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RESEARCH ARTICLE

Effect of optimal combination of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*) on proliferation and apoptosis of A549 lung cancer cells

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Supported by the National Natural Science Foundation of China: the Study on Molecular Mechanism of Anti-lung Cancer Angiogenesis of Astragalus-zedoariae in Experienced Prescriptions Based on TGF-β1 /MAPK / HIF-1α Signaling Pathway (No. 81673810)

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Abstract

OBJECTIVE: To investigate the effect of optimal combination (E) of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*) on proliferation and apoptosis of A549 lung cancer cells and the possible mechanism underpinning the action.

METHODS: A uniform design method was used to optimize the E of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*) in A549 lung cancer cells. MTS assay was applied to analyze the effect of the component formula of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*) on A549 cells viability in various uniform design groups. A549 cells with exponential growth in routine culture were exposed to $CoCl_2$ (200 μmol/L) to mimic hypoxic conditions. Group 0 was treated with RPMI-1640, the group $CoCl_2$ was treated with $CoCl_2$ (200 μmol/L), the group DDP + $CoCl_2$ was treated with 4 mg/L Cisplatin injection (DDP) + $CoCl_2$ (200 μmol/L), and the drug group was treated with various dose of E (0.5E, 1E, 2E) + $CoCl_2$ (200 μmol/L). All groups were cultured for 24 h. Cell apoptosis was measured by Annexin V-FITC/propidium iodide double staining and flow cytometry. Western blot assay and quantitative real-time polymerase chain reaction (qRT-PCR) were employed to detect the protein and mRNA expression of B-celllymphoma-2 (Bcl-2), Bcl-2-associated X protein (Bax) and cysteinyl aspartate specific proteinase-3 (caspase-3).

RESULTS: The E obtained by the uniform design was comprise of 200 mg/L Astragalus polysaccharide (X1) and 32 mg/L Curcumin (X3). Group DDP+ $CoCl_2$, group 1E + $CoCl_2$ and group 2E + $CoCl_2$ promoted the apoptosis of A549 cells ($P < 0.05$). Group 1E + $CoCl_2$ and group 2E + $CoCl_2$ had no statistically significant differences compared with the group DDP + $CoCl_2$ ($P > 0.05$). Compared with group 0, various doses of E + $CoCl_2$ could up-regulate the expression of Bax and caspase-3 and down-regulate the expression of Bcl-2 at protein and mRNA levels ($P < 0.05$).

CONCLUSION: Astragalus polysaccharide and Curcumin was the optimal combination of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Cur-*

cumae Phaeocaulis). E promoted the apoptosis of A549 cells. Combination of Astragalus polysaccharide and Curcumin increased the expression of Bax and caspase-3, and decreased the expression of Bcl-2 to initiate apoptosis in A549 cells under chemical-induced hypoxia.

Keywords: Lung neoplasms; A549 cells; Apoptosis; Astragalan; Huangqi (*Radix Astragali Mongolici*); Ezhu (*Rhizoma Curcumae Phaeocaulis*); Curcumin; Hypoxia

INTRODUCTION

Lung cancer has the highest death rate among all types of cancers. In 2017 the most common cause of cancer death was still lung cancer in the United States, which contributes to more than a quarter of cancer mortality among all tumor types.[1] In China, lung cancer is also the leading cause of cancer death for both males and females.[2] Non-small cell lung cancer (NSCLC) accounts for 80%-85% of all lung cancers. Cisplatin-based chemotherapy has been widely used for patients with NSCLC in recent years. Although chemotherapy and targeted therapy have been improved in recent decades, the efficacy of chemotherapy for NSCLC is modest at present, and the 5-year survival rate of NSCLC is still unsatisfactory.[3]

Traditional Chinese Medicine (TCM) has been used in treatment of lung cancer for many years in China. The method of promoting *Qi* and activating blood is commonly used in the treatment. The combination of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*) is one of the most common combinations in the method. The effective active ingredients of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*), such as Astragalus saponins, Astragalus polysaccharide, β-elemene and Curcumin, have been reported to have anti-cancer effects.[4-7] The research into the compatibility of Chinese herbs has risen from the herbal pieces level to the component formula level and the uniform experimental design has become a new valuable method in the compatibility research of Chinese medicine drugs.[8]

In this study, we used the uniform design method with a 4-factor and 8-level table to determine the optimal combination (E) of four components in Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*), namely Astragalus polysaccharide, Astragalus saponins, Curcumin and β-Elemene. Changes in the inhibition of A549 cell proliferation were observed as screening indices, and regression analysis was used to determine E. Using the chemical approach ($CoCl_2$) to simulate hypoxia, we analyzed the expression of Bcl-2, Bax and caspase-3 in A549 lung cancer cells treated with various doses of E.

MATERIALS AND METHODS

Drugs and reagents

Astragalus saponins and polysaccharide were purchased from Efebio Co., Ltd., (Shanghai, China). β-Elemene and Curcumin were purchased from the National Institutes for Food and Drug Control (Beijing, China). Antibody against Bax and antibody against β-actin were obtained from Abcam (Cambridge, UK). Antibody against caspase-3 was obtained from Cell Signaling Technology Inc. (Danvers, MA, USA). Antibody against Bcl-2, goat anti-rabbit IgG-horseradish peroxidase (HRP) and goat anti-mouse IgG-HRP were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Trizol reagent was obtained from Invitrogen (Carlsbad, CA, USA). Other reagents included a HiFiMMLVcDNA First Strand Synthesis kit, Ultra-pure RNA extraction kit. An Annexin V-FITC/PI Apoptosis Detection kit and UltraSYBR mixture were obtained from CWbio Co., Ltd., (Beijing, China). A Cell Titer 96® AQueous One Solution Cell Proliferation Assay (MTS) was purchased from Promega (Madison, WI, USA). Cisplatin injection was obtained from Hospira (Mulgrave, Australia). $CoCl_2$ was obtained from Sigma-Aldrich (St Louis, MO, USA).

Cell culture and treatments

The A549 human lung adenocarcinoma cell line was purchased from the Cell Center of the Chinese Academy of Medical Sciences (Beijing, China). The cells were cultured in Roswell Park Memorial Institute 1640 medium (RPMI-1640) (Gibco, NY, USA) containing 10% fetal bovine serum (Sijiqing, Hangzhou, China) and an antibiotic mixture of Penicillin-Streptomycin Solution (Pasching, Austria). The cells were seeded on culture plates for each experiment and grown at 37 ℃ with 5% CO_2. Astragalus saponins, Astragalus polysaccharide, Curcumin and β-Elemene were dissolved in dimethyl sulfoxide (DMSO) and diluted with RPMI-1640. The final concentration of DMSO never exceeded 5‰ (v/v). $CoCl_2$ was dissolved in sterile water for injection and diluted with RPMI-1640. Cisplatin was diluted with RPMI-1640.

Uniform design of the experiments and cell viability assay

In this study, the uniform design method was used to optimize the most effective component formula of Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*) in on effect of the proliferation of A549 lung cancer cells. A uniform design method with a 4-factor and 8-level table U8 (84) was used to optimize the proportions of four component in Huangqi (*Radix Astragali Mongolici*) and Ezhu (*Rhizoma Curcumae Phaeocaulis*), namely, Astragalus poly-