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Original Article

Associations between TLRs 2 and 4, and β-lactam antibiotics in COPD patients complicated with pulmonary infections

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the world. We aimed to investigate the associations between toll-like receptors 2 and 4 (TLR-2 and TLR-4) and β-lactam antibiotics in COPD patients complicated with pulmonary infections.

Methodology: A total of 156 COPD patients complicated with pulmonary infections were included. Their blood gas, airway resistance, health status, expression levels of TLR-2 and TLR-4, and pulmonary function were analyzed after treatment with β-lactam antibiotics.

Results: Blood gas indices oxygen saturation, partial pressure of oxygen, and partial pressure of carbon dioxide at one day before treatment, on the fifteenth day of treatment, and on the first day after the end of treatment showed significant differences ($p < 0.01$). Significant differences were also detected in airway resistance indices ($p < 0.01$). The differences in the mRNA expression levels of TLR-2 and TLR-4 were significant ($p < 0.05$). Downward trends were observed in the clinical pulmonary infection score and acute physiology and chronic health evaluation II score, which indicated alleviation of the disease. Pulmonary function indices recorded vital capacity (VC)/predicted VC (%), recorded forced vital capacity at 1 s (FEV1)/predicted FEV1 (%), and residual volume/total lung capacity were significantly different ($p < 0.05$).

Conclusions: β-Lactam antibiotics had obvious therapeutic effects on COPD patients complicated with pulmonary infections, probably by suppressing or attenuating TLR-2- and TLR-4-mediated inflammatory responses. It is necessary to comprehensively evaluate and choose appropriate antibiotics, aiming for maximum relief of the pain to help patients recover quickly.

Key words: antibiotics; association; infection; β-lactam; pulmonary disease; receptor.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a general term for chronic obstructive bronchitis and emphysema, typified by persistent small airway inflammation and progressive irreversible airflow restriction [1]. COPD triggers airway obstruction, poor airflow, and irreversible loss of pulmonary function [2]. COPD can trigger acute lung deterioration, speed up pulmonary function decline, and increase the risk of mortality. Besides, 10% of the global population suffer from this common global epidemic, and it is the third leading cause of death worldwide [3]. Pulmonary infections caused by viruses and bacteria are vital factors for an acute exacerbation of COPD and *Haemophilus influenzae*, Gram-negative *Moraxella* spp., *Streptococcus pneumoniae*, and *Pseudomonas* are the most common bacteria inducing infections in COPD patients [4]. Currently, appropriate application of antibiotics is the most common therapy for COPD complicated with pulmonary infections [5].

Toll-like receptors (TLRs) in mammals are a type of germline-encoded transmembrane receptors that can recognize conservative microbial structures, namely, the so-called pathogen-related molecular patterns [6]. TLR-2 and TLR-4 are related to COPD, asthma, pulmonary fibrosis and other chronic respiratory diseases [7]. Besides, the activation of TLRs induces inflammatory responses and contributes to the development of antigen-specific acquired immunity [8]. The TLR family is dominated by TLR-2 and TLR-4. TLR-4 is indispensable for the recognition of lipopolysaccharide (LPS), the dominant component of Gram-negative bacteria; whereas TLR-2 recognizes numerous ligands, including bacterial lipoids and lipoproteins [9]. Previous studies have shown that activating TLR-2 or suppressing TLR-4 are novel approaches for the treatment of COPD with pulmonary infections. At present, β-lactam antibiotics are the most widely used antibacterial agents in hospitals. They are included in 65% of all prescriptions for injectable

antibiotics in the United States, and are dominated by cephalosporins (nearly half of the prescriptions). β-Lactam antibiotics have good tolerance, good curative effects and wide applicability [10].

In this study, the expression levels of serum TLR-2 and TLR-4 before and after the application of β-lactam antibiotics were analyzed, with the aim to investigate the influences of β-lactam antibiotics on the expression levels of TLR-2 and TLR-4 in COPD patients with pulmonary infections.

Methodology

General data

A total of 156 COPD patients complicated with pulmonary infections (without other pulmonary diseases) who were treated in our hospital from August 2019 to August 2021 were enrolled. They were 47–75 years old, with an average age of 56.29 ± 3.45 years. After admission, the patients were informed of the treatment regimens and the drugs applied in detail, and they completed all examinations. All participants signed an informed consent and an observation consent, and the study was approved by the Ethics Committee of the hospital. The inclusion criteria were: 1) patients aged ≥ 47 years; 2) with no other lung diseases confirmed by preoperative examination; 3) patients whose indices examined and clinical manifestations met the diagnostic criteria of COPD stipulated in the COPD Foundation Guide to COPD Diagnosis and Treatment [11]; and 4) those whose diagnosis was supported by imaging, blood examinations, and sputum bacteria culture results, which confirmed the pulmonary infections. Exclusion criteria were: 1) patients with other lung diseases; 2) patients who had lung surgery; 3) patients with poor compliance and mental disorders; 4) those with severe heart, liver and kidney dysfunction; and 5) those who had undergone long-term treatment with antibiotics in the previous month.

Treatment methods

After admission, the patients underwent all necessary examinations for comprehensive evaluation. Then they received routine continuous inhalation of oxygen at a low flow, and were administered drugs for relieving cough and asthma, and routine bronchodilators (salbutamol + ipratropium bromide). The water-electrolyte balance of patients was also maintained. The patients were administered penicillin G sodium (North China Pharmaceutical Group Corp., Shijiazhuang, China, NMPH: H13020655), a type of β-lactam antibiotic, as an injection at a dose of 8 million units/day, 2-4 times. The injection rate was less than 500,000 units/min, to avoid toxic reaction of the central nervous system. All patients received continuous treatment for one month.

Blood gas analysis

After admission, 2 mL of radial artery blood was collected in the morning one day before treatment, on the 15th day of treatment, and on the first day after the end of treatment, respectively. Then, RAPIDLab 348EX blood gas analyzer (Siemens, Munich, Germany) was employed to detect indices such as oxygen saturation (SaO_2), partial pressure of oxygen (PaO_2), and partial pressure of carbon dioxide ($PaCO_2$).

Detection of pulmonary function and airway resistance indices

Routine ventilation function and impulse oscillometry (IOS) pulmonary function were measured by the Mastscreen IOS analyzer (Jaeger, Wuppertal, Germany). The IOS indices included total respiratory impedance at 5 Hz (Zrs5), respiratory resistance at 5 Hz (Rrs5), and respiratory resistance at 20 Hz (Rrs20). The routine ventilation function indices included vital capacity (VC), predicted VC, forced expiratory volume in 1 second (FEV1), predicted FEV1, residual volume (RV), and total lung capacity (TLC). The above-mentioned indices were measured one day before treatment, on the 15th day of treatment, and on the first day after the end of treatment.

Detection of TLR-2 and TLR-4

Fasting venous blood was collected from patients in the morning one day before treatment, on the 15th day of treatment, and on the first day after the end of treatment. Gene expressions were detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The detailed steps included blood collection (3 mL of blood), ribonucleic acid (RNA) extraction, reverse transcription of complementary DNAs (cDNAs), qRT-PCR and relative expression measurement. The patients' blood was directly stored in Tempus Blood RNA Tubes containing stabilizing reagents (Thermo Fisher Scientific, Waltham, USA) that can ensure the stable expression of RNAs for 4 to 5 days at room temperature or at least 7 days at 4 °C. RNAs were isolated within 3 days after collection using Tempus Spin RNA Isolation Kit (Thermo Fisher Scientific, Waltham, USA) as per the manufacturer's instructions. Afterwards, NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) was utilized to determine the content and quality of RNAs. In subsequent experiments, RNA

samples could be applied only after they were placed in aliquots on the denatured agarose gel stained by ethidium bromide for integrity evaluation. Only the samples displaying clear 28S and 18S ribosomal RNA (rRNA) bands were used for cDNA reaction. The concentration of the DNA template was 25 $ng \cdot \mu L^{-1}$. qRT-PCR was carried out using LightCycler® 480 SYBR Green I Master kit (Roche Diagnostics, Mannheim, Germany) and a Thermocycler (Bio-Rad, Hercules, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene. The primers used in this study included TLR-2-F: 5'-GGCATGTGCTGTCTGTT-3' and TLR-2-R: 5'-GCTTT CCGGCTTCCTTTT-3', TLR-4-F: 5'-GCTTTCCTGGCTTCCTTTT-3' and TLR-4-R: 5'-ATGCGGACACACACACACTTTCAAATA-3', and GAPDH-F: 5'-GCACCGTCAAGGCTGAAC-3' and GAPDH-R: 5'-TGGTGAAGACGCCAGTGGA-3'.

Scoring of disease

The Clinical Pulmonary Infection Score (CPIS) and Acute Physiology and Chronic Health Evaluation II (APACHE II) were employed to evaluate the degree and status of pulmonary infections in patients at one day before treatment, on the 15th day of treatment, and on the first day after the end of treatment. CPIS is mainly used to evaluate the white blood cell (WBC) count, SaO_2, tracheal secretion, chest X-ray film, pulmonary inflammatory infiltration, etc.; totaling 12 points, and the severity of COPD is proportional to the CPIS. APACHE II is primarily used to score age, chronic health status, etc.; with a total of score of 71 points, and the severity of COPD is directly proportional to the APACHE II score.

Pulmonary function test (PFT)

The recorded VC, predicted VC, recorded FEV1, predicted FEV1, RV and TLC of each patient were evaluated and measured by pulmonary function instrument at one day before treatment, on the 15th day of treatment and on the first day after the end of treatment.

Evaluation of treatment outcomes

After treatment, the curative effect on patients were evaluated as markedly effective (after antibiotic and routine treatment, clinical symptoms such as cough and expectoration completely disappeared, chest X-ray films returned to normal, and pulmonary function returned to normal), effective (after antibiotic and routine treatment, clinical symptoms such as cough and expectoration were evidently alleviated, the shadow on chest X-ray films was reduced compared with that before treatment, and pulmonary function was significantly enhanced), and ineffective (after antibiotic and routine treatment, clinical symptoms such as cough and expectoration were not relieved, the shadow of chest X-ray films showed no changes compared with that before treatment, and pulmonary function was not enhanced). The total number of effective cases was calculated as the sum of the number of markedly effective cases and the number of effective cases.

Statistical analysis

The collected data were analyzed using SPSS 26.0 software (IBM Inc., USA). Measurement data were expressed as $(\overline{x} \pm s)$, and compared by the one-way repeated measures analysis of variance before and after treatment. Numerical data were expressed as n (%). The significance level α was set as 0.05, which indicates a 5% risk of concluding a difference existing in all comparisons ($p < 0.05$).

Results

Blood gas analysis results

There were statistically significant differences in the blood gas indices, SaO_2, PaO_2, and $PaCO_2$, among patients at one day before treatment, on the 15th day of treatment and on the first day after the end of treatment ($p < 0.01$). Among blood gas indices, SaO_2 increased from 76.36 ± 13.34% at one day before treatment to 82.36 ± 10.62% on the 15th day of treatment, and further to 90.66 ± 6.67% on the first day after the end of treatment. PaO_2 rose from 49.25 ± 8.26 mmHg at one day before treatment, to 55.36 ± 7.89 mmHg on the 15th day of treatment, and to 60.52 ± 8.97 mmHg on the first day after the end of treatment. In addition, $PaCO_2$ gradually dropped from 91.32 ± 13.55 mmHg at the beginning of treatment to 83.13 ± 11.71 mmHg on the 15th day of treatment, and to 76.41 ± 12.17 mmHg on the first day after the end of treatment (Table 1).

Table 1. Results of blood gas analysis.

Index	1 day before treatment	On the 15th day of treatment	On the 1st day after the end of treatment	*p*
SaO_2	76.36 ± 13.34%	82.36 ± 10.62%	90.66 ± 6.67%	0.002
PaO_2	49.25 ± 8.26 mmHg	55.36 ± 7.89 mmHg	60.52 ± 8.97 mmHg	0.006
$PaCO_2$	91.32 ± 13.55 mmHg	83.13 ± 11.71 mmHg	76.41 ± 12.17 mmHg	0.001

$PaCO_2$: Partial pressure of carbon dioxide; PaO_2: partial pressure of oxygen; SaO_2: oxygen saturation.

Airway resistance index

According to statistical analysis results, airway resistance indices, Zrs5, Rrs5 and Rrs20, showed statistically significant differences among patients at one day before treatment, on the 15th day of treatment, and on the first day after the end of treatment ($p < 0.01$). Zrs5 was decreased from 0.52 ± 0.04 mmHg at one day before treatment to 0.43 ± 0.07 mmHg on the 15th day of treatment, and further to 0.34 ± 0.02 mmHg on the first day after the end of treatment. Rrs5 exhibited a gradual downward trend from 0.66 ± 0.04 mmHg at the beginning of treatment to 0.51 ± 0.03 mmHg and 0.39 ± 0.02 mmHg after treatment. Additionally, Rrs20 declined from 0.51 ± 0.03 mmHg at the beginning of treatment to 0.38 ± 0.04 mmHg and 0.22 ± 0.02 mmHg after treatment (Table 2).

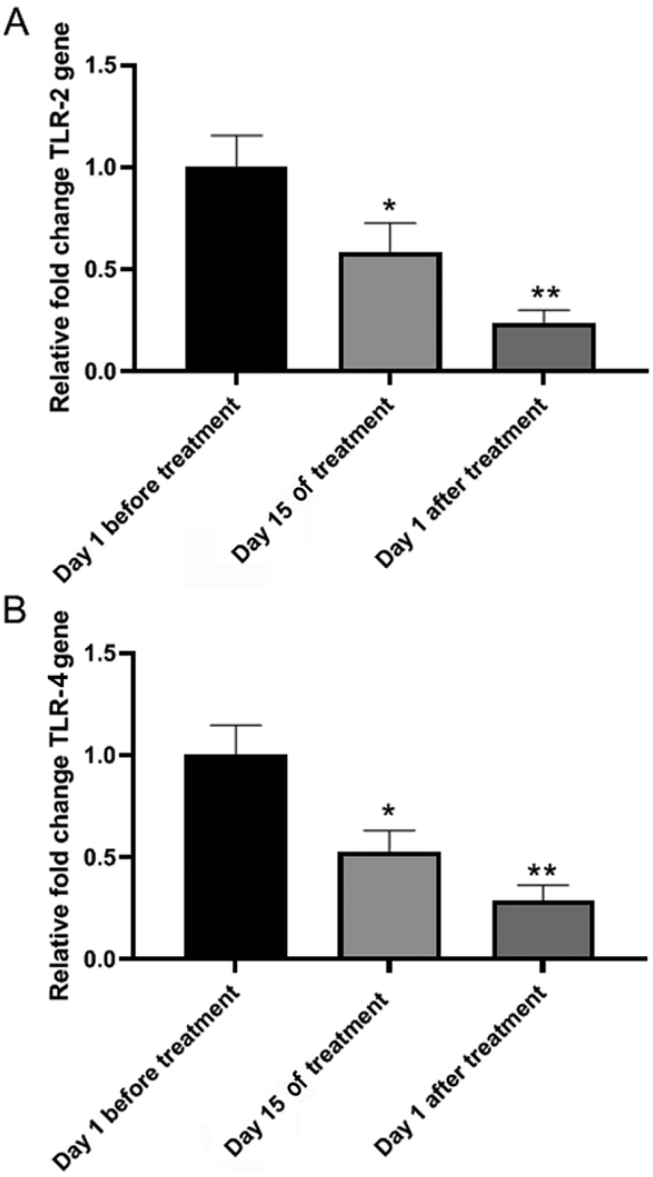
mRNA expression levels of TLR-2 and TLR-4 before and after treatment

There were statistically significant differences in the mRNA expression levels of *TLR-2* and *TLR-4* among patients at one day before treatment, on the 15th day of treatment and on the first day after treatment ($p < 0.05$). The mRNA expression level of *TLR-2* dropped from 3.67 ± 0.65 at one day before treatment to 1.98 ± 0.43 on the 15th day of treatment, and to 0.86 ± 0.14 on the first day after the end of treatment; and that of *TLR-4* declined from 4.56 ± 1.02 at one day before treatment, to 2.47 ± 0.87 on the 15th day of treatment, and to 1.32 ± 0.41 one day after treatment (Figure 1).

Scores of disease status

Downward trends were observed in the CPIS and APACHE II score; on one day before treatment, on the 15th day of treatment and on the first day after treatment; which reflected an improvement of the disease, and comparisons revealed that the differences in the scores were statistically significant ($p < 0.01$) (Table 3).

Figure 1. Relative mRNA expression levels of TLR-2 and TLR-4 before and after treatment.



TLR: Toll-like receptor.

Table 2. Airway resistance indices.

Index	1 day before treatment	On the 15th day of treatment	On the 1st day after the end of treatment	*p*
Zrs5	0.52 ± 0.04	0.43 ± 0.07	0.34 ± 0.02	0.000
Rrs5	0.66 ± 0.04	0.51 ± 0.03	0.39 ± 0.02	0.001
Rrs20	0.51 ± 0.03	0.38 ± 0.04	0.22 ± 0.02	0.000

Rrs5: Respiratory resistance at 5 Hz; Rrs20: respiratory resistance at 20 Hz; Zrs5: total respiratory impedance at 5 Hz.

Table 3. Scores of disease status.

Score	1 day before treatment	On the 15th day of treatment	On the 1st day after the end of treatment	*p*
CPIS	7.87 ± 2.15	5.56 ± 1.72	3.16 ± 0.44	< 0.01
APACHE II	19.15 ± 4.92	11.32 ± 2.74	6.12 ± 1.28	< 0.01

APACHE II: acute physiology and chronic health evaluation II; CPIS: clinical pulmonary infection score.

PFT indices

The comparison of results of pulmonary function before and after treatment indicated that there were statistically significant differences in recorded VC/predicted VC (%), recorded FEV1/predicted FEV1 (%), and RV/TLC ($p < 0.05$). Specifically, recorded VC/predicted VC rose from 67.53 ± 8.25% at one day before treatment to 76.12 ± 9.45% on the 15th day of treatment, and to 89.34 ± 11.42% on the first day after the end of treatment. Recorded FEV1/predicted FEV1 (%) gradually increased from 62.67 ± 7.22% at one day before treatment to 71.56 ± 6.24% on the 15th day of treatment, and to 83.32 ± 9.04% on the 1st day after the end of treatment. Moreover, RV/TLC displayed a gradual downward trend from 52.81 ± 6.22% at one day before treatment to 43.23 ± 4.87% on the 15th day of treatment, and to 32.62 ± 3.11% on the first day after the end of treatment (Table 4).

Treatment outcomes

All the patients were evaluated according to their clinical symptoms and imaging data at one month after treatment. Among the 156 patients, 117 (75.0%) were markedly effective cases, 29 (18.6%) effective cases, and 10 (6.4%) ineffective cases, with a total effective rate of 93.6%.

Discussion

COPD is characterized by the specific inflammatory responses of neutrophils, macrophages, and cluster of differentiation (CD)8+ T lymphocytes to long-term exposure to harmful gases (such as smoke from cigarette) [12]. Besides, the disease is also commonly featured with goblet cell proliferation and mucus hypersecretion, especially in the case of aggravation. Pulmonary infection is the leading cause of COPD deterioration [13]. In this study, the treatment with β-lactam antibiotics for one month evidently improved the respiratory function and blood oxygenation of COPD patients with pulmonary infections, and relieved their airway obstruction and clinical symptoms. Besides, the CPIS and APACHE II score significantly reduced, indicating the mitigation of the disease. Additionally, the patients' pulmonary function gradually returned to normal after treatment. Furthermore, the total effective rate of β-lactam antibiotics for the treatment of COPD complicated with pulmonary infections was over 90%, which is basically identical to the results of Thu *et al.* [14].

During ischemia and after reperfusion, injury-associated molecules are released by the damaged cells and these are endogenous danger signals that stimulate the expression of TLRs. The overexpression of TLR2 and TLR4 plays a key role in promoting renal ischemia-reperfusion [15]. In this study, the mRNA expression levels of TLR-2 and TLR-4 significantly decreased after treatment using β-lactam antibiotics, which is consistent with the study of Wu *et al.* [16]. TLR-2 and TLR-4 are type I transmembrane receptors expressed on the cell surface [17], but TLR-4 can be internalized or expressed in some cells under certain conditions. TLR2 and TLR4 signal mainly via 80 pathways that are myeloid differentiation (MyD) primary response gene-dependent or MyD-adaptor like-dependent [18]. When binding to ligands (such as bacterial peptidoglycan), TLR-2 forms a heterodimer with TLR-1 or TLR-6, and constitutes a functional complex by interacting with CD. On the contrary, TLR-4 binds to its ligands (such as bacterial lipopolysaccharide) to form a homodimer, and interacts with CD14 and/or MD2 (also known as lymphocyte TLR-2) [19]. This process initiates the recruitment of MyD to the Toll-IL-1 receptor domain in cells, and then activates the members of IL-1 receptor-related kinase and tumor necrosis factor receptor-related factors [20]. The activation of 88 transcription factors of the MAPK family and nuclear factor kappa-light-chain-enhancer of activated B cells results in the expression of 90 proinflammatory mediators. Moreover, TLR-2 and TLR-4 play wide-range roles in chronic respiratory diseases, including asthma and pulmonary fibrosis [21,22]. Hence, β-lactam antibiotics may relieve COPD complicated with pulmonary infections by reducing the expressions of TLR-2 and TLR-4 [23].

Conclusions

β-lactam antibiotics exhibit good clinical curative effects on patients with COPD complicated with pulmonary infections. This may be because β-lactam antibiotics can suppress or decrease TLR-2- and TLR-4-mediated inflammatory responses. However, the size of sample included in this study was small. Future

Table 4. PFT indices.

Index	1 day before treatment	On the 15th day of treatment	On the 1st day after the end of treatment	*p*
Recorded VC/predicted VC (%)	67.53±8.25	76.12±9.45	89.34±11.42	< 0.05
Recorded FEV1/predicted FEV1 (%)	62.67±7.22	71.56±6.24	83.32±9.04	< 0.05
RV/TLC (%)	52.81±6.22	43.23±4.87	32.62±3.11	< 0.05

FEV1: Forced vital capacity at 1 s; PFT: pulmonary function test; RV: residual volume; TLC: total lung capacity; VC: vital capacity.

research with larger sample size may increase the accuracy of the research. In the treatment of COPD complicated with pulmonary infection, it is necessary to comprehensively evaluate and choose appropriate antibiotics in consideration of all conditions of the patients, so as to relieve the patients' pain to the greatest extent and accelerate their recovery.

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