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Study of establishing disease-syndrome combined with animal model for immune thrombocytopenic purpura without additional conditions

Haiyan Lang [a], Ming Guo [a], Yuting Chu [b], Wei Ma [b], YaYue Zhang [b], Ling Zhang [b,**], Xinyi Chen [b,*]

[a] *Dongfang Hospital, Beijing University of Chinese Medicine, Beijing 100078, China*
[b] *Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 10070, China*

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Disease-syndrome combined animal model

Abstract *Objective:* To explore the feasibility of establishing the disease-syndrome combined animal model for immune thrombocytopenic purpura (ITP) without additional conditions.
Methods: Three batches of data related to the ITP model mice obtained by replication at different time were analyzed, and whether the APS-injected model mice replicated through the passive immune modeling method could simulate the pathogenesis and clinical characteristics of human ITP was evaluated according to the differentiation criteria for disease-syndrome combined model.
Results: The APS-injected replicated ITP model mice possessed the following traits: (1) Compared with the normal group, the platelet count was significantly decreased, and coagulation time was significantly increased in the model group ($P < 0.01$). (2) Compared with the normal group, the medullary thrombocytogenous megakaryocytes were significantly decreased ($P < 0.05$, 0.01, 0.001). (3) The APS-injected sites and other parts of the model mice had spontaneous hemorrhage. (4) Behavioral changing signs were observed 1 week after the modeling (i.e. low activity, delayed activity, poor appetite, skin petechia/hemorrhage and spontaneous hemorrhage at the injected sites or other parts), and were getting more and more severe.
Conclusion: According to the syndrome differentiation criteria for disease-syndrome combined model of ITP, the APS-injected animal model of ITP replicated through the passive immune

* Corresponding author.
** Corresponding author.
E-mail addresses: 15901017241@163.com (L. Zhang), chenxinyi0729@126.com (X. Chen).
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modeling method without additional conditions possesses the characteristics of disease-syndrome combined model. It provides an ideal tool for the development of traditional Chinese medicine pharmacology experiment.

Introduction

Disease-syndrome combination is an important diagnosis/treatment mode in traditional Chinese medicine (TCM), which is characterized by stressing on differentiation of syndromes and treatment.[1] In order to explore the action mechanism of medicinal herbs, the corresponding animal model should generally be established for verification. However, four diagnostic methods of TCM cannot be fully utilized on animal models (e.g. inquiry, pulse-taking and tongue inspection), thus the replicated animal model is basically just a disease model without any TCM syndromes.[2] To solve the problem, it is necessary to prepare a disease-syndrome or syndrome-disease model with other interventions based on chronic disease.

We hold the opinion that there is a gap between this model and disease occurrence and progress, for which the following idea is raised, i.e. now that a disease model can be replicated, it is possible to find out the TCM syndrome similar to human disease by observing some external signs of the model mice.[3] Based on this hypothesis, we made an overall analysis of the three batches of data related to the ITP model mice replicated without additional intervention, and evaluated whether the duplicated APS-injected ITP model mice prepared with the passive immune modeling method could simulate the occurrence and progress of disease and also clinical manifestations in human ITP according to the differentiation criteria for disease-syndrome combined model of ITP.

Differentiation criteria for disease-syndrome combined model

Identification criteria for disease model

The disease model of ITP mice must meet the following 4 criteria: (1) decrease in peripheral blood platelet count; (2) hemorrhage at the injection site or spontaneous skin petechial; (3) prolongation of blood coagulation time; (4) decrease in medullary thrombocytogenous megakaryocytes.

Identification criteria for the syndrome model

According to human ITP clinical characters associated with the behavior features of the experimental mice, and some quantitative determination indices, the external manifestations of human "failure of qi to control blood" syndrome were converted to that of "failure of qi to control blood" syndrome of the laboratory animals. The conversion indexes included: (1) hemorrhage at the injection site and other areas or spontaneous skin petechia (dim spot), which was the common features of the disease-syndrome; (2) decreased activity, similar to fatigue; (3) sluggishness, similar to limb weakness; (4) poor appetite, less intake of food and water, and loose stools, similar to spleen qi deficiency; (5) weight loss, similar to deficiency in the spleen which fails to control muscles.

Differentiation criteria for the disease-syndrome combined model

According to the evaluation indices for the disease-syndrome model, the differentiation criteria for the disease-syndrome combined model of ITP was specified as follows: more than 3 of the above 4 indices observed in the disease model (note: Index 1 was compulsory) associated with any 3 of the above 5 indices.

Methods

Preparation of anti-mice-platelet serum

The anti-mice-platelet serum was prepared through the following procedures: (1) The BALB/c mice were taken, anesthetized with ether, and the whole blood from the mouse heart was drawn and anti-coagulated with EDTA—Na_2. The platelet was separated, washed up and diluted with physiological saline. (2) The separated platelet was taken, mixed with an equivalent amount of complete Freund's adjuvant and incomplete Freund's adjuvant respectively into a water-in-oil form as the antigen. At Week 0, the antigen of complete Freund's adjuvant was injected into at least 4 sites on the sole, back and hypodermis of the *Cavia porcellus*. On Week 1, 2 and 4, the antigen of incomplete Freund's adjuvant was injected into the above 4 sites. On Week 5, the non-anticoagulatory whole blood was drawn from the heart of the guinea-pig. After 560 g × 10 min centrifuging, the supernatant was taken and the *C. porcellus* anti-mice-platelet serum (GP-APS)was obtained, and stored in a −20°C refrigerator for later use. (3) Improvement was done by referring to the ELISA method. The potency of anti-platelet-serum was determined (i.e. replacing the pure product of China-made lyophilized ELISA A protein by the antibody marked with alkaline phosphatase-protein A enzyme). (4) The APS was taken out from a −20°C refrigerator, and placed in a 56°C water bath for 30 min. Absorption was carried out for at least twice with an equal amount of BALB/c mice erythrocyte, which was diluted into APS solution (1:4) with physiological saline for later use.

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