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Effective constituents in Xiexin Decoction for anti-inflammation

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ABSTRACT

Aim of the study: To ascertain the effective constituents in Xiexin Decoction for anti-inflammation and the interactions of these constituents at the pharmacodynamic level.
Materials and Methods: Rats were administered oral Xiexin Decoction 1 h before intraperitoneal lipopolysaccharide. Nitric oxide production and Xiexin Decoction constituents in venous serum samples were quantified and the correlation between nitric oxide production and each constituent in serum was calculated. Raw264.7 cells were stimulated with lipopolysaccharide and one or more Xiexin Decoction constituents; cell viability and nitric oxide production was quantified.
Results: Xiexin Decoction significantly decreased nitric oxide production *in vivo*, which correlated well with rhein, baicalin, emodin and aloe-emodin. All the typical constituents of Xiexin Decoction, with the exception of physcione and chrysophanol, dose-dependently inhibited nitric oxide production *in vitro*. In an orthogonal designed *in vitro* study, rhein was the most powerful constituent, followed by baicalin then berberine and no synergy was found among these constituents.
Conclusions: Rhein was the most effective anti-inflammatory constituent in Xiexin Decoction followed by baicalin; no synergy was observed between rhein, baicalin and berberine at the pharmacodynamic level *in vitro*.

1. Introduction

Xiexin Decoction (XXD) is a traditional Chinese medicinal formula containing *Radix et Rhizoma Rhei* (*Rheum palmatum* L.), *Rhizoma Coptidis* (*Coptis chinesis* Franch) and *Radix Scutellaria* (*Scutellaria baicalensis* Georgi). It has been used for about 1700 years and is still widely used to treat inflammation-related diseases such as upper respiratory tract infection and gastritis. It has been reported to prevent lipopolysaccharide (LPS)-induced arterial hypotension in rats by inhibiting the expression of the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), as well as cytokine formation and prostaglandin E2 (PGE2) production (Lo et al., 2005a), and has been shown to attenuate inflammatory responses in LPS-exposed rat lungs (Lo et al., 2005b). These *in vivo* results were achieved by intravenous injection of XXD. In a previous study we set out to determine the *in vivo* anti-inflammatory effects of oral XXD. We found that oral XXD showed significant anti-inflammatory effects in four animal models of acute experimental inflammation including carrageenan and egg white-induced hind paw edema in rats, 2% acetic acid-induced inflammatory exudation in the abdominal cavity of mice and LPS-induced acute lung injury in mice. XXD also inhibited iNOS activity and reduced the production of inflammatory factors, such as nitric oxide (NO), tumor necrosis factor-α (TNF-α) and malondialdehyde (MDA), in a LPS-induced acute inflammation mouse model (Ma et al., 2006). We also found that *Radix et Rhizoma Rhei* was the most important anti-inflammatory component in XXD, followed by *Radix Scutellaria*, and that there was beneficial synergy between *Radix et Rhizoma Rhei* and *Radix Scutellaria* (Ma et al., 2007). However, the effective constituents in XXD for anti-inflammation have not been determined, which limits further research and development on this formula.

We know that, *Radix et Rhizoma Rhei* yields anthraquinones such as rhein, emodin, chrysophanol, physcione and aloe-emodin, *Rhizoma Coptidis* yields alkaloids such as berberine, coptisine, palmatine and jatrorrhizine, and *Radix Scutellaria* yields flavonoids such as baicalin, baicalein, wogonoside and wogonin (Fig. 1). All these anthraquinones, alkaloids and flavonoids can be detected *in vivo* after oral administration of XXD (Tan et al., 2007; Yan et al., 2007; Yan and Ma, 2007). Rhein (Wang et al., 2002), emodin (Wang et al., 2002; Li et al., 2005), aloe-emodin (Mijatovic et al., 2004), baicalin, baicalein and wogonin (Chen et al., 2001), and berber-

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ine (Kuo et al., 2004; Zhang et al., 2008) have been reported to have anti-inflammatory effects *in vivo* and *in vitro.* All the constituents cited above may contribute to the anti-inflamatory effects of XXD. However, the importance of each constituent in XXD needs to be investigated in a systematic way, so that the effective anti-inflammatory constituents can be clearly determined.

Chinese medicines, including XXD, are usually prescribed as a formula. The combination of several Chinese medicines is believed to increase the curative effects and decrease side effects due to drug–drug interactions. We found that there was a beneficial synergy between *Radix et Rhizoma Rhei* and *Radix Scutellaria in vivo* (Ma et al., 2007); however, these interactions may occur at the pharmaceutical, pharmacokinetic or pharmacodynamic level. Therefore, if interactions among the constituents of XXD occur at the pharmacodynamic level, this may be worthy of investigation.

In this study, both *in vivo* and *in vitro* experiments were carried out to ascertain the effective anti-inflammatory constituents in XXD and the potential interactions of these constituents at the pharmacodynamic level *in vitro*.

2. Materials and methods

2.1. Chemicals

Herbs in XXD including *Radix et Rhizoma Rhei* (*Rheum palmatum* L.), *Rhizoma Coptidis* (*Coptis chinesis* Franch) and *Radix Scutellaria* (*Scutellaria baicalensis* Georgi) were purchased from Shanghai Kang Qiao herbal pieces Co. Ltd. Authentication of these herbs was performed by Prof. Zhi-Li Zhao, Department of Botany, Shanghai University of Traditional Chinese Medicine. Authentication was performed by a comparison with appropriate voucher specimens at the herbaria and by performing both physical and chemical properties identification according to *The Pharmacopoeia of People's Republic of China* (*2000 edition*). All flavonoid, anthraquinone and alkaloid (except coptisine) standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Coptisine was purchased from the Wako Pure Chemical Ind. Ltd. (Japan). Dulbecco's modified Eagle's minimum essential medium (DMEM) was purchased from the Invitrogen Corporation. Fetal bovine serum (FBS) was purchased from Fumeng gene Co. Ltd. (Shanghai, China). Trypsinase was purchased from the Amresco Corporation. Culture-grade dimethyl sulfoxide (DMSO) was purchased from CalBiochem. Methanol, sodium pyruvate, penicillin, streptomycin sulfate, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tertazolium bromide (MTT), *E. coli* lipopolysaccharide (LPS), sulfanilamide, phosphoric acid, naphtylethylenediamine–HCl, and HEPES free acid were purchased from the Sigma Chemical Co. (USA). Water used in this study was produced using the Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade or better.

2.2. Animals

Male SD rats of grade II (Certificate No. SYXK 2004–2005), weighing 250 ± 20 g, were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). The rats were housed in an air-conditioned room at 22–24 °C with a 12 h dark/light cycle and were allowed food and water spontaneously. They were fasted for 12 h before the experiments. The animal study was performed according to the National Research Council's guidelines.

2.3. Cell culture

Raw264.7 murine macrophage cells were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Cells were cultured at 37 °C in DMEM medium supplemented with 10% heat-deactivated FBS, penicillin (100 U/mL), streptomycin sulfate (100 mg/mL), sodium pyruvate (1 mM) and HEPES (15 mM) in a humidified atmosphere of 5% CO_2 and passaged when about 85% confluence was achieved with trypsinase solution [0.25%, dissolved in phosphate buffer (PBS)].

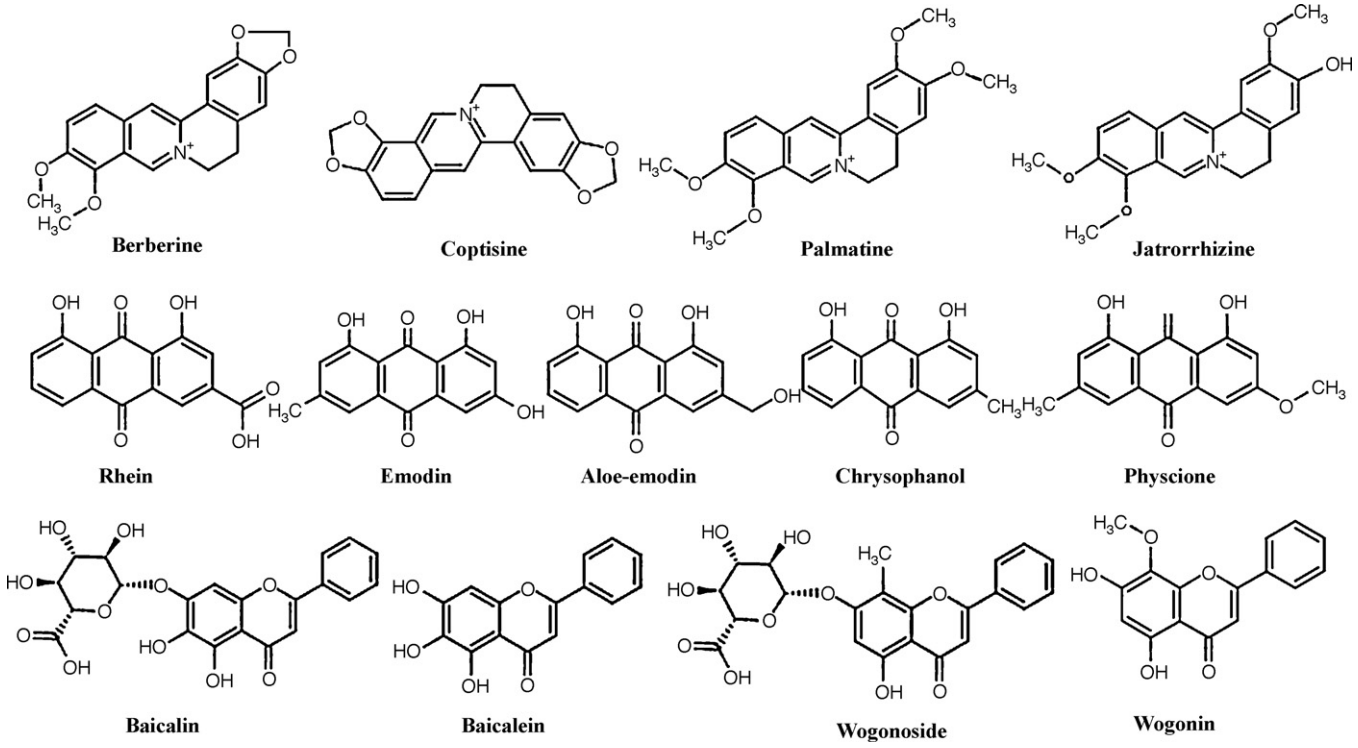

Fig. 1. Structures of the Xiexin Decoction constituents cited in this article (all obtained from http://sis.nlm.nih.gov/chemical.html).