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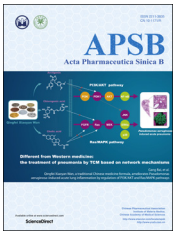

ORIGINAL ARTICLE

Qingfei Xiaoyan Wan, a traditional Chinese medicine formula, ameliorates *Pseudomonas aeruginosa*–induced acute lung inflammation by regulation of PI3K/AKT and Ras/MAPK pathways

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Abstract Gram-negative pathogen–induced nosocomial infections and resistance are a most serious menace to global public health. Qingfei Xiaoyan Wan (QF), a traditional Chinese medicine (TCM) formula, has been used clinically in China for the treatment of upper respiratory tract infections, acute or chronic bronchitis and pulmonary infection. In this study, the effects of QF on *Pseudomonas aeruginosa*–induced acute pneumonia in mice were evaluated. The mechanisms by which four typical anti-inflammatory ingredients from QF, arctigenin (ATG), cholic acid (CLA), chlorogenic acid (CGA) and sinapic acid (SPA), regulate anti-inflammatory signaling pathways and related targets were investigated using molecular biology and molecular docking techniques. The results showed that pretreatment with QF significantly inhibits the release of cytokines (TNF-α and IL-6) and chemokines (IL-8 and RANTES), reduces leukocytes recruitment into inflamed tissues and ameliorates pulmonary edema and necrosis. In

Abbreviations: ATG, arctigenin; CGA, chlorogenic acid; CLA, cholic acid; Dex, dexamethasone; DMSO, dimethylsulfoxide; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; GA, genetic algorithm; HE, hematoxylin and eosin; KEGG, Kyoto Encyclopedia of Genes and Genomes; LB, Luria–Bertani; LEV, levofloxacin; MAPK, mitogen activated protein kinase; NFATc1, nuclear factor of activated T cells c1; Ninj1, ninjurin1; PBS, phosphate-buffered saline; PI3K, phosphoinositide 3-kinase; QF, Qingfei Xiaoyan Wan; SARS, severe acute respiratory syndrome; SPA, sinapic acid; TCM, traditional Chinese medicine; TTBS, Tween 20/Tris-buffered saline; UPLC, ultra-performance liquid chromatography

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addition, ATG was identified as the primary anti-inflammatory agent with action on the PI3K/AKT and Ras/MAPK pathways. CLA and CGA enhanced the actions of ATG and exhibited synergistic NF-κB inactivation effects possibly *via* the Ras/MAPK signaling pathway. Moreover, CLA is speculated to target FGFR and MEK firstly. Overall, QF regulated the PI3K/AKT and Ras/MAPK pathways to inhibit pathogenic bacterial infections effectively.

1. Introduction

It may be a challenge to clarify traditional Chinese medicine (TCM) therapies because their mechanisms are usually unclear and many ingredients in these herbal formula may exert their effects by regulating multiple pathways and targets[1]. Recently, TCM has been considered to highlight targets in interconnected pathways that can be used to cure complex disease. The integration of TCM and "network" pharmacology provides an innovative approach for TCM theory of both reductionist and holistic medicine, and bridges traditional application and modern drug discovery[2,3]. TCM was widely used for the clinical treatment of infectious diseases for thousands of years in China, and it is based on different philosophies than those of Western medicine, which are based on antibiotics[4]. Now, TCM has also played an important role in the prevention and treatment of infection during several large respiratory disease outbreaks, such as severe acute respiratory syndrome (SARS) and acquired pneumonia[5,6]. Although the precise mechanisms of action remain unclear from the perspective of modern medicine, a therapeutic regimen combining Chinese and Western medicines has gained acceptance because it reduces resistance and toxicity.

Qingfei Xiaoyan Wan (QF) is a pill derived from a classic TCM prescription (Maxing Shigan decoction) and consists of eight common Chinese herbal medicines, Herba Ephedra, Gypsum Fibrosum, Pheretima, Fructus Arctii, Semen Lepidii, Bovis Calculus, Semen Armeniacae Amarum and Cornu Saigae Tataricae. QF is approved by the China Food and Drug Administration (No. Z12020757) and has been used clinically for the treatment of upper respiratory tract infection, acute bronchitis, acute exacerbation of chronic bronchitis and pulmonary infection with excellent clinical effects. In our previous studies, QF alleviates asthma and has anti-inflammatory activities[7,8]. Four types of NF-κB inhibitors, including arctigenin (ATG) derivatives, cholic acid (CLA) derivatives, chlorogenic acid (CGA), and sinapic acid (SPA), were screened[9] In this study, these anti-inflammatory compounds were quantified using an ultra-performance liquid chromatography (UPLC) system. The effects of QF on *Pseudomona aeruginosa*–induced lung infection in mice were investigated, and possible synergetic anti-inflammatory mechanisms for suppressing bacterial pneumonia by mutual regulation of the PI3K/AKT and Ras/MAPK pathways were identified.

2. Materials and method

2.1. Reagents and materials

ATG, SPA, CLA, CGA, arctiin, deoxycholic acid, glycocholic acid and deoxyglycocholic acid were obtained from Yifang S&T (Tianjin, China). Enzyme-linked immunosorbent assay (ELISA) assay kits of rat TNF-α, IL-6, IL-1β and RANTES, as well as human intracellular proteins (total p38, JNK and ERK), were obtained from Pierce/Endogen Co. (Rockford, Illinois, USA). Human TNF-α was purchased from PeproTech (Rocky Hill, NJ, USA). Dexamethasone (Dex) and levofloxacin (LEV) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Reagents for cell culture were obtained from Gibco BRL Life Technologies (Rockville, MD, USA). The NF-κB luciferase reporter plasmid pGL4.32 and *Renilla* luciferase reporter vector plasmid pRL-TK were obtained from Promega Co. (Fitchburg, WI, USA). The Lipofectamine 2000 transfection reagent was obtained from Invitrogen (Carlsbad, CA, USA). The anti-IκB-α antibody was obtained from Santa Cruz Biotechnology (San Diego, CA, USA). The *P. aeruginosa* PAK strain, a clinical isolate from the sputum of a patient suffering from bronchiectasis, was provided by Professor Mingqiang Qiao (College of Life Science, Nankai University, China). All other reagents used in this study were of analytical grade.

2.2. Preparation of QF and drugs

Commercial QF (batch No. 5230139) was donated by Tianjin Zhongxin Pharmaceutical Group Co. Ltd., Darentang Pharmaceutical Factory (Tianjin, China). The QF pills were powdered and suspended in distilled water. The QF suspension and LEV were directly diluted with physiological saline for administration to mice. The QF suspension, Dex, ATG, CLA, CGA and SPA were dissolved in dimethylsulfoxide (DMSO) for *in vitro* experiments, and the final concentration of DMSO added to the cells was less than 0.1%.

2.3. Animals and drug administration

Kunming mice (male, 18–22 g) were purchased from the Experimental Animal Center of the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Animal treatment and maintenance were performed in accordance with the Principle of Laboratory Animal Care (NIH Publication No. 85–23, revised 1985), and the Animal Ethics Committee of Nankai University approved the experimental protocol. All animals were housed in separate cages with food and water freely available under standard laboratory conditions of 23–26 °C with a 12 h light/12 h dark cycle. Sixty mice were randomly allotted into six groups, including an uninfected control group (Con) and five *P. aeruginosa* infected groups as follows: model group (Mod), positive control group (LEV) and low/medium/high dose QF groups (QF-L/QF-M/QF-H). LEV (7.5 mg/kg/d) and three doses