JTCM 中医杂志 Journal of Traditional Chinese Medicine

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J Tradit Chin Med 2017 June 15; 37(3): 412-420
ISSN 0255-2922

RESEARCH ARTICLE

Baicalin and rutin are major constituents in Shuanghuanglian injection involving anaphylactoid reaction

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Supported by National Science Foundation of China (the Research of Repair Mechanisms Based on the Neural Stem Cells Niche Regulation of Chinese Medicine After Brain Damage, No. 81373830) and National Science; Technology Major Projects for "Major New Drugs Innovation and Development" (Technology Reform of Shuanghuanglian Powder Injection, No. 2011ZX09201-201-15)

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Accepted: February 20, 2017

Abstract

OBJECTIVE: To identify the constituents in Shuanghuanglian injection (SHLI) that correlate with anaphylactoid reaction.

METHODS: Chemical fingerprints of 10 batches SHLI samples were determined by High Performance Liquid Chromatography (HPLC), and further investigated by similarity analysis. Combined with optical microscopy, both anaphylactoid experiments and confirmatory assay were displayed in Rat basophil leukemia cells (RBL-2H3) to obtain the histamine release inducing by SHLI. The content of histamine was tested by Enzyme-Linked Immuno Sorbent Assay method. Partial least squares regression (PLSR) method and HPLC-DAD-ESI-MS^n technology were conducted to analyze constituents in SHLI involving anaphylactoid reaction.

RESULTS: The results of spectrum and effect relationships showed that the eight constituents were positively correlated with anaphylactoid reaction. Among which, nearly 90% of them were identified as baicalin and rutin with PLSR and HPLC-DAD-ESI-MS^n. This result was in accordance with confirmatory assay on RBL-2H3 cells.

CONCLUSION: Baicalin and rutin from SHLI were the main constituents involving anaphylactoid reaction.

Keywords: Anaphylaxis; Purpura, schoenlein-henoch; Mast cells; Histamine release; Chromatography, high pressure liquid; Spectrometry, mass, electrospray Ionization; Shuanghuanglian injection

INTRODUCTION

Shuanghuanglian injection (SHLI) is made of extracts from Jinyinhua (*Flos Lonicerae*), Huangqin (*Radix Scutellariae Baicalensis*) and Lianqiao (*Fructus Forsythiae Suspensae*).[1] This prescription has been used for treating diseases, including acute respiratory tract infection, acute tonsillitis, faucitis and pneumonia.[2] Moreover, SHLI was designated as one of the national hospital emergency essential medicines by the State Administration of Traditional Chinese Medicine since 1992.[3] However, the widespread use of SHLI has caused a series of adverse drug reactions (ADRs) since 1996.[4-6]

Among which more than 70% of ADRs caused by SHLI were allergic reactions.[7-11] This was also the leading causes of death from SHLI.[12] Clinical exploration indicated that the characteristics of SHLI were in keeping with anaphylactoid reaction.[13-15]

Anaphylactoid reactions, similar to IgE-mediated allergic reactions, caused mast cell degranulation and releasing chemical mediators,[16] which induced acute allergic responses such as smooth muscle contraction, vasodilation, and increased vascular permeability.[17] An important mechanism differentiating anaphylactoid reactions from allergic reactions was no immune system involved, and the drugs directly stimulated mast cells or basophils degranulation.[18,19] Rat basophil leukemia cells (RBL-2H3), a histamine-secreting clone was isolated from RBL sub-clone RBL-IV (HR +) in 1976.[20] RBL-2H3 cells had similar granular content to that of mast cells and were commonly employed as a prototypic and convenient model to study mast cell degranulation. Like in mast cells, RBL-2H3 cells activated degranulation and released multiple mediators such as histamine, serotonin, and β-hexosaminidase.[21,22] Histamine, which was mostly stored in mast cells and basophils, was a prominent contributor to allergic diseases.[23] Previous studies of the potential allergens in SHLI mainly focused on several compounds in SHLI.[24-26] The major constituents involving the reaction had not been confirmed.[27-29] Our study aimed to find the potential constituents in SHLI with partial least squares regression (PLSR) and HPLC-DAD-ESI-MS^n method.[30]

MATERIALS AND METHODS

Chemicals and reagents

SHLI was obtained from the Second Chinese Medicine Factory of Harbin Pharm. Group Co., Ltd., (Batch No. 1111005, 1104023, 1102008, 1103110, 1101105, 1101002, 1012109, 1012115, 1011004 and 1012008). The ten batch numbers of SHLI were abbreviated as S1, S2, S3, S4, S5, S6, S6, S7, S8, S9 and S10 respectively. RBL-2H3 cells were rat basophil leukemia cells and purchased from the cell bank of Chinese academy of sciences (Shanghai, China). Fetal bovine serum and minimum essential medium were purchased from GIBCO (Grand Island, NY, USA). Histamine assay kit was purchased from Blue Gene Biotech Co., Ltd., (Shanghai, China). Compound 48/80 and HPLC grade acetic acid were purchased from Sigma (St. Louis, MO, USA). Triton X-100, PBS, and trypsin were purchased from Thermo Scientific (Waltham, MA, USA). MTS (cellTiter96 @AQueous) was purchased from Promega Corporation (Madison, WI, USA). Rutin and baicalin were purchased from National Institutes for Food and Drug Control (Beijing, China). Methanol (HPLC grade) was acquired from Fisher Corporation (Waltham, MA, USA). Agilent 1100 LC/MS D Trap XCT ESI instrument was acquired from Agilent Technologies Inc. (Matteoparo, CA, USA).

RBL-2H3 cell culture

RBL-2H3 cells were cultured in minimum essential medium (MEM) and supplemented with 10% fetal bovine serum at 37 ℃ and 5% CO_2. Cell in logarithmic growth period were used for experiments. Pre-experiments testing the effect of C48/80 as the positive control on basophil granulocytes showed that the optimal density and time period for inducing degranulation were 1×10^5 cells/mL and 30 min.

Cell viability

RBL-2H3 cells were suspended in fresh medium and seeded into 96-well plates at 1×10^3 cells per well in 100 μL medium. SHLI was added to obtain final dilutions of 0, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 mg/mL after an overnight incubation. The cells were then incubated for another 24 h. Subsequently, the culture medium was changed to 100 μL MEM supplemented with 20 μL MTS [3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (5 mg/mL) for another 1 h. Cell viability was determined at 492 nm by multifunctional microplate reader. The density of formazan formed by the untreated cells was taken as 100% of viability.

Determination of histamine release

RBL-2H3 cells in 48-well plate (1.0×10^5 cells/mL) after 24 h culture were washed three times with PBS. The flasks were divided into the negative control group, positive control group (compound 48/80, 10 μg/mL) and SHLI group (0; 0.10; 0.40 mg/mL). Then drugs were added and incubated at 37 ℃ for 30 min. Morphological changes in RBL-2H3 cells inducing by different concentrations of SHLI were observed by electrical microscope.

RBL-2H3 (300 μL) cell suspension was plated at 1×10^5 cells/mL in 48-well plates for 24 h. Then, the medium was removed and the wells were washed twice with PBS. SHLI sample solutions of 300 μL (0.40 mg/mL) of different batches were incubated at 37 ℃ for 30 min with the cells. Then histamine content in the supernatant was determined by Histamine-ELISA kit.[31] The procedure was according to manufacturer's instructions. The optical density was measured at 450 nm. The content of histamine was determined according to the standard curve.

Preparation of sample solution

SHLI sample of 10 mg was accurately weighed and dissolved in 10 mL of 50% methanol-water (v/v). The solution was stored at 4 ℃ and filtered through a 0.22 μm membrane prior to use.

HPLC conditions

The analysis was performed on an Agilent 1100 LC system. The separation was performed on an Agilent Extend-C18 (250 mm × 4.6 mm, 5 μm). The mobile phase was a mixture of methanol (A) and water containing 0.25% acetic acid (B) as the mobile phase with