Effects of Jiazhu decoction in combination with cyclophosphamide on breast cancer in mice


OBJECTIVE: To investigate the therapeutic effects of Jiazhu decoction (JZD) in combination with cyclophosphamide (CTX) on the growth of breast cancer in mice and to explore the possible molecular mechanisms of action.

METHODS: BALB/c mice were randomly divided into four groups of 10 (untreated model group, JZD group, CTX group, and JZD + CTX group) and subcutaneously injected with 4T1 mouse breast cancer cells. Tumors were allowed to establish for ~7 d before initiation of treatment with CTX (100 mg/kg every week by intraperitoneal injection) and/or JZD (0.015 mL of 1.65 g/mL crude drug, administered daily by gavage). The model group received equivalent volumes of vehicle on the same schedules. Tumor volumes were measured every 3 d. Mice were sacrificed after 3 weeks of treatment, and tumors were excised and subjected to RT-qPCR and western blot analysis to evaluate expression of the Wnt/β-catenin signaling pathway components β-catenin, c-Myc, and cyclin D1 at the mRNA and protein levels.

RESULTS: The mean tumor volume was smaller and the growth rate was slower in the CTX and JZD + CTX groups compared with the model group (P < 0.05), and in the JZD + CTX group compared with the CTX and JZD groups (P < 0.05). Tumor growth was inhibited by 35.4% and 48.1% by CTX and JZD + CTX treatment, respectively (P < 0.001). The expression of β-catenin, c-Myc, and cyclin D1 mRNA and protein in tumors was significantly lower in mice treated with JZD or JZD + CTX compared with the untreated mice (P < 0.05), and was significantly lower in mice treated with JZD + CTX compared with either JZD or CTX alone (P < 0.05).

CONCLUSION: JZD inhibited the growth of mouse breast cancer cells in vivo, possibly by reducing the expression of β-catenin, c-Myc, and cyclin D1. Combination therapy with JZD plus CTX had a more potent inhibitory effect on breast cancer growth compared with either agent alone.
INTRODUCTION

Breast cancer (BC) is the malignant tumor with the highest morbidity and mortality rate among women worldwide.1 In China, the morbidity rate of BC ranks first in women resident in both urban and rural areas, and the rate has increased in the past decade, raising a significant public health concern.2 At present, standard treatment for BC is surgery combined with radiotherapy, chemotherapy, endocrine therapy, and/or targeted therapy.3 Despite continuous improvement in BC treatment protocols, further advances are essential to lower mortality and morbidity.4 Even when BC is diagnosed and appropriately treated in the early stages of disease, there are still significant numbers of women suffer from either a local recurrence or metastatic disease.5 Furthermore, the incidence of adverse events resulting from surgery, chemotherapy, and radiotherapy, and the increasing emergence of drug resistance, have become important obstacles to improving BC therapy.6 Thus, the prevention and treatment of BC remains one of the major challenges facing modern medicine.

Traditional Chinese medicine (TCM) has unique advantages over modern medicine in maintaining the benefits of surgery, radiotherapy, and chemotherapy in BC.10 Professor Liu Shangyi is very good at using Chinese medicine to treat difficult and serious diseases, especially neoplastic disease.11,12 He has put forward a concept of "enrich yin for the situation of waning of Yin and Yang" for TCM treatment of BC.13,14 According to this treatment concept, my advisor Xie Su creates a formula called "Jiazhu decoction" (JZD), using couplet medicinals Biejia (Carapax Trionyxii) and Ezhu (Curcuma Rhizoma) as sovereign medicinals, to treat BC patients clinically. So far, JZD has been shown to have a certain effect in the treatment of BC. In this study, we aimed to evaluate the therapeutic effects of JZD plus cyclophosphamide (CTX) on the growth of mouse 4T1 BC cells in mice, and to explore the possible involvement of alterations in the Wnt/β-catenin signaling pathway in the mechanisms of action of JZD and CTX.

MATERIALS AND METHODS

Drugs and reagents

JZD was prepared with a Chinese medicinal formula consisting of Biejia (Carapax Trionyxii) [20170601] 20 g, Ezhu (Rhizoma Curcumae Phaeocaulis) [20170701] 10 g, Dihuang (Radix Rehmanniae) [20170601] 20 g, Shudihuang (Radix Rehmanniae Praeparata) [20170401] 20 g, Huangqi (Radix Astragali Mongolici) [20170701] 20 g, Donglingcao (Herba Isodi Rubescenti) [20170201] 20 g, and Maozhuacao (Radix Ranunculi Ternati) [20170101] 20 g, Lićco (Herba Humuli Scandentis) [20170501] 20 g, Baihe (Balbus Lili Lancifolii) [20170501] 20 g, Yi yiren (Semen Coicis) [20170601] 20 g, using Chinese herbal decocting pieces (Guiyang Ji Ren Tang Chinese Herbal Pieces Factory, Guizhou, China). CTX was obtained from Jiangsu Shengdi Pharmaceutical Co., Ltd. (16092625; Jiangsu, China). PCR primers were synthesized by Sangon Bio-tech Co., Ltd. (Shanghai, China). PowerUp SYBR Green Master Mix was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Rabbit anti-β-actin was from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China); horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG was from Beijing Zhong Shan Jin Qiao Biotechnology Co., Ltd. (Beijing, China); and rabbit monoclonal antibodies against β-catenin, cyclin D1, and c-Myc were from Abcam (Cambridge, UK). RIPA tissue/cell lysis buffer, BCA protein assay kit, and SDS-PAGE gel preparation kit were all purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The Hypersensitive Electrochemiluminescence (ECL) Kit was from Merck Millipore (Darmstadt, Germany).

Cell culture

The 4T1 mouse BC cell line was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in RPMI-1640 medium (Gibco, NY, USA) containing 10% fetal bovine serum (Sijiqing, Hangzhou, China), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified 5% CO2 incubator at 37 °C. Cells were passaged when they reached 90% to 95% confluence and were used for experiments when in a logarithmic growth phase.

Animals

Forty female specific pathogen-free BALB/c mice [6 weeks old, (20 ± 2) g body weight] were purchased from Beijing Hua Fu Kang Biotechnology Co. Ltd. (Beijing, China; certificate of quality SCXK [jing] 2014-0004). Mice were housed in the Clinical Medical Research Center, Affiliated Hospital of Guizhou Medical University at 22-24 °C with a relative humidity of 50% and a 12 h light/dark cycle. The mice were fed a normal diet and allowed access to water ad libitum.

Animal model and treatments

Mice were randomly divided into four groups designated the model, JZD, CTX, and JZD + CTX groups (n = 10 mice per group). Mouse were subcutaneously injected with 0.1 mL containing 107 4T1 BC cells in phosphate-buffered saline behind the forelimb arm-pit.15 When the tumor mass reached ~55-100 mm3 (ref.16), which occurred at approximately day 7 post-in-
jection, treatment was initiated. Starting on day 8, the CTX and JZD + CTX groups received an intraperitoneal injection of CTX 100 mg/kg in 0.1 mL normal saline once weekly for 3 weeks. The model and JZD groups received intraperitoneal injections of 0.1 mL normal saline on the same schedule. Starting on day 9, the JZD and JZD + CTX groups were administered 0.015 mL/g JZD (1.65 g/mL crude drug) by gavage once daily for 3 weeks. The model and CTX groups were administered 0.015 mL/g distilled water by gavage on the same schedule. The JZD mouse dose was allometrically scaled from the equivalent human dose based on body surface area.

Tumor measurement and inhibitory cancer ratio calculation
Tumor growth was assessed by measuring the long diameter (a) and short diameter (b) of the tumor with a Vernier caliper every 3 d. Volume (V) was calculated as ab^2/2. On day 22 of the experiment, mice were anesthetized, sacrificed, and weighed. The tumors were excised, weighed, portioned into pre-frozen tubes, and stored at −80 °C until analysis. The effects of treatment on tumor growth was expressed as the inhibitory cancer rate, calculated as: 

\[ P_{\text{growth}} = \frac{(M_{\text{model}} - M_{\text{treatment}})}{M_{\text{model}}} \times 100\% \]

Histopathology
Tumor samples were fixed in 10% buffered formalin, paraffin-embedded, cut into sections (5 μm thick), and stained with hematoxylin and eosin (HE) using standard procedures. Histopathological features were assessed under a light microscope.

Quantitative reverse-transcription polymerase chain reaction (RT-qPCR)
Total RNA was isolated from tumor samples using an Axygen Total RNA Extraction Kit according to the manufacturer’s instructions. Aliquots of total RNA were reverse transcribed using a PrimeScript™ RT Reagent Kit with gDNA Eraser and a ProFlex™ PCR System. qPCR was conducted using PowerUp™ SYBR™ Green Master Mix and an ABI Viia7Dx Real-Time PCR System. The PCR amplification conditions were: 95 °C for 2 min, 40 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 3 min, and a final extension of 72 °C for 7 min. Fluorescence was detected during the annealing phase. Target genes were quantified using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control. The primer sequences were: β-catenin forward 5’-TCAGCCGCTAGGACACC-GAT-3’ and reverse 5’-GATAAGCGGTGCTG-CAAA-3’; c-Myc forward 5’-CCCTATTTCCATCTGCGGACGAG-3’ and reverse 5’-GAGAAGGACG- TAGCGACC-3’; cyclin D1 forward 5’-TGACTGGGCGAAGTCTG-3’ and reverse 5’-CTCATCCCGCCCTTGGGATT-3’; and GAPDH forward 5’-AGGTGCTTGTTGAAAGGATTTG-3’ and reverse 5’-TGTAACCAGTAGTGGAGGTTCA-3’.

mRNA expression levels were calculated by the 2^-ΔΔCt method.

Western blot analysis
Tumor samples were lysed in RIPA Lysis Buffer containing a protease inhibitor mixture and sonicated with an Ultrasonic Crusher (HD3100, BANDELIN Electronic). Total protein concentration was measured using a BCA protein assay kit, and lyse samples with equivalent protein concentrations were separated by SDS-PAGE and electrotransferred to polyvinylidene difluoride membranes (0.45 μm, Millipore). The membranes were blocked with 5% skim milk in Tris-buffered saline/Tween-20 (TBST; 20 mM Tris, 140 mM NaCl, 0.1% Tween-20) for 2 h at room temperature and then incubated with the appropriate primary antibodies overnight at 4 °C. The next day, membranes were washed three times with TBST (10 min/wash) and incubated with HRP-conjugated goat anti-rabbit IgG for 1 h at room temperature. After three washes with TBST (10 min/wash), the membranes were incubated in ECL reagent. Protein bands were visualized in a CLINX imaging system and analyzed using ImageJ 1.48v software (National Institutes of Health, Bethesda, MD, USA). Relative protein expression was quantified by normalization to the internal reference protein β-actin.

Statistical analysis
Data were analyzed using SPSS 19.0 (SPSS Inc. Chicago, IL, USA). Quantitative data are expressed as the mean ± standard deviation (x ± s). A homogeneity test of variance was performed. When variances were equal, group means were compared by one-way analysis of variance followed by Dunnett’s t test (for comparisons between the model and treatment groups) or the Student-Newman-Keuls (SNK-q) test (for comparisons between two treatment groups). If variances were unequal, a nonparametric test was performed. P < 0.05 was considered statistically significant.

RESULTS

Tumor histopathology
HE staining of tumors from the untreated model group showed that most of the cancer cells were oval or round in shape, with a large nucleocytoplasmic ratio and strong staining of the nucleolus and chromatin (Figure 1A, B). Cells appeared to be actively dividing, arranged in a disorderly manner, and be poorly differentiated. Cancer cells showed a diffuse lamella-like solid distribution, with a small portion of glandular cavi- ty-like structure immersed in the striated muscle and surrounding tissues. Partial necrosis was evident (Figure 1A, B).
The mean tumor weight in the CTX and JZD + CTX groups were 45.0% and 9.0% of the JZD group, CTX group, and JZD + CTX group, respectively. To quantify the effects of the various treatments on tumor growth, we calculated the inhibitory cancer ratios. Inhibitory cancer rates were determined by comparing the mean tumor weights of the treated groups to the control group. The results showed that the JZD group had a significantly lower mean tumor weight compared to the model group (all P < 0.001, Dunnett’s t test). The mean tumor weight in the CTX and JZD + CTX groups were also significantly lower than that in the JZD group (both P < 0.001), whereas the mean tumor weight in the JZD + CTX and CTX groups were not significantly different.

**Tumor growth**

Tumor volumes were measured every 3 d for 21 d (Figure 2). Compared with the model group, JZD treatment led to significantly smaller tumors on days 6 and 9 of treatment (P < 0.05 for both), whereas CTX treatment resulted in significantly smaller tumors on days 12, 18, and 21 (P < 0.05 and P < 0.01), and JZD + CTX treatment led to significantly smaller tumors on days 6, 9, 18, and 21 (P < 0.05 and P < 0.001; Dunnett’s t test). In addition, on days 9, 18, and 21 of treatment, the mean tumor volume in the JZD + CTX group was significantly smaller than that in the CTX group (all P < 0.05) and the JZD group (P < 0.05, P < 0.01, and P < 0.001, respectively; SNK-q test) (Figures 2, 3).

**Inhibitory cancer rates**

To quantify the effects of the various treatments on tumor growth, we calculated the inhibitory cancer ratios compared with the model group. The inhibitory ratios of the JZD group, CTX group, and JZD + CTX group were 9.5%, 35.4%, and 48.1%, respectively (Table 1). The mean tumor weight in the CTX and JZD + CTX groups were significantly lower than that in the model group (both P < 0.001), whereas tumors in the JZD and model groups were comparable (Dunnett’s t test). The mean tumor weight in the CTX and JZD + CTX groups were also significantly lower than that in the JZD group (both P < 0.001), whereas the mean tumor weight in the JZD + CTX and CTX groups were not significantly different.

**β-catenin, c-Myc, and cyclin D1 mRNA expression in tumor samples**

RT-qPCR analysis (Figure 4) revealed that β-catenin, c-Myc, and cyclin D1 mRNA levels in tumors excised from the JZD, CTX, and JZD + CTX groups were all significantly lower than the mRNA levels in tumors from the model group (all P < 0.001, Dunnett’s t test). The expression of all three mRNAs was also lower in tumors from the JZD + CTX group than in either the JZD group or the CTX group (P < 0.01 and P < 0.001, respectively; SNK-q test). Notably, the relative expression of β-catenin mRNA in tumors from the JZD group was significantly lower than in tumors from the CTX group (P < 0.001), whereas c-Myc and cyclin D1 mRNA levels were comparable in the JZD and CTX groups (P > 0.05, SNK-q test).

**β-catenin, c-Myc, and cyclin D1 protein expression in tumors**

A similar analysis of protein expression levels by Western blotting (Figures 5, 6) revealed that β-catenin, c-Myc, and cyclin D1 protein levels were significantly lower in tumors from the JZD and JZD + CTX groups than in tumors from the model group (P < 0.05 and P < 0.001, respectively). In contrast, β-catenin and c-Myc protein levels were significantly lower in the CTX group than in the model group (P < 0.05), whereas cyclin D1 protein expression did not differ between these two groups. All three proteins were expressed at lower levels in the JZD + CTX group than in either the JZD group or CTX group (P < 0.05, P < 0.01, and P < 0.001; SNK-q test). β-Catenin protein levels were lower in the JZD group compared with the CTX group (P < 0.05), whereas expression of c-Myc and cyclin D1 proteins were comparable between these two groups (Figures 5, 6).

**DISCUSSION**

Intense research on the molecular biology of BC has revealed that the Wnt/β-catenin signaling pathway plays a crucial role in the growth of BC cells. Abnormal signaling in cancer cells is associated with several key modifications of this pathway, including altered Wnt expression level, enhanced stabilization or degradation of β-catenin, and nuclear translocation of β-catenin. Activation of the canonical Wnt/β-catenin signaling pathway occurs during the development of cancer, es-
Wnt binds to the transmembrane receptor Frizzled (Fz) and lipoprotein receptor-related protein 5/6 (LRP5/6), which induces Dsh (Discheveld, Dvl) to inhibit phosphorylation of β-catenin by the glycogen synthase kinase 3β (GSK-3β)/colorectal adenomatous polyposis protein (APC)/Axin complex. This prevents β-catenin degradation and induces its gradual accumulation in the nucleus, where it complexes with the transcription factor T cell factor (TCF) to promote the transcription of genes involved in stimulating cell proliferation and inhibition of apoptosis, such as cyclin D1 and c-Myc. Cyclin D1 is a key regulator of the G1 phase of the cell cycle through its activation of cyclin-dependent kinase 4/6. Accordingly, overexpression of cyclin D1 in cancer cells promotes G1 to S phase transition, DNA replication, and cell division, consistent with the uncontrolled proliferation characteristic of cancer cells. c-Myc, a downstream target gene of the β-catenin-TCF/LEF complex, plays a dual role in stimulating cell proliferation and inducing apoptosis. C-Myc also regulates the G1 to S phase transition; accordingly, high levels of this protein are associated with accelerated entry especially BC. Wnt binds to the transmembrane receptor Frizzled (Fz) and lipoprotein receptor-related protein 5/6 (LRP5/6), which induces Dsh (Discheveld, Dvl) to inhibit phosphorylation of β-catenin by the glycogen synthase kinase 3β (GSK-3β)/colorectal adenomatous polyposis protein (APC)/Axin complex. 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Figure 5 Western blot analysis of β-catenin, c-Myc, and cyclin D1 protein expression in tumors from each mouse group.

Representative blot of tumors from 1, the model group; 2, the JZD group; 3, the CTX group; and 4, the JZD + CTX group. JZD: Jiazhu decoction; CTX: cyclophosphamide. Model group: treated with normal saline and distilled water; JZD group: treated with normal saline and JZD (0.015 mL of 1.65 g/mL crude drug, administered daily by gavage) for 3 weeks; CTX group: treated with distilled water and CTX (100 mg/kg every week by intraperitoneal injection) for 3 weeks; JZD + CTX group: treated with JZD and CTX on the same dose and schedule above.

into the S phase of the cell cycle. The known close relationship between BC and the Wnt/β-catenin signaling pathway prompted us to investigate their expression as possible mediators of the therapeutic effects of JZD and/or CTX in the present study.

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Figure 6 Western blot analysis of β-catenin, c-Myc, and cyclin D1 protein expression in tumors from each mouse group.

Data are presented as the mean ± standard deviation of n = 10 mice group. JZD: Jiazhu decoction; CTX: cyclophosphamide. Model group: treated with normal saline and distilled water; JZD group: treated with normal saline and JZD (0.015 mL of 1.65 g/mL crude drug, administered daily by gavage) for 3 weeks; CTX group: treated with distilled water and CTX (100 mg/kg every week by intraperitoneal injection) for 3 weeks; JZD + CTX group: treated with JZD and CTX on the same dose and schedule above. P < 0.05, P < 0.01, P < 0.001 vs the model group; P < 0.05, P < 0.01, P < 0.001 vs the CTX group; P < 0.05, P < 0.01 vs the JZD group.

**REFERENCES**


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13 Han R. A brief analysis of Professor Liu Shangyi’s "when Yin and Yang both be eliminated, cultivating Yin to find a breakthrough". Zhong Yi Yao Xin Xi 2013; 30(4): 75-78.


27 Wen X, Zhou HL. Advances in research on chemical constituents and pharmacological effects of Bie jia (Carapa Gomas) Xi Bei Yao Xue Za Zhi 2008; 23(02): 122-124.


31 Liu P, Lin ZJ, Zhang B. Advances in research on chemical constituents and pharmacological effects of Bai Bei (Bulbus Lilii). Zhong Guo Shi Yan Fang Ji Xue Za Zhi 2017; 23(3): 201-211.


