretion and deranged mucociliary epithelial cells on the surface of the airway, which was in agreement with the main pathological changes of lung in bacterial pneumonia [16]. On the 7th day, the pathological changes in the model group and the blank control group were not improved. The left bronchial airway tissue exudation edema in the treatment group was significantly reduced, and the airway secretion was significantly reduced. The alveolar structure of the model group was severely damaged, and a large number of inflammatory factors were released, while the alveolar tissue in the treated group had no significant difference from the model group. There was no significant difference between the alveolar tissue and the model group on the first day of the treatment group. However, after 7 days of treatment, the alveolar structure damage was significantly improved and the alveolar structure damage was significantly reduced. The inflammatory response is reduced, which is closely related to the anti-inflammatory effects of methylprednisolone as demonstrated by several studies [17, 18].

Methylprednisolone is a glucocorticoid with significant anti-inflammatory effects, and is commonly used in the treatment of autoimmune diseases and inflammatory diseases [17, 18]. Azithromycin is commonly used as an anti-infection drug. It has broad spectrum antibacterial activity and is widely used in respiratory infections [19, 20]. In this study, the bacteriostasis of azithromycin was used to block the invasion of the lung infection. At the same time, methylprednisolone has an anti-inflammatory effect and can significantly reduce the bronchitis response and alveolar structure damage in mice. However, the complete recovery of lung lesions in mice was not observed in this study, which may be related to insufficient dosage of drug or unchangeable pathological changes caused by bacterial bronchopneumonia, which needs to be further studied.

In this study, it was also found that the levels of IL-6 and TNF-α in serum of mice with bronchopneumonia were significantly higher than those of normal mice (t=13.902, P=0.000), consistent with previous studies showing that TNF-α and IL-6 act as general markers of inflammation in pneumonia and regulate the expression of acute-phase proteins, thus integrating systemic response and local injury [21-23]. In the treated group, the levels of serum IL-6 and TNF-α on the 7th day were significantly lower than those on the 1st day, and significantly

lower than those in the model group and the blank control group. However, they were still significantly higher than those in the normal group. It was confirmed that methylprednisolone combined with azithromycin can effectively improve the inflammatory response of bacterial bronchopneumonia to achieve obvious therapeutic effect. However, our study has some limitations: (1) The immune function of mice after immunosuppression was not detected. Therefore, it is necessary to confirm whether the immune function of mice was suppressed after immunosuppression. (2) We did not address whether there would be a difference if the drugs were used alone or in combination.

In conclusion, challenge with Klebsiellato pneumoniae in an immunosuppressed state is an effective method for establishing a model of bacterial bronchopneumonia. Methylprednisolone combined with azithromycin can significantly improve the inflammatory response of pneumonia mice and can effectively reduce the lung injury.

Disclosure of conflict of interest

None.

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