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Chinese medicine formula "Weikang Keli" induces autophagic cell death on human gastric cancer cell line SGC-7901

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ABSTRACT

Weikang Keli (constitutes of *Root of Codonopsis pilosula*, *Rhizoma Atractylodis Macrocephalae*, *Rhizoma Curcumae Aeruginosae*, *Rhizoma Pinelliae*, *Actinidia chinensis Planch*, and *Rhodiola rosea*) is a well known Chinese herbal formula for gastric cancer therapy in clinical treatment. However, the detailed molecular mechanisms involved are still not fully understood. In this study, we found that Weikang Keli could induce patterns of autophagy in SGC-7901 cells, including intracellular vacuole formation, microtubule-associated protein 1 light chain 3 (LC3) conversion. Hoechst 33258 staining and Western blot analysis of apoptosis-related proteins showed that WK induced SGC-7901 cell death was not through apoptosis. *In vivo* study also revealed that i.g. administration of Weikang Keli once a day for 25 days could significantly reduce tumor volumes by about 50%. Collectively, the current data indicated that Weikang Keli induced gastric cancer cell death by autophagy effects.

Introduction

Despite its declining incidence, gastric cancer is still the second most common cause of death from cancer in Asia and worldwide (Kamangar et al. 2006; Leung et al. 2008). Gastric cancer is a serious threat to people's lives and health. Surgery remains the mainstay of any curative treatment; however, approximately two-thirds of patients diagnosed with gastric cancer have high possibilities of recurrence and metastasis after surgeries (Hundahl et al. 2000). Meanwhile, gastric cancer is not sensitive to radiotherapy, turning out to reply on the major treatment approach of chemotherapy. Most patients with advanced gastric cancer need to accept cytotoxic chemotherapy as one of the main treatments. Recently, platinum compounds, 5-fluorouracil and taxanes have been widely used in treatment of gastric cancer. Various attempts have been made to improve the objective response rate to chemotherapy, including chemotherapeutic agents in combination, however, the optimal combination regimen has remained elusive, and standard treatment remains a matter of debate (Ajani 2005). It is necessary to find new compounds and optimized combinational treatment for gastric cancer.

As Traditional Chinese Medicine can improve human immunity, attenuation function and synergies, it has been widely used in adjuvant therapies for cancer. A common view holds that the therapy against cancer shall integrate surgery, radiotherapy, chemotherapy and Traditional Chinese Medicine (Zhu and Si 2005). According to the understanding of the pathogenesis of gastric cancer in Traditional Chinese Medicine, based on the aim of tonifying spleen, invigorating the circulation of blood and detoxifying (Fu et al. 2011; Xu et al. 2003), a Chinese herbal compound, Weikang Keli (WK), constitutes six ingredients including *Root of Codonopsis pilosula*, *Rhizoma Atractylodis Macrocephalae*, *Rhizoma Curcumae Aeruginosae*, *Rhizoma Pinelliae*, *Actinidia chinensis Planch*, and *Rhodiola rosea*, can be used in the therapy against cancer. Studies have verified that sesquiterpene compounds extracted from *Rhizoma Atractylodis Macrocephalae* can induce cell differentiation of melanoma B16 and suppress cell migration via Ras/Erk and PI3K/Akt signaling pathways (Yan et al. 2011). The essential ingredient in *Rhizoma Curcumae Aeruginosae*, curcumol, can induce the apoptosis of lung cancer cell ASTC-a-1 through caspase non-dependent mitochondrial approach (Zhang et al. 2011). Normal butanol extracts of *Actinidia chinensis Planch* plays a key role in suppressing lung cancer cell A549, which is hold in the stage of G0–G1 with a significantly reduced ratio in the S stage (Wang et al. 2010). Ester plant hormone extracted from *Rhodiola rosea* plays a dual role of protection and treatment for cells. Experiments *in vivo*

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Table 1
The composition of WK.

Species	Chinese name	Plant part	Origin	Grams, g	%
Root of Codonopsis pilosula	Dang shen	Root	Gansu, China	10	13.3
Rhizoma Atractylodis Macrocephalae	Sheng bai zhu	Root, stem	Zhejiang, China	15	20.0
Rhizoma Curcumae Aeruginosae	E zhu	Root, stem	Guangxi, China	10	13.3
Rhizoma Pinelliae	Fa ban xia	Tuber	Sichuan, China	10	13.3
Actinidia chinensis Planch	Teng li gen	Root	Yunnan, China	15	20.0
Rhodiola rosea	Hong jing tian	Root, stem	Xizang, China	15	20.0
Total amount				75	100.0

and *in vitro* have confirmed that it can suppress the proliferation, migration and invasion of breast cancer cells, and can induce the generation of autophagy vesicles of human breast cancer cell MDA-MB-231 and mouse breast cancer cell V14 while shunning away the impact on the normal breast epithelial cells (Tu et al. 2008). WK has performed excellent efficacy in the clinical prevention and treatment of gastric cancer. However, the specific mechanism of WK remains not clarified. In this experiment, the human gastric cancer cell line SGC-7901 was taken as the object of study. Through *in vitro* and *in vivo* study, this paper intends to explore the mechanism of WK on suppressing the growth of gastric cancer cells.

Materials and methods

Cell line and animals

Human gastric cancer cell line SGC-7901 from Cell Bank of the Shanghai Institute of Biochemistry & Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, were cultured in RPMI-1640 medium (Invitrogen Corp, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA), 100 Units/ml penicillin, 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. BALB/c-nu mice were purchased from Shanghai SLRC Laboratory Animal Co., Ltd. (Shanghai, China). Mice were housed under specific pathogen-free conditions and provided with a standard rodent laboratory diet from Shanghai SLRC Laboratory Animal Co., Ltd.

Single herb and WK preparation

WK is composed of *Root of Codonopsis pilosula* (10 g), *Rhizoma Atractylodis macrocephalae* (15 g), *Rhizoma Curcumae aeruginosae* (10 g), *Rhizoma Pinelliae* (10 g), *Actinidia chinensis Planch* (15 g), and *Rhodiola rosea* (15 g). All medicinal plants used to prepare formulae were provided by Jiangsu Province Hospital on Integration of Chinese and Western Medicine (Nanjing, Jiangsu, China), plant parts, and origin used in the formula as Table 1. Aqueous extract of single herb and WK were prepared according to the following procedure: single herb or six medicinal materials were homogenized with a Waring blender, then soaked in the tenfold double distilled water for 24 h. The mixture was heated to 100 °C for 2 h, and the decoction was filtrated. The filtrates obtained from 2 cycles of the procedures were mixed, concentrated by heating and granulated by lyophilization. Total yield of the WK extract is 200 g lyophilized powder from water extract of 1 kg raw mixed herb. WK and its preparations were standardized, regulated, and quality-controlled according to the guidelines defined by Chinese State Food and Drug Administration (SFDA). Standardization of herbal extracts was done using HPLC fingerprinting with chemical standards (adenosine and lobetyolin) purchased from National Institute for the Control of Pharmaceutical and Biological Products in China.

HPLC analysis

HPLC fingerprint of single herb and WK was performed as following procedure: Extracts were separated by a Waters 2695 HPLC instrument (Waters, USA) equipped with a Alltima C_{18} (250 mm × 4.6 mm, 5 μm) column. The mobile phase consisted of water (I) and acetonitrile (II) at a flow rate of 1.0 ml min^{-1}. A gradient program was used as follows: 0 min, 5% II; 17 min, 10% II; 36 min, 17% II; 100 min, 60% II; 110 min, 100% II; 120 min, 100% II. The PDA detector scanned from 200 nm to 600 nm, the monitor wave length was set at 254 nm. Fig. 1 showed the HPLC fingerprint of WK extracts (Table 2).

Cell morphological assessment

SGC-7901 cells were cultured in RPMI-1640 till mid-log phase. Negative control (H_2O), positive control (hydroxychloroquine, HCQ), WK (0.005, 0.01 and 0.02 g/ml) was then added to the culture medium. The morphology of cells was monitored under an inverted microscope (Zeiss Axio Observer A1) at 2 h, 6 h, 12 h and 24 h.

Cytotoxicity assay

SGC-7901 cells were seeded in 96 well plates, 1×10^4 cells per well. The medium was replaced by serum-free medium containing different concentration of WK. After 24 h, 48 h and 72 h,

Table 2
Characterization of compounds in WK by HPLC.

Peak no.	RT (tR, min)	Compound name	Molecular formula	Classification[a]
1	13.38	Adenosine	$C_{10}H_{13}N_5O_4$	4
2	27.35	Di-(2-ethylhexyl) phthalate	$C_{24}H_{34}D_4O_4$	1
3	34.30	5-Hydroxy-4′,7-dimethoxy-flavone	$C_{17}H_{14}O_5$	1
4	35.95	(−)-Epicatechin	$C_{15}H_{14}O_6$	5
5	36.24	Salidroside	$C_{14}H_{20}O_7$	6
6	48.31	Stearic acid	$C_{18}H_{36}O_2$	1
7	49.43	n-Heneicosane	$C_{21}H_{44}$	1
8	54.74	Lobetyolin	$C_{20}H_{28}O_8$	1
9	73.23	Zeylanine	$C_{15}H_{20}O_4$	2
10	89.42	Codonolactone	$C_{15}H_{20}O_3$	1
11	105.49	Zedoarolide A	$C_{15}H_{20}O_6$	3

[a] (1) *Root of Codonopsis pilosula*; (2) *Rhizoma Atractylodis macrocephalae*; (3) *Rhizoma Curcumae aeruginosae*; (4) *Rhizoma Pinelliae*; (5) *Actinidia chinensis Planch*; (6) *Rhodiola rosea*.