EFFECTS OF TANSHINONE IIA COMBINED WITH MONTELUKAST ON COAGULATION, INFLAMMATION AND TH17/TREG CELL IMMUNITY IN CHILDREN WITH HENOCH-SCHONLEIN PURPURA

Ting Guo[1], Jizu Ling[1], Xu Fu[2], Zhongbin Tao[1], Yuning Li[1,*]
[1]Department of Pediatrics, The First Hospital of Lanzhou University, Lanzhou, PR China - [2]Lanzhou First People's Hospital, Lanzhou, PR China

ABSTRACT

Objective: *To investigate the effect of tanshinone IIA combined with montelukast on coagulation, inflammatory response and Th17/Treg cell immunity in children with Henoch-Schonlein purpura.*

Methods: *From January 2014 to January 2017, 84 children with anaphylactoid purpura were divided into an observation group (n = 42) and a control group (n = 42). The control group received routine treatment, while the observation group was treated with tanshinone IIA combined with montelukast based on routine treatment. Coagulation function, inflammatory reaction and Th17/Treg cell immune level were compared between the two groups.*

Results: *After treatment, the total effective rate was 95.23% in the observation group in comparison to 80.95% in the control group, a statistically significant difference (P<0.05). After treatment, the APTT level in both groups was significantly higher than that before treatment, while the FIB level in the observation group was significantly lower than that before treatment, also a statistically significant difference (P<0.05). After treatment, the level of IL-12 in both groups was significantly higher than that before treatment. Meanwhile, the level of IL-17 in the observation group was significantly lower than that in the control group (P<0.05), and the difference was statistically significant (P<0.05). Before treatment, no statistical difference was seen in Th17/Treg cell immunity (P>0.05). After treatment, the level of Th17 and Th17/Treg cells in both groups was significantly higher than that before treatment; moreover, the Treg cell level in the observation group was significantly higher than that in the control group (P<0.05), and the difference was statistically significant (P<0.05). After treatment, the CD_{19}^{+} level of the two groups was significantly lower than that before treatment, and the difference was statistically significant (P<0.05). Meanwhile, the level of $CD_3^{+}CD_4^{+}/CD_8^{+}$ in the observation group was significantly higher than that in the control group (P<0.05).*

Conclusion: *Tanshinone IIA combined with montelukast can improve coagulation function as well as reduce the inflammatory response and Th17/Treg cell immunity in children with purpura.*

Keywords: *Tanshinone IIA, montelukast, anaphylactoid purpura in children, coagulation function, inflammatory response, Th17/Treg cell immunity.*

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Introduction

Henoch-Schonlein purpura (HSP) is a type of autoimmune disease based on small-vessel inflammation. Its clinical manifestations mainly involve gastrointestinal mucosal haemorrhage, skin purpura and renal injury with joint swelling, pain and diffuse abdominal pain. This malady has a long course and is prone to recurrent attack(1). The treatment of HSP is mainly based on medication and treatment of symptoms, but because of its high incidence and poor prognosis, it has become the focus of paediatric research(2). The pathogenesis of HSP is mainly related to immune dysfunction, and no special drug treatment is currently available. The occurrence of HSP is related to an abnormal level of inflammatory mediators and cytokines in the body; therefore, improving bodily inflammation can be helpful in

clinical treatment of the disease[3]. Tanshinone IIA's effects include anti-atherosclerosis, repairing endothelial cells, optimizing coronary circulation and inhibiting thrombosis. Montelukast is an anti-inflammatory drug that can reduce allergic reactions and effectively relieve the symptoms caused by purpura. This study analysed the effects of tanshinone IIA combined with montelukast on coagulation, inflammatory response and Th17/Treg cell immunity in children with HSP[4].

Materials and methods

General information

From January 2014 to January 2017, 84 children with anaphylactoid purpura admitted to our hospital were randomly divided into an observation group (n = 42) and a control group (n = 42) for treatment. In the observation group, the course of the disease was 1-14 days, averaging (2.53±1.21) days.

The age of participants in this group ranged from 2 to 13 years, with an average age of (5.16±1.96) years. In the control group, the course of the disease was 1-13 days, with an average of (2.13±1.06) days. The age of participants in this group ranged from 3 to 14 years, with an average age of (5.94±2.65).

The general data for the two groups were comparable (P>0.05). Inclusion criteria comprised the first use of such drugs and the first diagnosis, consistent with the diagnosis of simple anaphylactoid purpura. In addition, participants were required to actively cooperate with the treatment and examination, and the family members of the children signed an informed consent. Children aged 2 to 14 years were selected. Exclusion criteria concerned patients with other diseases and infections, those receiving other drugs, patients with autoimmune diseases before onset or having serious systemic diseases and mental diseases who would not be able to cooperate with the treatment as well as those who had shown allergic reactions to therapeutic drugs. This study was approved by the hospital ethics committee.

Method

After admission, the patients were given anti-allergy, anti-infection, antihistamine, calcium and nutritional supplementation, improving circulation along with other symptomatic treatment. Patients with infection were given anti-infective drugs, while those with gastrointestinal reactions were given omela and other symptomatic treatment. In addition, those with abdominal pain and joint swelling were given glucocorticoid treatment, and those exhibiting damage to liver and kidney function received anticoagulant treatment. In comparison to the control group, the observation group was treated with tanshinone IIA combined with montelukast. Tanshinone IIA injection (Shanghai first biochemical pharmaceutical Co., Ltd, batch number: 140613, specification: 10mg/2ml) was given in the following doses: 20 mg / time to children under six years old, 25 mg/time to children between six and ten years old, and 40 mg/time to children over ten years old.

After dilution with 5% glucose solution, it was given by intravenous drip once a day for 14 days. Montelukast (Hangzhou Minsheng Binjiang Pharmaceutical Co., Ltd, batch number: 140256, specification: 10mg) was given in the following amounts: 4mg/time to children under six years old, 5mg/time to children between six and ten years old, and 10mg/time to children over ten years old. This medication was administered with warm water before bedtime, once a day, for one month.

Efficacy judgement and observation indicators

Significant effect

Purpura disappeared without joint pain, abdominal pain or other adverse reactions. In observing the therapeutic effect on purpura within one month, effective treatment was defined as no new purpura appearing within half a month, while ineffective treatment would show repeated purpura within one month and aggravated symptoms. The regression of purpura and the incidence of adverse reactions were recorded. Total effective rate = effective + effective.

The concentration of fibrinogen FIB was determined by enzyme-linked immunosorbent assay (ELISA). APTT was measured by semi-automatic coagulation analyser.

The indexes of inflammatory factors were detected by ELISA kit, and the operation was carried out in strict accordance with the instructions.

Th17/Treg cell immune level was measured by flow cytometry produced by U.S. Becton, Dickinson and Company (BD). CD_3^+, CD_{19}^+ and CD_4^+/CD_8^+ were detected by flow cytometry.

Statistical methods

SPSS 11.0 was used to process the data. The measurement data were expressed by ($\bar{x}\pm s$), t-test, percentage (%) and χ^2 test, with P<0.05 as the difference.

Results

Clinical efficacy

After treatment, the total effective rate was 95.23% in the observation group and 80.95% in the control group (P<0.05). See Table 1.

Group	Cases	Markedly effective	Effective	Invalid	Total effective rate
Observation group	42	35 (83.33)	5 (11.90)	2 (4.76)	40 (95.23)
Control group	42	10 (23.80)	8 (19.04)	8 (19.04)	34 (80.95)
χ^2					4.086
p					0.043

Table 1: Comparison of clinical effects [n (%)].

Coagulation function

Before treatment, no significant difference was seen between the APTT level and FIB level in the two groups (P>0.05).

After treatment, not only was the APTT level in both groups significantly higher than before treatment, but the observation group was significantly higher than the control group (P<0.05). In addition, FIB was significantly lower than before treatment, with the observation group showing statistically significantly lower values than the control group (P<0.05). See Table 2.

Group	Cases	APTT (s)		FIB (g /L)	
		Before treatment	After treatment	Before treatment	After treatment
Observation group	42	23.56±7.15	36.24±9.53*	4.64±0.49	2.57±0.13*
Control group	42	23.46±7.39	31.74±7.61*	4.56±0.38	3.65±0.49*
t		0.063	2.391	0.836	13.806
p		0.949	<0.001	0.406	<0.001

Table 2: Coagulation index ($\bar{x}\pm s$).
*Note: Compared with the same group before treatment *P<0.05.*

Inflammatory reaction

Before treatment, no statistical difference was shown in the serum levels of IL-12 and IL-17 between the two groups (P>0.05).

After treatment, the level of IL-12 in both groups was significantly higher than that before treatment; moreover, the level of IL-17 in the observation group was significantly higher than that in the control group (P<0.05), and the level of IL-17 in the observation group was statistically significantly lower than that before treatment (P<0.05). See Table 3.

Group	Cases	IL-12 (μg/L)		IL-17 (μg/L)	
		Before treatment	After treatment	Before treatment	After treatment
Observation group	42	172.56±52.41	232.45±63.56*	86.12±40.54	62.45±14.45*
Control group	42	171.23±21.65	150.55±68.45*	86.44±41.56	74.62±31.58*
t		0.152	5.682	0.035	2.271
p		0.879	<0.001	0.971	<0.001

Table 3: Comparison of serum inflammatory factors ($\bar{x}\pm s$).
*Note: Compared with the same group before treatment *P<0.05.*

Th17/Treg cellular immunity

Before treatment, no statistical difference in Th17/Treg cell immune indexes (P>0.05) was observed. After the treatment, the Th17 and Th17/Treg cell levels in both groups were significantly lower than those before treatment; moreover, the Treg cell level in the observation group was significantly higher than that in the control group (P<0.05). The difference was statistically significant (P<0.05). See Table 4.

Group	Cases	Th17/Treg		Th17 (%)		Treg (%)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	42	3.75±0.16	1.18±0.62*	13.46±1.23	7.23±0.36*	3.55±0.53	6.78±0.46*
Control	42	3.64±0.86	1.98±0.79*	13.55±1.54	10.96±0.69*	3.45±0.64	5.27±0.13*
t		0.814	5.162	0.295	31.060	0.779	20.471
p		0.417	<0.001	0.768	<0.001	0.437	<0.001

Table 4: Th17/Treg cell immune response comparison ($\bar{x}\pm s$).
*Note: Compared with the same group before treatment *P<0.05.*

Immune function index

Before treatment, no significant difference was remarked in the immune function between the two groups (P>0.05). After treatment, however, the CD19+ level of both groups was significantly lower than that before treatment, a difference that was statistically significant (P<0.05). In addition, the levels of CD_3^+, CD_4^+/CD_8^+were significantly higher in the observation group than in the control group (P<0.05). See Table 5.

Group	Cases	CD_{19}^+ (%)		CD_3^+ (%)		CD_4^+/CD_8^+	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation group	42	22.35±3.45	13.59±2.56*	55.65±4.94	59.62±3.45*	1.09±0.36	1.32±0.28*
Control	42	22.55±3.65	15.63±2.96*	55.15±4.85	57.65±3.96*	1.08±0.34	1.26±0.20*
t		0.258	3.378	0.468	2.430	0.130	1.130
p		0.797	<0.001	0.641	<0.001	0.896	<0.001

Table 5: Comparison of immune function indexes ($\bar{x}\pm s$).
*Note: Compared with the same group before treatment *P<0.05.*

Discussion

Henoch-Schonlein purpura (HSP) is a type of allergic disease of the capillaries. Because the body is allergic to a certain substance, the systemic arterioles are damaged, mainly affecting the capillaries of the skin and other organs. The pathological basis involves extensive inflammation of the small vessels. The clinical manifestations mainly include pain and swelling of the joints, skin purpura, different degrees of rash, gastrointestinal mucosal haemorrhage, abdominal pain, nephritis and infection[5]. HSP can cause pathological changes in multiple systems of the entire body, causing irreversible deformity in severe cases. Effective treatment should be taken promptly[6]. Because the inducing factors and aetiology of HSP are not clear, anti-inflammatory drugs are widely used to improve symptoms[7]. Tanshinone IIA is one of the components of Salvia miltiorrhiza, including many tanshinone derivatives that have the effect of promoting blood circulation, removing blood stasis and dysmenorrhea and offering pain relief, among which the effect of anti-inflammatory and vasodilation is significant[8]. Tanshinone IIA can inhibit the occurrence of thrombotic diseases, reduce the oxygen consumption of myocardium, improve hypoxia, inhibit platelet aggregation and reduce blood viscosity[9].

Montelukast, a selective leukotriene receptor antagonist, shows significant effects on inflammation, asthma and allergic reactions. It can improve vascular permeability, reduce the inflammatory reaction of small vessels and shorten the treatment time of purpura[10]. In children with HSP, the results of an associated change of capillary permeability can cause the dysfunction of the entire body's fibrinolysis and coagulation system and, in serious cases, can damage renal function[11]. This study shows that tanshinone IIA combined with montelukast special envoy can significantly improve the coagulation function of children, improve the therapeutic effect of allergic purpura, expand microvasculature, improve microcirculation, inhibit thrombosis and significantly reduce the recurrence rate. The results of this study showed that after treatment, the total effective rate for patients in the observation group was 95.23% as opposed to a total effective rate for patients in the control group of 80.95%. The level of IL-12 in the control group was lower than that in the observation group, and the level of IL-17 in the control group was higher than that in the observation group, indicating that tanshinone IIA combined with montelukast can also improve the coagulation function and the level of serum inflammatory factors, promoting recovery in the body.

Th17/Treg cells are two subpopulations of CD_4^+ T lymphocytes, which play an important role not only in maintaining the body's immunity but throughout the entire pathological process[12]. Th17 cell subsets are involved in autoimmune diseases through proinflammatory factors such as the IL-17 level, which can mediate the inflammatory response of local tissues[13]. Treg is a cell group having immunomodulatory ability, which secretes IL-10 and other cytokines and is closely related to the pathogenesis of various immune diseases[14]. The decrease of Treg cells can lead to a decrease in immunity[15]. The reaction degree of disease progression in children with purpura can manifest as an increase in the number of B-lymphocytes and an increase of CD_{19}^+. This study shows that tanshinone in combination with montelukast can improve levels of IL-12, Treg, CD_3^+ and CD_4^+/CD_8^+ and reduce the levels of IL-17, Th17, Th17/Treg and CD_{19}^+, an indication that tanshinone IIA combined with montelukast can significantly reduce the inflammatory response and improve the immune function of children with purpura.

In conclusion, tanshinone IIA combined with montelukast can improve the coagulation function along with reducing the inflammatory response and Th17/Treg cell immunity in children with purpura.

References

1) Qu P, Li WL. Compound DanShen Injection in Treating 41 Cases of Severe Preeclampsia Postpartum Hemorrhage. West J Tradit Chin Med 2019; 32: 113-115.
2) Gong YH, Xu ZL. The effect of hemoperfusion on children's Henoch Schonlein purpura and its influence on coagulation, inflammatory factors and immune function. Clin J Pract Hospital 2019; 16: 108-110.
3) Lópezmejías R, Castañeda S, Genre F, Remuzgo-Martínez S, Carmona FD, et al. Genetics of immunoglobulin-A vasculitis (Henoch-Schönlein purpura): An updated review. Autoimmun Rev 2018; 17: 301-315.
4) Ye LJ, Li JK, Xiao QW. Clinical analysis of Montelukast Combined with tanshinoneIIA sodium sulfonate in the treatment of simple Henoch Schonlein purpura in children. J Guangxi Med Univ 2018; 35: 134-137.
5) Schwarzová L, Pirník Z, Štecová N. Idiopathic thrombocytopenia refractery to therapy of cyclosporine A in clinical practice - case report. Ceska Slov Farm 2017; 66: 233-237.

6) Jiang X. Effects of Montelukast Combined with loratadine on serum TNF - α, IL-6 and OPN levels in children with Henoch Schonlein purpura. J Henan Univ (medical) 2018; 37: 32-34.
7) Wei Y, Li DS, Liu JJ, Zhang J, Zhao HE. Therapeutic effect and safety of montelukast sodium combined with budesonide in children with cough variant asthma: a Meta analysis. Zhongguo dang dai er ke za zhi 2016; 18: 1100-1105.
8) Wu SH, Liao PY, Chen XQ, Yin PL, Dong L. Add-on therapy with montelukast in the treatment of Henoch–Schönlein purpura. Pediatr Int 2014; 56: 315-322.
9) Jia MH, Chen XY, Zhang Y, Liao ZS. [Effect of nasal glucocorticoid combined with loratadine or montelukast on allergic rhinitis]. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2017; 31: 369-373.
10) Chen LF. The effect of budesonide combined with montelukast in the treatment of bronchial asthma. Chin J Primary Med Pharm 2017; 24: 3406-3410.
11) Salahshoor MR, Roshankhah S, Hosseni P, Jalili C. Genistein Improves Liver Damage in Male Mice Exposed to Morphine. Chin Med J (Engl) 2018; 131: 1598-1604.
12) Li YM. Cimetidine combined with montelukast sodium in the treatment of Henoch Schonlein purpura in children. J Pediatric Pharm 2019; 25: 34-37.
13) Hu AJ. Effect of Huaiqihuang granule combined with montelukast on cough variant asthma in children. J New Chin Med 2018; 50: 170-173.
14) Lim CH, Lee PC, Lim E, Tan J, Chan WH, et al. Correlation Between Symptomatic Gastro-Esophageal Reflux Disease (GERD) and Erosive Esophagitis (EE) Post-vertical Sleeve Gastrectomy (VSG). Obes Surg 2019; 29: 207-214.
15) Qin RJ, Wang HJ, Meng NA. Clinical observation of liangxuezhuyu decoction combined with montelukast sodium chewable tablets in the treatment of infantile anaphylactoid purpura. J Hainan Med Univ 2018; 50: 136-139.

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Corresponding Author:
Yuning Li
Email: dper71@163.com
(China)