Analysis of the Therapeutic Effect of Pidotimod Assisted Fluticasone Propionate in Children with Asthma and their Immune Function

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We attempt to study the therapeutic effect of pidotimod assisted fluticasone propionate in children with asthma and the improvement of their immune function. We selected 93 asthmatic children admitted to the hospital from May 2021 to December 2022 for the trial. Divided them into experimental group (46 cases) and reference group (47 cases) by computerized numbered random number table. All children received conventional therapy; the experimental group was treated with fluticasone propionate, while the reference group was treated with pidotimod in addition to the experimental group. All children received 90 d treatment, compared both groups on the treatment effect, immune function and peripheral blood levels of interferon regulatory factor 1 and signal transducer-activated transcription factor-1 and the incidence of adverse effects before and after treatment. Experimental group possessed shorter disappearance time of all clinical symptoms shorter than reference group (all $p<0.05$). After 90 d treatment, the cluster of differentiation 3^+, cluster of differentiation 4^+ and cluster of differentiation 4^+/cluster of differentiation 8^+ in the experimental group were (66.91±12.30) %, (45.23±4.56) % and (1.82±0.52) %, respectively, which were all higher than those in the reference group (61.45±11.73) %, (42.82±4.19) % and (1.54±0.40) %, while the cluster of differentiation 8^+ was ((28.37±2.42) %, which was lower than (30.06±2.75) % in the reference group (all $p<0.05$). After 90 d treatment, experimental group had lower interferon regulatory factor 1 and signal transducer-activated transcription factor-1 levels than reference group (both $p<0.05$). The incidence of nausea and vomiting, arthralgia, rash and dizziness were not significantly compared between both groups (all $p>0.05$). Pidotimod adjuvant to fluticasone propionate in children with asthma is effective and can improve their immune function and interferon regulatory factor 1 and signal transducer-activated transcription factor-1 expression levels without increasing the risk of adverse reactions, so it is worth promoting.

Key words: Asthma, pidotimod, fluticasone propionate, immune function, adverse reaction

Asthma is one of the mostly seen respiratory diseases in pediatrics. The symptoms are complex, mostly cough, shortness of breath and dyspnea, etc. Because of the timing of attacks at night and in the early hours of the morning and the tendency to have recurrent attacks, children's health is seriously affected[1]. Therefore, timely and effective treatment of children is essential to improve their condition and maintain their physical and mental health and is an urgent issue for pediatricians. Pidotimod is one of the most widely used immune boosters in clinical practice and has been reported to be effective in children with asthma, promoting symptomatic relief and improving immune function[2]. Fluticasone propionate has also been shown to be more effective in relieving symptoms in asthma patients and may have some anti-inflammatory effects[3]. However, little research has been done on the effect of the combination of these two drugs on the immune function of children with asthma. In this paper, we investigated the effects of pidotimod assisted fluticasone propionate on immune function in children with asthma. We selected 93 asthmatic children admitted to the hospital from May 2021 to December 2022 for the trial. Divided

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them into experimental group (46 cases) and reference group (47 cases) by computerized numbered random number table. Experimental group consisted of 26 males and 20 females; the age limit was from 2 y to 12 y old, mean (6.19±1.05) y old; asthma classification; mild 22 cases, moderate 17 cases and severe 4 cases. Reference group consisted of 28 males and 19 females, the age limit was from 2 y to 12 y old, mean (6.24±1.08) y old; asthma classification: Mild 23 cases, moderate 18 cases and severe 3 cases. The differences of the above information in both groups were small ($p>0.05$) and could be compared for follow-up. In inclusion criteria all children were consistent with the asthma diagnostic criteria established in the guidelines for the diagnosis and prevention of bronchial asthma in children (2016 edition)[4] ; all were first-onset; aged were from 2 y to 12 y old and no recent history of asthma treatment. Exclusion criteria combined with severe abnormalities in other organ functions; combined with primary immune system disorders and/or organic pathologies; allergy to drugs related to this study. The guardians of the children all signed consent forms and the study was approved by the Hospital Medical Ethics Committee. All subjects were given conventional treatment, including asthma, oxygen and anti-infection, etc. The experimental group was treated with fluticasone propionate inhalation aerosol (Glaxo Welcome SA, approval number: H20130190) at a dose of 125 μg/time, 2 times/day. After receiving the same treatment as experimental group, the reference group was treated with pidotimod (Sunstone (Tangshan) Pharmaceutical Co., Ltd, approval number: HH200010091) at a dose of 400 mg/time, 2 times/day. All subjects were treated continuously for 90 d. Specimen acquisition and processing 6 ml of venous blood was collected from all subjects 1 d before and 90 d after treatment, divided into 2 tubes and stored at -20° in a refrigerator. Compared both groups on the treatment effect, immune function and peripheral blood levels of Interferon Regulatory Factor 1 (IRF1) and Signal Transducer-Activated Transcription Factor-1 (STAT1) and the incidence of adverse reactions before and after treatment. The assessment of treatment effect was achieved by the time of disappearance of clinical symptoms, covering four items; shortness of breath, cough, dyspnea and croup. Immune function tests was done by collecting 3 ml of venous blood from all children 1 d before treatment and 90 d after treatment and Cluster of Differentiation (CD) 3^+, $CD4^+$ and $CD8^+$ were measured using an EPICSXL flow cytometer (purchased from Beckman Coulter, United States of America) and calculated $CD4^+/CD8^+$. IRF1 and STAT1 assay was done by collecting 3 ml of venous blood from all children before morning meal 1 d before and 90 d after treatment. After anticoagulation with ethylenediaminetetraacetic acid, Peripheral Blood Mononuclear Cells (PBMC) were extracted by Ficoll density gradient centrifugation and isolated. Total Ribonucleic Acid (RNA) was extracted from PBMC and complementary Deoxyribonucleic Acid (cDNA) was obtained with the aid of a reverse transcription kit. Polymerase Chain Reaction (PCR) was used to complete the detection of the above two index levels. 20 μl of PCR reaction system consisting of cDNA 0.5 μl, 2×SYBR Green PCR Master Mix 10 μl, upstream and downstream primers 0.5 μl respectively and nuclear-free water 8.5 μl. The reaction conditions were 95° for 60 s, 95° for 5 s, 60° for 30 s, 60° for 30 s, 40 cycles. IRF1 upstream primer: 5-GCGGCACTGGGCACGGCT-3, downstream primer: 5-GGTGGCAAGCACCAAGAGAAC-3. STAT1 upstream primer: 5-GTGACGTGGACATCCGCAAAG-3, downstream primer: 5-TCCGAGACACCTCGTCAAAC-3. Relative expression of IRF1, STAT1 was calculated by the $2^{-\Delta\Delta Ct}$ method. There were four adverse reactions, which were nausea and vomiting; arthralgia; skin rash and dizziness. Adopted Statistical Package for the Social Sciences (SPSS) 24.0 software to analyses the data. Adverse reactions were described as [n, (%)] in the outcome indicators of this study, and the Chi-square (χ^2) test was performed. The rest of the indicators were described by ($\bar{x}\pm s$) and t-test was performed $p<0.05$ means that the difference is statistically significant. Experimental group had shorter disappearance time of all clinical symptoms than reference group (all $p<0.05$) as shown in Table 1. After 90 d treatment, experimental group had higher $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ than reference group, but lower $CD8^+$ than reference group (all $p<0.05$), as shown in Table 2. After 90 d treatment, experimental group had lower IRF1 and STAT1 levels than reference group (all $p<0.05$), as

shown in Table 3. The incidence of all adverse reactions was not significant in both groups (all p>0.05), as shown in Table 4. Asthma has a high prevalence worldwide and its incidence is increasing as a result of changing lifestyles and a deteriorating living environment, which directly affects the health of patients and places a burden on their families and society[5]. Asthma can occur at all ages and is most common in children[6]. Because children are often at a critical stage of growth and development, and because their immune systems are not yet fully developed, the condition can become life-threatening if not treated aggressively and effectively. Fluticasone propionate is one of the glucocorticoids and has a high affinity for the glucocorticoid receptor, which allows for effective control of asthma in children through a variety of anti-allergic and anti-inflammatory actions[7]. Pidotimod has been widely used and proven to boost the immune system and prevent infections[8,9]. In addition, under normal physiological conditions, the body is always in a dynamic balance between T lymphocyte Type 1 (Th1) and Th2, which plays a key role in the maintenance of the body's immune function. When the above balance is disrupted, it can lead to a series of immune-related diseases, such as the weakening of Th1 and the enhancement of Th2, which can easily lead to a chronic inflammatory response and thus trigger asthma[10]. This also provides a theoretical basis for the treatment of asthma with pidotimod. It is hypothesized that the addition of pidotimod to fluticasone propionate treatment may help to improve the outcome in children with asthma and is of some research value. The results of this paper demonstrate that experimental group had shorter disappearance time of all clinical symptoms than reference group. This is similar to the study reported by Jin *et al.*[11] and confirms that the use of pidotimod with fluticasone propionate in children with asthma is effective in relieving clinical symptoms. The reason for this is that fluticasone propionate, one of the commonly used therapeutic agents for asthma, exerts potent local anti-inflammatory and anti-allergic effects, while reducing airway hyper responsiveness, thus effectively and rapidly reducing asthma symptoms. Pidotimod is a synthetic immune booster with oral bioactivity, which promotes both specific and non-specific immune responses. Therefore, the combination of the two drugs can inhibit airway hyper responsiveness and inflammation in different ways, thus enhancing the therapeutic effect[12]. In addition, after 90 d treatment, experimental group had higher $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ than reference group, but lower $CD8^+$ than reference group. This is highly consistent with the study reported by Wang *et al.*[13], reflecting that the dosing regimen in the experimental group significantly enhanced immune function in children with asthma. To extrapolate the reason, fluticasone propionate, as a glucocorticoid, is mainly used topically and cannot completely improve the immune dysfunction of patients. However, pidotimod promotes the activation of natural killer cells, stimulates neutrophil proliferation, enhances macrophage as well as neutrophil phagocytosis and acts as a suppressor of Th2 cell function, ultimately improving the immune function of the body[14]. In addition, IRF1 is a transcription factor that mediates the regulation of gene expression through a variety of signaling pathways and plays a crucial role in the process of cellular immunity and differentiation, and its high expression may contribute to the development of asthma[15]. STAT1 is abnormally highly expressed in the epithelial cells of asthmatic patients, thus promoting the development of Intercellular Cell Adhesion Molecule-1 (ICAM), which positively regulates Th2 cell function and leads to increased airway inflammation in asthma[16]. After 90 d treatment, experimental group had lower IRF1 and STAT1 levels than reference group. It is speculated that one of the mechanisms of action of the treatment regimen in the experimental group may be related to the inhibition of IRF1 and STAT1 expression. However, the mechanism of action is still not fully understood and needs to be confirmed by further studies, which provides an important direction for future research. The comparison of the incidence of all adverse reactions between both groups was not significant. This reflects the feasibility of the treatment regimen in the experimental group, which does not increase toxic side effects. In conclusion, pidotimod adjuvant to fluticasone propionate in the treatment of children with asthma can achieve significant results, which are conducive to the relief of clinical symptoms in children, and can improve their immune function and inhibit the expression of IRF1 and STAT1 levels, with better safety and higher clinical

promotion value. However, there are still shortcomings in this study, such as the lack of comparative analysis of children with different asthma classifications, which could be considered in future studies to provide a more comprehensive basis for the clinical treatment of children with asthma.

TABLE 1: COMPARISON OF THE DISAPPEARANCE TIME OF VARIOUS CLINICAL SYMPTOMS BETWEEN BOTH GROUPS (x̄±s, d)

Group	Cases	Shortness of breath	Cough	Breathing difficulties	Growling sound
Experimental	46	2.43±0.47	5.28±1.04	2.78±0.45	5.17±0.38
Reference	47	3.12±0.58	6.43±1.22	3.66±0.52	6.02±0.55
t value	-	6.295	4.887	8.719	8.653
p value	-	<0.001	<0.001	<0.001	<0.001

TABLE 2: COMPARISON OF T-LYMPHOCYTE SUBSETS BETWEEN BOTH GROUPS (x̄±s)

Group	Cases	$CD3^+$ (%)		$CD4^+$ (%)		$CD8^+$ (%)		$CD4^+/CD8^+$	
		1 before treatment	After 90 d treatment	1 d before treatment	After 90 d treatment	1 d before treatment	After 90 d treatment	1 d before treatment	After 90 d treatment
Experimental	46	57.03±10.12	66.91±12.30	36.87±3.48	45.23±4.56	32.89±3.15	28.37±2.42	1.24±0.36	1.82±0.52
Reference	47	57.08±10.17	61.45±11.73	36.91±3.50	42.82±4.19	32.53±3.18	30.06±2.75	1.25±0.37	1.54±0.40
t value		0.024	2.191	0.055	2.655	0.548	3.144	0.132	2.914
p value		0.981	0.031	0.956	0.009	0.585	0.002	0.895	0.005

TABLE 3: COMPARISON OF IRF1 AND STAT1 LEVELS BETWEEN BOTH GROUPS (x̄±s)

Group	Cases	IRF1		STAT1	
		1 d before treatment	After 90 d treatment	1 d before treatment	After 90 d treatment
Experimental	46	2.67±0.25	1.14±0.17	0.48±0.07	0.34±0.02
Reference	47	2.70±0.24	1.50±0.20	0.49±0.08	0.40±0.04
t value		0.59	9.344	0.641	9.118
p value		0.556	<0.001	0.523	<0.001

TABLE 4: COMPARISON OF ADVERSE REACTIONS BETWEEN BOTH GROUPS [n, (%)]

Group	Cases	Nausea and vomiting	Joint pain	Rash	Dizziness
Experimental	46	2 (4.35)	0 (0.00)	2 (4.35)	1 (2.17)
Reference	47	1 (2.13)	1 (2.13)	3 (6.38)	2 (4.26)
χ^2 value		0.367	0.989	0.189	0.323
p value		0.545	0.32	0.664	0.57

Conflict of interests:

The authors declared no conflict of interests.

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