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Effect of Xinxibao Spleen Combined with Aminopeptide on Erythrocyte Immunity and Toll-Like Receptors Signaling Pathways in Adjuvant Treatment of Children with Recurrent Respiratory Tract Infections

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ABSTRACT

The objective was this study to investigate the effect of spleen aminopeptide combined with xinxibao on erythrocyte immunity and Toll-like receptors (TLRs) signaling pathways in adjuvant treatment of children with recurrent respiratory tract infections (RRTI). A total of 138 children with RRTI admitted to our hospital from May 2017 to May 2019 were selected as the study subjects and randomly divided into 3 groups with 46 cases in each group. The 3 groups were given conventional treatment. On this basis, control group A was given spleen aminopeptide adjuvant treatment, the control group B was given xinxibao adjuvant treatment, while the observation group was given xinxibao combined with spleen aminopeptide adjuvant treatment. Statistical comparison was made on the clinical efficacy, clinical symptom improvement time and trace element calcium (Ca), zinc (Zn) content, peripheral blood T lymphocyte subsets ($CD4^+$, $CD8^+$), erythrocyte immunity indicators [Cooperative Tumor Erythrocyte Rosette (ATER), Erythrocyte Immune Adherence Enhancing Factor (FEER), Erythrocyte Immune Adherence Inhibiting Factor (FEIR)], Toll-like Receptor 2 (TLR2), Toll-like Receptor 4 (TLR4) level related to the TLRs signaling pathway before treatment, 2 weeks and 4 weeks after treatment. The observation group had higher total effective rate of clinical treatment than the control groups A and B, faster disappearance of high fever, cough, and pulmonary rales than the control groups A and B, showing statistically significant difference ($P<0.05$); after 2, 4 weeks of treatment, the observation group had higher contents of trace elements Ca and Zn than the control groups A and B, lower TLR2 and TLR4 levels than the control groups A and B, showing statistically significant difference ($P<0.05$); after 2, 4 weeks of treatment, the observation group had higher ATER, FEER, $CD4^+$, $CD4^+/CD8^+$ levels, lower FEIR and $CD8^+$ than the control groups A and B, showing statistically significant difference ($P<0.05$). It is concluded that the combination of spleen aminopeptide and xinxibao in adjuvant treatment of RRTI can alleviate children's symptoms, enhance peripheral T lymphocyte subsets, erythrocyte immunity, supplement trace element content, regulate TLRs signaling pathway, and further improve the therapeutic effect.

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Authors' Contribution
XL and RZ planned the study. YX, HY and LG counted the treatment data. FN and DL analyzed and MLN treated the data. All authors discussed the results and wrote the manuscript.

Key words
Recurrent respiratory tract infections, Xinxibao, Spleen aminopeptide, Trace element content, Immunity, TLRs signaling pathway.

INTRODUCTION

Recurrent respiratory tract infection (RRTI), a common respiratory disease in pediatrics, occurs more often in children aged 2-6 years. The protracted disease can attack repeatedly, with one-year occurrence frequency of bronchitis, cold, pneumonia and tonsillitis beyond the normal range and incidence rate in about 30% of children with respiratory infections (Toivonen *et al*., 2016; Santamaria *et al*., 2019; Jurkiewicz and Zielnik-Jurkiewicz, 2018). Studies have confirmed a certain relationship between the onset of RRTI and immunity. At present, clinical treatment of the disease is mainly through immunomodulation (Zhang *et al*., 2019). Spleen aminopeptide is a new type of oral multifunctional cellular immunopotentiator. After entering the human body, it can enhance the body immunity by enhancing lethal effect, replication effect, synergistic effect and proliferation effect of T lymphocytes (Tian *et al*., 2018). Xinxibao is a zinc supplement product that can enhance the body's immunity (Gammoh and Rink, 2017). In addition, studies have pointed out that TLRs signaling pathway is involved in the occurrence and development of respiratory inflammatory

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diseases, which can activate the body's innate immune cells and produce immune effects (Garziano, 2017). Based on this, this study treats RRTI children with spleen aminopeptide and xinxibao for the first time, and analyzes their effects on erythrocyte immunity and TLRs signaling pathway in the children. The results are as follows.

MATERIALS AND METHODS

Clinical data

After reviewed and approved by the ethics committee of our hospital, 138 RRTI children admitted to our hospital from May 2017 to May 2019 were selected as the study subjects. Inclusion criteria: (1) all meet the relevant diagnostic criteria of RRTI (Hai-Feng *et al*., 2014); (2) The children and their family members gave informed consent to this study and signed an informed consent form voluntarily. Exclusion criteria: (1) Patients complicated with respiratory diseases such as bronchiectasis, bronchial asthma, tuberculosis, *etc*.; (2) Patients complicated with dysfunction of vital organs such as heart, liver, and kidney; (3) Patients with primary immunodeficiency disease; (4) Those in allergic constitution, or having contraindications to the drugs used in this study; (5) Those intolerable to severe side effects during treatment; (6) Those with poor treatment compliance and cannot cooperate with this research. The subjects were randomly divided into 3 groups, each with 46 cases. The clinical data of the 3 groups (gender, age, course of disease, weight, number of respiratory infections, and respiratory infection site) are balanced and comparable (P>0.05), as shown in Table I.

Treatment methods

All three groups were given conventional treatment, and symptomatic and supportive treatments such as expectoration, cough relieving, asthma relieving, and oxygen inhalation were given in light of the children's condition; on this basis, the control group A was given spleen aminopeptide (Zhejiang Fengan Biological Pharmaceutical Co., Ltd., National Medicine Permission Number H10970214) adjuvant treatment, 2 mg/time, dissolved in 10 ml cold water and taken orally once a day; control group B was given xinxibao (Jinan Health Source Biotechnology Co., Ltd., National Medicine Permission Number G20140656) adjuvant treatment, 1 tablet for 1-3 years old, 2 tablets for 4-7 years old, chewed before meals, 3 times a day; observation group was given spleen aminopeptide combined with xinxibao adjuvant treatment. The usage and dosage of spleen aminopeptide, xinxibao were the same as those in the control groups A and B. All 3 groups were continuously treated for 1 month.

Detection method

Cubital venous blood (5 ml) was drawn from all the 3 groups on empty stomach, subject to 3000 r/min high-speed centrifugation to separate serum. DS-3C automatic trace element analyzer provided by Jining Dongsheng Electronics Equipment LTD. was used to analyze calcium (Ca), zinc (Zn) content. DxFLEX flow cytometer provided by Beckman Coulter Commercial Enterprise (China) Co., Ltd. was used to determine Toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4), peripheral blood T lymph subsets ($CD4^+$, $CD8^+$), erythrocyte immunity indicator [Cooperative Tumor Erythrocyte Rosette (ATER), Erythrocyte Immune Adherence Enhancing Factor (FEER), Erythrocyte Immune Adherence Inhibiting Factor (FEIR)] levels strictly following the instrument operating instructions.

Table I.- Comparison of clinical data of the 3 groups of children with recurrent respiratory tract infection.

Clinical data	Control group A (n=46)	Control group B (n=46)	Observation group (n=46)	t	P
Gender	28/18	25/21	26/20	0.415	0.813
Age (years)	2.5-6(4.25±1.02)	2-6(4.08±0.97)	3-6(4.41±0.79)	1.443	0.240
Course of disease (year)	2-5(3.37±0.75)	1-5(3.12±1.20)	1-5(2.94±0.92)	2.259	0.108
Weight (kg)				0.410	0.815
≤20	19(41.30)	22(47.83)	21(45.65)		
≥20	27(58.70)	24(52.17)	25(54.35)		
No. of respiratory infections (times/year)	8-14(10.54±1.49)	7-14(10.37±1.72)	7-13(10.12±1.56)	0.809	0.447
Respiratory tract infection site				0.410	0.815
Upper respiratory tract	24(52.17)	27(58.70)	25(54.35)		
Lower respiratory tract	22(47.83)	19(41.30)	21(45.65)		

Content group A received spleen aminopeptide treatment; content group B received Xinxibao adjuvant treatment and observation group received Xinxibao combined with spleen aminopeptide adjuvant treatment.

Table II.- Effect of xinxibao administered with spleen aminopeptide adjuvant on clinical efficacy and clinical symptom improvement time of children with recurrent respiratory tract infection [n(%)].

	Control group A (n=46)	Control group B (n=46)	Observation group (n=46)	*F*	P
Clinical efficacy (n(%))					
Clinically cured	6(13.04)	5(10.87)	18(39.13)		
Markedly effective	12(26.09)	13(28.26)	10(21.74)		
Effective	18(39.13)	16(34.78)	15(32.61)		
Ineffective	10(21.74)	12(26.09)	3(6.52)		
Total effective rate	36(78.26)	34(73.91)	43(93.48)		0.038
Clinical symptom improvement time ($\bar{x}$±s, d)					
Disappearance time of high fever	3.34±0.37	3.50±0.43	2.72±0.31	56.05	<0.001
Disappearance time of cough	4.98±0.30	5.12±0.34	3.94±0.25	213.923	<0.001
Disappearance time of pulmonary rales	4.37±0.34	4.52±0.38	3.31±0.28	177.407	<0.001

For details of groups, see Table 1.

Table III.- Effect of xinxibao administered with spleen aminopeptide adjuvant on the trace elements in children with recurrent respiratory tract infection ($\bar{x}$±s).

	Control group A (n=46)	Control group B (n=46)	Observation group (n=46)	F	P
Ca (mmol/L)					
Before treatment	2.04±0.26	2.05±0.25	2.02±0.24	0.172	0.843
2 weeks after treatment	2.20±0.12	2.15±0.13	2.31±0.15	17.186	<0.001
4 weeks after treatment	2.27±0.10	2.25±0.12	2.37±0.13	13.811	<0.001
Zn (μmol/L)					
Before treatment	7.45±1.03	7.42±1.01	7.41±1.01	0.019	0.981
2 weeks after treatment	10.87±1.09	11.98±1.12	12.84±1.15	35.753	<0.001
4 weeks after treatment	12.42±1.18	13.15±1.20	14.29±1.24	28.050	<0.001

For details of groups, see Table 1.

Observation indicators

The following observation indicators were followed: (1) clinical efficacy and efficacy criteria: clinically cured: no recurrence within 1 year; markedly effective: the number of respiratory tract infections is reduced by more than 2/3 within 1 year with significantly improved symptoms; effective: the number of respiratory tract infections is reduced by 1/2~2/3 within 1 year, with symptoms and signs improved to some extent; ineffective: the number of respiratory infections in 1 year has been reduced by less than 1/2, with insignificant change in symptoms and signs after treatment. Total effective rate = (clinically cured + markedly effective + effective)/46×100% (Somnath *et al*., 2017). (2) Clinical symptom improvement time, including the disappearance time of high fever, cough, and pulmonary rales. (3) The content of trace elements Ca and Zn before treatment, 2 weeks and 4 weeks after treatment. (4) Erythrocyte immunity indicators before treatment, 2 weeks and 4 weeks after treatment, including ATER, FEER, FEIR. (5) Peripheral blood T lymphocyte subsets before treatment, 2 weeks, and 4 weeks after treatment, including $CD4^+$, $CD8^+$, $CD4^+/CD8^+$. (6) TLR2 and TLR4 levels before treatment, 2 weeks and 4 weeks after treatment.

Statistical methods

Data is analyzed through SPSS21.0, measurement data is represented by ($\bar{x}$ ±s) and tested by t, count data is represented by n (%) and tested by χ^2. SNK-q test is used for pairwise comparison, P<0.05 indicates statistically significant difference.

RESULTS

Clinical efficacy

The observation group has higher total effective rate of clinical treatment than the control groups A and B, showing statistically significant difference (P<0.05). In comparison of the control groups A and B, the difference is not statistically significant (P>0.05), as shown in Table II.

Clinical symptom improvement time

The observation group has faster disappearance of high fever, cough, and pulmonary rales than the control

groups A and B, showing statistically significant difference (P<0.05). In comparison of the control groups A and B, the difference is not statistically significant (P>0.05), as shown in Table II.

Trace elements

After 2, 4 weeks of treatment, the observation group has higher contents of trace elements Ca and Zn than the control groups A and B, showing statistically significant difference (P<0.05). Control groups A, B show no statistically significant difference in Ca content (P>0.05), while control group B has higher Zn content than control group A, showing statistically significant difference (P<0.05), as shown in Table III.

Erythrocyte immunity

After 2, 4 weeks of treatment, the observation group has higher ATER, FEER levels, lower FEIR than the control groups A and B, showing statistically significant difference (P<0.05). Control group has higher AATER, FEER levels and lower FEIR than control group B, showing statistically significant difference (P<0.05), as shown in Table IV.

Table IV.- Effect of xinxibao administered with spleen aminopeptide adjuvant on erythrocyte immunity indicators in children with recurrent respiratory tract infection ($\bar{x}$±s, %).

	Control group A (n=46)	Control group B (n=46)	Observation group (n=46)	F	P
ATER					
Before treatment	57.14±4.17	55.74±4.20	56.32±4.15	1.307	0.274
2 weeks after treatment	60.12±3.47	58.27±3.05	62.47±3.56	17.975	<0.001
4 weeks after treatment	63.14±3.07	61.74±3.02	64.89±3.16	12.048	<0.001
FEER level					
Before treatment	59.46±5.78	58.98±6.07	60.47±6.14	0.739	0.479
2 weeks after treatment	64.74±5.09	62.19±4.78	67.89±5.14	14.966	<0.001
4 weeks after treatment	67.21±5.29	64.32±5.37	70.47±5.72	14.591	<0.001
FEIR level					
Before treatment	49.12±3.24	48.97±2.98	48.35±3.21	0.775	0.463
2 weeks after treatment	38.13±2.49	42.47±2.31	35.94±2.56	84.261	<0.001
4 weeks after treatment	34.59±2.58	36.12±2.73	32.29±2.97	22.368	<0.001

ATER, Cooperative tumor erythrocyte rosette; FEER, Erythrocyte immune adherence enhancing factor; FEIR, Erythrocyte immune adherence inhibiting factor.
For details of groups, see Table 1.

Table V.- Effect of xinxibao administered with spleen aminopeptide adjuvant on peripheral blood T lymphocyte subsets in children with recurrent respiratory tract infection ($\bar{x}$±s).

	Control group A (n=46)	Control group B (n=46)	Observation group (n=46)	F	P
$CD4^+$ (%)					
Before treatment	27.49±5.26	28.14±5.19	28.52±5.14	0.462	0.631
2 weeks after treatment	34.96±4.58	32.10±4.47	37.41±4.92	14.958	<0.001
4 weeks after treatment	38.45±3.24	35.85±4.13	40.27±3.61	16.779	<0.001
$CD8^+$ (%)					
Before treatment	32.26±4.79	32.85±4.68	31.74±4.72	0.634	0.532
2 weeks after treatment	27.89±3.12	29.50±3.04	25.19±3.25	22.159	<0.001
4 weeks after treatment	25.83±3.14	27.66±3.07	23.92±3.01	17.028	<0.001
$CD4^+$ / $CD8^+$					
Before treatment	0.85±0.15	0.86±0.14	0.90±0.12	1.710	0.185
2 weeks after treatment	1.25±0.17	1.09±0.19	1.49±0.21	51.270	<0.001
4 weeks after treatment	1.49±0.17	1.30±0.14	1.68±0.15	70.166	<0.001

For details of groups, see Table 1.
TLR, toll-like receptor.

Table VI.- Effect of xinxibao administered with spleen aminopeptide adjuvant on TLRs signaling pathway related factors of children with recurrent respiratory tract infection ($\bar{x}$±s, %).

	Control group A (n=46)	Control group B (n=46)	Observation group (n=46)	F	P
TLR2					
Before treatment	11.07±1.85	10.57±1.79	10.92±1.74	0.941	0.393
2 weeks after treatment	5.74±0.59	6.48±0.63	4.52±0.56	127.702	<0.001
4 weeks after treatment	1.17±0.19	1.57±0.24	0.84±0.13	166.740	<0.001
TLR4					
Before treatment	6.94±0.98	6.51±0.84	6.67±0.72	2.984	0.054
2 weeks after treatment	3.25±0.47	3.76±0.50	2.98±0.41	33.885	<0.001
4 weeks after treatment	1.36±0.19	1.57±0.21	0.97±0.14	128.182	<0.001

For details of groups, see Table 1.
TLR, toll-like receptor.

Peripheral blood T lymphocytes

After 2 weeks and 4 weeks of treatment, the observation group has higher $CD4^+$, $CD4^+/CD8^+$ levels, lower $CD8^+$ level than control groups A, B, showing statistically significant difference (P<0.05). Control group A has higher $CD4^+$, $CD4^+/CD8^+$ levels, lower $CD8^+$ than control group B, showing statistically significant difference (P<0.05), as shown in Table V.

TLRs signaling pathway related factors

After 2 weeks and 4 weeks of treatment, the observation group has lower TLR2, TLR4 levels than control groups A, B, showing statistically significant difference (P<0.05). Control group A has lower TLR2, TLR4 levels than control group B, showing statistically significant difference (P<0.05), as shown in Table VI.

DISCUSSION

RRTI is a common disease in children, which has repeated attacks over the years in a mixture of old and new illness, seriously affecting physical and mental health of the children and increasing mental and economic burden of the parents (de Oliveira *et al*., 2019; Li *et al*., 2019). It is currently believed that in addition to the anatomical and physiological characteristics of the respiratory tract in children, RRTI is also closely related to the low immunity of children (Koatz *et al*., 2016; Saad *et al*., 2016). Therefore, clinical treatment of RRTI is mostly given through immunomodulation to achieve the purpose of healing.

Spleen aminopeptide is a compound preparation of nucleotides and peptides extracted from fresh animal spleen. Studies have pointed out that the drug has certain curative effects on RRTI and can effectively regulate the body immunity (Wu *et al*., 1997). With the continuous deepening of clinical research, it is found that when the body lacks trace element zinc, the function of helper T lymphocytes will be impaired, leading to decline of body immunity, which is also one of the pathogenesis of RRTI (Ma *et al*., 2018). With zinc, selenium and iodine protein powder as its main ingredient, xinxibao can quickly increase the blood zinc content in the body after administration. Based on this, in this study, spleen aminopeptide was combined with xinxibao for the first time to treat RRTI children. The results indicate that the observation group has higher total effective rate of clinical treatment than the control groups A and B, faster disappearance of high fever, cough, and pulmonary rales than the control groups A and B, suggesting that the combination of spleen aminopeptide and xinxibao in the treatment of RRTI children can promote children's symptom improvement and further enhance the treatment effect. As a new type of immunomodulator, spleen aminopeptide is rich in essential trace elements, amino acids, and immunoregulatory factors for the human body, which can enhance lethality of T lymphocytes, enhance the synergy, replication and proliferation effects, promote the release of lymphokines and interferons, thereby exerting strong antibacterial and anti-infection effects, enhancing the body's immune function (Tian *et al*., 2018). Zinc can stabilize immune function. When the body lacks zinc, body fluid and cellular immunity abnormality will occur. The weight of immune organs such as thymus and spleen can be reduced by about 30%. Thymus atrophy causes decreased thymus hormone activity and reduced number of T lymphocytes. Spleen atrophy means weakening immune response of the body. Moreover, the lack of zinc can also inhibit nucleic acid and protein synthesis, delay children's growth and development,

and reduce the body's immunity. After administration, xinxibao can quickly take effect, effectively replenish zinc content, and promote biological enzyme activity, thus smoothly synthesizing proteins, sugars, fats, nucleic acids, etc. while enhancing T cell function and strengthening the body's immunity (Dąbrowska-Leonik *et al*., 2018). Therefore, spleen aminopeptide combined with xinxibao in the treatment of RRTI children has a synergistic effect to further enhance the therapeutic effect. In addition, because spleen aminopeptide and xinxibao can supplement trace elements and enhance the function of T lymphocytes, in this study, the observation group has obviously increased trace elements Ca, Zn, $CD4^+$, $CD4^+/CD8^+$ and significantly decreased $CD8^+$ after 2 weeks and 4 weeks of treatment.

In the past, T lymphocytes were the main indicators of immune status of the body in clinical research, and there is rare research on the role of erythrocyte immunity in RRTI. Erythrocyte has effective defense effect on the body, and also plays a positive role in enhancing immune response of T lymphocytes (Maggini *et al*., 2018). This study analyzed erythrocyte function of RRTI patients after combination therapy of spleen aminopeptide and xinxibao. The results show that the observation group has higher ATER and FEER levels, lower FEIR than the control groups A and B after 2 weeks and 4 weeks of treatment, suggesting that spleen aminopeptide combined with xinxibao can significantly improve the erythrocyte immunity of RRTI children, which also confirms from the perspective of erythrocyte that the combination of spleen aminopeptide and xinxibao can enhance the body's immune regulation.

As a group of transmembrane proteins that can identify pathogenic microorganisms and cell wall products, TLRs can resist the invasion of pathogenic microorganisms and endogenous immune substances, and play an important role in the pathogenesis of respiratory diseases, especially immune-related diseases, thus having close relation with onset of respiratory infections (Kostakou *et al*., 2019; Huang *et al*., 2019). The TLRs signaling pathway is a type of transmembrane non-catalytic single receptor that can recognize pathogen-derived molecules, thereby activating the body's innate immunocytes and producing complex immunological effects (Roozbehkia *et al*., 2017). In the TLRs family, TLR2 and TLR4 are closely related to respiratory diseases, which play an important role in identifying infectious pathogens, regulating early host inflammation, immune response, and pathogenesis. In this study, TLRs signaling pathway related factors TLR2 and TLR4 are used as indicators to investigate whether spleen aminopeptide combined with xinxibao can improve immune function through TLRs signaling pathway (Xie *et al*., 2018). The results show that the observation group has lower TLR2 and TLR4 than the control groups A and B after 2 and 4 weeks of treatment, suggesting that the combination of spleen aminopeptide and xinxibao can significantly enhance RRTI children's immunity, lower serum TLR2 and TLR4 levels (Xie *et al*., 2018). The reason is that both spleen aminopeptide and xinxibao can correct the immunity dysregulation in RRTI children, improve the immune function, enhance the body's anti-infection capacity, and inhibit the inflammation and immune response of RRTI children, thereby regulating TLRs signaling pathway and finally achieving the purpose of disease recovery.

CONCLUSION

To conclude, the combination of spleen aminopeptide and xinxibao in adjuvant treatment of RRTI can improve children's symptoms, enhance peripheral T lymphocyte subsets and erythrocyte immunity, supplement trace element content, regulate TLRs signaling pathways, and further improve the therapeutic effect. However, this study has small sample size, and clinical multi-center, multi-channel sampling is needed for further confirmation.

Statement of conflict of interest

The authors have declared no conflict of interests.

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