Tropical Journal of Pharmaceutical Research July 2021; 20 (7): 1505-1510
ISSN: 1596-5996 (print); 1596-9827 (electronic)
© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i7.25

Original Research Article

Effect of a combination of interferon-α and ambroxol on children with pneumonia, and on serum amyloid A: A randomized controlled study

Zhuangzhuang Li[1], Lingling Xie[1]*, Jinguang Wu[1], He Wang[2], Shugen Wang[1]
[1]Department of Pharmacy, [2]Department of Pediatrics, Cangzhou Central Hospital, Cangzhou, China

For correspondence:* ***Email:* *xielingling007@126.com;* ***Tel:*** *+86-15333275533*

Sent for review: 19 April 2021 *Revised accepted: 28 June 2021*

Abstract

Purpose: *To investigate the effect of combination of interferon-a with ambroxol on children with pneumonia, and its effect on serum amyloid A (SAA).*
Methods: *A total of 140 children who presented with pneumonia in Cangzhou Central Hospital from January 2019 to November 2020 were selected and randomly assigned to control group and study group. All the children were treated with conventional therapy against cough and phlegm, anti-infection, oxygen inhalation, antipyretic, water and electrolyte correction, and nutritional support. The control group received aerosol inhalation of ambroxol + physiological saline, in addition to conventional treatment. The study group was given aerosol inhalation of recombinant human interferon-α1b + ambroxol + physiological saline. The two groups were treated continuously for 7 days. The time taken for clinical symptoms to subside in each group, as well as serum levels of amyloid A (SAA), interleukin 6 (IL-6), immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG) before treatment and 7 days post-treatment were evaluated. Clinical efficacy and incidence of adverse reactions after 7 days of treatment were also obtained and compared.*
Results: *The time taken for symptom disappearance was significantly shorter in the study group than in control group, and inflammation indices were lower in the study group than in control group ($p < 0.05$). Levels of IgA, IgM, and IgG in the two groups after treatment were significantly elevated, but they were markedly higher in the observation group than in control group ($p < 0.05$). Clinical treatment efficacy was superior in the study group, relative to control ($p < 0.05$). However, no statistical disparity in incidence of adverse reactions was witnessed between the two groups ($p > 0.05$).*
Conclusion: *A combination of interferon-α1b and Ambroxol may be a boon in the treatment of children with pneumonia due to its efficacy and effect on inflammatory response and SAA levels.*

Keywords: *Interferon-α1b, Ambroxol, Pediatric pneumonia, Serum amyloid A*

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Pediatric pneumonia is one of the commonest pediatric diseases. It occurs predominantly in infants and young children, due to factors such as pathogen infection or inhalation of amniotic fluid and oil, as well as allergic reactions and other inflammatory reactions in the lungs. It manifests mainly as fever, cough, pulmonary wet rales, difficulty in breathing and shortness of

© 2021 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

breath, and it is considered as one of the death-related diseases in infants and young children [1,2]. The targets used for controlling pediatric pneumonia include infection controls, relief of symptoms, correction of hypoxia, and prevention of progression of the disease [3]. Ambroxol is a frequently used clinical expectorant which substantially improves respiratory tract function [4].

Definite outcomes of the use of recombinant human interferon-α1b in pediatric pneumonia have been widely documented [5]. However, not much is known about the effect of combined use of recombinant human interferon-α1b and Ambroxol on pediatric pneumonia. This study is unique in the sense that it applied a combination of recombinant human interferon-α1b and Ambroxol on children with pneumonia, with a view to investigating its impact on inflammatory and immune indicators.

METHODS

Study participants

Children with pneumonia who were admitted to *Cangzhou Central Hospital* from January 2019 to November 2020 were enrolled. The included children were: those who met the clinical diagnostic criteria for pneumonia, children aged more than 5 months, and who were tolerant to the study drugs, and those whose parents/guardians voluntarily participated in the study and signed informed consent. Patients who had pulmonary tuberculosis, asthma, bronchial foreign body and other respiratory diseases and pulmonary dysplasia; and those with congenital heart disease, severe liver and kidney insufficiency and sepsis were excluded. Moreover, patients with epilepsy and other central nervous system diseases, and those who used glucocorticoid within one month prior to the study were excluded.

In all, a total of 140 children were included. They were randomly assigned into control group and study group using the random number table method, 70 children each. This study was commenced following clearance from the ethical committee of our institution. The baseline data were homogenous in the two groups (p > 0.05; Table 1).

Treatments

All children were given conventional symptomatic treatment such as relieving cough and reducing sputum, anti-infection treatment, oxygen inhalation, antipyretics, correction of water and electrolytes, and nutritional support. The control group received conventional basic treatment: for those aged ≤ 12 months, 15 mg nebulized Ambroxol inhalation (Ruiyang Pharmaceutical Co. Ltd; SFDA approval number H20173342; specification: 4ml: 30mg) +2 mL of physiological saline were provided; for those aged > 12 months, 30 mg of Ambroxol (Ruiyang Pharmaceutical Co. Ltd; SFDA approval no. H20173342; specification: 4ml: 30mg) + 2 mL of physiological saline were given. In the observation group, those aged ≤12 months were given inhalation of recombinant human interferon-α1b (Shenzhen Kexing Biological Engineering Co. Ltd; SFDA approval number S10960058; specification: 10 μg/bottle) at a dose of 1.0 μg/kg + 15 mg of Ambroxol (Ruiyang Pharmaceutical Co. Ltd.; SFDA approval no. H20173342; specification: 4 ml: 30 mg) + 2 mL of physiological saline.

Those aged > 12 months were administered atomized inhalation of recombinant human interferon-α1b (Shenzhen Branch Xing Biological Engineering Co. Ltd; SFDA approval number S10960058; specification: 10 μg/piece) at a dose of 1.0 μg/kg + 30 mg of Ambroxol (Ruiyang Pharmaceutical Co. Ltd; SFDA approval no. H20173342; specification: 4 ml: 30 mg) + 2 mL of physiological saline. Both groups received atomized inhalation of oxygen at a flow rate of 6 L/min, 10 min at a time, twice a day. Both groups were treated for 7 days.

The protocol has been approved by the Medical Science Research Ethics Committee of *Cangzhou Central Hospital*, with the Approved No. of 2016-16(004) and followed the international guidelines for human studies [6].

Table 1: Baseline information on patients in the two groups

Group	Gender		Age (years)	Course of disease (days)	Severity	
	Male	*Female*			*Severe*	*Mild*
Control	42	28	4.05±1.08	4.26±1.10	45	25
Study	38	32	3.80±1.02	4.08±0.95	50	20
χ^2/t	0.467		1.408	1.036	0.819	
P-value	0.495		0.161	0.302	0.366	

Determination of outcome indices

Time taken before disappearance of clinical symptoms

Time taken before disappearance of clinical symptoms such as fever, cough, shortness of breath, wet rales, and lung X-ray shadows were recorded and compared.

Inflammation indicators

Inflammation indicators before treatment and 7 days after treatment including serum levels of amyloid A (SAA) and interleukin 6 (IL-6) were detected; 2 ml of blood was drawn intravenously on an empty stomach early in the morning, and the serum was separated within 2 hours, and frozen at -80 ℃. The IL-6 was detected using enzyme-linked immunosorbent assay (ELSA), the kit was purchased from Shanghai Jingguan Biological Products Co., Ltd., and the operation was carried out in strict accordance with the instructions. SAA was determined by immunoscattering rate turbidimetric method, the instrument was IMMACE double optical path immunoturbidity analyzer, and the kit was purchased from Bexkuman-coulter of the United States.

Immunity factors

Immunity factors before treatment, and 7 days after treatment were measured including serum immunoglobulin A (IgA), immunoglobulin M (IgM) and immunoglobulin G (IgG); The levels of serum IgA, IgG and IgM were measured by the nephelometric method using the IMMAGE 800 device (Beckman Coulter, Inc., Brea, CA, USA).

Clinical efficacy

Clinical efficacy after 7 days of treatment was classified as *cured* i.e. clinical symptoms disappeared, absence of lung X-ray shadow, and normal results from relevant laboratory indicators; *improved* i.e. clinical symptoms were mitigated, reduction in lung X-ray shadows, significantly improved laboratory indicators; or *ineffective*: i.e. no significant relief from the clinical symptoms, or the symptoms got even worse.

Incidence of adverse reactions

Incidence of adverse reactions post-medication was recorded and analyzed.

Statistical analysis

The SPSS 20.0 was software used for statistical evaluation. Counting data are presented as numbers and percentages [n (%)], and were compared with the X^2 test, while ordinal data were compared using rank sum test. Measurement data are expressed mean ± standard deviation (SD), and were examined with *t*-test for determination of differences between groups. Values of $p < 0.05$ were declared as significant differences.

RESULTS

Time taken for clinical symptoms to subside

A remarkably shorter time taken for symptoms to subside in the study group than the control group was observed ($p < 0.05$), as presented in Table 2.

Inflammation indices

Statistically significant reductions were seen in serum levels of SAA and IL-6 in the two groups after treatment, when compared the corresponding pre-treatment levels. However, the levels of these inflammation parameters were lower in the study group ($p<0.05$). These results are shown in Table 3.

Levels of immunity indices

As shown in Table 4, the levels of IgA, IgM, and IgG in the two groups after treatment were markedly elevated, but they were higher in the study group than in the control group ($p < 0.05$).

Table 2: Comparison of time taken for clinical symptoms to subside between the two groups

Group	Fever	Cough	Shortness of breath	Wet rale	Lung X-ray shadow
Study	2.65±0.72	4.40±1.05	4.02±1.04	5.45±1.60	6.04±1.56
Control	3.50±1.06	5.24±1.25	5.06±1.32	6.66±2.06	7.11±1.80
t	5.539	4.305	5.178	3.881	3.758
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

Values are mean ± SD

Table 3: Comparison of Inflammation indices between the two groups

Group	SAA		IL-6	
	Before treatment	***After treatment***	***Before treatment***	***After treatment***
Study group	142.23±12.43	75.57±8.64	48.67±1.65	31.03±0.79
Control group	140.05±25.54	86.76±8.76	49.23±1.79	20.76±1.35
t	0.642	7.609	1.925	54.934
P-value	0.522	<0.001	0.056	<0.001

Values are mean ± SD

Table 4: Comparison of levels of some immunity indices between the two groups (g/L)

Group	Time	IgA	IgM	IgG
Control	Before treatment	5.30±1.42	0.82±0.20	0.80±0.22
	After treatment	7.55±2.05*	1.18±0.33*	1.16±0.32*
Study	Before treatment	5.18±1.60	0.80±0.18	0.78±0.20
	After treatment	9.03±2.80*#	1.30±0.34*#	1.32±0.36*#

**P* < 0.05, compared with value before treatment; #*p* < 0.05, compared with control group

Table 5: Comparison of clinical efficacy between the two groups [n (%)]

Group	Cured	Improved	ineffective
Control	26 (37.14)	35 (50.0)	9 (12.86)
Study	46 (65.71)	21 (30.0)	3 (4.29)
Z		3.459	
P-value	0.001		

Table 6: Comparison of incidence of adverse reactions between the two groups

Group	Nausea	Vomiting	Diarrhea	Rash	Total incidence
Control	1	1	0	1	3 (4.29%)
Study	2	1	1	1	5 (7.14%)
χ^2					0.530
P-value					0.466

Clinical efficacy

Table 5 shows that clinical efficacy in the study group was markedly superior to that of the control group ($p < 0.05$).

Adverse events

During the treatment period, there were 2 cases of nausea, 1 case of vomiting, 1 case of rash, and 1 case of diarrhea in the study group, while in the control group, there were nausea, vomiting, and rash (one case each). None of these adverse events had a detrimental effect on the treatment, and they all disappeared on their own after the medications were stopped. No statistically significant differences in the incidence of adverse reactions were found between the two groups ($p > 0.05$). These data are presented in Table 6.

DISCUSSION

Serum amyloid A (SAA) arises from tissue amyloid A secreted by hepatocytes. Healthy people exhibit lower levels of SAA, while the level rises rapidly when the body is exposed to inflammation or active disease. Similar to C-reactive protein, it belongs to the acute phase reactive protein. However, in contrast, under inflammatory stimulation, SAA accumulates more significantly than C-reactive protein, and the serum level of SAA rises at a much faster rate than that of C-reactive protein [7,8]. IL-6 is a pro-inflammatory factor secreted by Th2 cells. It stimulates a large number of phagocytes and effector molecules to gather at the site of infection to eliminate pathogens. If the antigen is not cleared in time, it will cause cytokine release levels to exceed the physiological levels, thereby leading to inflammatory infiltration [9,10]. Accumulating evidence have shown that serum SAA and IL-6 levels in children with pneumonia are elevated [11,12].

Several trials have demonstrated that pediatric pneumonia is directly linked to immune function [13,14]. Thus, the levels of serum IgM, IgG, and IgA in children with pediatric pneumonia are strikingly decreased [15]. Ambroxol is a mucolytic agent which contains the bioactive compound bromocyclohexylamine, with good bronchial and lung tissue affinity. Its mechanism of action in

children with pneumonia can be explained thus: (1) stimulation of the secretions from mucous and serous glands so as to dilute sputum, promote cilia movement, and expel sputum; and (2) promotion of the synthesis of alveolar type Ⅱ cells and the release of surface-active substances to reduce the surface tension of the alveoli, inhibit contraction of smooth muscles of the respiratory tract and improve lung function, ultimately resulting in antitussive effect [16,17]. In addition, Ambroxol inhibits the growth of bacteria or viruses; this can increase the airway concentration of antibacterial drugs, and improve the anti-infective effect, thereby reducing local inflammation [18].

In this study, substantial mitigation of clinical symptoms, as well as clinical efficacy and desirable changes in related laboratory indices were observed in the two groups post-treatment. Nonetheless, the findings in this study revealed that the clinical symptoms in children treated with aerosol inhalation of combination of recombinant human interferon-α1b and Ambroxol subsided faster than those in the control group. This suggests that combined treatment using recombinant human interferon-α1b and Ambroxol further reduced the clinical symptoms of pneumonia in children. Serum SAA and IL-6 levels in the study group after treatment were found to be strikingly lower than the corresponding control group values. Thus, it can be reasonably assumed that the combination treatment with recombinant human interferon-α1b and Ambroxol plays a pivotal role in reducing the inflammation in children. Furthermore, a considerably lower serum levels of IgA, IgM, and IgG in the study group after treatment than those in the control group was noticed.

Therefore, it can be speculated that the combined treatment using recombinant human interferon-α1b and Ambroxol can effectively improve immunity in the children and promote functional immunity restoration. Moreover, an excellent clinical efficacy was seen in the study group in contrast to the control group, indicating that the combination of interferon-α1b and Ambroxol further improved the efficacy of treatment. Thus, the combined treatment option is beneficial and clinically acceptable. These aforementioned benefits may be attributed to the fact that interferon is a very special bioactive protein with multiple functions. It exerts potent and broad-spectrum antiviral effects, as well as favorable regulatory effect on immunity. Interferon a1b, one of the subtypes of interferons, has extremely potent activity, and it is directly deposited and distributed in every tissue of the respiratory system after inhalation. Therefore, the concentration of interferon is greatly increased, resulting in rapid effectiveness in removal of pathogens, inhibition of inflammation, enhancement of immunity, mitigation of clinical symptoms and suppression of the disease.

Moreover, the current study revealed that there were no serious adverse reactions during the treatment of the two groups of children, suggesting that the combination of interferon-α1b and Ambroxol is safe. Therefore, the combination of interferon-α1b and Ambroxol may be a boon in the treatment of children with pneumonia due to its innumerable benefits in terms of efficacy, inflammatory response, and serum SAA levels.

Limitations of the study

The current study is limited by a small sample size and absence of a long-term follow-up. Hence, long-term data and more participants will be needed to draw definite conclusions and use in clinical settings.

CONCLUSION

Altogether, we recommend the combination of interferon-α1b and Ambroxol as a more excellent route for the treatment of children with pneumonia, as it shows robust outcomes in producing better efficacy, yielding lower inflammatory reactions and adverse events.

DECLARATIONS

Conflict of interest

No conflict of interest is 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.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution,

and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Oketch JW, Kamau E, Otieno GP, Otieno JR, Agoti CN, Nokes DJ. Human metapneumovirus prevalence and patterns of subgroup persistence identified through surveillance of pediatric pneumonia hospital admissions in coastal Kenya, 2007-2016. BMC Infect Dis. 2019; 19 (1): 757.
2. Cheng CY, Cheng SY, Chen CC, Pan HY, Wu KH, Cheng FJ. Ambient air pollution is associated with pediatric pneumonia: a time-stratified case-crossover study in an urban area. Environ Health. 2019; 18(1): 77.
3. McIntosh K. A New Way of Managing Pediatric Pneumonia. Clin Infect Dis. 2019; 69 (11): 1935-1936.
4. Meng F, Cheng J, Sang P, Wang J. Effects of Bronchoalveolar Lavage with Ambroxol Hydrochloride on Treating Pulmonary Infection in Patients with Cerebral Infarction and on Serum Proinflammatory Cytokines, MDA and SOD. Comput Math Methods Med. 2020; 2020: 7984565.
5. Mehta S, Mukherjee S, Balasubramanian D, Chowdhary A. Evaluation of neuroimmunomodulatory activity of recombinant human interferon α. Neuroimmunomodulation. 2014; 21(5): 250-6.
6. Department of Health, Education, and Welfare; National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report. Ethical principles and guidelines for the protection of human subjects of research. J Am Coll Dent 2014; 81: 4-13.
7. Liu Q, Li Y, Yang F, Xu T, Yao L, Sun J, Liang W. Distribution of serum amyloid A and establishment of reference intervals in healthy adults. J Clin Lab Anal. 2020; 34 (4): e23120.
8. De Buck M, Gouwy M, Struyf S, Opdenakker G, Van Damme J. The ectoenzyme-side of matrix metalloproteinases (MMPs) makes inflammation by serum amyloid A (SAA) and chemokines go round. Immunol Lett. 2019 Jan; 205:1-8.
9. Su G, Ding L, Zhang Z. The Effect of Interleukin-6 Gene Polymorphism on Pediatric Pneumonia. Iran J Public Health. 2019; 48 (11): 2035-2040.
10. Abstracts of the 22nd International Symposium on Intensive Care and Emergency Medicine. Brussels, Belgium, 19-22 March 2002. Crit Care. 2002; 6 Suppl 1: S1-127.
11. Wang M, Zhu Q, Fu J, Liu L, Xiao M, Du Y. Differences of inflammatory and non-inflammatory indicators in Coronavirus disease-19 (COVID-19) with different severity. Infect Genet Evol. 2020; 85: 104511.
12. Vietri L, Fui A, Bergantini L, d'Alessandro M, Cameli P, Sestini P, Rottoli P, Bargagli E. Serum amyloid A: A potential biomarker of lung disorders. Respir Investig. 2020; 58 (1): 21-27.
13. Bouras M, Asehnoune K, Roquilly A. Contribution of Dendritic Cell Responses to Sepsis-Induced Immunosuppression and to Susceptibility to Secondary Pneumonia. Front Immunol. 2018; 9: 2590.
14. Roquilly A, McWilliam HEG, Jacqueline C, Tian Z, Cinotti R, Rimbert M, Wakim L, Caminschi I, Lahoud MH, Belz GT, Kallies A, Mintern JD, Asehnoune K, Villadangos JA. Local Modulation of Antigen-Presenting Cell Development after Resolution of Pneumonia Induces Long-Term Susceptibility to Secondary Infections. Immunity. 2017; 47 (1): 135-147.e5.
15. Lee WJ, Huang EY, Tsai CM, Kuo KC, Huang YC, Hsieh KS, Niu CK, Yu HR. Role of Serum Mycoplasma pneumoniae IgA, IgM, and IgG in the Diagnosis of Mycoplasma pneumoniae-Related Pneumonia in School-Age Children and Adolescents. Clin Vaccine Immunol. 2017; 24 (1): e00471-16.
16. He W, Xiao W, Zhang X, Sun Y, Chen Y, Chen Q, Fang X, Du S, Sha X. Pulmonary-Affinity Paclitaxel Polymer Micelles in Response to Biological Functions of Ambroxol Enhance Therapeutic Effect on Lung Cancer. Int J Nanomedicine. 2020; 15: 779-793.
17. Cao DW, Hou MX, Zhang XR. Ambroxol alleviates ventilator-induced lung injury by inhibiting c-Jun expression. Eur Rev Med Pharmacol Sci. 2019; 23 (11): 5004-5011.
18. Deretic V, Timmins GS. Enhancement of lung levels of antibiotics by ambroxol and bromhexine. Expert Opin Drug Metab Toxicol. 2019; 15 (3): 213-218.