Journal of Ethnopharmacology ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Quantitative evaluation of the *in vitro* effect and interactions of active fractions in Yaotongning-based formulae on prostaglandin E_2 production

Li-Jun Ni [a], Xiao-Ling Xu [a], Li-Guo Zhang [a,*], Wan-Zhong Shi [b,**]

[a] School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China
[b] Shuguang Hospital Affiliated to Shanghai University of TCM, 185 Puan Road, Shanghai 200021, China

ARTICLE INFO

Article history:
Received 29 October 2013
Received in revised form
11 April 2014
Accepted 6 May 2014

Keywords:
Yaotongning Capsules (YTNC)
Effect of active fractions on anti-inflammation
Interactions of active fractions
Prostaglandin E_2 (PGE_2)

ABSTRACT

Ethnopharmacological relevance: Traditional Chinese medicine (TCM) formula of Yaotongning Capsules (YTNC) is a common remedy to treat rheumatism (RA) in China and possesses diverse biological activities including anti-inflammation. However the effects of component material medicines (CMMs) in YTNC and different combinations of the CMMs on the efficacy of YTNC and the interactions of these CMMs have been being unclear due to ten CMMs and too many compounds involved in YTNC. Moreover, many TCM formulae are available for treating RA according to TCM theory. It is unknown if the YTNC prescription is better than other TCM formulae for treating RA or better efficacy could be obtained when some CMMs in YTNC are replaced by other herbs. Quantitatively investigate the in vitro effect of active fractions from the CMMs of YTNC and other eight herbs commonly used in the TCM formulae for RA treatment on anti-inflammatory activity of different combinations of the active fractions, the interactions of the active fractions to evaluate the reasonability, advantage (or disadvantage) of the YTNC prescription and to see if the prescription could be improved from the point of anti-inflammation.

Materials and Methods: Twenty-six active fractions, which were categorized as alkaloids, flavonoids, saponins, volatile oils/aqueous extracts and polysaccharides were prepared to design TCM samples by combining some of the active fractions, based on the YTNC formulating principle, combination chemistry concept and the importance of the active fractions in YTNC. The anti-inflammatory activities of the samples were evaluated by their half-maximal inhibitory concentration (IC_{50}) values that inhibiting the production of prostaglandin E_2 (PGE_2) in ANA-1 murine macrophages (ANA-1 cells). The cells plated in 96-well plates were classified into blank group and test sample group. Each group was stimulated with lipopolysaccharides (LPS, 1 mg/mL) for 2 h. ANA-1 cells were pretreated with different concentrations of test samples prior to the addition of arachidonic acid (10 μmol/L). The supernatants were collected and measured using PGE_2 ELISA Kit, and the cytotoxicity was assayed by cell counting Kit-8 (CCK8)-based test. The interactions of the active fractions in YTNC were evaluated by comparing the experimental IC_{50} values of the samples derived from YTNC to their corresponding additive IC_{50} values. The effect of each active fraction on cellular anti-inflammation–PGE_2 secretion inhibition activity, and the reasonability, advantages (or disadvantages) of YTNC were evaluated based on the comparison of IC_{50} values of the samples.

Results: The disassembled formulae consisted of some active fractions of YTNC and the whole prescription of YTNC consisted of the all active fractions from YTNC all demonstrate cellular anti-inflammatory activity, and there were no significant differences between these formulae. The vehicle of YTNC Chinese rice wine exhibits the ability to enhance the cellular anti-inflammation of YTNC. Synergistic effect exerts in the combination of alkaloids, flavonoids and saponins of YTNC, antagonistic or additive effects occur in the other combinations of active fractions from YTNC. The anti-inflammatory activities of some TCM samples which include some active fractions from the eight selected herbs are significantly higher than the samples derived from YTNC. The flavonoids of *Carthamus tinctorius*, the volatile oils of *Cinnamomum cassia* and *Angelica pubescens* perform better in cellular anti-inflammation than the flavonoids and volatile oils in YTNC.

Conclusions: The prescription of YTNC is reasonable in the view of anti-inflammation. The saponins and polysaccharides from the CMMs of YTNC have better anti-inflammatory activities than the saponins and polysaccharides from the other eight herbs. Reducing the varieties of YTNC CMMs and replacing the flavonoids

* Corresponding author. Tel./fax: +86 21 64253045.
** Corresponding author. Tel.: +86 21 53829018; fax: +86 21 33080118.
E-mail addresses: zlgfyt@163.com (L.-G. Zhang), shwzhshug@aliyun.com (W.-Z. Shi).

http://dx.doi.org/10.1016/j.jep.2014.05.009
0378-8741/

and volatile oils of YTNC with the flavonoids of *Carthamus tinctorius* and the volatile oils of *Cinnamomum cassia* (or *Angelica pubescens*) would improve the safety and anti-inflammatory activity of YTNC. Synthetically evaluating various pharmacological activities of TCM formulae designed in the present work may lead to develop more effective and safer TCM using YTNC as prototypes.

1. Introduction

Rheumatism (RA) affects human body's bones, joints and soft tissue, and often comes with arthrodynia and limitation in function, which normally leads to changes in gait patterns owing to pain (Kaufman et al., 2001). These symptoms concern with the inflammation occurring on relative bone joints and bring a lot of pain and inconvenience to patients. Modern medicine usually treats the disease by suppressing pain, diminishing inflammation and improving human body's immunity etc. (Belch et al., 1988; Simon et al., 1999; Bauerová et al., 2011). There are many options for treating RA in terms of Traditional Chinese Medicine (TCM) theory because different TCM formulae are prescribed for same disease due to individual differences of pathogenesis and progression of patients. In clinical, this practice is referred as treating same disease with different methods (Wang et al., 2009a). After sorting and categorizing the TCM prescriptions for treating RA during recent 10 years, our research team found that there were 154 TCM formulae including Yaotongning Capsules (YTNC) (Fang, 2013), which exhibits a clear anti-inflammatory and analgesic effect, and the capability of strengthening immune activity and activating blood circulation (Pharmacopeia Committee of PR China, 2010), and has been used clinically to treat RA for decades in China.

According to TCM formulating theory, a TCM formula is usually consisted of one or two principal component material medicines (CMMs, i.e., the fundamental elements of TCM formulae) and other CMMs that assist the effects of the principal one as adjuvant parts (Anonymous, 1999). It is believed that reasonable combination of CMMs can exert synergic effects and toxicity reduction. *Strychnos nux-vomica* L., seed (Ma Qian Zi) is the principal medicine of YTNC. It has analgesic and anti- inflammatory etc. functions (Chen et al., 2012). The other nine material medicines *Ephedra sinica* Stapf, stalk (Ma Huang), *Glycyrrhiza uralensis* Fisch., root (Gan Cao), *Atractylodes lancea* (Thunb.) DC., root and rhizome (Cang Zhu), *Boswellia sacra* Flueck., resin (Ru Xiang), *Commiphora myrrha* (Nees) Engl., resin (Mo Yao), *Cyathula officinalis* K. C. Kuan, root (Chuan Niu Xi), *Eupolyphaga sinensis*, whole insect (Tu Bie Chong), *Buthus martensii* Karsch, whole insect (Quan Xie), *Bombyx mori* L. infected by *Beauveria bassiana* (Bals.) Vuill, whole insect (Jiang Can) are crushed into powder and mixed with the powder of *Strychnos nux-vomica* to intensify its action and provide extra functions such as protentiation of immunological response or invigoration of blood circulation. As the vehicle of YTNC, Chinese rice wine is taken with YTNC by the patients to improve the drug absorption. However the effects of the CMMs on the efficacy of YTNC and the interactions of these CMMs have been being unclear due to ten CMMs and too many compounds included in YTNC. Moreover, it is unknown if the YTNC prescription is better than other TCM formulae for treating RA or better efficacy could be obtained when some CMMs in YTNC are replaced by other herbs.

Recently, the synergistic/antagonistic effects of some TCM samples consisted of two to three of crude extracts or several main components have been elucidated successfully by molecular pharmacology (Wang et al., 2008; Ma et al., 2009; Lau et al., 2012; Wang et al., 2012a). The studies provide a potential way to rapidly investigate the effect of different combinations of active compounds (or extracts) on the pharmacological activities of TCM and the interactions of selected compounds. However the TCM samples in the published literatures were designed by combining some main inclusions (or crude extracts) in the formulae in equal ratio or the ratios determined by orthogonal experimental tables without considering the formulating principle of the investigated TCM. The samples designed by this way are not able to properly represent the actual compositions and functions of different CMMs in the TCM formulae. Active fractions, which are purified from CMMs and have similar molecular skeletons such as alkaloids, flavonoids, saponins and polysaccharides, will be good fundamental elements for constructing TCM samples because the interfering factors inhibiting the activity of TCM and the in-active substances such as starch and tannins can be removed by this way (Zhang et al., 2007a). Moreover, the reported studies evaluated the pharmacological activities of TCM samples by comparing their significant differences between the samples' test values, rather than by quantitative evaluation indices such as half-maximal inhibition concentration (IC_{50}) values. Sometimes the actual activity of TCM samples may be covered by the comparisons of the significant differences. And more importantly, just investigating a TCM alone cannot demonstrate whether the TCM is better than other TCMs for treating the same disease or if the TCM's formula could be improved.

Basing on above considerations, besides the ten CMMs and the vehicle of YTNC - Chinese rice wine, other extra 8 herbs (*Panax notoginseng* (Burkill) F. H. Chen, stalk (San Qi), *Taxillus chinensis* (DC.) Danser, stalk and twig (Sang Ji Sheng), *Angelica pubescens* Maxim., root (Du Huo), *Carthamus tinctorius* L., flower (Hong Hua), *Davallia mariesii* T. Moore ex Baker, root and rhizome (Gu Sui Bu), *Panax ginseng* C.A.Mey., root (Ren shen), *Epimedium sagittatum* (Siebold & Zucc.) Maxim., whole plant (Yin Yang Huo), *Cinnamomum cassia* (Nees & T.Nees) J. Presl, twig (Gui Zhi), which are frequently applied in the other 153 TCM formulae for treating RA and have similar functions to those in YTNC, were also selected as CMMs of this study. To represent the actual active material foundation of TCM as far as possible and avoid false positive/negative results that might be caused by directly using herb powders or crude extracts in cellular experiments due to their poor solubility, we purified 26 active fractions from the 19 CMMs in the categories of alkaloids, flavonoids, saponins, volatile oils/aqueous extracts and polysaccharides. As reported in the literatures, the 26 active fractions have the pharmacological activities including analgesic, anti-inflammatory and immune activity, activating blood circulation and metabolism etc. functions (see Table 1) that are similar to YTNC's. Therefore they may be effective parts of TCM formulae for treating RA and be suitable for the elements used to design TCM samples. According to the formulating principle of YTNC and the importance of the active fractions in the prescription of YTNC, and by means of the concept of combination chemistry, different TCM samples were designed by combing some of the 26 active fractions in proper way.

Modern pharmacological study shows that the production of RA is related to the responses of organism defense, especially the inflammatory cytokines in cells such as prostaglandin E_2 (PGE_2). The higher the concentration of PGE_2 in cells is, the more severe inflammation is (Sung et al., 2012; Yoon et al., 2013). Therefore comparing the half-maximal inhibitory concentration (IC_{50}) values of the TCM samples that inhibit the production of prostaglandin E_2 (PGE_2) in cells will help us to investigate the effect of the active

Please cite this article as: Ni, L.-J., et al., Quantitative evaluation of the *in vitro* effect and interactions of active fractions in Yaotongning-based formulae on prostaglandin E_2 production. Journal of Ethnopharmacology (2014), http://dx.doi.org/10.1016/j.jep.2014.05.009