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EXPERIMENTAL STUDY

Effect of Liuweidihuang pill and Jinkuishenqi pill on inhibition of spontaneous breast carcinoma growth in mice

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Abstract

OBJECTIVE: To investigate the preventing and treating action of Liuweidihuang pill (LP) and Jinkuishenqi pill (JP) on spontaneous breast carcinoma in mice.

METHODS: A model of spontaneous breast carcinoma was derived from 11.5-month-old female Kunming breeding mice following the delivery of several litters. The mice were randomly divided into five groups: model control group (C), Liuweidihuang pill high-dose group (LH; 4.6 g·kg^{-1}·d^{-1}), Liuweidihuang pill low-dose group (LL; 2.3 g·kg^{-1}·d^{-1}), Jinkuishenqi pill high-dose group (JH; 4.6 g·kg^{-1}·d^{-1}) and Jinkuishenqi pill low-dose group (JL; 2.3 g·kg^{-1}·d^{-1}). Cancer tissue volume was measured by water immersion. Histopathology was analyzed by hematoxylin and eosin staining. Vascular endothelial growth factor (VEGF), extracellular signal-regulated kinase (ERK) and cyclin D1 protein expression in cancer tissue was assayed by western blotting.

RESULTS: Compared with the control group, cancer tissue volume and weight were lower in the LP and JP groups, and survival time was longer. The expression of VEGF, ERK and Cyclin D1 were inhibited in the LP and JP groups ($P < 0.05$), and cell differentiation was increased. Tumor weights and volumes and VEGF, ERK and Cyclin D1 expression in LL or LH were significantly lower than in JL and JH ($P < 0.01$).

CONCLUSION: Both LP and JP could restrain cancer growth and promote cancer cell differentiation; moreover, LP was more effective than JP The likely mechanism of action was *via* inhibition of VEGF, ERK and cyclin D1.

Key words: Breast neoplasms; Pathology; Vascular endothelial growth factors; Extracellular signal-regulated MAP kinases ; Cyclin D1; Liuweidihuang pill; Jinkuishenqi pill

INTRODUCTION

Breast cancer (BC) is a common malignancy in women and its mortality rate has increased over the past 30 years. The major treatment for breast carcinoma is still surgery, while chemotherapy, radiation therapy, and hormone and immune therapy serve as auxiliary ap-

proaches. However, these therapies often give rise to many complications, and seriously affect patient quality of life.

Clinical practices using Traditional Chinese Medicine (TCM) have displayed significant advantages in managing breast carcinoma. Through regulating the *Yin-Yang* balance of the body, it can improve the quality of life for breast carcinoma patients by increasing survival and improving viscera function and immunity.[1,2]

Breast cancer is caused by healthy *Qi* insufficient, long-term deficit of liver and kidney *Qi* and *Yin-Yang* unbalance. Clinical treatment often use fundoscopic (replenishing *Qi* and blood), nourishing liver and kidney, reinvigoration, meantime paying attention to the *Yin-Yang* balance, with emphasis on *Yang*; strengthening healthy *Qi* and eliminating pathogenic factors, with emphasis on strengthening healthy *Qi*. For strengthening healthy *Qi*, more attention should be paid to the balance between *Yin* and *Yang*, regulating *Qi* and blood with emphasis on *Yang*, so as to striving in *Yang* from *Yin* and striving in *Yin* from *Yang*. [3-6]

Liuweidihuang pill (LP) and Jinkuishunqi pill (JP) are provided for this type of disease.[7,8] LP is not cloying, and has the function of strengthening healthy *Qi*, dispelling exogenous pathogens, eliminating toxicants, and simultaneously applying purging-tonifying therapy.[9,10] Treatment with JP, a classical *Yang*-reinforcing prescription, seeks to supplement *Yang* with the combination of appropriate *Yin* so as to achieve the effect of reinforcing *Yang* from *Yin*. The prescription is widely used by recent generations because of its significant effects.[11,12]

The general principle of breast cancer prevention and treatment is to support immune function, disperse pathogens, nourish *Yin* and supplement kidney function.[3,4]

This study, based on TCM, explored the effect of NY and invigorating the kidney during the treatment of spontaneous breast carcinoma using Liuweidihuang Wan and a classical WY prescription, Jinkuishenqi pill. As cyclin D1, vascular endothelial growth factor (VEGF) and extracellular signal-regulated kinase (ERK) play very important roles in the occurrence and development of breast carcinoma,[13,14] we also explored the effect of the different treatments on these targets.

MATERIALS AND METHODS

Reagents

Liuweidihuang pill (batch number 211021283) and Jinkuishunqi big honeyed pills (batch number Z11020054) were purchased from Beijing Tong Ren Tang Technologies Co., Ltd., (Beijing, China). Trizol was obtained from Invitrogen (Seattle, Washington City in USA). VEGF, cyclin D1 and ERK monoclonal antibodies were from Santa Cruz (Los Angeles, California City in USA). Horseradish peroxidase-labeled goat anti-rabbit IgG monoclonal antibody was from Beijing Zhongshan Golden Bridge Co., Ltd.(Beijing, China). Methanol and phosphate buffer were domestic analytical reagents.

Animals

Three-hundred and forty female Kunming breeding mice (aged 11.5 months, average weight 22 g) were provided by the animal center of Jiangxi TCM (production license No. SCSK-2011-0001). The Animal Ethics Committee of National Research Institute for Family Planning Beijing approved the animal experimentation protocols, and all animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals established by the Chinese Council on Animal Care. The animals were housed in accordance with the guidelines for care and use of animals in scientific research-registered animal facilities. The animals were maintained in cabin-type isolators at standard environmental conditions (22-25 ℃, 40%-70% humidity) with a 12:12 h dark/light photo cycle. Trained technicians palpated the mammary glands of all animals every 3 days and noted the location and size of all nodules using standard techniques.[5,6] At 11-18 months, 20%-30% of animals eventually developed a mammary tumor.

Following tumor development, each mouse was distributed to one of five groups using a random number table, as follows:[15,16] control group (C), Liuweidihuang pill high-dose group (LH; 4.6 $g \cdot kg^{-1} \cdot d^{-1}$), Liuweidihuang pill low-dose group (LL; 2.3 $g \cdot kg^{-1} \cdot d^{-1}$), Jinkuishunqi pill high-dose group (JH; 4.6 $g \cdot kg^{-1} \cdot d^{-1}$) and Jinkuishunqi pill low-dose group (JL, 2.3 $g \cdot kg^{-1} \cdot d^{-1}$). The stock concentration of LP and J was 24 g/100 mL, and was administered *via* intragastric administration. The mice in the control group were given physiological levels of saline to the agonal stage (symptoms: skeletonization, dispirited demeanor, significantly reduced dietary intake, slow reactions, decreased heart rate, faint breath or the occurrence of periodic breath) and then killed, recording the period between the beginning of the tumor and euthanasia. Tumors were then excised and weighed. Final diagnosis of the breast cancer was determined by histopathological analysis. The final volume of cancer tissue was measured by the water immersion method.

The inhibitory cancer ratio was calculated as follows:

Inhibitory cancer ratio = P inhibitory = $(M_{model} - M_{treatment}) / M_{model} \times 100\%$

Histopathology

Mammary tumors were collected in 10% buffered formalin. Fixed and paraffin-embedded tissues were cut at 5-μm thickness, stained with hematoxylin and eosin following standard procedures, and examined under a light microscope.[17]