Tropical Journal of Pharmaceutical Research October 2023; 22 (10): 2201-2207
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i10.23

Original Research Article

Effect of the combination of oseltamivir, artificial cow-bezoar, chlorphenamine maleate, and interferon nebulization on immune function in children with influenza

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Sent for review: 20 March 2023 *Revised accepted: 8 October 2023*

Abstract

Purpose: *To determine the effect of combining oseltamivir, artificial cow-bezoar, chlorphenamine maleate, and interferon inhalation on immune function and serum amyloid A (SAA) levels in children with influenza.*
Methods: *A total of 114 children with influenza treated at the Second Affiliated Hospital of Hainan Medical University, Hainan Province, China from December 2019 to December 2022 were randomly divided into two groups, viz, study group (n = 57) and control group (n = 57). Control group received oseltamivir sodium chloride infusion, artificial cow-bezoar, and chlorphenamine maleate granules. Study group was treated with interferon alpha-1b in addition to control group treatment. Their clinical symptoms, duration of symptoms, immune function, SAA and C-reactive protein (CRP) levels were determined before and after treatment. Adverse reactions were also recorded.*
Results: *Study group had a significantly shorter duration of fever, cough, sore throat, and nasal congestion after 5 days of treatment than control group ($p < 0.05$). The study group also showed higher CD3+ and CD8+ levels and lower CD4+ and CD4+/CD8+ levels than control group after treatment. However, both groups showed lower levels of SAA, CRP, and SAA/CRP after treatment than before treatment ($p < 0.05$). Furthermore, the levels of SAA, CRP, and SAA/CRP were lower in study group than in control group after treatment ($p < 0.05$). The incidence of adverse reactions in the study group after treatment was significantly lower than in the control group ($p < 0.05$).*
Conclusion: *Quadruple therapy using oseltamivir, artificial cow-bezoar, chlorphenamine maleate, and interferon inhalation significantly shortens symptoms, boost immunity, lower SAA levels, and reduce side effects in children with influenza.*

Keywords: *Influenza, Oseltamivir, Artificial cow-bezoar, Chlorphenamine maleate, Interferon, Serum amyloid A, Immune function, Quadruple therapy*

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INTRODUCTION

Common cold is an acute respiratory illness caused by influenza virus. Compared with other respiratory virus infections, its clinical symptoms and signs are more severe, and it has explosive and seasonal characteristics [1]. Children, especially those in the initial phases of their educational journey, have immature immune functions and weaker anti-infection abilities,

making them susceptible to influenza virus infections. After infection with influenza virus, individual's immune response is affected, leading to an excessive activation of inflammatory response. This heightened inflammatory response is unfavorable for clearing the virus, and it increases the risk of organ damage [2]. Therefore, timely and effective treatment for childhood influenza is necessary.

Oseltamivir is a newly developed neuraminidase inhibitor and is the only anti-influenza virus drug administered intravenously. It exerts its antiviral effects by inhibiting neuraminidase [3]. Oseltamivir has demonstrated favorable outcomes in preventing and managing childhood influenza. However, many types of pathogens cause childhood influenza, and the efficacy of using drugs alone is not satisfactory. Interferon α-2b is a broad-spectrum antiviral drug that has inhibitory effects on both RNA and DNA viruses, with high specificity and good efficacy [4]. Previous clinical studies on the use of oseltamivir, phenylephrine, and interferon nebulization for treatment of influenza in children are limited. Hence, this research seeks to investigate the impact of triple therapy involving oseltamivir, phenylephrine, and interferon nebulization on the serum amyloid A (SAA) level and immune function in children with influenza.

METHODS

Patients

A total of 114 children diagnosed with influenza and treated at the Second Affiliated Hospital of Hainan Medical University, Hainan Province, China from December 2019 to December 2022 were selected for this study. The children were randomly assigned to study group (n = 57) and control group (n = 57). This study was approved by the hospital ethics committee (approval no. LW2022723) and followed international guidelines for human studies. Informed consent was obtained from the children's parents or guardians.

Inclusion criteria

Those included were children with body temperature higher than 37.5 ^{o}C on admission; clinical symptoms such as fever, cough, sore throat, and headache; no history of antiviral drug use; and diagnosed according to the diagnostic criteria in the "Expert Consensus on Integrated Traditional Chinese and Western Medicine Diagnosis and Treatment of Viral Pneumonia in Children" [5].

Exclusion criteria

Those excluded were children with severe liver, kidney, cardiovascular, or hematologic disorders; allergy or drug allergy, and presence of lung infection on chest X-ray. Others excluded were children with mental abnormalities and children who had received antiviral drug treatment before enrollment.

Treatments

Control group received treatment involving an injection of Peramivir chloride sodium (Guangzhou Nanshin Pharmaceutical Co. Ltd, National Drug Approval Number: H20130029, 100 mL per specification), in combination with phenylephrine and chlorpheniramine maleate granules (Beijing CR Sanjiu Pharmaceutical Co., Ltd., National Drug Approval Number: H11022051). These granules contained 0.125 g of artificial cow-bezoar and chlorphenamine maleate, with each dose providing 5 mg of synthetic bovine bezoar and 0.5 mg of chlorpheniramine maleate. Peramivir chloride sodium was administered intravenously once daily for 30 min. Phenylephrine and chlorpheniramine maleate granules were administered orally three times per day based on the child's weight: 0.5 - 1.0 g per dose for children weighing 10 - 15 kg, 1.0 - 1.5 g per dose for those weighing 16 - 20 kg, 1.5 - 2.0 g per dose for those weighing 21 - 25 kg, and 2.0 - 2.5 g per dose for those weighing over 25 kg.

Study group received interferon α-1b (Shanghai Institute of Biological Products, National Drug Approval Number: S20053003, 10 μg: 1 mL per specification) in addition to the same treatment as control group. The interferon α-1b was administered via inhalation, at a dose of 30 μg mixed with 1 mL of sterile water, twice daily. Both groups were treated for 5 days. Throughout the treatment process, children were closely monitored for clinical symptoms, signs, and any potential adverse drug reactions. If any adverse reactions are identified, appropriate intervention measures are promptly implemented.

Evaluation of parameters/indices

Duration of symptoms

The duration of symptoms including fever, cough, sore throat, and nasal congestion in both groups of patients were recorded. 3 mL of venous blood was collected from the elbow in the morning from both groups of patients before and after treatment, and centrifuged at 2000 r/min for 10 min at -20 ^{o}C.

Flow cytometry

A flow cytometer was used (Thermo Fisher, model Attune NxT) to determine CD3+, CD4+, CD8+, and CD4+/CD8+ levels. The SAA, CRP, and SAA/CRP levels before and after treatment were compared in both groups of patients.

Enzyme-linked immunosorbent assay

Fasting venous blood was collected from the elbow in the morning from patients and a Beckman Coulter automatic biochemical analyzer (Jinan Taiyi Biotechnology Co., Ltd., model BK-600) was used to perform enzyme-linked immunosorbent assay (ELISA) for SAA and CRP. Adverse reactions during the treatment period in both groups of patients were recorded.

Statistical analysis

Statistical Package for Social Sciences (SPSS) 21.0 software was used for statistical analysis. Continuous variables are expressed as mean ± standard deviation (SD) and compared within and between groups using t-tests. Categorical variables are expressed as n (%) and compared between groups using χ^2 tests. *P*-value < 0.05 was considered statistically significant.

RESULTS

Duration of major symptoms

Table 1 shows the general data of children with influenza between the two groups. There were no statistically significant differences in the general information between the two groups ($p > 0.05$) as shown in Table 1. Compared with control group, the duration of fever, cough and other main symptoms in study group were shorter ($p < 0.05$), as shown in Table 2.

Immune function

Before treatment, there were no significant differences in $CD3^+$, $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ between the two groups ($p > 0.05$); after treatment, $CD3^+$ and $CD8^+$ levels were significantly increased, and $CD4^+$ and $CD4^+/CD8^+$ were significantly decreased in the two groups, and the levels of $CD3^+$ and $CD8^+$ in the study group were significantly higher than those in control group, and $CD4^+$ and $CD4^+/CD8^+$ were significantly lower than those in control group ($p < 0.05$; Table 3).

SAA and CRP levels

Before treatment, there was no significant difference in SAA and CRP levels between the two groups ($p > 0.05$). After treatment, SAA, CRP and SAA/CRP levels in the two groups were significantly lower than those before treatment ($p < 0.05$). After treatment, SAA, CRP levels and SAA/CRP in study group were significantly lower than those in control group ($p < 0.05$).

Incidence of adverse reactions

After treatment, the study group displayed a significantly lower total adverse reaction rate of 3.51 % in comparison to control group, significantly lower than 15.79 % in control group, ($\chi^2 = 4.930$, $p < 0.05$).

Table 1: Comparison of general data of children with influenza between the two groups (n = 57)

Group	Gender (case (%))		Age (years)	Disease duration (h)
	Male	Female		
Study	24 (42.11)	33 (57.89)	4.28±0.52	26.34±5.17
Control	22 (38.60)	35 (61.40)	4.47±0.47	25.38±4.89
χ^2/t	0.146		1.917	0.954
P-value	0.703		0.058	0.342

Table 2: Comparison of duration of main symptoms between the two groups (n = 57)

Group	Fever (h)	Cough (days)	Sore throat (days)	Nasal congestion (days)
Study	36.27±7.45*	2.48±0.76*	1.48±0.36*	1.45±0.38*
Control	42.18±6.75	3.74±0.84	1.94±0.75	2.33±0.48
t value	4.438	0.398	4.175	10.852
P-value	0.000	0.000	0.000	0.000

Table 3: Comparison of immune function between the two groups before and after treatment (n = 57)

Group	$CD3^+$/%		$CD4^+$/%		$CD8^+$/%		$CD4^+/CD8^+$	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***
Study	58.45±2.56	69.49±2.49*	41.74±2.45	31.65±2.47*	26.48±1.47	33.46±2.15*	1.72±1.32	0.94±0.26*
Control	57.98±2.67	61.42±2.36*	41.26±2.48	35.26±2.41*	26.89±1.38	29.37±1.98*	1.76±1.25	1.23±0.19*
T	0.959	17.760	1.040	7.898	1.535	10.565	0.166	6.799
P-value	0.340	0.000	0.301	0.000	0.128	0.000	0.868	0.000

Note: *$P < 0.05$, compared with that before treatment in the same group

Table 4: Comparison of SAA and CRP levels before and after treatment between the two groups (n = 57)

Group	SAA (mg/L)		CRP		SAA/CRP	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***	***Before treatment***	***After treatment***
Study	17.38±2.29	10.35±1.48*	3.17±0.56	1.45±0.44*	5.48±1.34	7.14±0.97*
Control	18.14±2.13	14.28±1.72*	3.20±0.44	1.70±0.32*	5.66±1.27	8.41±1.26*
T	1.835	13.076	0.318	7.355	0.736	2.089
P-value	0.069	0.000	0.751	0.001	0.463	0.039

Note: *$P < 0.05$, compared with that before treatment in the same group

Table 5: Adverse reactions during treatment in the two groups (n=57 (%))

Group	Vomiting	Nausea	Abdominal pain	Diarrhea	Total Occurrence
Study	0 (0.00)	0 (0.00)	1 (1.75)	1 (1.75)	2 (3.51)
Control	3 (5.26)	2 (3.51)	1 (1.75)	3 (5.26)	9 (15.79)
X^2 value					4.930
P-value					0.026

DISCUSSION

Influenza is caused by a type of orthomyxovirus known as influenza virus. The immune system of infants is not yet fully developed and their ability to resist infection is relatively poor, making them more susceptible to influenza [6]. Influenza virus infection in children usually presents with more severe clinical symptoms and exhibits the epidemiological characteristics of sudden outbreaks and rapid spread. If not treated promptly, it may lead to viral pneumonia, and elevate the likelihood of multi-organ dysfunction and systemic inflammatory response syndrome [7].

Peramivir is currently the only intravenous anti-influenza virus drug available in China. It is a neuraminidase inhibitor with a good affinity for neuraminidase [8] and effectively suppresses neuraminidase activity. According to related surveys, peramivir is used in patients of any age and exhibits a relatively high safety profile with no reported adverse reactions [9]. However, the therapeutic effect of a single antiviral drug is unsatisfactory and requires the use of other drugs to improve clinical efficacy. Granules for children, containing a combination of artificial cow-bezoar, chlorphenamine maleate, chlorpheniramine maleate, artificial bezoar, and other constituents, have been utilized in this study. It relieves symptoms such as headache, runny nose, and nasal congestion caused by infantile influenza. Artificial cow-bezoar and chlorphenamine maleate effectively inhibit the synthesis and secretion of prostaglandins, with analgesic and antipyretic effects. Chlorpheniramine maleate antagonizes histamine, thereby reducing symptoms of nasal congestion and runny nose, artificial bezoar clears heat, detoxifies and calms [10]. The combination of peramivir and artificial cow-bezoar and chlorphenamine maleate and chlorpheniramine maleate granules is a common clinical treatment for infantile influenza.

Interferon I, a clinically used immunomodulatory drug, exhibits both antiviral and antitumor properties. Although it does not have a direct effect on the virus, it affects cells through cell surface receptors and produces antiviral proteins that cause antiviral proliferation. In addition, interferon itself enhances the vitality and combat effectiveness of T-lymphocytes and macrophages, thereby achieving an immunomodulatory effect [11]. Treatment of influenza patients with interferon nebulization allows the drug to enter the body directly from the respiratory tract. Furthermore, interferon may be adsorbed to the nasal and respiratory mucosa and quickly transported throughout the body via the submucosal capillaries, resulting in a rapid drug effect. The combination of multiple drugs for the treatment of influenza in children has become a widely accepted clinical treatment method. Therefore, the objective of this study was to examine the effects of the triple combination of Peramivir, artificial cow-bezoar, chlorphenamine maleate, and interferon nebulization on the SAA levels and immune function in children with influenza.

The results of this study show that the triple combination therapy of Peramivir, Artificial cow-bezoar, chlorphenamine maleate, and interferon nebulization significantly shortened the duration of clinical symptoms such as fever, cough, and sore throat in children with influenza while reducing adverse reactions. This indicates that the combined use of the drugs significantly

improved clinical efficacy. The reason for this may be that interferon α-1b and Peramivir have a synergistic effect which results in a better antiviral effect, and regulates the immune function of children, promoting disease recovery.

Clinical research has demonstrated that immune function plays a pivotal role in the pathogenesis of influenza among children. Notably, CD3+ and CD4+ cells are key components of the body's cellular immunity, and fluctuations in their levels impact the immune regulation of influenza in children. However, it's important to note that CD8+ is a cytotoxic T cell that exerts an inhibitory effect on CD4+. Typically, studies have shown that the total number and subpopulation levels of T lymphocytes are maintained in a delicate balance, and any shifts in these levels result in immune dysfunction, which is detrimental to overall human health. The findings of this study reveal that the administration of triple combination therapy involving Peramivir, Artificial cow-bezoar and chlorphenamine maleate, and interferon nebulization resulted in significant increases in the serum levels of CD3+ and CD8+ in children with influenza. Conversely, there were significant decreases in CD4+ and CD4+/CD8+ levels. Importantly, the efficacy of this triple therapy outperformed that of Peramivir and Artificial cow-bezoar and chlorphenamine maleate treatment when administered individually.

These results indicate that the triple combination therapy holds the potential to enhance the immune function of children affected by influenza, ultimately proving to be an effective approach. SAA and CRP are inflammatory factors released by the liver. When the body is infected, it is released into the bloodstream in large quantities, thereby enhancing the phagocytic function of white blood cells and keeping the body in an inflammatory state, thereby exacerbating tissue damage. After treatment, the serum levels of SAA, CRP, and SAA/CRP were significantly reduced in both groups of children, with study group being lower than control group, indicating that the triple combination therapy of Peramivir, Artificial cow-bezoar and chlorphenamine maleate, and interferon nebulization was more effective than treatment with Peramivir, Artificial cow-bezoar, and Chlorphenamine maleate alone, and the combination therapy significantly reduces inflammation levels in children with influenza. It also accelerates recovery, affirming the viability of this drug combination approach.

Limitations of this study

First, the relatively short follow-up period of six months may not capture the long-term effects of the triple therapy. A more extended follow-up would provide a clearer understanding of the treatment's sustained impact and any potential late-onset adverse reactions. Second, while the study demonstrated positive outcomes, the sample size was modest, and it was conducted in a single center. Expanding the study to involve multiple centers with larger and more diverse populations could enhance the generalizability of the findings.

Third, although adverse reactions were monitored and found to be significantly lower in study group compared to control group, a more comprehensive investigation of potential side effects and safety concerns over an extended period is warranted. Lastly, the study primarily focused on clinical and laboratory parameters. It would be valuable for future research to incorporate patient-reported outcomes and assess the economic implications of this triple therapy to provide a more comprehensive evaluation of its effectiveness and cost-effectiveness.

CONCLUSION

The quadruple combination therapy of peramivir, artificial cow-bezoar chlorphenamine maleate, and interferon nebulization lowers the duration of clinical symptoms in children with influenza, improves immune function, and reduces the levels of SAA and CRP inflammatory factors. While this study offers promising insights into the benefits of quadruple therapy for children with influenza, the limitations underscore the need for further research, including longer-term investigations, larger and more diverse populations, and a broader assessment of outcomes and safety profiles.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

This study was approved by the Ethics Committee of the Second Affiliated Hospital of

Hainan Medical University, Hainan Province, China (approval no. LW2022723).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xianjie Wu and Zhimian Liang contributed equally.

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