

# Synergistic inhibitory effect of wogonin and low-dose paclitaxel on gastric cancer cells and tumor xenografts

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**Objective:** To investigate the synergistic inhibitory effects of wogonin (WOG) and chemotherapeutic drugs on growth of gastric cancer cells and tumor xenografts.

**Methods:** The IC<sub>50</sub> values of WOG, cisplatin (CDDP) and paclitaxel (PTX) in four gastric cancer cell lines were determined by MTS assay. Hoechst staining and the median effect method of Chou-Talalay were used to assess the apoptosis of cells and the interaction of two drugs, respectively. BGC-823-derived xenografts in nude mice were established to investigate the effects of WOG combined with chemotherapeutic drugs *in vivo*.

**Results:** WOG, CDDP and PTX inhibited the growth of BGC-823, MGC-803, MKN-45 and HGC-27 gastric cancer cells in a dose-dependent manner. WOG combined with CDDP or PTX synergistically inhibited the growth of all gastric cancer cell lines *in vitro*. In BGC-823, MGC-803, HGC-27 and MKN-45 cell lines, synergisms between WOG and PTX were shown when the fraction affected (Fa) values were <0.45, <0.90, <0.85 and <0.60. While WOG and CDDP had a synergistic inhibitory effect when the Fa values were >0, >0, >0.65 and >0.10. From the results of *in vivo* experiments using tumor xenografts, WOG and low-dose PTX showed better efficacy than either drug alone. The inhibitory percentages of tumor weight were 61.58%, 20.29%, and 22.28% for the combination, WOG-alone, and low-dose PTX-alone groups, respectively. Notably, WOG combined with CDDP displayed very high toxicity.

**Conclusions:** A synergistic inhibitory effect on growth was observed when WOG was combined with low-dose PTX in gastric cancer cells and tumor xenografts. These findings provide evidence for the design of a clinical trial to test the combination of WOG with low-dose PTX in human gastric cancer.

**Keywords:** Wogonin (WOG); paclitaxel (PTX); gastric cancer; synergistic effect



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## Introduction

Gastric cancer is the fourth most common malignant tumor in the world. The mortality from gastric cancer is preceded only by that of lung cancer. Approximately 40% of patients with gastric cancer miss the opportunity to undergo radical curative surgery at the time of diagnosis, while 50% of patients experience recurrence and metastasis after surgery (1). Therefore, chemotherapy plays an important role in the treatment of this cancer.

Cisplatin (CDDP) and paclitaxel (PTX) are frequently used in the systemic chemotherapy of patients with gastric cancer, and combination chemotherapy with two or three drugs is common with superiority in the first- or second-line treatment of gastric cancer (2). Although great achievements have been made in the treatment of gastric cancer, the response rate of combination chemotherapy is rarely over 50% (3). However, the toxicity of combination chemotherapy is greater than that of single drugs (4). Thus, the development of optimal chemotherapeutic regimens

with high efficacy and low toxicity is necessary.

Wogonin (WOG) is a flavonoid extracted from the root of *Scutellaria baicalensis Georgi*. It is a phytochemical that has been widely used in the treatment of allergy, inflammation, hypertension, and cardiovascular diseases (5). Recently, *in vitro* and *in vivo* experiments have demonstrated that WOG can inhibit the growth of malignant tumors such as bladder cancer (6), myelogenous leukemia (7), hepatocellular carcinoma (8), gastric cancer (9), and glial tumors (10) through inducing Ca<sup>2+</sup>-dependent apoptosis (11), attenuation of NF-κB activity (12), and enhancement of the expression of p53 up-regulated modulator of apoptosis (PUMA), all of which could mediate its cytotoxicity (13).

Importantly, WOG inhibits the growth of malignant tumor cells but does not influence the proliferation of normal cells such as epithelial and peripheral blood cells (14), and prostate cells (13). Accordingly, regimens combining WOG and common chemotherapeutic drugs may achieve better efficacy with lower toxicity levels. Zhao *et al.* have previously reported that WOG can strengthen the efficacy of low doses of the chemotherapy drug fluorouracil without increasing toxicity in a nude mouse model of gastric cancer (9). However, there have been no investigations related to the effects of combining WOG and other common chemotherapeutic drugs such as CDDP and PTX in gastric cancer. Therefore, this study was conducted to investigate whether WOG synergizes the effects of either CDDP or PTX in gastric cancer *in vitro* and *in vivo*.

## Materials and methods

### Drugs and cell culture

WOG (kindly provided by Professor Qinglong Guo, China Pharmaceutical University), CDDP (Qilu Pharmaceutical Co., Ltd., China) and PTX (Hainanhaiyao Co., Ltd., China) were freshly prepared using sterile physiological saline before each use.

Human gastric cancer BGC-823, MGC-803, and MKN-45 cell lines (kindly provided by Professor Youyong Lv, Peking University Cancer Hospital & Institute) and HGC-27 cell line (purchased from the Shanghai Institute for Biological Sciences, CAS) were maintained in RPMI-1640 medium (Gibco) with 10% heat-inactivated fetal bovine serum (Gibco) under a humidified atmosphere of 5% CO<sub>2</sub> and 95% air at 37 °C.

### Cell proliferation assay

Cell proliferation was analyzed by CellTiter 96<sup>®</sup> AQueous One Solution Cell Proliferation Assay (MTS assay, Promega) in three replicates according to the manufacturer's instructions. Briefly, cells (5,000/well) were grown in RPMI-1640 medium (100 μL/well) in 96-well plates and exposed to various concentrations of the experimental drug. After 48 h of drug exposure, 20 μL of MTS was added to the medium and cells were incubated in the CO<sub>2</sub> incubator at 37 °C for 2-4 h. The absorbance was then read at 490 nm by a plate reader. Absorbance at 490 nm was directly proportional to the viable cell number in culture (15) and thus could be used to determine relative growth using the following calculation:  $[(A_{\text{treated}} - A_{\text{zero}}) / (A_{\text{control}} - A_{\text{zero}})] \times 100\%$ . The A<sub>treated</sub> reflected the living cell number in treated wells, the A<sub>control</sub> depicted the living cell number of untreated wells, and the A<sub>zero</sub> reflected background absorbance, which is subtracted to correct the error.

### Assessment of combination effects of two drugs

Based on the median effect method of Chou-Talalay (16), the IC<sub>50</sub> values of individual drugs was obtained. The combination index (CI) was calculated by the following formula (17):  $CI = (D)_1 / (DX)_1 + (D)_2 / (DX)_2 + [k(D)_1(D)_2 / (DX)_1(DX)_2]$ , where  $k=0$  for mutually exclusive drugs; (D)<sub>1</sub> and (D)<sub>2</sub> were the concentration of the separate drugs in their combination; (DX)<sub>1</sub> and (DX)<sub>2</sub> were individual drug concentrations that result in a cell inhibition ratio of X%. These concentrations were calculated by the formula:  $D = D_m [Fa / (1 - Fa)]^{1/m}$ , where D<sub>m</sub> is the concentration required to attain an inhibition of 50%, fraction affected (Fa) represents the respective proliferation inhibition parameters for the separate drugs (e.g., an Fa of 0.5 is a proliferation inhibition of 50%), and *m* is the slope of the median effect plot. CI >1, CI =1, and CI <1 represent antagonism, additive effect, and synergism, respectively. Then, effects of the combination were displayed using forms of Fa-CI plots, which were the CI versus Fa and showed the evolution of drug interactions (synergism, antagonism, additive effects) (18).

### Detection of cell apoptosis

Cells grown on coverslips in 12-well plates were exposed to individual drugs or two drug simultaneously for 48 h, then incubated with Hoechst 33258 (Hoechst Staining Kit, Beyotime, China). Fluorescence microscopy was used

to observe cell shape captured from six random visual fields. The ratio of apoptotic cells to total cell number was calculated.

**Xenograft model in nude mice**

BGC-823 cells ( $1 \times 10^6$ ) suspended in phosphate buffered saline (0.1 mL) were subcutaneously injected into the right oxter of 6-week-old female BALB/c athymic *nu/nu* mice (Vital River, China). Seven days after injection, when tumor volumes were about  $50 \text{ mm}^3$ , all mice ( $n=50$ ) were randomly divided into 10 groups ( $n=5$  per group) and treated intraperitoneally with different drugs as follows: control group (physiological saline, once a day for 2 weeks); WOG 60 mg/kg group (once a day for 2 weeks); PTX 10 mg/kg group (once a week for 2 weeks); PTX 20 mg/kg group (once a week for 2 weeks); CDDP 3 mg/kg group (once a week for 2 weeks); CDDP 6 mg/kg group (once a week for 2 weeks); WOG (60 mg/kg) plus PTX (10 mg/kg) group; WOG (60 mg/kg) plus PTX (20 mg/kg) group; WOG (60 mg/kg) plus CDDP (3 mg/kg) group; and WOG

(60 mg/kg) plus CDDP (6 mg/kg) group. The length and width of tumors as well as mouse weight were measured twice per week from day 0 to 16, where day 0 was the day of initial treatment and day 16 was the day of sacrifice. Tumor volume was calculated by the formula  $V = L \times W^2 \times \pi/6$  ( $V$ , volume;  $L$ , length;  $W$ , width of tumor).

**Statistical analysis**

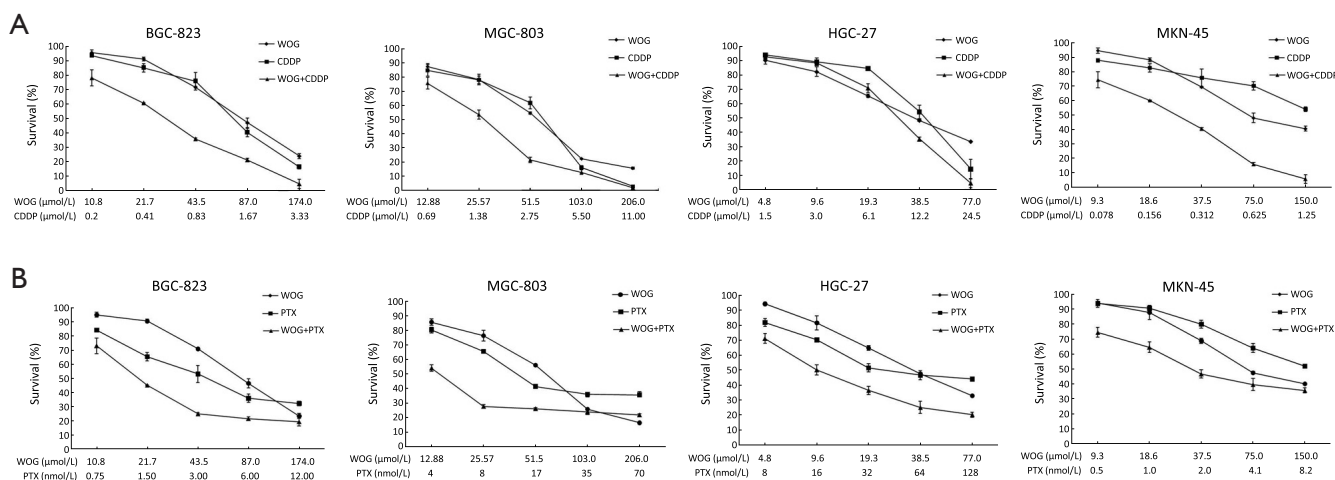
We performed statistical analysis using the Student's *t*-test with SPSS software (SPSS Inc., Chicago, IL, USA). The statistical significance of the data was accepted when the P value was less than 0.05.

**Results**

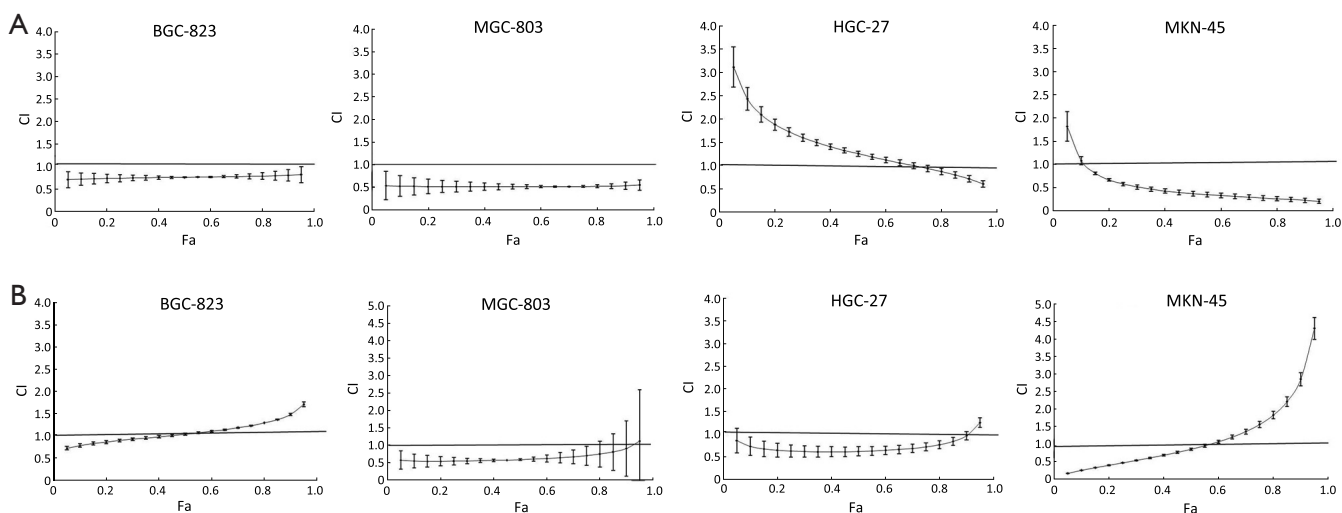
**WOG, CDDP, and PTX inhibit growth of gastric cancer cells in a dose-dependent manner**

The effect of each drug on gastric cancer cell growth was first tested to evaluate cell-line-specific sensitivity. The  $IC_{50}$  values for WOG, CDDP, and PTX in BGC-823, MGC-803, MKN-45, and HGC-27 cell lines are shown in Table 1. The data demonstrated that the growth of all cell lines was inhibited by any of the drugs used independently in a dose-dependent manner, although different cells display different sensitivity to the same drug (Figure 1). Compared with any individual drug, the growth of cells treated with a combination of WOG and either CDDP or PTX was further inhibited in a dose-dependent manner (Figure 1).

Cell line	$IC_{50}$ ( $\mu\text{mol/L}$ )		
	WOG	PTX	CDDP
BGC-823	$82.44 \pm 2.60$	$4.45 \pm 0.42$	$1.29 \pm 0.02$
MGC-803	$52.68 \pm 6.63$	$20.41 \pm 0.8$	$2.30 \pm 0.15$
HGC-27	$35.38 \pm 1.32$	$57.11 \pm 5.12$	$11.24 \pm 0.77$
MKN-45	$87.59 \pm 2.99$	$8.40 \pm 0.35$	$1.92 \pm 0.48$



**Figure 1** Inhibition of WOG and CDDP/PTX on four cell lines. Cells were exposed to various concentrations of WOG, CDDP and PTX alone or in combination for 48 h. Cell viability was detected by MTS assay. A and B were WOG combined with CDDP and PTX, respectively.



**Figure 2** Inhibition of the mean-effect of combination treatment on BGC-823, MKN-45, MGC-803 and HGC-27 cell lines. After exposed to combination of WOG and CDDP/PTX at a fixed ratio (WOG  $IC_{50}$ :CDDP/PTX  $IC_{50}$  were 1:1) for 48 h. The values of CI were calculated by the prescribed method. A and B were WOG combined with CDDP and PTX, respectively. CI <1 indicates synergism.

**Table 2** Summary of CI value and the concentration of the separate drugs in combination at 50% Fa

Drug administration		Fa =0.5			
		BGC-823	MGC-803	HGC-27	MKN-45
WOG + CDDP	CI	0.76±0.01	0.51±0.05	1.61±0.10	0.37±0.05
	Regimen				
	WOG ( $\mu\text{mol/L}$ )	28.68	11.91	34.36	23.12
	CDDP ( $\mu\text{mol/L}$ )	0.55	0.64	7.25	0.19
WOG + PTX	CI	1.05±0.03	0.60±0.03	0.74±0.11	0.86±0.03
	Regimen				
	WOG ( $\mu\text{mol/L}$ )	39.17	17.79	13.42	46.87
	PTX ( $\mu\text{mol/L}$ )	26.25	5.93	22.34	2.58

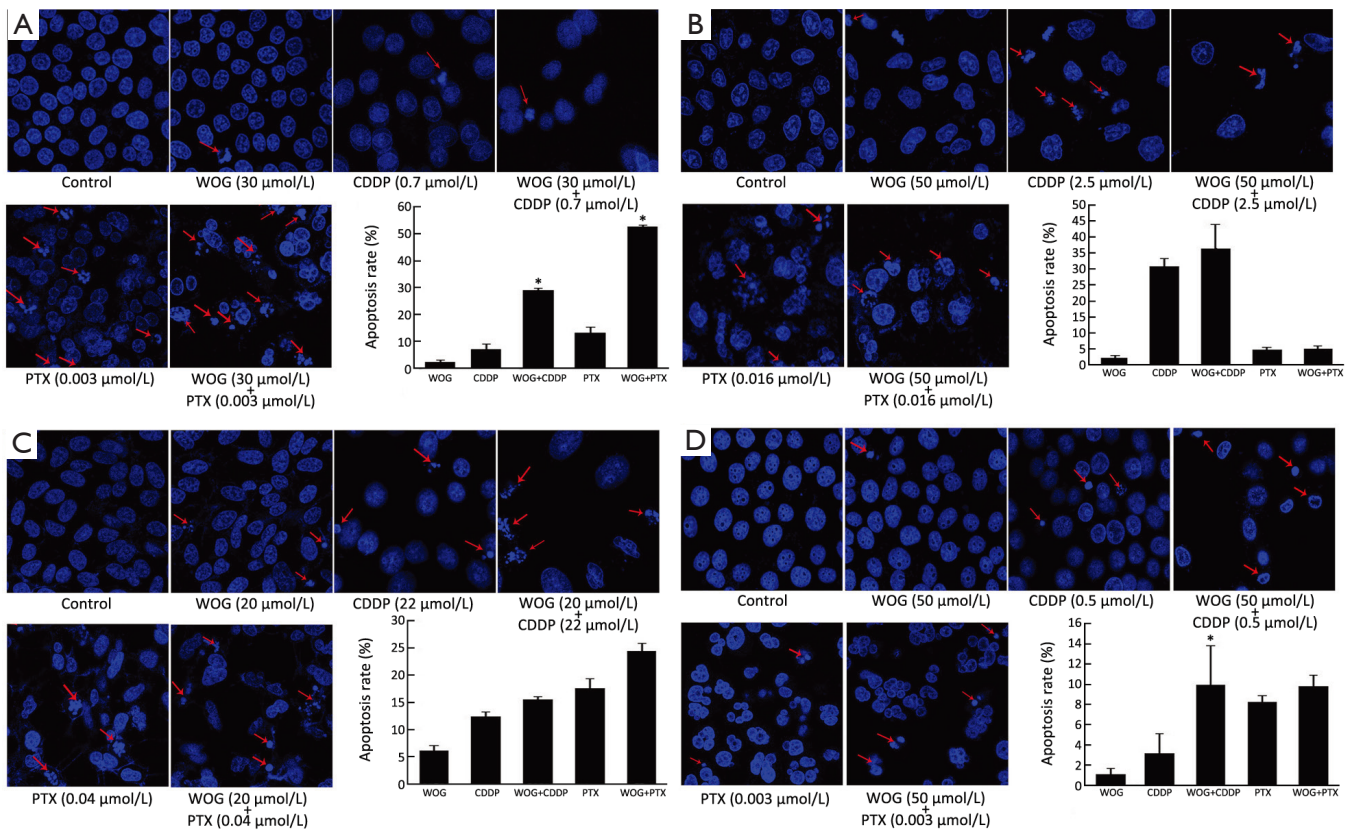
### Analysis of synergistic effects between WOG and CDDP/PTX *in vitro*

In BGC-823 and MGC-803 cell lines, WOG and CDDP induced significant synergistic growth inhibition (CI <1). While in HGC-27 and MKN-45 cell lines, WOG and CDDP had a synergistic inhibitory effect when Fa values were >0.65 and >0.1, respectively (Figure 2A). In BGC-823, MGC-803, HGC-27, and MKN-45 cell lines, synergism between WOG and PTX was seen when Fa values were <0.45, <0.9, <0.85, and <0.6, respectively (Figure 2B), suggesting that there might be antagonism between WOG and high-dose PTX.

Table 2 summarizes the values of CI and the concentration

of the individual drugs in their combination when Fa =0.5. In BGC-823 cell lines, WOG plus PTX showed antagonism (CI >1), which was also observed for WOG plus CDDP in HGC-27 cell lines. In the combination of WOG and CDDP, the concentrations of WOG were 33.75%, 22.53%, and 26.54% of the WOG  $IC_{50}$ , while the concentrations of CDDP were 42.48%, 28.83%, and 9.89% of the  $IC_{50}$  of CDDP in BGC-823, MGC-803, and MKN-45 cell lines, respectively. In the combination of WOG and PTX, the concentrations of WOG were 31.23%, 34.48%, and 53.49% of the WOG  $IC_{50}$ , while the concentrations of PTX were 29.03%, 39.12%, and 33.08% of the PTX  $IC_{50}$  in MGC-803, HGC-27, and MKN-45 cell lines, respectively.





**Figure 3** Apoptosis assessment on different cell lines. A, B, C and D were changes of nucleolus morphology and average apoptosis ratio of drugs alone or in combination on BGC-823, MGC-803, HGC-27 and MKN-45, respectively. \*,  $P < 0.05$  combination group compared with single-agent treatments.

**Apoptosis is induced by WOG and CDDP/PTX**

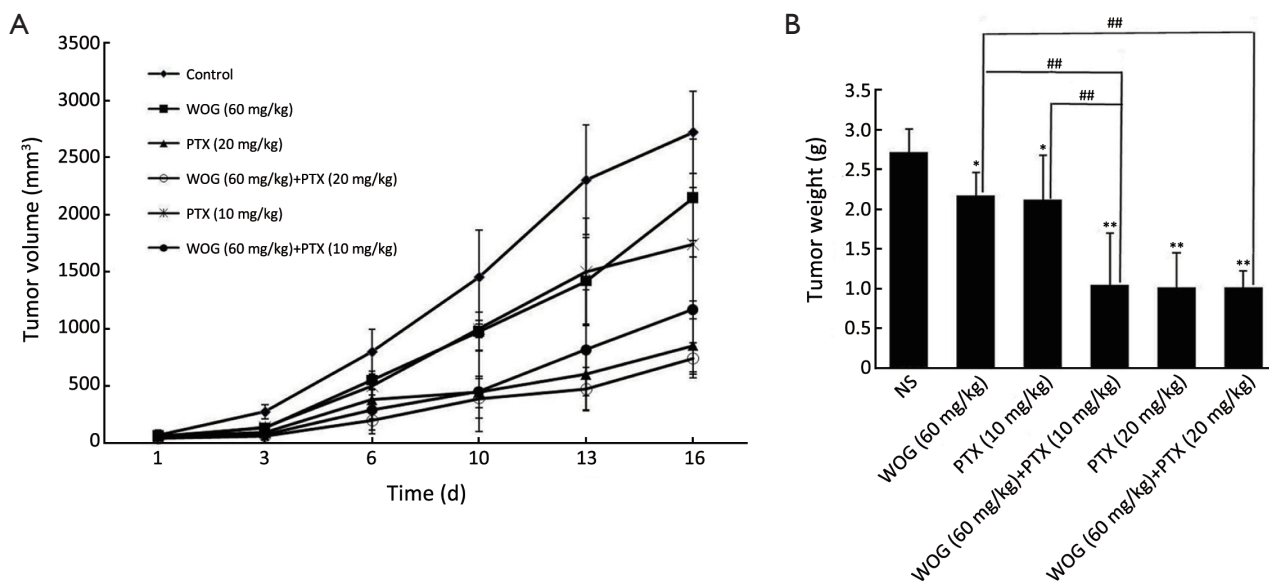
We chose drug concentrations that had a synergistic effect when used in combination therapy for follow-up experiments. First, apoptosis was evaluated in BGC-823, MGC-803, HGC-27, and MKN-45 cells that were exposed to single drugs or the combination treatments for 48 h. The cells were stained and evaluated for nuclear shape under an inverted fluorescence microscope (Figure 3). While untreated cells displayed evenly dispersed blue fluorescence within their nuclei, treated cells showed chromatin condensation or dense staining fragmentation called apoptotic bodies, which implied an early apoptotic event (19) (Figure 3). Meanwhile, the total number of cells in the single-drug-treated groups decreased compared with untreated groups. Importantly, a more obviously reduction was observed in combination groups. This was particularly more remarkable in the BGC-823 cell line ( $P < 0.05$ , Figure 3A). However, there were similar trends in the other three cell

lines ( $P > 0.05$ ). These results suggest that the combination of WOG and either CDDP or PTX could enhance the induction of apoptosis in gastric cancer cells.

**Inhibitory effect of drug combination on growth of BGC-823 tumor xenografts**

Because WOG combined with PTX/CDDP significantly inhibited the growth of different gastric cancer cell lines, the effect of combination treatment on the growth of gastric cancer cell line-derived tumor xenografts in nude mice was explored.

The effect of combining WOG with CDDP was first tested. While there was no significant difference in tumor volumes before treatment ( $P > 0.05$ ), after treatment all five mice exhibited poor mental state and inactivity. In the next 2-3 d, mice became thinner and their skin lost luster, and they soon all died. To counteract the toxicity, we reduced the dose of CDDP by half, but the result was not changed.



**Figure 4** Inhibitory effects of WOG and PTX on BGC-823 xenografts in nude mice. A was tumor volume (mm<sup>3</sup>); B was tumor weight (g). \*, P<0.05 compared with control; \*\*, P<0.01 compared with control; ##, P<0.01 combination group compared with corresponding single-agent groups.

**Table 3** Summary of inhibition of WOG and PTX on BGC-823 xenografts

Dose (mg/kg)	Body weight (g)		Number of animals		Tumor weight (g)	IR (%)
	Incipience	Terminal	Incipience	Terminal		
Control	22.1±0.8	23.9±0.7	5	5	2.72±0.28	
WOG =60	21.5±1.1	22.9±1.1	5	5	2.17±0.29 <sup>*</sup>	20.29
PTX =10	21.8±0.6	23.2±1.1	5	5	2.12±0.56 <sup>*</sup>	22.27
PTX =20	21.0±1.3	22.5±0.7	5	5	1.02±0.43 <sup>**</sup>	62.53
WOG + PTX =60+10	19.9±1.3	21.4±1.9	5	5	1.05±0.65 <sup>**##</sup>	61.58
WOG + PTX =60+20	20.9±1.4	21.4±0.8	5	5	1.01±0.21 <sup>**#</sup>	62.75

IR, inhibitory ratio of tumor weight; <sup>\*</sup>, P<0.05 compared with control; <sup>\*\*</sup>, P<0.01 compared with control; <sup>##</sup>, P<0.01 combination group compared with corresponding single-agent groups; <sup>#</sup>, P<0.01 combination group compared with WOG 60 mg/kg group.

In contrast, the above situation did not happen in groups where mice were treated with either WOG or CDDP independently. These observations suggest that WOG and CDDP cannot be used together because of much greater toxicity than single agents.

In the study of treatment with a combination of WOG and PTX, treated groups, either single drugs or the combination, all showed an inhibitory effect on the growth of BGC-823 xenografts when compared with the non-treated control group. The combination treatment groups

displayed even more obvious inhibition than corresponding single-drug groups. By the 16th day after the initial treatments, the mean tumor volume of the WOG plus PTX (10 mg/kg) group was much smaller than that of either the PTX-alone (10 mg/kg) or WOG-alone groups (1,171 mm<sup>3</sup>, 1,740 mm<sup>3</sup>, and 2,148 mm<sup>3</sup>, respectively) (P<0.05, *Figure 4A*). A similar trend was demonstrated among the WOG plus PTX (20 mg/kg) group, and the PTX-alone (20 mg/kg) or WOG-alone groups (P>0.05, *Figure 4A*). The mean tumor weights demonstrated that the combination treatments

were more efficient than single drugs (Figure 4B). Indeed, in the WOG plus PTX (10 mg/kg) group, the inhibitory ratio (IR) of tumor weight was 61.58% while it was 20.29% and 22.28% in the WOG-alone and PTX-alone (10 mg/kg) groups, respectively. Interestingly, one mouse treated with PTX (20 mg/kg) presented with hemiplegia, which is an indicator of neurotoxicity. No weight loss was observed before and after treatments (Table 3).

## Discussion

In China, the incidence and mortality of gastric cancer are twice that of the world average (20). For patients with advanced gastric cancer, chemotherapy plays an important role. The most desirable chemotherapeutic schedule aims to achieve a higher response rate, reduced toxicity, and delayed appearance of drug resistance. To date, the efficacy of existing chemotherapeutic drugs has not been satisfactory. Thus, the development of strategies that increase their anticancer activity is urgently needed. WOG is a promising new finding for anticancer therapy because it induces cytotoxic effects in many cancer cell lines and also animal tumor models, but, interestingly, WOG exhibits little toxicity towards normal tissues (21). Its main mechanism of action involves prolonged activation PLCr1 via H<sub>2</sub>O<sub>2</sub> signaling, which leads to sustained elevation of cytosolic Ca<sup>2+</sup> in malignant, but not normal, cells, and this subsequent Ca<sup>2+</sup> overload leads to disruption of the mitochondrial membrane and cell death. Because of differences in mechanisms of action and toxicity profiles, the combination of WOG with conventional chemotherapy drugs boasts enormous clinical potential.

Our study investigated the antitumor effects of WOG combined with either of two chemotherapy drugs, PTX and CDDP, on different gastric cancer cell lines *in vitro* and on BGC-823 xenografts *in vivo*. As previously reported (9), treatment with any of the drugs alone inhibited the growth of gastric cancer cells in a dose-dependent manner. According to the IC<sub>50</sub> values, the HGC-27 cell line was the most sensitive to WOG, while the BGC-823 cell line was the most sensitive to CDDP and PTX.

Using a fixed ratio of WOG to PTX/CDDP (WOG IC<sub>50</sub>:PTX/CDDP IC<sub>50</sub>=1:1), we found that combination treatments had higher inhibitory rates on proliferation than monotherapies, within a certain range of concentration, on the four cell lines tested. This suggested that combination treatments were more efficacious than monotherapies.

The median-effect analysis method of Chou and

Talalay is an emerging approach to evaluate combination effects of chemotherapeutic drugs *in vitro*. It can judge whether or not the interaction of drugs is synergistic or dynamic (16). According to this method, the combination of WOG and CDDP/PTX in the four cell lines tested all showed synergism in different ranges of Fa. For most of the cell lines, the dose required for drugs when used in combination treatments was lower than their IC<sub>50</sub>. These results demonstrate that identical effects could be acquired in combination therapies with lower doses of each drug compared with monotherapies. Therefore, individual toxicities associated with higher doses could be reduced. All these results provide evidence supporting the use of WOG in combination with chemotherapy drugs for clinical application.

As for a mechanism of action, WOG has been shown to induce extensive apoptosis in many malignant tumors such as hepatocellular carcinoma (22), ovarian cancer, prostate cancer (13), and murine sarcoma (23). Thus, triggering apoptosis is considered one of the mechanisms used by WOG to execute its anti-tumor effects. Indeed, Lee has reported that WOG increases expression of P53, a tumor suppressor protein, and its target genes, *p21*, *p27*, and *PUMA*, to promote cell apoptosis (13). Zhao has also demonstrated that WOG induces apoptosis by inhibiting the activity of NF-κB (9), as well as that of anti-apoptotic proteins such as Bcl-2 and Bcl-x. In our study, Hoechst staining revealed a more obvious apoptotic phenotype in cells receiving combination therapies than in those treated with monotherapies, especially in the BGC-823 cell line. Accordingly, apoptotic rates were highest in the combination groups in comparison with the single-drug or no-treatment control groups. These results suggest that further stimulating apoptosis is one way by which combination therapies can attain better efficacy.

To date, the effect of combining WOG and CDDP/PTX in gastric cancer cells has not been reported. Lee has previously found that compared with CDDP alone, the combination of WOG and CDDP did not enhance inhibitory effects in Jurkat and HL-60 cells (24). However, in our studies, the efficacy of combination treatments was significantly better than that of CDDP alone. These disparate findings may be because of metabolic differences critical to WOG and/or CDDP sensitivity among different cell lines (25). It has also been reported that WOG inhibits P-glycoprotein, one of the ABC transporter family proteins (26). Thus, it has been proposed that combination treatments exhibit synergistic effects probably because

WOG can decrease the excretion of chemotherapeutic drugs by suppressing P-glycoprotein.

In this study, WOG combined with CDDP/PTX demonstrated significant anti-tumor activity *in vitro*. However, although the *in vitro* results provide information about cytotoxicity, the usefulness of these experiments is limited for evaluating the efficacy of drugs (25). Thus exploration *in vivo* was encouraged. To this end, WOG and PTX showed inhibition when used separately on both BGC-823 xenografts and MKN-45 xenografts (data not shown). However, combination of WOG and CDDP displayed toxic synergism. Indeed, no mice in this combination group survived, while survival rates were not influenced in monotherapy groups. The lethality of WOG combined with CDDP was also apparent in MKN-45 xenografts (data not shown). It is well described that treatments that increase therapeutic efficacy *in vitro* have the potential for markedly enhanced toxicity *in vivo* because of accumulation of drugs. Moreover, metabolic differences between cells and animals may change the synergistic relationships of two drugs (25). These reasons may explain why the synergy of WOG and CDDP observed *in vitro* was not verified in animals. Thus, in summary, WOG and CDDP are not good partners in the treatment of gastric cancer.

WOG combined with low-dose PTX, on the other hand, obtained the same efficacy as with high-dose PTX, which was much better than that of low-dose PTX- or WOG-alone groups. The IR were more than 60% in both the WOG plus PTX (10 mg/kg) and PTX (20 mg/kg) groups, while it was about 20% in single-drug groups. The use of PTX in the clinic is limited by its side effects such as allergic reactions, hematological toxicity, and neurotoxicity. In our experiment, one of the five mice treated with PTX alone (20 mg/kg) presented with hemiplegia, which is indicative of neurotoxicity. In contrast, the combination of WOG and PTX was not related to any toxicity; indeed, no differences in body weight and survival rate were noted in mice of this treatment group. Instead, it was found that the combination of WOG and low-dose PTX has a satisfactory synergistic effect *in vivo*.

On the whole, this is the first study in which the inhibitory effects of WOG combined with CDDP/PTX on the growth of different gastric cancer cells and on BGC-823 xenografts were compared. Both combination treatments showed significant inhibitory effects *in vitro*, but only the combination of WOG and low-dose PTX was efficacious and safe when used for animal experiments. This study provides evidence supporting the potential clinical efficacy of combination treatment involving low-dose PTX and WOG

to elicit a synergistic anti-tumor effect in gastric cancer.

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*Disclosure:* The authors declare no conflict of interest.

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