Journal of Ethnopharmacology 135 (2011) 561–568

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

The inhibitory activities of the components of Huang-Lian-Jie-Du-Tang (HLJDT) on eicosanoid generation via lipoxygenase pathway

Huawu Zeng[a,1], Shengshan Dou[a,1], Jing Zhao[a], Siyang Fan[a], Xing Yuan[a], Shuanglai Zhu[a], Li Li[a], Weidong Zhang[a,b], Runhui Liu[a,*]

[a] Department of Natural Product Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, PR China
[b] Department of Natural Product Chemistry, School of Pharmacy, Shanghai Jiaotong University, Shanghai 200240, PR China

ARTICLE INFO

Article history:
Received 21 December 2010
Received in revised form 22 March 2011
Accepted 27 March 2011
Available online 2 April 2011

Keywords:
Huang-Liang-Jie-Du-Tang (HLJDT)
Oren-gedoku-to
Baicalein
Coptisine
Arachidonic acid cascade
Lipoxygenase
Leukotriene A_4 hydrolase
Cyclo-oxygenase

ABSTRACT

Aim of the study: Huang-Lian-Jie-Du-Tang (HLJDT) is a traditional Chinese medicine with anti-inflammatory use. In the present study, the effects of its component herbs and pure components were observed on eicosanoid generation to find out the contributory components and their precise targets on arachidonic acid (AA) cascade.

Materials and methods: By monitoring leukotriene B_4 (LTB_4), 5-hydroxyeicosatetraenoic acid (5-HETE), and 12-hydroxy-5,8,10-heptadecatrienoic acid (12-HHT), we compared the effects of HLJDT, HLJDT free of one or two component herbs, and water extract of four single component herbs of HLJDT (*Rhizoma coptidis*, *Radix scutellariae*, *Cortex phellodendri* and *Fructus gardeniae*) on eicosanoid generation in rat elicited peritoneal macrophages. In addition, thirteen pure compounds from HLJDT (baicalin, baicalein, wogonoside, wogonin, berberine, magnoflorine, phellodendrine, coptisine, palmatine, jateorrhizine, crocin, chlorogenic acid, and geniposide) were tested in the macrophages. Furthermore, the efficacies of these thirteen compounds were evaluated on cell-free purified enzymes: leukotriene A_4 hydrolase (LTA_4H), 5-, 15-lipoxygenase (5-, 15-LO), and cyclo-oxygenase-1/2 (COX-1/2). Moreover, the possible synergetic effect on LO pathway derived LTB_4 generation between the active components was also tested in rat peritoneal macrophages.

Results: Our experiments showed that *Rhizoma coptidis* and *Radix scutellariae* were responsible for the suppressive effect of HLJDT on eicosanoid generation. Some of the pure components including baicalein, baicalin, wogonoside, wogonin, coptisine, and magnoflorine inhibited eicosanoid generation in rat macrophages via LO pathway of AA cascade. Further experiments on cell-free purified enzymes confirmed that *Radix scutellariae* derived baicalein and baicalin showed significant inhibition on 5-LO and 15-LO, while *Rhizoma coptidis* derived coptisine showed medium inhibition on LTA_4H. On the other hand, no significant inhibition of thirteen components on COX-1/2 was observed. Moreover, the slight synergetic inhibition on LTB_4 between baicalein and coptisine was proved in the rat peritoneal macrophages.

Conclusions: Baicalein and coptisine, the active components of HLJDT, for the first time are found to interfere with arachidonic acid cascade via inhibition on different points of LO pathway. This finding makes the mechanism of HLJDT clearer and achieves its safer therapeutic application.

1. Introduction

Huang-Lian-Jie-Du-Tang (HLJDT; oren-gedoku-to in Japanese), a classic formula to clear "heat" and "poison", is an aqueous extract of four herbal materials, *Rhizoma coptidis*, *Radix scutellariae*, *Cortex phellodendri* and *Fructus gardeniae*. A patent medicine in pill form, based on an improved formula of HLJDT, has got the permission of Chinese state food and drug administration to access to market (drug approval number Z20025356). It is allowed to be used as over-the-counter (OTC) to treat a variety of inflammatory diseases mainly related with ears, nose, and throat.

In our previous study, HLJDT exerted anti-inflammatory activity by interfering with lipoxygenase (LO) pathway of arachidonic

Abbreviations: HLJDT, Huang-Lian-Jie-Du-Tang; AA, arachidonic acid; LTB_4, leukotriene B_4; 5-HETE, 5-hydroxyeicosatetraenoic acid; 12-HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; LTA_4H, leukotriene A_4 hydrolase; LO, lipoxygenase; COX, cyclo-oxygenase; OTC, over-the-counter; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NDGA, nordihydroguaiaretic acid; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; PGB_2, prostaglandin B_2; LC–MS/MS, liquid chromatography–tandem mass spectrometry; EIA, enzyme immunoassay; HPLC, high performance liquid chromatography.

* Corresponding author. Tel.: +86 21 81871245; fax: +86 21 81871245.
E-mail address: lyliurh@126.com (R. Liu).

[1] These authors contributed equally to this work.

0378-8741/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2011.03.055

acid (AA) cascade, and many pure compounds (including baicalein, baicalin, wogonoside, wogonin, berberine, coptisine, jatrorrhizine, palmatine, and geniposide) had been identified in HLJDT (Dou et al., 2009; Zeng et al., 2009). More pure compounds (e.g. chlorogenic acid, magnoflorine, phellodendrine, crocin, etc.) have been reported to exist in the herbal materials of HLJDT (Han et al., 2007; Ma et al., 2009; Chen et al., 2010; Ye et al., 2010). Most of these constituents can be classified into three structural groups, alkaloids (e.g. berberine, palmatine, and coptisine), flavonoids (e.g. baicalin, baicalein, wogonoside, and wogonin), and iridoids (e.g. geniposide and shanzhiside). To achieve accurate evaluation of HLJDT efficacy and provide a better understanding of its mechanism of working, it is necessary to find out the contributory components and their precise targets on AA cascade. To our knowledge, no corresponding comparison of the efficacies of these constituents on AA cascade was performed.

In this study, the inhibitory activities of HLJDT and its component herbs on eicosanoid generation were compared in rat peritoneal macrophages by monitoring leukotriene B_4 (LTB_4), 5-hydroxyeicosatetraenoic acid (5-HETE), and 12-hydroxy-5,8,10-heptadecatrienoic acid (12-HHT) with high performance liquid chromatography (HPLC) methods. Based on our pervious study and referred to the previous reported literatures, the thirteen pure components of HLJDT: baicalin, baicalein, wogonoside, wogonin, berberine, magnoflorine, phellodendrine, coptisine, palmatine, jateorhizine, crocin, chlorogenic acid, and geniposide were selected and evaluated at the level of intact cells. Then, to elucidate their mechanism of action and identify their targets, the effects of these pure components from HLJDT on leukotriene A_4 hydrolase (LTA_4H), 5-LO, 15-LO, and cyclo-oxygenase (COX)-1/2 were determined. Moreover, the possible synergetic effect on the generation of LO pathway derived LTB_4 between the active components baicalein and coptisine was also tested in rat peritoneal macrophages.

2. Materials and methods

2.1. Materials

Rhizoma coptidis (Rhizoma of *Coptis chinensis* Franch.), *Radix scutellariae* (Radix of *Scutellaria baicalensis* Georgi.), *Cortex phellodendri* (Cortex of *Phellodendron chinense* Schneid.), and *Fructus gardeniae* (Fructus of Gardenia Jasminoides Ellis.) were purchased from Bozhou (Anhui province, China) and authenticated by Professor HanMing Zhang (Second Military Medical University, Shanghai, China). The voucher specimens were stored in the School of Pharmacy of Second Military Medical University, Shanghai, China (No. HL20070523, HQ20070523, HB20070523, ZZ20070523, respectively).

Arachidonic acid, thioglycolate broth, the calcium ionophore A23187, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and nordihydroguaiaretic acid (NDGA) were purchased from Sigma Chemical Co. (St Louis, MO, USA). NS-398 and bestatin were purchased from Cayman Chemicals Inc. (Ann Arbor, MI, USA). Other reagents were from Sinopharm Chemical Reagent Company Ltd. (Shanghai, China).

Baicalin, baicalein, wogonoside, wogonin, berberine, geniposide, chlorogenic acid, magnoflorine, crocin, jatrorrhizine, palmatine, coptisine and phellodendrine were purchased from Phytomarker Ltd. (Tianjin, China). The purities of all the standards (shown in Fig. 1) were about 97–98%.

Arachidonic acid and the calcium ionophore A23187 were dissolved in ethanol. MTT and thioglycolate broth were prepared with water. NDGA, NS-398, and bestatin were used as reference drugs, the component substances of HLJDT and the reference drugs were prepared as stock solution with dimethyl sulfoxide (DMSO). All the stock solutions above were stored frozen and diluted prior to use.

2.2. Sample preparation

Extract of HLJDT was prepared from four medicinal herbs *Rhizoma coptidis*, *Radix scutellariae*, *Cortex phellodendri*, and *Fructus gardeniae* (3:2:2:3) as previously described (Dou et al., 2009). The freeze-dried water extracts of the single component herbs of HLJDT and HLJDT free of one or two component herbs were prepared in the same way. The dry powder of extract was dissolved in water at a concentration 10 mg/ml, the fluid was stirred at 37 °C for 1 h. After centrifugation, the supernatant was sterilized by filtration through a 0.2 μM filter and stored at −20 °C prior to use.

2.3. Animals

Sprague–Dawley rats (160–200 g) from Shanghai Laboratory Animal Company Ltd. (Shanghai, China) were maintained in controlled conditions of 22 ± 1 °C and given *ad libitum* access to food and water. All animal treatments were strictly in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals. The experiments of rats were carried out with the permission of using experimental animal [SYXK (Shanghai) 20070003].

2.4. Cell viability

Rat peritoneal macrophages were seeded into 96-well plates. Adherent peritoneal macrophages were washed twice with phosphate-buffered saline (PBS) and incubated with the compounds tested for 18 h, then MTT was added to give a final concentration of 0.5 μg/ml. Cells were incubated for 4 h and 100 μl DMSO was added per well. The amount of formazan accumulated in the growth medium was assessed at 570 nm using a microplate reader. Conditions were considered toxic if the cells' ability to metabolize MTT to formazan was lowered by more than 20% as compared with control.

2.5. Rat peritoneal macrophages

Rats were injected *i.p.* with 3 ml thioglycolate broth 3 days before use. Rat peritoneal macrophages were prepared partially based on previous report (Zeng et al., 2009). The macrophages collected were resuspended in PBS (pH = 7.4, 2 mM $CaCl_2$, and 0.5 mM $MgCl_2$) at a concentration of 2×10^7 cells/ml. After pretreated with drugs for 20 min, the aliquots (0.5 ml) were incubated with AA (50 μM) and A23187 (5 μM) for 15 min. The reactions were stopped by adding 1 ml cold alcohol, then internal stands prostaglandin B_2 (PGB_2) 200 ng was added to each sample. Eicosanoids formed were extracted with solid-phase extraction (Sep-Pak C_{18} 200 mg, Waters Corporation, MA, USA). The samples were evaporated to dryness with centrifugal vacuum concentrator (Labconco Corp., Kansas City, MS, USA) and stored frozen for analysis.

2.6. Assay of AA metabolites

The AA metabolites were detected with HPLC, liquid chromatography–tandem mass spectrometry (LC–MS/MS) and enzyme immunoassay (EIA) methods as indicated in Section 3 and figures.

HPLC method: A LC-2010A Chromatography system (Shimadzu, Tyoto, Japan) equipped with double wavelength ultraviolet (UV) detector was used for all analysis. The system was controlled by LC solution software (Shimadzu). The separation was performed by a