



Evaluation of the cytokine levels and immune response status of montelukast, loratadine and tanshinone combination therapy for Henoch–Schonlein purpura

Wu–Qiong Duan[✉], Chang–Huai Zhou, Ming–Hai Luo

Department of Pediatrics, Ankang Central Hospital in Shanxi Province, Ankang City, Shanxi Province, 725000

ARTICLE INFO

Article history:

Received 12 Jun 2017

Received in revised form 19 Jun 2017

Accepted 3 Jul 2017

Available online 14 Jul 2017

Keywords:

Henoch-Schonlein purpura

Montelukast

Loratadine

Tanshinone

Immune response

ABSTRACT

Objective: To evaluate the cytokine levels and immune response status of montelukast, loratadine and tanshinone combination therapy for Henoch-Schonlein purpura. **Methods:** A total of 80 patients with Henoch-Schonlein purpura who were treated in Ankang Central Hospital between May 2014 and January 2017 were collected and divided into montelukast group, loratadine group, tanshinone group and combined treatment group according to the random number table, 20 cases in each group. Serum levels of inflammatory factors, Th17/Treg cellular immunity indexes before and after treatment were compared among four groups of patients. **Results:** Before treatment, differences in serum levels of inflammatory factors and Th17/Treg cellular immunity indexes were not statistically significant among four groups of patients. After treatment, serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels in combined treatment group were lower than those in montelukast group, loratadine group and tanshinone group; serum IL-17 level was lower than that in montelukast group, loratadine group and tanshinone group while IL-10 and TGF- β levels were higher than those in montelukast group, loratadine group and tanshinone group. **Conclusions:** Montelukast, loratadine and tanshinone combination therapy for Henoch-Schonlein purpura helps to reduce systemic inflammatory response and balance Th17/Treg cell immunity.

1. Introduction

Henoch-Schonlein purpura is the hypersensitivity vasculitis that invades the skin and tissue organ cell artery/capillary, it tends to occur in children and adolescents, it may be accompanied by abdominal pain and joint pain in addition to the typical purpura performances, but blood routine shows the platelet count does not reduce[1,2]. The cause of anaphylactoid purpura is not clear, the severe cases can cause the injury or even failure of multiple important viscera, and therefore, early sensitive and reliable treatment is needed. Montelukast and loratadine are the most common western medicines for the treatment of anaphylactoid purpura, which help to reduce its clinical symptoms; tanshinone is an extract compound of dangingseng, which has the effect of promoting blood circulation to remove blood stasis and has been

proven to be able to improve the condition of Henoch-Schonlein purpura[3–5]. At present, monotherapy of these three drugs has been clinically applied, but the efficacy differs, and there are limitations in terms of reversing the disease, so many current scholars have put forward the combination of drugs with different mechanisms of action in order to enlarge the final results. In the study, the effect of different drug compatibility on Henoch-Schonlein purpura condition was explored in order to provide practical evidence for the treatment of patients with similar disease.

2. Information and methods

2.1 General information

A total of 80 patients with Henoch-Schonlein purpura who were treated in Ankang Central Hospital between May 2014 and January 2017 were selected as the research subjects, and the family members of patients signed the informed consent. According to the random number table method, they were divided into montelukast group, loratadine group, tanshinone group and combined treatment group, 20 cases in each group. Montelukast group included 11 men

[✉]Corresponding author: Wu-Qiong Duan, Department of Pediatrics, Ankang Central Hospital in Shanxi Province, Ankang City, Shanxi Province, 725000.

Tel: 13891515960

Fund Project: Social Development Key Project of Shaanxi Province No: 1024k11-01-01-03.

and 9 women that were 7-41 years old, and with disease course of 2-10 d; loratadine group included 10 men and 10 women that were 7-37 years old, and with disease course of 1-12 d; tanshinone group included 12 men and 8 women that were 6-45 years old, and with disease course of 2-13 d; combined treatment group included 11 men and 9 women that were 5-39 years old, and with disease course of 2-13 d. The differences in gender, age, and disease course were not statistically significant among the four groups ($P>0.05$), and the hospital ethics committee approved the study.

2.2 Diagnostic criteria for Henoch-Schonlein purpura

(1) The purpura of both lower extremities; (2) with abdominal pain and joint pain; (3) blood routine examination showed mild-moderate increase of hemocyte, and normal or elevated eosinophilic cells; (4) erythrocyte sedimentation rate might accelerate and immunoglobulin IgA might increase; (5) might be associated with kidney damage.

2.3 Inclusion and exclusion criteria

Inclusion criteria: (1) diagnosed for the first time and not receiving systematic treatment before; (2) cooperating with whole examination and treatment and with complete data. Exclusion criteria: (1) associated with other autoimmune diseases; (2) associated with systemic infectious diseases; (3) associated with basic kidney diseases such as acute and chronic glomerulonephritis and hydronephrosis; (4) allergic to montelukast, loratadine and tanshinone.

2.4 Therapy

Montelukast group received montelukast monotherapy, specific as follows: montelukast tablets (Mudanjiang Hengyuan Pharmaceutical Co., Ltd., approved by H20060366) 4 mg/time, 1 time/d for those < 6 years old; 5 mg/d, 1 time/d for those 6 years old, for 1 month of continuous treatment. Loratadine group were treated with loratadine monotherapy, specific as follows: loratadine tablets (Shandong Tianshun Pharmaceutical Co., Ltd., approved by H20051688) 5 mg/time, 1 time/d for those with body weight < 30 kg; 10 mg/d, 1 time/d for those with body weight \geq 30 kg, for 1 month of continuous treatment. Tanshinone group received tanshinone monotherapy as follows: tanshinone injection (Shanghai No. 1 Biochemical & Pharmaceutical Co., Ltd., approved by H31022558) 20 mg in 5% glucose liquid 50 mL, by intravenous drip, 1 time/d, for 7 d of continuous treatment.

The combined treatment group received combined treatment of montelukast, loratadine and tanshinone, and the dosage and usage of each drug were the same as monotherapy group.

2.5 Observation indexes

Before and after treatment, 5 mL fasting peripheral venous blood was extracted from four groups of patients at the same time point and anti-coagulated with heparin sodium (Jilin Yinglian Biopharmaceutical Co., Ltd., approved by H22021911), 3 mL was centrifuged at low speed to get upper serum, which was frozen in -70°C environment with the other 2 mL peripheral blood. Enzyme-linked immunosorbent assay (ELISA) was used to determine serum levels of inflammatory cytokines high mobility group box 1 (HMGB1), interleukin-8 (IL-8), interleukin-17 (IL-17), interleukin-23 (IL-23) and interleukin-33 (IL-33) content. ELISA was used to detect serum levels of Th17/Treg cellular immunity-related indicators, including Th17 cytokine interleukin-17 (IL-17) as well as Treg cytokines interleukin-10 (IL-10) and transforming growth factor β (TGF- β).

2.6 Statistical processing

Statistical software was SPSS 20.0 and the data were processed by professionals. Inflammatory factors and Th17/Treg cellular immune indexes belong to measurement data and were in terms of mean \pm standard deviation, comparison among groups was by variance analysis and pair-wise comparison between groups was by LSD method. Statistics $P<0.05$ indicated statistical significance in differences.

3. Results

3.1 Inflammatory factors

Comparison of serum inflammatory factors HMGB1 (ng/mL), IL-8 (ng/L), IL-14 (ng/L), IL-23 (ng/L) and IL-33 (ng/L) levels among four groups before and after treatment was as follows: before treatment, differences in serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels were not statistically significant among four groups of patients ($P>0.05$). After treatment, serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels in four groups were lower than those before treatment, serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels in combined treatment group were lower than those in montelukast group, loratadine group and tanshinone group, and differences were statistically significant ($P<0.05$). After treatment, differences in serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels were not statistically significant among montelukast group, loratadine group and tanshinone group ($P>0.05$), shown in Table 1.

Table 1.

Comparison of serum inflammatory factor levels among four groups before and after treatment.

Groups	n	Time	HMGB1	IL-8	IL-14	IL-23	IL-33
Montelukast group	20	Before treatment	11.28 \pm 1.76	341.28 \pm 40.57	167.83 \pm 19.25	241.28 \pm 29.76	563.28 \pm 67.91
		After treatment	8.51 \pm 0.94 [*]	215.47 \pm 26.38 [*]	116.71 \pm 15.48 [*]	153.24 \pm 17.93 [*]	346.28 \pm 41.61 [*]
Loratadine group	20	Before treatment	11.53 \pm 1.84	340.65 \pm 39.25	165.92 \pm 18.76	240.63 \pm 28.49	557.45 \pm 65.23
		After treatment	8.76 \pm 0.92 [*]	209.16 \pm 25.34 [*]	113.24 \pm 14.95 [*]	161.24 \pm 18.64 [*]	352.47 \pm 43.84 [*]
Tanshinone group	20	Before treatment	11.19 \pm 1.79	335.72 \pm 41.18	162.29 \pm 19.14	245.35 \pm 27.28	560.94 \pm 62.18
		After treatment	8.23 \pm 0.92 [*]	214.32 \pm 26.79 [*]	115.83 \pm 14.76 [*]	158.39 \pm 20.14 [*]	361.28 \pm 40.76 [*]
Combined treatment group	20	Before treatment	11.42 \pm 1.83	342.74 \pm 39.83	160.35 \pm 18.74	241.18 \pm 25.43	561.24 \pm 61.29
		After treatment	5.02 \pm 0.67 ^{**}	102.36 \pm 13.28 ^{**}	72.13 \pm 8.94 ^{**}	94.36 \pm 10.15 ^{**}	203.46 \pm 24.88 ^{**}

Note: compared with same group before treatment, ^{*} $P<0.05$; compared with monotherapy group after treatment, ^{**} $P<0.05$.

Table 2.

Comparison of serum Th17/Treg cellular immune index levels among four groups before and after treatment (pg/mL).

Groups	n	Time	IL-17	IL-10	TGF- β
Montelukast group	20	Before treatment	73.28 \pm 8.91	54.37 \pm 6.12	1 532.37 \pm 18.223
		After treatment	55.97 \pm 7.63*	68.23 \pm 7.11*	1 715.45 \pm 183.26*
Loratadine group	20	Before treatment	72.94 \pm 8.65	52.19 \pm 7.53	1 509.26 \pm 159.32
		After treatment	56.93 \pm 6.24*	66.98 \pm 6.91*	1 703.48 \pm 198.23*
Tanshinone group	20	Before treatment	71.36 \pm 8.59	53.56 \pm 6.29	1 499.27 \pm 163.58
		After treatment	58.34 \pm 6.12*	67.29 \pm 8.23*	1 762.38 \pm 198.21*
Combined treatment group	20	Before treatment	73.13 \pm 8.46	54.12 \pm 5.87	1 517.63 \pm 189.23
		After treatment	48.27 \pm 5.63**	87.34 \pm 9.11**	2 132.37 \pm 260.59**

Note: compared with same group before treatment, * $P < 0.05$; compared with monotherapy group after treatment, ** $P < 0.05$.

3.2 Th17/Treg cellular immune indexes

Comparison of serum Th17/Treg cellular immune indexes IL-17, IL-10 and TGF- β levels among four groups before and after treatment was as follows: before treatment, differences in serum IL-17, IL-10 and TGF- β levels were not statistically significant among four groups of patients ($P > 0.05$). After treatment, serum IL-17 levels in four groups were lower than those before treatment while IL-10 and TGF- β levels were higher than those before treatment, serum IL-17 level in combined treatment group was lower than that in montelukast group, loratadine group and tanshinone group while IL-10 and TGF- β levels were higher than those in montelukast group, loratadine group and tanshinone group, and differences were statistically significant ($P < 0.05$). After treatment, differences in serum IL-17, IL-10 and TGF- β levels were not statistically significant among montelukast group, loratadine group and tanshinone group ($P > 0.05$), shown in Table 2.

4. Discussion

Montelukast, loratadine and tanshinone are the drugs for Henoch-Schonlein purpura that have already been applied in clinical practice, their mechanisms of action differ, and they can all partially improve the patient's condition. Montelukast is the leukotriene receptor antagonist that can inhibit the increase of blood vessel permeability due to the overrelease of leukotriene; loratadine is a second-generation antihistamine drug, which reduces allergic reactions by selectively antagonizing peripheral amine H₁ receptors; tanshinone is the extract of Chinese traditional medicine salvia miltiorrhiza that has many pharmacological actions such as enhancing fibrinolytic enzyme activity, inhibiting platelet aggregation, anti-inflammatory, anti-allergic and stabilizing mast cell membrane[6-8]. The monotherapy of these drugs can partially alleviate the clinical symptoms of Henoch-Schonlein purpura, but the effect is limited and unable to completely reverse the illness, many scholars have currently recommended the combination of three drugs to control the disease and optimize treatment outcome, but there is few related

clinical research at present. In the study, the patients with Henoch-Schonlein purpura who received monotherapy and the combination of three drugs were selected as the research subjects, and the curative effects were compared to clarify the effects of different drug compatibility.

The essence of Henoch-Schonlein purpura is a kind of inflammation, and the abnormal expression of a lot of inflammatory mediators causes the activation of mast cells, eosinophils and other allergic cells, resulting in the increased blood vessel damage and permeability as well as the emergence of purpura symptoms[9-10]. HMGB1 has a strong inflammatory effect on the outside of the cells, which can activate and chemise inflammatory cells, further induce the generation of other inflammatory factors, and form inflammatory cascade reaction[11]. IL-8 is one of the important viscera inflammation chemokines, which plays an important role in the inflammatory response and immune response, and has been proven to be involved in systemic lupus erythematosus, kawasaki disease, crohn's disease and other autoimmune diseases. IL-14 and IL-23 are unusually highly expressed in various inflammatory diseases, and can cause the generation of other inflammatory mediators and amplify the inflammatory response[12]. IL-33 promotes the generation of inflammatory mediators, activates mast cells and activates the Th2 immune response, and it is closely associated with the development of Henoch-Schonlein purpura[13]. In the study, serum levels of above inflammatory factors were compared among groups before and after treatment, and it was found that compared with those before treatment, serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels are lower in each group after treatment, illustrating that various drug compatibility can decrease the systemic inflammatory response in patients with Henoch-Schonlein purpura; further compared with three monotherapy groups, combined treatment group were with lower serum HMGB1, IL-8, IL-14, IL-23 and IL-33 levels after treatment, confirming that the combined therapy of three drugs may exert more significant anti-inhibition effect, and the curative effect is more significant, which is speculated to be directly related to the inhibiting effect of three drugs on Henoch-Schonlein purpura through different channels.

Recent studies have shown that the Th17/Treg cellular immune

imbalance is one of the important immunological mechanisms that cause Henoch-Schonlein purpura[14,15]. Th17 cells belong to the pro-inflammatory CD4⁺T cell subgroup, there is Th17 cell hyperfunction in patients with Henoch-Schonlein purpura, and the cytokine IL-17 secreted by it is increasing accordingly. IL-17 has powerful inflammatory effect, and can raise neutrophils to local damaged blood vessels, further stimulate a variety of cells to secrete pro-inflammatory factors, and IL-17 can stimulate the vascular endothelium to secrete adhesion molecules, and mediate inflammatory cell infiltration[16]. Treg cells are another kind of CD4⁺T cells that antagonize each other with Th17 cells, and the activated Treg cells exert immunosuppression and immune regulation, and can secrete IL-10, TGF- β and other factors to inhibit the action of the IL-17. In the study, serum levels of Th17/Treg cytokines were compared among groups before and after treatment, and it was found that compared with those before treatment, serum IL-17 levels were lower while IL-10 and TGF- β levels were higher in each group after treatment, showing that various drug compatibility can reverse Th17/Treg cellular immune imbalance to different extent; further compared with three monotherapy groups, combined treatment group were with lower serum IL-17 level, and higher IL-10 and TGF- β levels after treatment, confirming that the combination of three drugs can more effectively inhibit the pro-inflammatory effect of Th17 cells, and enhance the immunosuppressive action of Treg cells, and it is also one of the fundamental mechanisms for combined treatment to optimize Henoch-Schonlein purpura.

Montelukast, loratadine and tanshinone combination therapy for patients with Henoch-Schonlein purpura can significantly reduce the systemic inflammatory response, and also optimize Th17/Treg cellular immune status. Compared with single drug therapy, the combination of three drugs has better clinical effect and is worth popularization and application in clinical practice in the future.

References

- [1] Sasaki E, Shibata M, Kato A, Hamano N, Katsuki T, Tada M, et al. An adult case of severe steroid-resistant Henoch-Schönlein purpura nephritis treated with intravenous cyclophosphamide and tonsillectomy. *CEN Case Rep* 2016; **5**(2): 212-218.
- [2] Feng D, Huang WY, Hao S, Niu XL, Wang P, Wu Y, et al. A single-center analysis of Henoch-Schonlein purpura nephritis with nephrotic proteinuria in children. *Pediatr Rheumatol Online J* 2017; **15**(1): 15.
- [3] 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**(3): 315-322.
- [4] Chen Xiaofan. Effect of montelukast sodium combined with loratadine in the treatment of 32 children with allergic purpura. *Shanghai Med Pharm J* 2017; **38**(3): 31-34.
- [5] Fan Mao-xiao, He Fei, Ding Lan, Gu Shuai-shuai. Effect of sodium tanshinone II A sulfonate injection on purpura subsiding and renal involvement in patients with allergic purpura. *Chin J Clin Res* 2016; **29**(2): 262-265.
- [6] Duan X, Yu D, Yu C, Wang B, Guo Y. Henoch-Schönlein purpura following high-voltage electric burn injury: A case report and review of the literature. *Exp Ther Med* 2016; **11**(2): 540-542.
- [7] Tian Ying, Wang Yu-chuan. Efficacy of montelukast sodium combined with tanshinone in children with anaphylactoid purpura. *China J Modern Med* 2014; **24**(13): 70-74.
- [8] Yang Xiao-fei, Xing Wei-wei, Yang Hu. A clinical observation of Montelukast combined with loratadine treatment of 28 children with anaphylactoid purpura. *Chin J Dermatovenereol* 2015; **29**(11): 1210-1213.
- [9] Shah R, Ramakrishnan M, Vollmar A, Harrell A, Van Trump R, Masoud A. Henoch-schönlein purpura presenting as severe gastrointestinal and renal involvement with mixed outcomes in an adult patient. *Cureus* 2017; **9**(3): e1088.
- [10] Oshima Y, Hoshino J, Suwabe T, Hayami N, Yamanouchi M, Sekine A, et al. Multicentric Castleman's disease associated with IgA vasculitis (Henoch-Schönlein purpura) responding well to tocilizumab: a case report. *Clin Rheumatol* 2017; **36**(3): 729-733.
- [11] Gan Shiwei, Zhao Linsheng, Wang Yan. Expression of high mobility group protein 1 in children with anaphylactoid purpura and its clinical significance. *J Chongqing Med Univ* 2014; **39**(12): 1767-1770.
- [12] Liu Li, Huang Yan-Ping, Fang Xia-Ling, Zhang Yuan-Yuan, Chen Ning, Hou Hong-Hong. Effects of hemoperfusion treatment on serum IL-23 and IL-17 levels in children with Henoch-Schonlein purpura. *Chin J Contemp Pediatr* 2015; **17**(8): 796-800.
- [13] Yue Jia, Han Zi-ming, Han Mei-gui, Huang Qian, Zhang Fang-min. Expression and clinical significance of serum interleukin-33 and T-cell immunoglobulin domain and mucin domain protein-1 in children with Henoch-Schonlein purpura nephritis. *J Xinxiang Med Univ* 2015; **32**(7): 653-656.
- [14] Donadio ME, Loiacono E, Peruzzi L, Amore A, Camilla R, Chiale F, et al. Toll-like receptors, immunoproteasome and regulatory T cells in children with Henoch-Schönlein purpura and primary IgA nephropathy. *Pediatr Nephrol* 2014; **29**(9): 1545-1551.
- [15] Sasaki E, Shibata M, Kato A, Hamano N, Katsuki T, Tada M, et al. An adult case of severe steroid-resistant Henoch-Schönlein purpura nephritis treated with intravenous cyclophosphamide and tonsillectomy. *CEN Case Rep* 2016; **5**(2): 212-218.
- [16] Zhang Ting-Ting, Ji Xiao-Fei, Li Shao-You, Zhao Bo, Duan Jiang, Xiong Jing-Jing, et al. Detection of Th17, Treg cells and IL-17, IL-23 levels in children with Henoch-Schonlein purpura. *Chin J Immunol* 2016; **32**(18): 1801-1804.