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Original article

Hemolytic assay for Huangqi Injection

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ABSTRACT

Objective Huangqi Injection is a preparation with an extract of *Astragali Radix* which has a long history of being used as a tonic to strengthen the body's immunity. Anaphylaxis and hemolysis are two main adverse drug reaction (ADR) of injections. Our study was aimed to establish an approach for the (ADR) prediagnosis of Huangqi Injection. **Methods** An *in vitro* model for anaphylactoid assay of Huangqi Injection based on the release rate of histamine and β-hexosaminidase of RBL-2H3 cells induced by injections and a colorimetric method based on the detection of hemoglobin resulted in the erythrocyte hemolysis for prediagnostic assaying the hemolytic ADR of injections were established. **Results** Both histamine and β-hexosaminidase are the anaphylactiod mediators, but β-hexosaminidase release induced by Huangqi Injection could not be determined by spectrophotometry due to the interference of the injection itself. In addition, normal hemolysis and abnormal hemolysis were discovered during the experiment. The fingerprints and tannins in different batches of injections showed obvious differences, indicating that the content of tannins was related to abnormal hemolysis and higher histamine-secreting from RBL-2H3 cells. **Conclusion** The results indicate that the hemolytic assaying method is not only suitable for prediagnostic assaying of hemolytic ADR of herbal medicine injection, but also partly reflects the anaphylaxis of herbal injections, and tannins may be the major factors causing abnormal hemolysis.

Key words

adverse drug reaction; anaphylaxis; fingerprint; hemolysis; Huangqi Injection; RBL-2H3

1. Introduction

The roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Hsiao) or *A. membranaceus* (Fisch.) Bge. Maxim. of family Leguminosae, also known as *Huangqi* in China, have a long history of being used as tonic to strengthen the body immunity (Lai et al, 2014). Recent years, owing to the superiority of quick absorption and effect, herbal medicine injections were widely developed (Bai et al, 2012). Huangqi Injection is a preparation of with the extract of *Astragali Radix* which complied with the *Quality Standard of Chinese State Drug Administration* and was officially listed in the *Drug Standard of China*. The major components are astragalosides, and the other pharmacological ingredients include polysaccharides, flavones, and amino acids (Wang et al, 2009). Modern pharmacological research has shown that Huangqi Injection can enhance myocardial contractility, improve circulation, protect myocardial cells, and regulate immunity which is capable of restoring the immune functions in cancer subjects, as well as helping patients to improve the quality of life

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and therapeutic response clinically (Liu et al, 2010).

But due to the complex ingredients, the quality of the injections is hardly to be controlled. The epidemic investigation about adverse drug reaction (ADR) of herbal medicines has shown that ADR caused by herbal medicine injections accounts for 72.27% (Han et al, 2007). Recently, more and more reports indicate that ADR of herbal medicine injections is serious and SFDA has noticed to stop the production of seven kinds of herbal injections (SFDA, 2006). Anaphylaxis and hemolysis are two main ADR of injections. Thus the establishment of prediagnostic methods for ADR of injections is imperative for the development and supervision of herbal medicine injections.

Clinically Huangqi Injection was reported to possess acute anaphylactic and hemolytic ADR. Hemolysis is one of properties of saponins which can lead to hemolytic ADR of herbal medicine injections containing saponins and it was the major barrier which restricted the development of herbal medicine injections. Observation *in vitro* was the major method for hemolytic assay according to *Chinese Pharmacopeia*, which was found with low accuracy and difficult to be quantitative. Previously the hemolysis prediagnosis of Xuesaitong Injection with active ingredients of ginsenosides was studied (Dou et al, 2011) and the major components causing anaphylaxis of Xuesaitong Injection were determined with HPLC technique. As a large number of clinical studies have been carried out and published, it was essential to evaluate the ADR and safety of Huangqi Injection. Continuously, a simple and precise approach was discussed based on the measurement of hemolysis degree with spectrophotometry technique for determining hemolytic reaction of Huangqi Injection, as well as the major factor causing hemolysis was discussed. Meanwhile, the approach for assaying anaphylaxis implemented on the cell model was also discussed and optimized. To our knowledge, this is the first research on systematic study of the ADR of Huangqi Injection, with the aim at providing new experimental thoughts and methods for safety control of Chinese materia medica (CMM) injections.

2. Materials and methods

2.1 Materials

Agilent 1260 series HPLC was purchased from Agilent Technologies, Inc. (USA). Chromatographic C-18 column (250 mm × 4.6 mm, 5 μm) was made from Kromasil Corporation (Sweden). UV–vis spectrophotometer was purchased from Unique Co. (Shanghai, China). Freeze-drying machine was purchased from Labconco Corporation (England). Centrifuge was purchased from Thermo Electron Corporation (Germany). Pipettors were purchased from Effendorf (Germany). Multimode Microplate Reader was purchased from Tecan Group Ltd. (Austria). Mill-Q-plus filter systems (USA).

RBL-2H3 cells were purchased from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) which were produced from American Type Culture Collection (ATCC). Fetal bovine serum was made in Gibco Invitrogen (New Zealand). Histamine Assay Kit was purchased from IBL International GMBH (Germany). 4-Nitrophenyl *N*-acetyl-β-*D*-glucosaminide was purchased from Sigma-Aldrich, Co. (USA). DMEM, Triton X−100, PBS, and trypsin were purchased from Thermo Scientific Co. (China). Huangqi Injections were purchased from Zhengda Qingchunbao Pharmaceutical Co., Ltd. (batch No. 1103222, 1108182, and 906202, China); Harbin Zhenbao Pharmaceutical Co., Ltd. (batch No. A20110916, A20110714, A20111002, A20090808, A20101206, and A2010121145, China); Shenwei Pharmaceutical Co., Ltd. (batch No. 9123142, China); Shengtai Pharmaceutical Co., Ltd. (batch No. 20080925, China); Diaojiuhong Pharmaceutical Co., Ltd. (batch No. 1009031, China). All other chemical reagents were purchased from Kermel Chemical Co. (China).

Rabbits for experiment were purchased from Dalian Medical University. The rabbits were fed *ad libitum* with standard feed and water in the course of the study. All experimental procedures were in accordance with institutional animal care guidelines.

2.2 Methods

RBL-2H3 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 100 U/mL penicillin streptomycin at 5% CO_2 and 37 ^{o}C. Cells were subcultured using trypsin when reaching 80% confluency. Cell density, precision, and repeatability of the method were evaluated respectively. RBL-2H3 cell suspension (100 μL) was plated at different concentration (1, 2, 3, 4, 5, 6, and 7 × 10^5 cells/mL) in 24 plates with additional 300 μL medium and cultured for 24 h. Then, the medium was removed and washed twice with PBS. Triton X-100 (400 μL, 0.1%) was incubated at 37 ^{o}C for 30 min with the cells and then according to the histamine assay method to compare the absorbance. The absorbance was inversely proportional to the logarithm of the concentration of histamine. According to the standard curve of histamine, the absorbance of histamine released from RBL-2H3 cells treated by Triton X-100 had better in range of 0.1−0.3. According to the result, the optimum cell density was confirmed at the concentration of 5 × 10^5 cells/mL.

Precision was assessed with the diluted Huangqi Injection in certain content for six times and repeatability was assessed for six times. The relative standard deviation (RSD) was used to evaluate the precision and repeatability, which showed that all the RSD were less than 3%.

RBL-2H3 cell suspension (100 μL) was plated at 5 × 10^5 cells/mL in 24 plates with 300 μL culture medium and cultured for 24 h. Then, the medium was removed and washed twice with PBS. The diluted Huangqi Injection solutions (400 μL, three-quarters diluented solution) were incubated at 37 ^{o}C for 30 min with the cells, and then the contents of histamine and β-hexosaminidase in the supernatant were determined.

The histamine release assay of RBL-2H3 induced by sample solution was assayed using a histamine enzyme-linked