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Ex vivo and *in vivo* effect of Chinese herbal pill Yi Shen Juan Bi (YJB) on experimental arthritis

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ABSTRACT

Aim of this study: Chinese herbal pill Yi Shen Juan Bi (YJB), which is a compound herbal drug, has traditionally been used as an anti-rheumatic drug in Chinese local clinics. Thus, we investigated the therapeutic effect of the YJB in rheumatoid arthritis (RA), using Freund's complete induced adjuvant arthritis (AA) in rat model.
Methods: For that purpose, macrophage derived cytokine tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) were measured in *ex vivo* by enzyme linked immunosorbent assay (ELISA). We also assayed the effect of YJB on peritoneal macrophage derived nitric oxide (NO) in *ex vivo* by Griess reaction. Prostaglandin E (PGE) in metapedes was assayed by ultraviolet spectrophotometer method. Further synovial Bax level was examined by Western blot analysis.
Results: YJB significantly decreased the production of peritoneal macrophages derived TNF-α, IL-1 and NO. YJB also significantly decreased prostaglandin E (PGE) and upregulated the Bax expression in AA rat's synovium.
Conclusion: YJB is a potential anti-rheumatic agent targeting the inflammatory and immunomodulatory response of macrophages while down regulating the PGE and up-regulating the pro-apoptotic Bax expression. Such characteristics of YJB on AA may be advantageous to the treatment of clinical rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of synovial tissue and the destruction of cartilage and bone in the joints (Choy and Panayi, 2001). Macrophages play an important role in RA, as the rheumatoid synovium is intensively infiltrated by macrophages and their numbers correlate with clinical scores and articular destruction in RA (Mulherin et al., 1996; Tak et al., 1997).

Yi Shen Juan Bi (YJB) is a compound anti-rheumatic herbal drug in pill form, patent number: ZL200510040550 (Perera et al., 2010a,b). Previous investigations in our laboratory, indicated that administration of the YJB significantly inhibited the swelling of the adjuvant-non-injected footpad of AA rats and collagen-induced arthritis (CIA) in rats while without significant effect on rats' secondary organ indexes (Peng et al., 2010; Perera et al., 2010a,b). Further YJB significantly inhibited inflammations of adjuvant arthritis (AA) by decreasing the production of serum tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and inducible nitric oxide synthase (iNOS) (Perera et al., 2010a,b). Also we confirmed that YJB could significantly down-regulate the anti-apoptotic Bcl-2 and up-regulate the pro-apoptotic caspase 3 expressions *in vivo* (Perera et al., 2010a,b). In addition YJB could significantly inhibit the expression of the p65 submit of NF-κB and COX-2 in the ankle synovial membrane in CIA rats (Peng et al., 2010).

Thus the present work was carried out in order to verify a possible action of YJB on macrophages derived cytokines and immunomodulatory mediators. For that purpose, the effect of YJB has been examined on macrophages derived cytokines TNF-α and IL-1 in *ex vivo* by enzyme linked immunosorbent assays (ELISA). We also assayed the effect of YJB on nitric oxide (NO) production in macrophage in *ex vivo* by Griess reaction. We evaluated the effect of YJB on prostaglandin E (PGE) in rat's metapedes by ultraviolet spectrophotometer method. In addition, rat's synovial Bax level was examined by Western blot analysis.

2. Materials and methods

2.1. Experimental animal

Male Sprague–Dawley (SD) rats, weighing 180–220 g were purchased from Experimental Animal Center of Second Military

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Table 1
Drug formula of YJB with major effective compounds.

Plant name	Composition (g)	Major effective compounds
Rehmannia glutinosa	262.5	Catalpol (Zhang et al., 2008)
Eupolyphaga sinensis	210	Octacosanol (Lu and Jiang, 1992)
Angelica sinensis	210	Butylidene phthalide, ligustilide and n-butylidene-phthalide (Benny and Vanitha, 2004)
Bombyx batryticatus	210	4-*O*-Methylglucose (Kikuchi et al., 2004)
Herba epimedii	210	Icariin (Wu et al., 2003)
Herba erodii	262.5	Geraniol and quercetin (Haimin et al., 2008)
Buthus martensi	31.3	Toxin BmK alpha IV (Chai et al., 2006)
Corydalis yanhusuo	210	Tetrahydropalmatine and corydaline (Yuan et al., 2004)
Scolopendra subspinipes	31.5	Chitosan, glucosamine and D-hydroxylysine (Xiao and Liu, 2005)
Panax ginseng	210	Panaxadione (Sugimoto et al., 2009)
Polistes mandarinus (stir-baking)	210	8-Hydroxyquinoline-4-one, p-dihydroxybenzene, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid and thymidine (Wei et al., 2008)
Cynanchum paniculatum	262.5	Anodyne (Sugama and Hayashi, 1988)
Rhizoma drynariae	31.5	Naringin (Wong and Rabie, 2006)
Polygonum cuspidatum	262.5	Phytoalexin resveratrol (Ferrero et al., 1998)
Pyrola rotundifolia	31.5	Oleanolic acid and ursolic acid (Liu, 1995)
Millettia reticulata	262.5	Epicatechin and naringenin (Fang et al., 2010)
Zaocys dhumnades (stir-fried with wine)	210	Hydrolysates (Jie et al., 2002)
Humulus scandens	262.5	Friedelanone and linoleic acid (Li et al., 2010)
Rehmannia glutinosa (dried)	210	Catalpol (Zhang et al., 2008)

Medical University, China. They were kept in a temperature-controlled environment (22 ± 2 °C), 55 ± 5% relative humidity with a 12 h:12 h light-dark cycle and fed with standard chow, for at least 1 week before any manipulations. All experimental protocols were approved by the Animal Care and Use Committee of China Pharmaceutical University, in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Preparation formula of YJB (Perera et al., 2010a,b)

The herbs were authenticated by the pharmacognosy expert at the Department of Pharmacognosy, China Pharmaceutical University, China (Table 1).

2.3. Method of preparation of YJB (Perera et al., 2010a,b)

Scorpio and Scolopendra were pulverized to fine powder and then sifted. Radix Angelica and Rhizoma Corydalis were heated under reflux with 60% ethanol separately per 1 h and then combined both extracts and filtered. Filtrate was recovered from the ethanol and concentrated to thin extract with a relative density of 1.2 (50 °C). The other ingredients were decocted in water three times. Subsequently, the decoction was combined with the filtrate and concentrated to the relative density of 1.20 (50 °C). Ethanol was added to prepare a solution containing 50% of ethanol and kept stilled. Then separated the supernatant and recovered the ethanol and concentrated to a thin extract with a relative density of 1.2 (50 °C). Fine powder, thin extract and a quantity of excipients were mixed well and made into concentrated pills. Finally, the pills were dried and polished.

2.4. Other drug used

Cyclophosphamide (CPA), product of Jiangsu Hengrui Pharmaceutical Co., Ltd., was dissolved in distilled water before use.

2.5. Reagents and assay kits

Lipopolysaccharide (LPS) and 5-diphenyl-2H tetrazolium bromide (MTT) were purchased from Sigma chemical Company. ELISA kits of rats TNF-α, IL-1 and TNF-α were purchased from Jingmei Biotech Company. All other reagents were of analytical grade and commercially available.

2.6. Drug treatment and induction of AA

SD rats were divided separately into 6 groups randomly: the normal control group, the model group, the positive control group of western medicine (CPA, 7 mg/kg), and YJB high dose (YJB-H, 2.4 g/kg), the middle dose (YJB-M, 1.2 g/kg) and low dose (YJB-L, 0.6 g/kg). Each rat was injected intradermally with 0.1 ml of Freund's complete adjuvant (Sigma product) in to the left hind metatarsal footpad of rat for induced inflammation except the normal control group. On day 14 after the immunization, medicines were given to each treatment group by intragastric administration once daily for 14 days. For model group rats were treated with sodium carboxyl methyl cellulose and the normal control rats were given an equal volume of distilled water.

2.7. Measurement of IL-1, TNF-α and NO (Li et al., 2002)

After continuous intragastric administrations of drugs for 14 days, rats were killed by cervical dislocation. Peritoneal macrophages (PM) of RA rats were collected in D-Hanks' medium. Then PM were resuspended in RPMI-1640 medium at 2×10^5 cells/ml and the cell suspension was seeded onto 24-well culture plate at a total volume of 1 ml per well. After incubation for 2 h at 37 °C in 5% CO_2 atmosphere, supernatants were removed and the adherent cells were washed with D-Hanks' medium containing 5% neonatal bovine serum for 3 times. Thus the monolayer of PM was obtained. LPS, with a final concentration of 5 mg/l, was added to each well and RPMI-1640 was also added to make a final volume per well up to 1 ml. Then the plate was incubated at 37 °C in air with 5% CO2 for 48 h. After centrifugation ($500 \times g$, 10 min) all the supernatants were collected and the IL-1 and TNF-α activities were determined by the ELISA assay according to the manufacturer's instruction.

To assay NO, 100 μl of each culture supernatant (corresponding to 2×10^5 macrophage) was incubated at room temperature for 10 min with 100 μl of Griess reagent (0.5% sulfanilamide, 0.05% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4) (Schmidt and Kelm, 1996). The absorbance (*A*) in each well was determined by EJ301 ELISA micro well reader at the wave-length of 540 nm.

2.8. Assay of PGE (Lu and Zhang, 1993)

PGE located in right metapedes of AA rats was analyzed by ultraviolet spectrophotometer method. After continuous intragastric