Original Article
Efficacy of Sheng Xuexiaoban capsules combined with glucocorticoids in treating primary immune thrombocytopenia

Wenping Wu, Linjuan Xu, Gang Wang

Department of Hematology, People's Hospital of Quzhou, Quzhou, Zhejiang Province, China

Received July 8, 2020; Accepted July 29, 2020; Epub October 15, 2020; Published October 30, 2020

Abstract: Objective: To explore the efficacy of Sheng Xuexiaoban capsules combined with glucocorticoids in treating primary immune thrombocytopenia (PITP). Methods: Eighty-two patients with PITP were allocated into an observation group and a control group by a random number table, with 41 cases in each group. Patients in the observation group were treated with Sheng Xuexiaoban capsules combined with glucocorticoids, while those in the control group were treated with glucocorticoids alone. After 3 months of treatment, platelet count, clinical efficacy, regulatory T (Tr) cells and inflammatory factors in the peripheral blood were compared. Results: The platelet count increased in both groups after treatment (P<0.05), and was higher in the observation group (P<0.05). The total effective rate in the observation group (95.12%) was significantly higher than in the control group (70.73%; P<0.05). After treatment, percentage of CD3⁺, CD4⁺ and CD4⁺/CD8⁺ all increased in both groups (P<0.05), while CD8⁺ decreased (P<0.05). The observation group showed higher CD3⁺, CD4⁺ and CD4⁺/CD8⁺, but lower CD8⁺ percentage than the control group (all P<0.05). After 3 months of treatment, IL-10 in both groups was higher than that before treatment (P<0.05), and IL-17 in the observation group was higher than that in the control group (P<0.05). Conclusion: Sheng Xuexiaoban capsules combined with glucocorticoids is superior to glucocorticoids alone in treating PITP; which regulates immune function in vivo and inhibits inflammation. Therefore, it is worthy of further clinical application.

Keywords: Sheng Xuexiaoban capsule, glucocorticoid, primary immune thrombocytopenia, efficacy observation

Introduction

Primary immune thrombocytopenia (PITP) is a disease characterized by destruction and underproduction of platelets, and results in hemorrhage; with an annual morbidity of 1.9-6.4/100,000 in children and 3-4/100,000 in adults [1, 2]. This disease shows a polarization trend, that is, it occurs mostly in people under 14 and over 60 years old, and 9/10 of patients over the age of 75 are male [3]. Although PITP is benign, it has an acute attack and can cause severe bleeding or even death. Moreover, repeated and protracted PITP is easily transformed into chronic ITP, which seriously affects the physical and mental health and life quality of patients [4]. The mechanisms of PITP still remain unknown, but a large number of studies have shown that the pathogenesis of PITP is closely related to the dysfunction of the immune system [5, 6]. The most recognized pathogenesis of PITP is antibody-mediated humoral immunity, and the key to the pathogenesis is the production of antibodies against platelet membrane glycoproteins in vivo [7, 8]. Antigen-antibody complexes, formed by the combination of antibody and platelet membrane glycoproteins, bind to macrophage Fcy receptors and are phagocytized and cleared by macrophages [9]. Based on the above mechanisms, glucocorticoids are recommended by the Chinese Society of Hematology in 2016, which can reduce the generation of antibodies and complexes while inhibiting the phagocytic system of macrophages, and thus will increase capillary permeability, thereby stimulating bone marrow hematopoiesis and the release of peripheral blood [10]. At present, the treatment of PITP focuses on the correction of the immune disorder and regulation of cytokines to improve immune function so as to relieve clinical symptoms [11]. As the preferred drug
for PITP, glucocorticoids are capable of suppressing the immune system and inhibiting the production of platelet antibodies to increase platelets. However, there are side effects and adverse reactions in their clinical application and even recurrence of PITP during the withdrawal of treatment [12-14]. In traditional Chinese medicine, the pathogenesis of PITP is explained as “blood-heat and inordinate bleeding”, thus, the treatment takes cooling blood and stopping bleeding as its principle. Previous studies have found that traditional Chinese medicine combined with hormones has a significant effect on reducing toxicity and increasing efficiency in the treatment of PITP [15]. Sheng Xuexiaoban capsules are composed of indigo naturalis, cortex moutan, forsythia, hairyvein agrimony and licorice; which achieve efficacy by clearing heat, cooling blood, and stopping bleeding. According to two domestic studies, the total effective rate of Sheng Xuexiaoban capsules plus glucocorticoids in the treatment of PITP was higher that of glucocorticoids alone (96.67% vs 70.00%, 85.70% vs 73.30%) [16, 17]. Therefore, it’s more effective for Sheng Xuexiaoban capsules combined with glucocorticoids to treat PITP. Previous studies have shown that this treatment has the functions of anti-inflammation, sterilization, inhibition of capillary infiltration and regulation of immune function, but the specific immunoregulatory mechanisms have not been made clear [18]. In this study, besides the clinical efficacy, the immune mechanisms of Sheng Xuexiaoban capsules combined with glucocorticoids for PITP were further studied and discussed.

Materials and methods

Clinical data

Eighty-two patients with PITP admitted to the People’s Hospital of Quzhou from March 2017 to December 2018 were divided into an observation group and a control group by a random number table, with 41 cases in each group. Patients in the observation group were treated with Sheng Xuexiaoban capsules combined with glucocorticoids, while those in the control group were treated with glucocorticoids alone. All patients enrolled were aged 18-75 years old, with an average age of 64.5±6.0 years old. This study was approved by the Ethics Committee of People’s Hospital of Quzhou. All patients included in this study signed an consent form.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients diagnosed with PITP according to the Hemostasis and Thrombology Group of Chinese Society of Hematology in 2016 [19]; (2) Patients over 18 years old. Exclusion criteria: (1) Patients with severe heart and lung diseases; (2) Patients with hepatic and renal insufficiency; (3) Patients with previous thrombotic diseases; (4) Patients taking other thrombopoietic drugs during treatment; (5) Patients allergic to drugs; (6) Pregnant and lactating women; (7) Patients with malignant tumors; (8) Patients with osteoporosis.

Methods

Patients in the control group took prednisolone acetate tablets (1 mg/kg/d) (Harbin Pharmaceutical Group Fourth Pharmaceutical Factory, China) orally on an empty stomach in the morning, while those in the observation group were additionally treated with Sheng Xuexiaoban capsules (0.45 g each, Hao Qi Jun Pharmaceutical Co., Ltd., Shaanxi, China), three times a day, half an hour after meals, 4 capsules each time. Withdrawal of glucocorticoids: The platelets of the patient were detected every 3 days. If the platelets increased more than 2 times or reached a normal level, the total amount of glucocorticoids was reduced by 10% each time by 5-10 mg/d, for 6 consecutive courses (2 weeks/course). The patients were followed up for 3 months to evaluate the clinical efficacy.

Outcome measures

Platelet count: At admission and 3 months after treatment, the fasting venous blood from the elbow was collected in the morning from all patients and sent to the testing room, where platelets were detected by a hematology analyzer.

Efficacy evaluation: Markedly effective: no bleeding after treatment and the platelet count ≥100*10^9/L; effective: no bleeding after treatment and the platelet count ≥30*10^9/L; ineffective: bleeding after treatment and the...
Sheng Xuexiaoban capsules combined with glucocorticoids in treating PITP

platelet count <30*10^9/L. The total effective rate = the number of (markedly effective + effective) cases/total number of cases *100% [20].

Detection of regulatory T cells (Tr cells) and inflammatory factors in peripheral blood: 5 mL of fasting blood was collected from all patients in the morning at admission and 3 months after treatment. FACSCanto II flow cytometer (BD Company, USA) was used to detect the expression of FITC-labeled anti-human CD3, CD4 and CD8 monoclonal antibodies. Serum and plasma were separated using a centrifuge at 3,300 rpm/min. The separated plasma was mixed with 40 μL phosphate buffer solution containing protease inhibitor (Haibiao Technology Co., Ltd., Xiamen, China) and was stored in a freezer at -80°C. Interleukin-10 (IL-10) and interleukin-17 (IL-17) levels were determined by enzyme-linked immunosorbent assay (Bio RAD Biotechnology Co., Ltd., Shanghai, China) with a full-automatic multifunctional microplate reader (Thermo Company, USA).

Side effects of glucocorticoids: Infection, osteoporosis, Cushing’s syndrome, gastric ulcer, elevated blood pressure and blood glucose may occur after treatment.

Statistical methods

The SPSS 17.0 was applied to analyze the data. Continuous variables were expressed as mean ± standard deviation (X ± sd). Data consistent with a normal distribution and variance homogeneity were analyzed by the independent samples t-test. Paired t test was used for comparison before and after treatment. The counting data were expressed as percentage and analyzed by Pearson chi-square test. P<0.05 was considered statistically significant.

Results

General data and baseline data

There was no statistical difference in sex, age, platelet count and bleeding site between the two groups, indicating group comparability (P>0.05), as shown in Table 1.

Comparison of platelet count

There was no statistical difference in platelet count between the two groups before treatment (P>0.05). The platelet count was significantly increased after 3 months of treatment, and the observation group was higher than the control group (P<0.05). The difference before and after treatment in the observation group was more significant than that in the control group (P<0.05). See Table 2.

Comparison of clinical curative efficacy

Comparison of the curative efficacy showed that the total effective rate in the observation group (95.12%) was significantly higher than that in the control group (70.73%; P<0.05), as shown in Table 3.

Comparison of Tr cell subsets in peripheral blood

Before treatment, there was no statistical difference in CD3+, CD4+, CD8+ and CD4+/CD8+...
Sheng Xuexiaoban capsules combined with glucocorticoids in treating PITP

Table 2. Comparison of platelet count (X ± sd)

<table>
<thead>
<tr>
<th>Item</th>
<th>Platelets count (10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td></td>
</tr>
<tr>
<td>Observation group (n=41)</td>
<td>17.73±6.64</td>
</tr>
<tr>
<td>Control group (n=41)</td>
<td>16.93±7.16</td>
</tr>
<tr>
<td>t</td>
<td>0.582</td>
</tr>
<tr>
<td>P</td>
<td>0.599</td>
</tr>
<tr>
<td>Three months after treatment</td>
<td></td>
</tr>
<tr>
<td>Observation group (n=41)</td>
<td>130.44±56.47^a</td>
</tr>
<tr>
<td>Control group (n=41)</td>
<td>94.73±51.40^a</td>
</tr>
<tr>
<td>t</td>
<td>2.747</td>
</tr>
<tr>
<td>P</td>
<td>0.007</td>
</tr>
<tr>
<td>Difference before and after treatment</td>
<td></td>
</tr>
<tr>
<td>Observation group (n=41)</td>
<td>112.71±58.84</td>
</tr>
<tr>
<td>Control group (n=41)</td>
<td>77.80±44.25</td>
</tr>
<tr>
<td>t</td>
<td>12.264</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: ^P<0.05, compared within the same group before treatment.

Comparison of IL-10 and IL-17

Before treatment, there was no significant difference in IL-10 and IL-17 between the two groups (P>0.05). While after 3 months of treatment, IL-10 was significantly higher than that before treatment in both groups (P<0.05), and IL-17 was significantly higher than that before treatment in the observation group (P<0.05). After treatment, IL-10 and IL-17 in the observation group were significantly higher than those in the control group, and the difference before and after treatment in the observation group was more significant than that in the control group (P<0.05). See Table 5.

Discussion

Glucocorticoids achieve more than 70% efficacy in treating PITP, but patients with recurrence and who are refractory to treatment still account for 11-35% [7, 10]. Therefore, reducing the incidence of relapsed and refractory PITP has become a priority. In traditional Chinese medicine, the pathogenesis of PITP lies in blood stasis caused by blood-heat bleeding, and Yin deficiency and internal heat. Therefore, the treatment is based on the principle of cooling blood and stopping bleeding, as well as dispersing stasis and eliminating plaque. The effectiveness of Sheng Xuexiaoban capsules is based on the above principles. A study revealed that Sheng Xuexiaoban can directly stimulate megakaryocytes to proliferate, regulate immune function and increase CD4^+ in vivo. Moreover, Sheng Xuexiaoban plays a pivotal role in cooling blood and stopping bleeding, dispersing stasis and eliminating plaques, and meanwhile raising the number of platelets [21]. The effective ingredients of Sheng Xuexiaoban capsule, paenol, have been reported to reduce capillary permeability and regulate immune function [22]. A domestic study found that the total effective rate of Sheng Xuexiaoban capsules plus glucocorticoids in the treatment of PITP is higher than that of glucocorticoids alone (96.67% vs 70.00%) [16]. In the present study, the combined therapy also achieved a significant curative effect. After treatment, the elevation of platelets in the observation group was statistically higher than that in the control group, and the clinical effective rate (95.12%) was significantly higher than that in the control group (70.73%). This suggests that Sheng Xuexiaoban capsules combined with glucocorticoids improves the clinical efficacy, which is correlated with above mechanisms. In this study, the efficacy of glucocorticoids alone was lower than that in previous clinical reports, which may be related to the smaller sample size enrolled.

The occurrence of PITP is not only related to humoral immunity but also to cellular immunity [23]. Besides, Tr cells play an important role in the occurrence and development of autoimmune diseases, with varied number and func-
Table 3. Comparison of curative efficacy (n, %)

<table>
<thead>
<tr>
<th>Item</th>
<th>Observation group (n=41)</th>
<th>Control group (n=41)</th>
<th>( \chi^2 )</th>
<th>Comparison of curative efficacy (n, %)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markedly effective</td>
<td>21 (51.22)</td>
<td>11 (26.83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effective</td>
<td>18 (43.90)</td>
<td>18 (43.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ineffective</td>
<td>2 (4.88)</td>
<td>12 (29.27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The total effective rate</td>
<td>39 (95.12)</td>
<td>29 (70.73)</td>
<td>8.613</td>
<td></td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 4. Comparison of Tr cell subsets in peripheral blood (\( \bar{x} \pm sd \))

<table>
<thead>
<tr>
<th>Item</th>
<th>CD3(^+)%</th>
<th>CD4(^+)%</th>
<th>CD8(^+)%</th>
<th>CD4(^+)%/CD8(^+)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation group (n=41)</td>
<td>65.32(\pm3.86)</td>
<td>32.84(\pm3.16)</td>
<td>28.15(\pm2.74)</td>
<td>0.178(\pm0.005)</td>
</tr>
<tr>
<td>Control group (n=41)</td>
<td>65.48(\pm3.92)</td>
<td>32.86(\pm3.12)</td>
<td>27.86(\pm2.58)</td>
<td>0.19(\pm0.004)</td>
</tr>
<tr>
<td>t</td>
<td>0.196</td>
<td>0.028</td>
<td>0.498</td>
<td>0.575</td>
</tr>
<tr>
<td>P</td>
<td>0.845</td>
<td>0.978</td>
<td>0.620</td>
<td>0.567</td>
</tr>
<tr>
<td>Three months after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation group (n=41)</td>
<td>73.34(\pm4.61)^a</td>
<td>42.56(\pm3.84)^a</td>
<td>21.51(\pm1.86)^a</td>
<td>1.978(\pm0.008)^a</td>
</tr>
<tr>
<td>Control group (n=41)</td>
<td>69.07(\pm4.23)^a</td>
<td>38.42(\pm3.34)^a</td>
<td>24.35(\pm2.31)^a</td>
<td>1.579(\pm0.013)^a</td>
</tr>
<tr>
<td>t</td>
<td>4.361</td>
<td>5.203</td>
<td>6.118</td>
<td>165.821</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Difference before and after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation group (n=41)</td>
<td>8.02(\pm0.75)</td>
<td>9.72(\pm0.68)</td>
<td>6.64(\pm0.88)</td>
<td>0.811(\pm0.010)</td>
</tr>
<tr>
<td>Control group (n=41)</td>
<td>3.59(\pm0.31)</td>
<td>5.57(\pm0.22)</td>
<td>3.50(\pm0.26)</td>
<td>0.400(\pm0.016)</td>
</tr>
<tr>
<td>t</td>
<td>&lt;68.460</td>
<td>37.079</td>
<td>21.797</td>
<td>139.103</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: ^aP<0.05, compared within the same group before treatment.
Sheng Xuexiaoban capsules combined with glucocorticoids in treating PITP

The expression of CD4⁺ Tr cells accounts for 5-15% in the peripheral blood of healthy individual [26, 27]. CD4⁺ cells assist B cells to produce antibodies, and are the most important cells in the regulation of the immune system in autoimmune diseases [28]. CD8⁺ T cells mediates platelet destruction and participates in the occurrence of PITP [29, 30]. Besides, CD3⁺ has been found to be down-regulated in patients with chronic ITP due to the mediation of cytotoxicity-related factors [31]. Above all, CD3⁺ and CD4⁺ decrease while CD8⁺ increases in patients with PITP [21], consistent with our findings. After treatment, the expression of CD3⁺ and CD4⁺ increased and CD8⁺ decreased in the two groups compared with before treatment, and the observation group showed more significant improvement than the control group. Macrophages are important in immune system diseases, and IL-10 and IL-17 exert anti-inflammatory effects in vivo [32-34]. A large number of inflammatory factors are secreted due to the immune dysfunction caused by PITP, so the anti-inflammatory factors, IL-10 and IL-17, are present with low expression. We also noticed that decreased IL-10 and IL-17 in patients with PITP became elevated in the two groups after treatment, especially in the observation group. The expression of IL-17 in the observation group was more improved than that in the control group, which may be related to the regulation of immune function and the reduction of immune response by Sheng Xuexiaoban capsules.

A study revealed that the treatment of PITP with traditional Chinese medicine combined with hormones had significant curative effects by reducing toxicity and enhancing treatment efficiency [15]. The evident increase of platelet count and the fast withdrawal of hormones after treatment with Sheng Xuexiaoban capsules combined with glucocorticoid contribute to lower incidence of side effects in the observation group.

The sample size of this study is small and needs to be further expanded. In addition, it is necessary to prolong the observation duration to study the long-term recovery in both groups.

To sum up, Sheng Xuexiaoban capsules combined with glucocorticoids improves the clinical treatment efficacy, regulates the immune system, and reduces the incidence of side effects in patients with PITP.
Sheng Xuexiaoban capsules combined with glucocorticoids in treating PITP

function and inhibits inflammation, which is worthy of clinical application.

Acknowledgements
This work was supported by the Sanmenxia Science and Technology Bureau Foundation (2019060324).

Disclosure of conflict of interest
None.

Address correspondence to: Gang Wang, Department of Hematology, People’s Hospital of Quzhou, No. 2 Zhongloudi, Kecheng District, Quzhou 324000, Zhejiang Province, China. Tel: +86-1367661-0498; E-mail: wanggang32qz@163.com

References
[19] Thrombosis and Hemostasis Group, Hematology Society, Chinese Medical Association. Consensus of Chinese experts on diagnosis and
Sheng Xuexiaoban capsules combined with glucocorticoids in treating PITP


