Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 105 (2006) 346–351
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Effects of Scutellariae Radix on gene expression in HEK 293 cells using cDNA microarray

Chia-Sheng Chen [a], Nae-Jing Chen [a], Li-Wei Lin [b], Chia-Chang Hsieh [c,d], Guang-Wei Chen [a], Ming-Tsuen Hsieh [b,*]

[a] *Institute of Chinese Medical Sciences, China Medical University, 91 Hsueh Shih Road, Taichung 404, Taiwan, R.O.C.*
[b] *Institute of Chinese Pharmaceutical Sciences, China Medical University, 91 Hsueh Shih Road, Taichung 404, Taiwan, R.O.C.*
[c] *School of Medicine, Kyorin University, Tokyo, Japan*
[d] *Jen-Te Junior College of Medicine, Nursing and Management, Miaoli, Taiwan, R.O.C.*

Received 21 February 2005; received in revised form 3 November 2005; accepted 3 November 2005
Available online 6 January 2006

Abstract

The aim of the present study was to elucidate the molecular mechanisms underlying the anti-inflammatory effect of Scutellaria Radix (SR). The complementary DNA (cDNA) microarray method was used to survey the effects of SR on the changes of gene expression profile in HEK293 cells. Based on differential expression, 66 genes were selected for further analysis from 9600 candidate genes in the microarray; 23 genes were validated by RT-PCR. The broad spectrum of the differentially expressed genes, including those associated with inflammation, immune response, energy metabolism, as well as others, such as ISGF3G, IL6ST, CD98, ATP5G2, PHKG2, YB-1 and SLC7A4, indicate overall cellular response to SR treatment. Our results suggest that the anti-inflammatory effect of SR may be related to IL6ST down-expression, and over-expression of CD98. Moreover, SR-related improvement in immune response may be related to the ISGF3G over-expression.

Keywords: Scutellariae Radix; HEK293 cells; gene expression; cDNA microarray

1. Introduction

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, or other disease states. A common feature of these conditions is the enhanced formation of cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), IL-6 and interferon-γ (IFN-γ) (Tracey and Cerami, 1994). Although the actual benefit of reduction in body temperature of febrile patients remains uncertain, antipyretic therapy is recommended under some conditions (Mackowiak, 2000). Antipyretic drugs include corticosteroids, acetaminophen, aspirin and the other nonsteroidal anti-inflammatory drugs (NSAIDs). The corticosteroids suppress fever by blocking cytokine transcription and down regulating the synthesis of cytokine receptors. Acetaminophen blocks the conversion of arachidonic acid to prostaglandin E2 by inhibiting cyclooxygenase (Vane and Botting, 1995). Unfortunately, however, these anti-inflammatory and antipyretic drugs have side effects, and they may cause peptic ulcer, inhibition of platelet function, and liver or renal dysfunction (Roberts and Jason, 2001). Several Chinese medicines have been used as anti-inflammatory and antipyretic agents, but their modes of action at molecular levels are largely unknown.

Scutellaria baicalensis Georgi (Labiatae), a traditional Chinese medicine, has been used for the treatment of infectious diseases by physicians. Early reports suggest that the roots of *Scutellaria baicalensis* (Scutellariae Radix) possess anti-inflammatory and antipyretic properties (Lin et al., 1980). In our previous study, we found that Scutellariae Radix (SR) mainly exerts its anti-inflammatory and antipyretic effects through the nitrogenic or prostaglandinergic pathways in the central nervous system (Hsieh et al., 2001). The anti-inflammatory molecular mechanisms and gene expression of SR remain unclear, however.

Complementary DNA microarray provides an efficient tool for solving the difficulties related to expression quantification

* Corresponding author. Tel.: +886 4 22054755; fax: +886 4 22053958.
E-mail address: mthsieh@mail.cmu.edu.tw (M.-T. Hsieh).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.11.012

for large numbers of genes (Chen et al., 1998, 2001; Alizadeh et al., 2000; Yoneda et al., 2001). In a previous study, we used the cDNA microarray technique to analyze differential gene expression in the scopolamine-treated rat hippocampus (Hsieh et al., 2003). Recent papers also describe the study of differential gene expression in human embryonic kidney 293 (HEK 293) cells cultured in Chinese herbs (Tsai et al., 2003; You et al., 2003). Therefore, this HEK 293 cell line was utilized as a substrate in this study. Microarray techniques originally developed by Dr. Konan Peck of Academia Sinica were adopted where labeled DNA was used for the coupling and estimation of mRNA (Chen et al., 1998), with this method applied to examine the effect of SR on changes in the expression profile in HEK293 cells. In addition, expression of several genes was confirmed by semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) analysis.

2. Materials and methods

2.1. Preparation of SR extract

Scutellaria baicalensis Georgi root was purchased from the Hsie-Lung Chinese herbal store, Taichung, Taiwan. The plant was identified by Dr. Yuan-Shiun Chang of the Institute of Chinese Pharmaceutical Sciences, China Medical University and a voucher specimen (ICPS 001-036) has been deposited in the Institute of Chinese Pharmaceutical Sciences, China Medical University. The water extract of SR (SRE) was obtained-using distilled water, and then filtered and concentrated to dryness in a vacuum rotary evaporator. About 300 mg of the dried SRE was grounded and then shaken with 30 ml DMEM medium (Dulbecco's Modified Eagle Medium, Hyclone) for 60 min, followed by centrifugation (3600 rpm, 15 min) to remove insoluble ingredients. The supernatant was passed sequentially through 0.45- and 0.22-μm filters for sterilization. The SRE stock solution (10 mg/ml) was stored at −20 °C until use.

2.2. Culture of HEK 293 cells

The human embryonic kidney 293-cell line (HEK 293 cell) was obtained from the Institute of Biomedical Science, Academia Sinica, Taiwan. These cells were plated onto culture dishes (150 mm diameter) and cultured in 20 ml DMEM medium containing 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin (Sigma–Aldrich Chemical Co., Ltd, USA). After 24 h incubation at 37 °C, cells were grown until 80–85% confluent, and the medium aspirated. For the experiments, the confluent cells were treated with 18 ml of fresh DMEM and 2 ml of SRE stock solution at an SRE concentration of 1 mg/ml for 48 h by incubation at 37 °C. The experiment was performed using three different batches of SRE.

2.3. Human cDNA microarray on nylon membrane

A cDNA collection containing 9600 clones was from Dr. Konan Peck. Putative gene clones were obtained from the IMAGE Consortium libraries (Lennon et al., 1996) through its distributor (Research Genetics, Huntsville, AL). Most of the clones had GenBank (Benson et al., 1994) partial-sequence tags (EST) from dbEST (Boguski et al., 1993, 1994). A total of 9600 clones containing mostly known genes were selected for tailored microarray. All the clones were carefully selected and manipulated to avoid systematic error.

2.4. mRNA extraction and cDNA labeling

Total SRE RNA (1 mg/ml) with treated and untreated HEK293 cells was extracted as described in the TRI-reagent protocol (Molecular Research Center Inc.). Messenger RNA (mRNA) was purified from total RNA using Dynal beads. For each standard labeling, a 2-μg mix of mRNA was annealed with 50 μM random hexmer in a total volume of 20 μl. The cDNA synthesis was performed in a 50 μl mixture containing 25 mM each of dATP, dCTP and dGTP, 2 mM dTTP, 1 mM Biotin-16-dUTP, 0.1 M DTT and 200 units of Superscript RT II. The mixture was incubated for 90 min at 42 °C and the process terminated by heating to 94 °C for 5 min. The RNA was degraded by addition of 5.5 μl of 3 M NaOH and incubated at 50 °C for 30 min. The mixture was neutralized by addition of 5.5 μl of 3 M acetic acid. The cDNA labeling was precipitated by the addition of 38 μl of distilled water, 50 μl of 7.5 M ammonia acetate, 1 μl of glycogen (20 μg/μl) and 760 μl of absolute alcohol. The sample was then incubated for 30 min at −80 °C, and centrifuged at 13,000 rpm for 15 min. The pellet was washed with 75% ethanol and centrifuged at 13,000 rpm for 5 min, and then the pellet was dissolved in 20 μl of autoclaved H_2O.

2.5. Microarray hybridization and signal analysis

The membrane carrying the 9600 EST clones was prehybridized in 5 ml of 1× hybridization buffer [4× SSC, 0.1% *N*-lauroylsarcosine, 0.02% SDS, 1% BM blocking reagent (Boehringer Mannheim)], and 10 μg/ml salmon sperm DNA (GibcoBRL) at 63 °C for 1.5 h. The probe was mixed with 2 μl of 10 μg/μl poly d (A), 2 μl of 10 μg/μl human Cot-1 DNA (GibcoBRL), and 25 μl of 2× hybridization buffer to a final volume of 50 μl. The probe mixture was denatured at 95 °C for 5 min and then cooled on ice. The membrane was sealed with the probe mixture in the hybridization bag, incubate at 95 °C for 5 min and then at 63 °C for 16–18 h (overnight). The membrane was washed twice with 5 ml of 2× SSC, 0.1% SDS for 5 min at room temperature, and then three times (15 min per) with 5 ml of 0.1× SSC and 0.1% SDS at 63 °C. The membrane was blocked with 4 ml of 1% BM blocking reagent containing 20% dextran sulfate at room temperature for 1 h, incubated with a 5 ml mixture containing 700×-diluted Streptavidin-β-galactosidase (1.38 U/ml; GIBCO BRL), 10,000×-diluted anti-digoxigenin alkaline phosphatase (0.075 U/ml enzyme activity; Boehringer Mannheim), 50% polyethylene glycol 8000 (Sigma), and 0.3% BSA in 1× TBS buffer at room temperature for 1.5 h, followed by mini-Q water wash. The reaction was stopped with 1× PBS containing 20 mM EDTA for 20 min and the product air dried. A color image was generated using a UMAX PowerLook 3000 flatbed