Effects of Herbal Extracts Used in Oriental Medicines on Heme Oxygenase-1 Expression

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Effects of twenty-three aqueous herbal extracts used in oriental medicines on heme oxygenase (HO)-1 expression were estimated in a mouse hippocampal cell line, HT22. HO-1 is one of the cytoprotective enzymes activated various stimuli, and Western blot analysis was used for evaluated HO-1 expression. Six aqueous extracts such as Rhei Rhizoma, Paeoniae Radix, Uncariae Ramulus et Uncus, Theae Folium, Prunellae Spica, and Coptidis Rhizoma significantly increased HO-1 expression in HT22 cells at the concentration of 300 ㎍/ml. In Addition, four aqueous extracts including Eucommiae Cortex, Moutan Cortex Radicis, Ginseng Radix Rubra, and Scutellariae Radix moderately increased HO-1 expression. These results would be usefulfor the isolation and identification of their neuroprotective principles.

Key words : Heme oxygenase-1, HT22, Oriental medicine

Introduction

Heme oxygenase (HO) has attracted particular interest as it is finely up-regulated by stress conditions and generates products that might have important biological activities[1]. HO is the enzyme that controls the initial and rate-limiting steps in heme catabolism, catalyzing the cleavage of the heme ring to form ferrous iron, carbon monoxide (CO) and biliverdin[2]. Subsequently, biliverdin is rapidly metabolized into bilirubin by cytosolic biliverdin reductase, and free iron is sequestered by ferritin[2]. Three isoforms of HO have been identified as referred to HO-1, HO-2 and HO-3. Although HO-2 and HO-3 are constitutively expressed, HO-1 is inducible in many cell types, including human endothelial cells[3]. Numerously studies have shown that HO-1 has the cytoprotective and anti-inflammatory effects[4,5].

HT22 cells have been used as one of the useful models for studying the mechanism of oxidative glutamate toxicity[6]. This immortalized mouse hippocampal cell lacks ionotrophic glutamate receptors,and glutamate in this system inhibits cystine transport and leads to glutathione depletion[7]. In addition, the overexpression of HO-1 in HT22 cells has been a useful evidence for searching the drug with neuroprotective effects[8]. In the present study, we describe the effects of aqueous herbal extracts used in oriental medicines on HO-1 expression in HT22 cells.

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Material and Methods

1. Materials

Oriental herbal drugs used in this study were purchased from University Oriental Drugstore, Iksan, Korea in March, 2006, and were authenticated by matching with herbarium specimen (the Herbarium of the College of Pharmacy, Wonkwang University, Korea). Each oriental herbal drug (50 g) was extracted with hot water (500 ml) for 2 h. Each hot water soluble mixture obtained was filtered and freeze dried to yield the aqueous extracts, which were then stored at -70 ℃ until use. The chemicals and cell culture materials were obtained from the following sources: Dulbecco's Modified Eagle's medium (DMEM), antibiotics from Gibco BRL (Gaithersburg, MD, USA); BCA protein assay kit from Pierce (Rockford, IL, USA) horseradish peroxidase-conjugated secondary antibodies from Santa Cruz Biotechnology, Inc.; ECL substrate solution from Amersham Pharmacia Biotech (Piscataway, NJ, USA). The other chemicals were obtained from Sigma Co. (St Louis, MO, USA).

2. Cell cultures and sample treatments

HT22 cell line is a subclone of the HT4 hippocampal cell line. Cells were received from Dr. In Hee Mook (Ajou University, Suwon, Korea) and were grown at 37 ℃ in a humidified incubator under 5% CO_2 and 95% air in DMEM supplemented with heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. Sample extracts were dissolved in distilled water. Curcumin, a positive control, was dissolved in DMSO, and its final concentration was kept at 0.1%. This final concentration showed no relevant effects on cellular growth and survival in our assay.

3. Western blotting analysis for HO-1 expression

HT22 cells (3 x 10^6 cells/3 mL in 60 mm dish) were incubated with sample extracts or the HO-1 inducer curcumin(20 μM) for 12 h, and then cells were collected and washed with phosphate-buffered saline (PBS). After centrifugation, cell lysis was carried out at 4 ℃ by vigorous shaking for 15 min in RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris - HCl (pH 7.4), 50 mM glycerophosphate, 20 mM NaF, 20 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4 and protease inhibitors]. After centrifugation at 14,800 × g for 15 min, the supernatant was separated and stored at -70 ℃ until use. The protein concentration was determined by using the BCA protein assay kit. After addition of sample loading buffer, protein samples were electrophoresed on a 12.5% SDS-polyacrylamide gel. Proteins were transferred topolyvinylidene difluoride blots at 300 mA for 6 h. The blots were blocked for 1 h at room temperature in fresh blocking buffer (0.1% Tween 20 in Tris-buffered saline, pH 7.4, containing 5% non-fat dried milk). Dilutions (1:1000) of primary antibodies weremade in PBS with 3% non-fat dried milk. Following three washes with PBST (PBS and 0.1% Tween 20), the blots were incubated with horseradish peroxidase-conjugated secondary antibodies in PBS with 3% non-fat dried milk for 1 h at room temperature. The blotswere washed again three times in PBST buffer, and transferred proteins were incubated with ECL substrate solution for 1 min according to the manufacturer's instructions followed by visualization with X-ray film.

Results and Discussion

In the present study aiming at the determination of effects of traditional oriental medicines on heme oxygenase-1 (HO-1) expression, twenty-three aqueous herbal extracts were investigated. The results of HO-1 expression of these extracts in HT22 cells are presented in Fig. 1. Among the tested samples, the aqueous extracts of Rhei Rhizoma, Paeoniae Radix, Uncariae Ramulus et Uncus, Theae Folium, Prunellae Spica, and Coptidis Rhizomashowed significantly inducing expression of HO-1 at the concentrations of both 100 and 300μg/ml for 12 h incubation. Relative quantities of HO-1 protein induced by above six aqueous herbal extracts was also determined using a densitometer (Kodak Digital Science 1D Analysis Software, Rochester, New York, USA), and it demonstrated apparently their HO-1 inducing effects(Fig. 2).

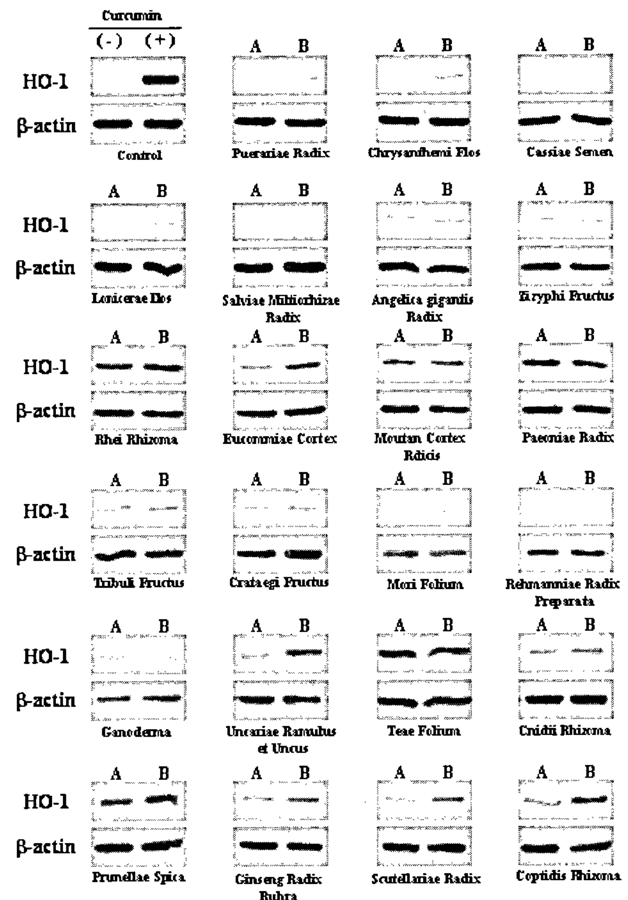

Fig. 1. HO-1 expression in HT22 cells exposed to twenty-three aqueous herbal extracts. Cells were incubated with various indicated aqueous extracts or the HO-1 inducer curcumin (20 μM) for 12 h, and HO-1 protein expression was evaluated using anti-HO-1 antibody as described under Materials and Methods. Western blot represents three independent experiments. (-) : control, (+) : curcumin 20 μM, A : 100 μg/ml, B : 300 μg/ml.

Diarylheptanoid curcumin, one of the major constituents of the rhizome of *Curcuma longa* L. (Zingiberaceae), has known to HO-1 inducer[4]. Curcumin used as a positive control and it also showed significant HO-1 protein expression (Fig. 1). In Addition, four aqueous extracts including Eucommiae Cortex, Moutan Cortex Radicis, Ginseng Radix Rubra, and Scutellariae Radix exhibited the moderate inducing effects of HO-1 protein in HT22 cells.

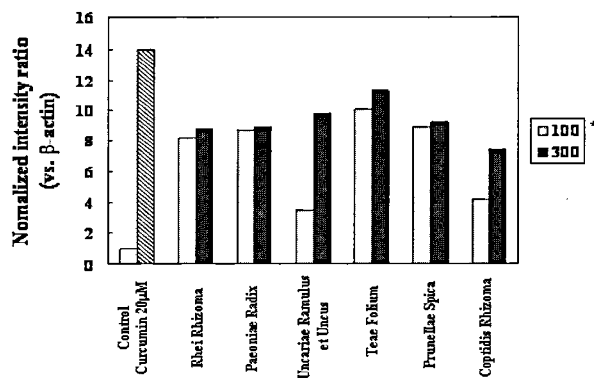

Fig. 2. Relative quantities of HO-1 protein induced by six aqueous herbal extracts. Summarized data by densitometer showing the intensity ratio of HO-1 protein to -actin under control condition and during treatments of six aqueous extracts (Rhei Rhizoma, Paeoniae Radix, Uncariae Ramulus et Uncus, Theae Folium, Prunellae Spica, and Coptidis Rhizoma). ; * μg/ml

Induction of the HO-1 is considered a generalized response to oxidative stress and a cytoprotective response[9]. HO-1 (EC 1.14.99.3) catalyzes the degradation of heme into carbon monoxide, free iron and biliverdin, using molecular oxygen, NADPH and cytochrome P-450. Its cytoprotective property has been explained by the fact that HO-1 generates bile pigments with antioxidant properties[10].Carbon monoxide may be cytoprotective because of its cyclic GMP-mediated modulation of vascular tone and neurotransmission[11]. It is also well established that the overexpression of HO-1 in HT22 cells, a mouse neuroblastoma of hippocampal origin, is involved in the neuroprotective activity[8]. Therefore, the finding of HO-1 inducer from natural products would be valuable.

In summary, six aqueous extracts such as Rhei Rhizoma, Paeoniae Radix, Uncariae Ramulus et Uncus, Theae Folium, Prunellae Spica, and Coptidis Rhizoma significantly increased HO-1 expression in HT22 cells, and these results would be useful for the isolation and identification of their neuroprotective principles.

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