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Regulation of non-classical immune parameters in immune thrombocytopenic purpura mice by a spleen-invigorating, qi-replenishing and blood-containing formula

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β-endorphin (β-EP);
Vasoactive intestinal peptide (VIP);
Salivary IgA (SIgA)

Abstract *Objective:* This study investigated the regulatory effect of non-classical immune parameters on immune thrombocytopenic purpura (ITP) mice by a spleen-invigorating, qi-replenishing and blood-containing formula (SQBF).
Method: A total of 80 BALB/c mice were randomly divided into four equal groups (20 mice each): control group, model group, prednisone group and spleen-invigorating, qi-replenishing and blood-containing (SQBF) group. Mice in the model group, prednisone group, and SQBF group were administered anti-platelet serum to induce ITP. The dynamic variations of platelet counts in ITP mice were measured with an automatic blood analyzer before modeling and 48 h, and 8, 12 and 15 days following APS injection. Levels of β-endorphin (β-EP), vasoactive intestinal peptide (VIP) and salivary IgA (SIgA) were detected by enzyme-linked immunosorbent assay (ELISA) on 15th day of experiment.
Results: SQBF enhanced peripheral blood platelet counts in ITP mice similar to that of prednisone, and both groups showed a statistically significant response compared with the model group ($P < .01$). The SQBF significantly decreased β-EP levels compared with the model and prednisone intervention groups ($P < .05$), significantly increased the levels of VIP and SIgA in

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ITP mice compared with the model group ($P < .05$) and had significant protective effects on the thymus of ITP mice compared with the model group ($P < .01$).
Conclusions: The SQBF had a similar effect to prednisone with regards to enhancing peripheral blood platelet counts in ITP mice. Furthermore, it decreased β-EP levels and increased VIP and SIgA, and protected the thymus. This shows that, on base of the brain-gut axis functions, some non-classical immune vascular active factors or neurotransmitters are also involved in immune responses, and also have relationship with the onset of ITP and bleeding and/or hemostasis. It needs further study to determine whether a change in these active factors is related to immediate hemostasis.

Introduction

The pathogenesis of immune thrombocytopenic purpura (ITP) is generally believed to be caused by abnormalities of immune cells and humoral immunity that lead to the generation of antibodies to platelets (PAIgG, PAlgM). These bind to platelet membrane glycoproteins, causing the destruction of platelets in the reticuloendothelial system. This causes a decrease in platelet numbers, a shortening of the platelet life span and change in platelet function.[1–3] Currently, adrenocortical hormones such as prednisolone, prednisone, dexamethasone and hydrocortisone[4] represent the first-line therapy for ITP. Shortly after receiving standardized treatment, the platelet count of some ITP patients increases significantly, with a significant improvement in bleeding tendency. However, the long-term and repeated use of adrenocortical hormones causes adverse drug reactions, including central obesity, hyperglycemia, edema, hypertension and bone necrosis. Furthermore, hormone dependence and ineffectiveness may also develop, reducing the clinical therapeutic effects of adrenocortical hormones.[5] Therefore, we aimed to identify a single drug or combination of drugs from conventional Chinese herbal medicine that might provide clinical benefit to patients in the place of hormone therapy. A prospective central randomized controlled clinical trial (register ID: ChiCTR-TRC-13003602) reported that a spleen-invigorating, qi-replenishing and blood-containing formula (SQBF) might be an alternative treatment for ITP because it had efficacy in treating chronic ITP-afflicted patients with hormone dependence and ineffectiveness. The main therapeutic effects of SQBF were symptom relief, rapidly mitigating bleeding tendency and slowly increasing platelet counts. This study investigated whether the therapeutic effects of this formula were related to immune regulation.

Materials

Animals

Fifty guinea pigs weighing 250 g (25 males and 25 females) were purchased from the Tian Rui Experimental Animal Farm in Xing Ping, Shanxi Province, China [License number: SCXK (Shan Xi) 2012-001; common breeding environment]. Eighty BALB/c mice weighting 18—22 g (40 males and 40 females) were purchased from the Laboratory Animal Center of the Fourth Military Medical University [License number: SCXK (Shan Xi) 2014-002; specific pathogen free breeding].

Drugs

SQBF

SQBF consists of Radix Astragali Preparata (*Radix Astragali seu Hedysari Praeparatae*), Salviae miltiorrhizae (*Radix Codonopsis pilosulae*), Poria (*Poria*), Largehead Atractylodes Rhizome (*Rhizoma Atractylodis Macrocephalae*), Donkey-hide Gelatin (*Colla Corii Asini*) and Radix Rubiae (*Radix Rubiae*), prepared and provided by Xi'an Xinghua Drug Research Institute based on the pharmaceutical standards for new drugs. Drug preparation: the water extraction process was adopted, and the preparation process was as follows: Donkey-hide Gelatin (*Colla Corii Asini*) was ground into fine powder, and screened filtered through an 80-mesh sieve. Other drugs were soaked in water overnight, and decocted twice. First, eight cups (400 mL/cup) of cold water were added to the herbs in SQBF except Donkey-hide Gelatin (*Colla Corii Asini*) and boiled 2 h. The liquid was strained and kept as the decoction. Then six cups of water were added to the previously cooked herbs and they were boiled for 2 h. The two decoctions were subsequently blended, condensed into a thick paste without dregs, dried at 80°C to be powder which was screened through an 80-mesh sieve. The final medication was obtained by blending this powder and Donkey-hide Gelatin (*Colla Corii Asini*). Quality standards are according to the classification and reporting requirements for Chinese medicine, for natural medicines in the "drug registration management method". Radix Astragali Preparata (*Radix Astragali seu Hedysari Praeparatae*) was conducted using the thin-layer chromatography TLC identification method and High Performance Liquid Chromatography Evaporative Light Scattering Detector (HPLC-ELSD) methods, respectively. Results of thin-layer chromatography showed that the thin layer identification of Radix Astragali Preparata (*Radix Astragali seu Hedysari Praeparatae*) was consistent with the standards under the category of Radix Astragali Preparata (*Radix Astragali seu Hedysari Praeparatae*) in the *Chinese Pharmacopoeia 2010 edition*. Integral precision