Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(6) 2555–2561

DOI: 10.1177/0300060518820412
journals.sagepub.com/home/imr
SAGE

Effect of montelukast combined with methylprednisolone for the treatment of mycoplasma pneumonia

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Abstract

Objective: To study the effect of the leukotriene receptor agonist montelukast combined with methylprednisolone on inflammatory response and peripheral blood lymphocyte subset content in children with mycoplasma pneumonia.

Methods: Seventy-four children were enrolled and randomly divided into a standard treatment group and a montelukast plus methylprednisolone group. Serum levels of inflammatory cytokines and corresponding cytokines of T lymphocyte subsets were measured, and peripheral blood was collected to determine the T cell subset content.

Results: At 3 days and 7 days after treatment, serum MCP-1, PCT, ICAM-1, CXCL8, CRP, IFN-γ, and IL-17 levels and peripheral blood Th1 and Th17 content were significantly decreased in both groups, while serum IL-4 and TGF-β levels and peripheral blood Treg and Th2 content were significantly increased. However, serum MCP-1, PCT, ICAM-1, CXCL8, CRP, IFN-γ, and IL-17 levels and peripheral blood Th1 and Th17 content were significantly lower while serum IL-4 and TGF-β levels and peripheral blood Treg and Th2 content were significantly higher in the montelukast plus methylprednisolone group compared with the control group.

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Conclusion: Montelukast combined with methylprednisolone for the treatment of mycoplasma pneumonia can inhibit inflammatory responses and regulate levels of Th1/Th2 and Th17/Treg cells.

Keywords
Mycoplasma pneumonia, montelukast, glucocorticoid, inflammatory response, immune response, children

Date received: 25 June 2018; accepted: 29 November 2018

Introduction

Mycoplasma pneumoniae (MP) is a common pathogenic bacterium that causes community-acquired pneumonia in children. The condition is self-limiting in most children, but bronchiolitis obliterans can occur after macrolide antibiotic treatment, and severe cases can develop into potentially life-threatening pneumonia.[1,2] Activation of the systemic inflammatory response is a key feature of severe pneumonia, and the abnormal secretion of various inflammatory mediators is an important contributing factor to disease progression.[3] Methylprednisolone is an intermediate-acting glucocorticoid preparation that inhibits inflammatory and immune responses, and montelukast is a leukotriene receptor antagonist that can inhibit the inflammatory response mediated by leukotriene.[4,5] In the present study, montelukast combined with methylprednisolone was used for the treatment of children with mycoplasma pneumonia, and the resulting changes in inflammatory mediators and peripheral blood lymphocyte subset content were analyzed.

Patients and Methods

Research subjects

Seventy-four children with severe mycoplasma pneumonia who were treated in Huai'an Second People's Hospital between June 2014 and October 2016 were enrolled in this study. All children presented with clinical symptoms of fever, cough, and wheezing. Mycoplasma pneumonia was confirmed by X-ray and positive serum mycoplasma IgM. A random number table was used to divide the children into two groups, each containing 37 patients. The intervention group received standard treatment combined with montelukast and methylprednisolone, and included 22 males and 15 females aged 5–12 years. The control group received standard treatment and included 21 males and 16 females aged 5–13 years. There was no significant difference in patient demographics between the two groups. Ethical approval was obtained from the Ethic Committee of Huai'an Second People's Hospital, and all participants and their parents or guardians provided written informed consent prior to participation.

Therapy

Both groups of patients received standard therapy including mucosolvan (1.2–1.6 mg/kg) to reduce phlegm and azithromycin for infection, given as 10 mg/kg/d in 100 mL of 5% glucose injection, by intravenous drip for 3 days). The intervention group also received montelukast combined with methylprednisolone as follows: methylprednisolone 2 mg/kg/d in 100 mL of 5%

glucose injection, by intravenous drip, for 5 days; oral administration of montelukast (Singulair, Shandong Lunan Beite Pharmaceutical Co., Ltd., Lanshan District, Linyi City, Shandong Province, China), 4 mg for children 2–5 years old and 5 mg for children 6–12 years old, once daily in the evening. Both groups were treated for 7 consecutive days.

Serum cytokine assay

Before treatment and at 3 d and 7 d after treatment, 3 mL of peripheral venous blood was collected from each patient and centrifuged to separate serum. Enzyme-linked immunosorbent assay kits (Beyotime Biotechnology, Shanghai, China) were used to detect MCP-1, PCT, ICAM-1, CXCL8, CRP, IFN-γ, IL-4, IL-17, and TGF-β in the isolated serum.

Peripheral blood T cell subset assay

Before treatment and at 3 d and 7 d after treatment, 3 mL of peripheral venous blood was collected from each patient and anticoagulated with EDTA prior to incubation with monoclonal antibodies against CD4, IFN-γ, IL-4, IL-17, and TGF-β (Immunoway, Beijing, China). The Th1 ($CD4^+IFN\text{-}\gamma^+$), Th2 ($CD4^+IL\text{-}4^+$), Th17 ($CD4^+IL\text{-}17^+$), and Treg ($CD4^+TGF\text{-}\beta^+$) content were subsequently determined by flow cytometry (Beckman Coulter, CytoFLEXS, NY, USA).

Statistical methods

One-way ANOVA was used to compare serum cytokine levels and peripheral blood T cell content between the two groups. Values of $P<0.05$ were considered to indicate statistical significance. SPSS version 21.0 software (IBM Corp, Armonk, NY, USA) was used for all statistical analyses.

Results

Serum cytokine levels

Before treatment, differences in serum MCP-1, PCT, ICAM-1, CXCL8, and CRP levels were not statistically significant between the two groups of patients ($P>0.05$). At 3 d and 7 d after treatment, the serum levels of all cytokines were significantly lower in both groups than the levels before treatment ($P<0.05$) and, furthermore, were significantly lower in the intervention group compared with the control group ($P<0.05$), as shown in Table 1.

Peripheral blood T lymphocyte subsets and corresponding cytokine levels

Before treatment, differences in peripheral blood Th1, Th2, Th17, and Treg content were not statistically significant between the two groups of patients. At 3 d and 7 d after treatment, the peripheral blood Th1 and Th17 content in both groups was significantly lower compared with before treatment, while the Th2 and Treg content was significantly higher ($P<0.05$). Furthermore, at 3 d and 7 d after treatment, the peripheral blood Th1 and Th17 content of the intervention group was significantly lower than that of the control group, while the Th2 and Treg content was significantly higher ($P<0.05$), as shown in Table 2.

Before treatment, differences in peripheral blood IFN-γ, IL-4, IL-17, and TGF-β levels were not statistically significant between the two groups of patients. At 3 d and 7 d after treatment, however, the peripheral blood IFN-γ and IL-17 content of both groups was significantly lower than that before treatment while the IL-4 and TGF-β content was significantly higher ($P<0.05$). Furthermore, at 3 d and 7 d after treatment, the peripheral blood IFN-γ and IL-17 content of the intervention group was significantly lower than that of

Table 1. Serum levels of inflammatory markers before and after treatment ($\bar{x} \pm s$).

Groups	n	Time	MCP-1 (ng/L)	PCT (μg/L)	ICAM-1 (μg/L)	CXCL8 (ng/L)	CRP (mg/L)
Intervention group	37	Before treatment	174.5 ± 22.3	16.9 ± 2.2	352.3 ± 52.4	93.5 ± 11.2	38.9 ± 6.2
		3 d after treatment	$96.4 \pm 13.6^{\triangle\blacktriangle}$	$8.3 \pm 1.1^{\triangle\blacktriangle}$	$201.3 \pm 34.6^{\triangle\blacktriangle}$	$48.6 \pm 6.2^{\triangle\blacktriangle}$	$17.6 \pm 2.2^{\triangle\blacktriangle}$
		7 d after treatment	$52.9 \pm 11.2^{\triangle\blacktriangle}$	$4.2 \pm 0.7^{\triangle\blacktriangle}$	$142.6 \pm 22.7^{\triangle\blacktriangle}$	$30.2 \pm 4.7^{\triangle\blacktriangle}$	$9.5 \pm 1.1^{\triangle\blacktriangle}$
Control group	37	Before treatment	177.1 ± 20.5	17.2 ± 2.3	357.1 ± 42.1	95.1 ± 11.8	39.6 ± 4.9
		3 d after treatment	$129.7 \pm 17.3^{\blacktriangle}$	$12.8 \pm 1.9^{\blacktriangle}$	$285.2 \pm 33.6^{\blacktriangle}$	$75.2 \pm 9.3^{\blacktriangle}$	$23.7 \pm 3.5^{\blacktriangle}$
		7 d after treatment	$93.5 \pm 11.7^{\blacktriangle}$	$9.5 \pm 1.1^{\blacktriangle}$	$219.3 \pm 27.4^{\blacktriangle}$	$57.5 \pm 7.1^{\blacktriangle}$	$14.2 \pm 1.9^{\blacktriangle}$

$^{\triangle}$: Comparison between intervention group and control group, $P<0.05$; $^{\blacktriangle}$: comparison between before treatment and after treatment, $P<0.05$

Table 2. Peripheral blood T lymphocyte subset content before and after treatment ($\bar{x} \pm s$).

Groups	n	Time	Th1	Th2	Th17	Treg
Intervention group	37	Before treatment	17.53 ± 2.41	5.62 ± 0.84	4.41 ± 0.67	0.78 ± 0.11
		3 d after treatment	$10.32 \pm 1.88^{\triangle\blacktriangle}$	$7.88 \pm 0.93^{\triangle\blacktriangle}$	$2.15 \pm 0.35^{\triangle\blacktriangle}$	$1.42 \pm 0.19^{\triangle\blacktriangle}$
		7 d after treatment	$7.59 \pm 0.93^{\triangle\blacktriangle}$	$9.51 \pm 1.15^{\triangle\blacktriangle}$	$1.58 \pm 0.20^{\triangle\blacktriangle}$	$1.95 \pm 0.22^{\triangle\blacktriangle}$
Control group	37	Before treatment	17.91 ± 2.52	5.49 ± 0.86	4.39 ± 0.69	0.80 ± 0.10
		3 d after treatment	$13.95 \pm 2.15^{\blacktriangle}$	$6.76 \pm 0.89^{\blacktriangle}$	$3.42 \pm 0.52^{\blacktriangle}$	$1.18 \pm 0.18^{\blacktriangle}$
		7 d after treatment	$10.25 \pm 1.77^{\blacktriangle}$	$7.91 \pm 0.89^{\blacktriangle}$	$2.57 \pm 0.36^{\blacktriangle}$	$1.39 \pm 0.20^{\blacktriangle}$

$^{\triangle}$: Comparison between intervention group and control group, $P<0.05$; $^{\blacktriangle}$: comparison between before treatment and after treatment, $P<0.05$

the control group, while the IL-4 and TGF-β content was significantly higher ($P<0.05$), as shown in Table 3.

Discussion

Mycoplasma pneumoniae is pathogen lacking a cell wall, and can cause bronchial and capillary bronchial epithelial damage and airway inflammation, leading to increased airway secretions and the obstruction of capillary bronchus following infection of the respiratory tract. Macrolide antibiotics are commonly used for the treatment of mycoplasma pneumoniae infection and are effective in the majority of children. However, the condition can progress rapidly in severe cases, in which macrolide antibiotic therapy alone is insufficient to exert a curative effect.[6,7] Excessive activation of the airway inflammatory response represents an important step in progression to severe mycoplasma pneumonia, and combination therapy using drugs with different mechanisms of action to suppress the inflammatory reaction is key to the treatment of this

Table 3. Levels of cytokines corresponding to peripheral blood T lymphocyte subsets before and after treatment ($\bar{x} \pm s$).

Groups	n	Time	IFN-γ	IL-4	IL-17	TGF-β
Intervention group	37	Before treatment	5.94 ± 0.78	0.41 ± 0.08	84.41 ± 10.25	1.48 ± 0.19
		3 d after treatment	$2.52 \pm 0.34^{\triangle\blacktriangle}$	$0.71 \pm 0.11^{\triangle\blacktriangle}$	$36.41 \pm 6.24^{\triangle\blacktriangle}$	$3.65 \pm 0.67^{\triangle\blacktriangle}$
		7 d after treatment	$1.89 \pm 0.22^{\triangle\blacktriangle}$	$0.92 \pm 0.13^{\triangle\blacktriangle}$	$20.37 \pm 3.84^{\triangle\blacktriangle}$	$5.03 \pm 0.77^{\triangle\blacktriangle}$
Control group	37	Before treatment	6.02 ± 0.91	0.44 ± 0.07	86.21 ± 10.77	1.52 ± 0.20
		3 days after treatment	$3.94 \pm 0.52^{\blacktriangle}$	$0.58 \pm 0.08^{\blacktriangle}$	$59.62 \pm 8.76^{\blacktriangle}$	$2.77 \pm 0.39^{\blacktriangle}$
		7 days after treatment	$2.74 \pm 0.42^{\blacktriangle}$	$0.72 \pm 0.09^{\blacktriangle}$	$36.41 \pm 5.28^{\blacktriangle}$	$3.36 \pm 0.51^{\blacktriangle}$

$^{\triangle}$: Comparison between intervention group and control group, $P<0.05$; $^{\blacktriangle}$: comparison between before treatment and after treatment, $P<0.05$

severe form of the disease.[8] Montelukast is a leukotriene receptor antagonist, capable of antagonizing the binding of leukotriene to its receptor as well as the activation of downstream inflammatory responses,[9] while methylprednisone is an intermediate-acting glucocorticoid that exerts significant anti-inflammatory and immunosuppressive effects[10] Zhou (2017) previously reported that the use of methylprednisolone for refractory mycoplasma pneumoniae pneumonia in children was associated with improved clinical outcomes.[5] Other studies have reported the effectiveness of montelukast and methylprednisolone for the treatment of mycoplasma pneumonia,[11]but the efficacy of combination therapy using both agents remains unclear. In the present study, the effect of montelukast combined with methylprednisolone on inflammatory and immune responses was evaluated.

Overactivation of the inflammatory response is an important characteristic of severe mycoplasma pneumonitis, and also represents an important phase in the development of the disease. During this process, the secretion of MCP-1, PCT, ICAM-1, CXCL8, CRP and other inflammatory mediators increases significantly. MCP-1 and CXCL8 are important chemokines that can promote inflammatory cell chemotaxis to inflammatory lesions and increase the number of infiltrated inflammatory cells in the lesion.[12] PCT is a precursor of calcitonin, and a variety of parenchymal cells in the body can overexpress PCT when stimulated by pro-inflammatory mediators, resulting in its secretion into the circulation where it represents a sensitive indicator of the degree of inflammation.[13] ICAM-1 can mediate adhesion between inflammatory cells and vascular endothelium, and promote inflammatory cell infiltration to the site of inflammation.[14] CRP is a non-specific acute-phase protein synthesized by hepatocytes, and the degree of activation of the inflammatory response generally correlates with the level of secreted CRP.[15] To evaluate the effect of montelukast combined with methylprednisolone on the inflammatory response in the development of mycoplasma pneumonia, the levels of these inflammatory mediators were determined in the present study. The results showed that serum MCP-1, PCT, ICAM-1, CXCL8, and CRP levels in both groups were significantly decreased after treatment, with levels in the intervention group significantly lower than those in the control group. This finding indicates that routine anti-infective, phlegm-reducing, and other symptomatic treatment can inhibit activation of the inflammatory response to a certain extent, but that combined use of

montelukast and methylprednisolone can more effectively inhibit the inflammatory response in the development of mycoplasma pneumonia.

The abnormal activation of the inflammatory response in children with severe mycoplasma pneumonia is closely related to that observed in disorders of the immune response.[16] The $CD4^+$ T subset is an important T cell subset that resists and eliminates pathogens, and can be further divided into Th1, Th2, Th17, Treg and other subsets according to different patterns of cytokine secretion. Th1 cells mainly secrete INF-γ, IL-2, TNF-α, and other cytokines,[17,18] while Th17 cells mainly secrete IL-17, IL-22, and IL-23, and are typically involved in the cellular immune response and pathogen elimination.[19] The IL-4, IL-5, TGF-β, and IL-10 cytokines secreted by Th2 and Treg have the effect of inhibiting the inflammatory response, and can antagonize the differentiation, maturation, and secretory function of Th1 and Th17.[20,21] During the progression of mycoplasma pneumonia, Th1 and Th17 are significantly activated, and the cytokines they secreted are capable of eliminating pathogens while also causing tissue damage; The activation of Th2 and Treg can inhibit the function of Th1 and Th17 after the pathogen is cleared to prevent tissue damage. In the present study, the analysis of $CD4^+$ T cell subset content in peripheral blood showed that the Th1 and Th17 content in both groups significantly decreased while the Th2 and Treg content significantly increased after treatment, and that the Th1 and Th17 content of the intervention group was significantly lower than that of the control group while the Th2 and Treg content was significantly higher. Further analysis of cytokine levels corresponding to $CD4^+$ T cell subsets indicated that peripheral blood IFN-γ and IL-17 content in both groups significantly decreased while IL-4 and TGF-β content significantly increased after treatment, and that the IFN-γ and IL-17 content of the intervention group was significantly lower than that of the control group while IL-4 and TGF-β content was significantly higher. This finding further indicates that conventional treatment can regulate the immune response to a certain extent but that montelukast in combination with methylprednisolone can more effectively regulate the balance of Th1/Th2 and Th17/Treg cells.

Montelukast combined with methylprednisolone appears to be more efficacious for the treatment of mycoplasma pneumonia, and can modulate both the inflammatory response during disease progression and the balance of Th1/Th2 and Th17/Treg cells.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This study is supported by the Science-technology Support Projects of Huai'an City, China (grant number HAS2013020).

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