Lung cancer is the leading cause of malignancy-related death in the Western world.\(^1\,^2\) The morbidity and the lethality of the lung adenocarcinoma increase drastically in China, and lung cancer has become the main cause of death among malignancies.\(^3\)

The two major histological types of lung cancer are non-small cell lung cancer (NSCLC) accounting for about 85% of cases and small cell lung cancer (SCLC) accounting for 15% of cases.\(^4\) The most active chemotherapeutic agent for the treatment of NSCLC and SCLC is cisplatin (CDDP), which is used in a doublet with other agents, such as paclitaxel, gemcitabine, and docetaxel.\(^5\) However, the overall 5-year survival from lung cancer remains dismal at around 16%.\(^6\) The response rate in NSCLC from CDDP alone is about 20% and in combination with a second agent improves to about 26%.\(^7\) Therefore, it is urgent to explore an effective therapeutic approach.

**ABSTRACT**

Objective: To observe the effect of the combination of Wenxia Changfu Formula (温下肠腑方, WCF) with cisplatin (CDDP) on inhibiting non-small cell lung cancer (NSCLC) in vitro and in vivo and explore its mechanism from its effect on cell cycle. Methods: In vitro, WCF-containing serum was prepared and the rhubarb b1, emodin, and aconitine were detected qualitatively by high-performance liquid chromatogram (HPLC). A549 cell lines were treated with blank control (dimethyl sulfoxide), normal serum, normal serum with CDDP (1.25, 2.5, and 5.0 \( \mu \text{g/mL} \), respectively), WCF-containing serum plus different doses of CDDP (1.25, 2.5, and 5.0 \( \mu \text{g/mL} \), respectively). The inhibitory effect was detected by 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The cell cycle was detected by flow cytometry. The protein and mRNA expressions of cyclin D1, proliferating cell nuclear antigen (PCNA), retinoblastoma (Rb), and p16 were observed with immunofluorescence and RT-PCR, respectively. In vivo, nude mouse xenograft model was established and grouped into the control, CDDP, WCF, and combination groups. The combination's inhibition of tumor growth and influence on the spleen, thymus, and thymus gland weight were observed. Results: The inhibitory rate of the combination against A549 cell lines excelled the CDDP alone significantly \((P<0.05)\); the combination showed a synergistic inhibitory effect \((Q=1.19)\). Compared with the monotherapy, the combination increased the cell percentage in G0/G1 phase and decreased the cell percentage in S phase significantly \((P<0.05)\); the protein and mRNA expressions of cyclin D1, PCNA, and Rb were significantly reduced; the protein and mRNA expressions of p16 were significantly enhanced. Compared with the monotherapy, the combination inhibited the tumor growth significantly in vivo and reduced the weight of tumor \((P<0.05)\); compared with the CDDP group, the spleen and thymus gland weight of the combination group were enhanced significantly \((P<0.05)\). Conclusions: The combination of WCF with CDDP significantly inhibited the A549 cell lines proliferation in vitro and the growth of the tumor in vivo; it inhibited effectively the atrophy of the immune organ caused by chemotherapy. The combination inhibited overproliferation of A549 cell lines by arresting the G0/G1 phase of cell cycle and affecting the protein and mRNA expressions of cell cycle-related proteins, cyclin D1, etc.

**KEYWORDS** Chinese medicine, Wenxia Changfu Formula, chemotherapy, inhibitory effect, cell cycle, cell cycle-related proteins, non-small cell lung cancer
The combination of Chinese medicine (CM) with chemotherapy plays an active role in the treatment of tumor.\(^{(8)}\) Researches reported Wenxia Changfu Formula has (温下肠腑方, WCF) significant effect of inhibiting S180, H22, and Lewis transplantable tumor, improving the immune function, and inducing apoptosis of cells.\(^{(9-12)}\) This project combined WCF with CDDP and studied its intervention effect on lung cancer in vitro and in vivo. It was a pilot study on the effect of inhibiting proliferation and its mechanism.

**METHODS**

**Reagents**

CDDP was purchased from Qilu Pharmaceutical Co., Ltd., China. Cell cycle kits was purchased from Becton Dickinson, USA. Primary antibody: a mouse antihuman cyclin D1, p16, proliferating cell nuclear antigen (PCNA), Rb monoclonal antibody, secondary antibody: FITC-IgG, immunofluorescence staining kits were all from Abcam Co., Ltd., UK.

**Preparation of WCF**

WCF is composed of Radix Aconiti Praeparatae (12 g), Radix et Rhizoma Rhei (6 g), Panax ginseng (12 g), and Angelica sinensis (9 g), which were all purchased from the Jianlian Company of Traditional Crude Drugs (Jinan, China). The mixture of Radix Aconiti Praeparatae and Panax ginseng were macerated for 1 h and then decocted for 2 h; Angelica sinensis was added and decocted for 0.5 h, Radix et Rhizoma Rhei was added and decocted for 1/4 h for the first and the second time. The filtrates were mixed and condensed. The yield of WCF extract was 1 g/mL stored at 4 ℃ and filtered before use.

**Preparation of Drug-Containing Serum**

Ten male Wistar-Kyoto rats (certification No. SCXK 20080004), age 14 weeks, weight 280–320 g were purchased from the Laboratory Animal Center of Shandong University (Jinan, China). The mixture of Radix Aconiti Praeparatae and Panax ginseng were macerated for 1 h and then decocted for 2 h; Angelica sinensis was added and decocted for 0.5 h, Radix et Rhizoma Rhei was added and decocted for 1/4 h for the first and the second time. The filtrates were mixed and condensed. The yield of WCF extract was 1 g/mL stored at 4 ℃ and filtered before use.

**Cell Culture**

NSCLC A549 cell lines were purchased from the Shanghai Institute of Biochemistry and Cell Biology (SIBCB), China Academy of Sciences (CAS). The cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% penicillin, and streptomycin in a humidified incubator with 5% CO₂ at 37 ℃.

**MTT Assay**

A549 cells lines were seeded in 96-well plates. After 12 h, A549 cell lines were grouped into normal serum, normal serum with 1.25, 2.5 and 5.0 μg/mL CDDP, WCF-containing serum, WCF-containing serum with 1.25, 2.5 and 5.0 μg/mL CDDP groups, respectively. Samples added only 3-(4,5-dimethylthiazo(-z-y1)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were used as blank controls. After being incubated respectively for 24, 48, and 72 h, 20 μL of 5 mg/mL MTT solution was added, and cells were cultured at 37 ℃ for 4 h. Next, culture medium was removed, and 150 μL DMSO was added to each well and agitated for 15 min. The absorbance of the samples was measured at 570 nm. At least, 3 independent experiments were conducted. To confirm the synergism of WCF-containing serum and CDDP, Jin’s calculation formula was used to determine the combination index (Q) (with Q<1.15, indicating synergism, 0.85<Q<1.15 indicating an additive effect, Q<0.85 indicating antagonism).\(^{(14)}\)