https://doi.org/10.31925/farmacia.2022.5.9 **ORIGINAL ARTICLE**

THE EFFECT OF METHYLPREDNISOLONE COMBINED WITH MACROLIDE ANTIBIOTICS ON *MYCOPLASMA PNEUMONIAE* PNEUMONIA IN CHILDREN

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Abstract

This study aimed to evaluate the clinical efficacy of the combination of methylprednisolone (MP) and macrolide antibiotics (MA) in the treatment of *Mycoplasma pneumoniae* pneumonia (MPP) in children. Sixty children with MPP were selected as research subjects and were randomly divided into an experimental group and a control group, with 30 patients in each group. The children in the experimental group were treated with MP + MA, and those in the control group were treated only with MA. The treatment efficacy, improvement of clinical symptoms, improvement in the quality of life, and adverse reactions of the two groups of children were compared before and after the treatment. The combination of MP and MA shower increased efficacy compared with the treatment with MA alone ($p < 0.05$) translated by a shorter time of clinical symptoms alleviation, improved in the quality of life, improve in the levels of tumour necrosis factor α (TNF-α), high-sensitivity C-reactive protein (Hs-CRP), and CD4+ levels. Moreover, in the experimental group it was observed a lower rate of adverse reactions after treatment compared with those in the in the control group ($p < 0.05$). In conclusion, for the treatment of MPP in children, the combined treatment with MP and MA showed a superior effect, which could improve the clinical treatment effect, relieve the clinical symptoms, effectively shorten the hospital stay, improve the prognosis, promote the improvement in quality of life of children, and enhance the immune function of children. Therefore, it is worthy of clinical application and promotion.

Rezumat

Acest studiu și-a propus să evalueze eficacitatea clinică a combinației de metilprednisolon (MP) și antibiotice macrolide (AM) în tratamentul pneumoniei cu *Mycoplasma pneumoniae* (MPP) la copii. Au fost selectați șaizeci de copii cu MPP și au fost împărțiți aleatoriu într-un grup experimental și un grup de control, 30 de pacienți *per* grup. Copiii din lotul experimental au fost tratați cu MP + AM, iar cei din lotul de control au fost tratați doar cu AM. Eficacitatea tratamentului, ameliorarea simptomelor clinice, îmbunătățirea calității vieții și reacțiile adverse ale celor două grupuri de copii au fost comparate înainte și după tratament. Asocierea de MP și AM a crescut eficacitatea față de tratamentul cu AM în monoterapie ($p < 0,05$), fenomen explicat printr-un timp mai scurt de atenuare a simptomelor clinice, îmbunătățirea calității vieții, scăderea factorului de necroză tumorală α (TNF-α), a proteinei C reactive (Hs-CRP) și optimizarea nivelului de CD4+. Mai mult, în lotul experimental s-a observat o rată mai mică de reacții adverse după tratament, în comparație cu cele din grupul de control ($p < 0,05$). În concluzie, pentru tratamentul MPP la copii, asocierea de MP și AM a arătat un efect superior, care ar putea îmbunătăți efectul tratamentului clinic, ameliora simptomele clinice, scurta durata spitalizării, îmbunătăți prognosticul, promova creșterea calității vieții copiilor și îmbunătățirea funcției imunitare a copiilor. Prin urmare, este demn de utilizare și promovare clinică.

Keywords: methylprednisolone, macrolide antibiotics, *Mycoplasma pneumoniae* pneumonia, immunologic function

Introduction

Pneumonia is a very common respiratory disease and common in children. Its main pathogen is *Mycoplasma pneumonia*. The main clinical symptoms include headache, fever, and frequent cough [1, 2]. Currently, no clear mechanism of pathogenesis for *Mycoplasma pneumoniae* pneumonia (MPP) has been elucidated. Some scholars have pointed out that the onset of MPP is closely related to the invasion of *Mycoplasma pneumoniae* and the cytokine-mediated inflammatory response [3, 4]. *Mycoplasma pneumoniae* is considered to harm the organ system of the body, causing patients to have a series of adverse reactions [5]. Studies have shown that macrolide antibacterial drugs (MA) have strong cell permeability and can effectively inhibit protein synthesis in pathogen cells and is currently the first choice for the treatment of mycoplasma diseases without cell wall structure. When MA is used to treat children with MPP, erythromycin lactobionate is often used as the first-line drug [6, 7]. Interesting is that the studies that evaluated the efficacy of erythromycin lactobionate and azithromycin in the treatment of children with MPP showed that the

control effect of lung infection has not been completed [8]. Some studies considered that methylprednisolone (MP) has a good effect in the treatment of children with MPP, and proved that children can tolerate high-dose drug shock treatment [9, 10]. In this study, 60 cases of children with MPP were selected and they were divided into an experimental group and a control group. Two medication methods were adopted to treat children with MPP, so as to explore its clinical treatment effect and to see if the combined treatment with MA and MP is more efficient than the treatment with MA alone.

Materials and Methods

Subjects included in the study

Sixty children with MPP admitted to the Department of Paediatrics, Zhejiang Provincial People's Hospital, Zhejiang, China from July 2019 to October 2020 were selected as the study group. The study was approved by the Ethical Committee of the hospital and for each child included in the study, a legal tutor signed an informed content for participation in the study.

The children were randomly divided into an experimental group (n = 30) and a control group (n = 30). There were 17 boys and 13 girls in the experimental group, with a mean age of 6.5 ± 2.8 years old. In the control group were 15 boys and 15 girls with a mean age of 6.8 ± 2.7 years old. At the beginning of the study the clinical data of children in two groups were similar.

The inclusion criteria were defined as follows: children diagnosis with MPP; children with complications such as atelectasis and pleural effusion; and children whose family members were informed of this study and volunteered to participate.

The exclusion criteria were defined as follows: children with low immune function; children combined with severe coagulation dysfunction and mental disorders; patients with tuberculosis infection, congenital heart disease, and other diseases; patients treated with other drugs within the past 1 month; and children with incomplete clinical data.

Administration Scheme

The children in the control group received only MA for symptomatic, anti-infective, and supportive treatment. The scheme was as follows.

The intravenous infusion of erythromycin lactobionate (China Hunan Kelun Pharmaceutical Co., Ltd., National Medicine Standard H43020028) was dissolved at doses of 15 mg/kg bw into 100 mL of 5% glucose solution, and 1 mL of glucose solution was used to prepare 1 mg of erythromycin lactobionate. The prepared solution was injected by intravenous infusion, once every 12 hours till the condition gradually stabilized, for 5 days. After erythromycin treatment, for 4 days the patients receive no treatment and then they received azithromycin (Zhejiang Asia-Pacific Pharmaceutical Co., Ltd., China, Zhunzi H20103069), orally, 10 mg/kg bw/day for another 5 days.

On this basis, children in the experimental group followed the same antibiotic scheme but received also in the first 5 days MP (China National Pharmaceutical Group Rongsheng Pharmaceutical Co., Ltd., National Medicine Standard H20030727), in doses of 2.0 mg/kg bw/day.

Observation indicators

After treatment, the fever time, lung rales disappearing time, cough relief time, hospital stay, and healing time of children in two groups were recorded and compared. The clinical treatment effect, the incidence of adverse reactions, the quality of life scores before and after treatment, and tumour necrosis factor α (TNF-α), high-sensitivity C-reactive protein (Hs-CRP) and $CD4^+$ T cells percentage were compared between the two groups.

Peripheral blood samples were collected from each participant before and after treatment. The blood collected on polymer gel for serum separation was centrifuged at 3000 × g for 10 min and the serum collected was used to determine TNF-α and hs-CRP levels. Peripheral blood samples collected on EDTA were used to determine $CD4^+$ T cell percentage.

The determination of TNF-α and hs-CRP in serum samples were determined by chemiluminescent immunometric assays using the specific kits for the equipment IMMULITE® 1000 system (Siemens Healthcare Diagnostics Inc., United Kingdom). The detection limit for TNF-α was 1.7 - 1000 pg/mL. The reference range for hs-CRP was 0.2 - 100 mg/L and the analytical sensitivity was 0.1 mg/L.

The counting of $CD4^+$ T cells was done using the Dynal T4 Quant Kit/Reagent Kit for Sysmex Analysers (Invitrogen, Oslo, Norway) and the corresponding data management software from Sysmex Europe (Norderstedt, Germany). The procedure was done according to the manufacturer's instructions. Briefly, 250 µL of blood collected on EDTA was diluted with 250 µL of washing buffer before being incubated for 10 minutes with 50 µL of magnetic beads coated with anti-CD14 antibody (CD14 Dynabeads®). After 3 minutes of incubation on the magnet rack, the beads with monocytes were removed. The monocyte-free supernatant was incubated for 10 minutes at 50 rpm with 50 µL CD4 Dynabeads® in a rotator tube. After incubation, the beads containing $CD4^+$ T cells were separated and washed on the magnet rack twice for 3 minutes each time. Then 100 µL lysis solution was added to release $CD4^+$ T cell nuclei and kept for 10 minutes. After lysis, the tube was placed in the magnet rack once more to remove the beads, for 3 minutes. The collected supernatant was added into a new tube

and the nuclei of the $CD4^+$ T cells were counted using a pocH-100i in the white blood cell channel.

Treatment effect evaluation

Cure: the inflammation was obviously absorbed, the clinical symptoms such as fever, shortness of breath, and cough disappeared and the lung rales disappeared.

Obviously effective: the inflammation was absorbed to a certain extent, the symptoms of fever, shortness of breath, and cough were improved greatly and lung rales disappeared.

Effective: the fever, cough, shortness of breath, and other symptoms had been improved, and there was a slight rale in the lungs.

Ineffective: no improvement in the child's clinical symptoms, or even worsening of the symptoms.

Quality of life evaluation

The quality of life scale was adopted to evaluate the quality of life of patients. The evaluation included mental state, living conditions, sleep and rest, mobility, concentration, energy, drug dependence, thought, self-esteem, pain, dependence on medical methods, and learning ability. Each item was scored from 0 to 5 points, and the total score was 60 points. The quality of life was proportional with the score obtained.

Adverse reactions mainly include gastrointestinal reactions such as loss of appetite, nausea, vomiting, and diarrhoea, as well as dizziness and skin rash.

Statistical analysis

Data analysis was done using SPSS 20.0 software. The count data were expressed as percentages, using Chi-squared ($\chi 2$) test; and the measurement data were expressed as media ± standard deviation ($\bar{x} \pm s$), using Student's t-test. A value of $p < 0.05$ meant that there was a statistically significant difference.

Results and Discussion

Clinical treatment effect

The treatment efficacy rate for the control and experimental group were 76.67% and 96.67%, respectively, showing that the experimental group presents a better clinical treatment effect in contrast to the control group ($p < 0.05$) (Table I).

Table I

Clinical treatment effect

Group	Cure (n)	Obviously effective (n)	Effective (n)	Ineffective (n)	Efficacy rate (%)
Experimental group	17	7	5	1	96.67
Control group	11	8	4	7	76.67
p value	-				0.012

Improvement of clinical symptoms

The disappearance of lung rales, lung lesion absorption, antipyretic administration period, cough disappearance, hospitalization, and healing period expressed in days in the experimental group were visibly shorter in contrast to those in the control group ($p < 0.05$) (Table II).

Table II

Improvement of clinical symptoms (days)

Group	The disappearance of lung rales (days)	Lung lesion absorption (days)	Antipyretic administration period (days)	Cough disappearance (days)	Hospital stay (day)	Healing period (days)
Experimental group	6.15 ± 2.06*	7.43 ± 1.43*	3.06 ± 1.66*	4.76 ± 1.62*	9.64 ± 1.93*	12.08 ± 1.48*
Control group	8.46 ± 1.98	9.78 ± 1.61	4.81 ± 1.87	5.93 ± 2.08	13.87 ± 2.17	16.85 ± 1.91

*$p < 0.05$ compared with the control group

Serological test results

The treatment downregulates the levels of TNF-α and hs-CRP in the experimental group compared with the levels before treatment ($p < 0.05$), while upregulate the $CD4^+$ T cells percentage compared with the levels before treatment ($p < 0.05$). Compared with the levels in the control group after treatment, the improvement in serological parameters was better in the experimental group compared with the control group ($p < 0.05$) (Figure 1).

Score on quality of life

Before treatment, there were no differences in the score on the quality of life of the two groups of children. The treatment significantly increased the quality of life score in the experimental group compared with the control group ($p < 0.05$) (Table III).

Adverse reactions

The data in Table IV showed that after the treatment, the incidence of adverse reactions in the control group was significantly increased (26.67%) compared with those in the experimental group (10.00%) ($p < 0.05$).

Children with MPP can cause serious damage to the respiratory tract. If it is not treated in time, it will greatly harm patient health [11]. In clinical practice, the MA treatment is considered the first-line treatment, but drug resistance is the main factor that limits the therapeutic effect. The onset of MPP is closely related to the excessive cell-mediated

immune inflammatory response [12], so immunity is adopted during treatment. Suppressive treatment is considered to be an effective measure. MP is a medium-acting glucocorticoid, which has anti-allergic, anti-inflammatory, and immune-regulating effects [13]. In the clinic, MP has been utilized orally and intravenously in the treatment of severe mycoplasma infection, and the effect was promising [14, 15].

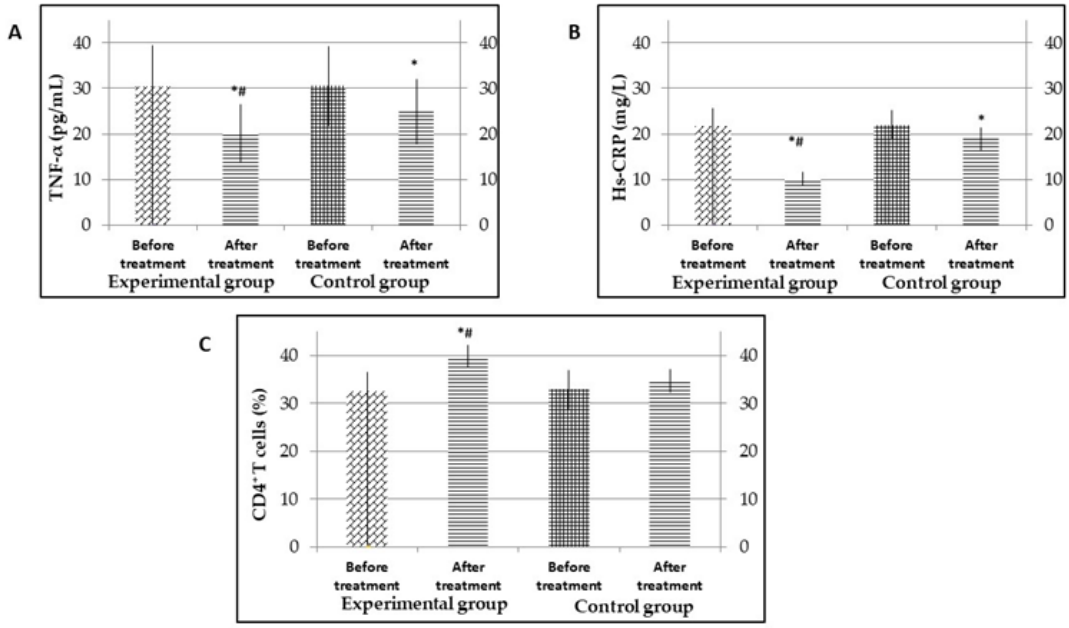

Figure 1.

The levels of TNF-α, hs-CRP, and $CD4^+$ T cells before and after the treatment in the two groups

* $p < 0.05$ compared with the level before treatment; # $p < 0.05$ compared with the control group after treatment

Table III

Changes in quality of life score before and after the treatment

Group	Before the treatment	After the treatment
Experimental group	35.25 ± 4.98	49.90 ± 4.54
Control group	34.94 ± 5.22	43.32 ± 4.08
p value	0.617	0.031

Table IV

Incidence of adverse reactions of children in two groups after the treatment

Group	Nausea and vomiting (n)	Loss of appetite (n)	Rash (n)	Dizzy (n)	Diarrhoea (n)	Incidence rate (%)
Experimental group	0	2	1	0	0	10.00
Control group	1	3	2	1	1	26.67
p value	-					0.015

In this study, the selected children had typical clinical manifestations, such as persistent high fever, which was largely related to an excessive inflammatory response. The results of this study showed that the disappearance time of lung rales and lung lesions absorption, antipyretic administration period, cough disappearance, hospital stay, and healing period in the experimental group were much shorter in contrast to those in the control group ($p < 0.05$). The levels of TNF-α and hs-CRP after the treatment in the experimental group were significantly lower in contrast to those before the treatment ($p < 0.05$), and were lower than those in the control group ($p < 0.05$). In addition, the clinical treatment effect of the experimental group was higher than that of the control group ($p < 0.05$). The results revealed that a combination of MP and MA for the treatment of children with MPP had a superior curative effect, which was closely related to MP inhibiting the body's excessive inflammatory response, inhibiting cytokine production, and reducing capillary permeability. Moreover, the results of the serological index test showed that the CD4+ level of the experimental group of children after treatment was remarkably better compared with that before the treatment ($p < 0.05$), and better than that in the control group. Such results suggested that MP can effectively

improve the patient's immunity. In addition, the score on the quality of life of the children in the experimental group after treatment was higher than that of the control group, which showed that the MP + MA could quickly alleviate the patient's clinical symptoms and adverse physical signs, promote the mental state of patients and improve the quality of life, shorten the length of hospitalization, and improve their survival.

Regarding the mechanism through which the combination of MA and MP can better inhibit *Mycoplasma pneumonie* infection-induced inflammation, new *in vitro* studies have shown their implication in the regulation of miR-499a-5p/STAT3 axis [16]. *Mycoplasma pneumonie* infection can up-regulate STAT3-mediated signalling pathway [17] that is involved in inflammation and down-regulate the levels of miR-499a-5p that is involved in alleviating inflammation in infection-related diseases [18].

Conclusions

To sum up, in the clinical treatment of children with MPP, the treatment of MP combined with MA had a significantly superior effect, which can improve the clinical treatment effect, alleviate clinical symptoms, shorten hospital stays, and promote patient improvement in the quality of life, and enhance the immunologic function of children. Therefore, it is worthy of clinical application and promotion.

Conflict of interest

The authors declare no conflict of interest.

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