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Preliminary Evaluation of the Interactions of *Panax ginseng* and *Salvia miltiorrhiza* Bunge with 5-Fluorouracil on Pharmacokinetics in Rats and Pharmacodynamics in Human Cells

Chenxin Gu,* Jinping Qiao,* Meilin Zhu,* Jinglei Du,* Weihu Shang,*
Wei Yin,* Wei Wang,* Mei Han*,†,a and Weidong Lu‡,a

*Key Laboratory of Radiopharmaceuticals, Ministry of Education
College of Chemistry

†College of Nature Resources and Technology
Beijing Normal University, Beijing 100875, P. R. China

‡Dana-Farber Cancer Institute, Harvard Medical School
Boston, MA 02215, USA

Abstract: An increasing number of cancer patients are using herbs in combination with conventional chemotherapeutic treatment. It is therefore important to study the potential consequences of the interactions between herbs and anticancer drugs. The effects of extracts from *Panax ginseng* (*PGS*) and *Salvia miltiorrhiza* Bunge (*SMB*) on the pharmacokinetics of 5-fluorouracil (5-FU) were performed *in vivo* and detected by high performance liquid chromatography (HPLC), while, an ATP assay was used to study the pharmacodynamic interactions *in vitro*. The results of the pharmacokinetic experiments showed a significant increase in the elimination half-life ($t1/2(k_e)$) of 5-FU in the *PGS*-pretreated group and in the area under the curve (AUC) in the *SMB*-pretreated group compared with the control group. However, after *SMB* pretreatment, weight loss was observed in rats. The results of pharmacodynamic experiments showed that neither *PGS* nor *SMB*, when used alone, directly inhibited cancer cell growth at 0.1–100 μg/ml. Moreover, *PGS* had a synergistic cytotoxic effect with 5-FU on human gastric cancer cells but not on normal gastric cells. The results imply that when combined with 5-FU, *PGS* may be a better candidate for further study. This study might provide insights for the selection of herbal-chemotherapy agent interactions.

Correspondence to: Dr. Mei Han, Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University; College of Nature Resources and Technology, Beijing Normal University, No.19 XinJieKouWai Street, HaiDian District, Beijing 100875, P. R. China. Tel: (+86)-10-6220-7786, Fax: (+86)-10-6220-6031, E-mail: hanmei@bnu.edu.cn
[a]These authors contributed equally to this work.

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Introduction

Cancer is a well-recognised global phenomenon. The traditional approaches to cancer therapy are surgery, radiotherapy and chemotherapy. Recently, the combination of herbal medicine and conventional chemotherapeutic agents has attracted increasing attention (Martinez *et al.*, 2008; Wargovich *et al.*, 2010; Saquib *et al.*, 2011). The interactions between herbal medicines and drugs can be characterized as either pharmacokinetic or pharmacodynamic in nature. Pharmacokinetic interactions result from alterations in pharmacokinetic parameters of a conventional drug caused by an herbal medicine. Pharmacodynamic interactions may occur when the components of herbal medicines have either synergistic or antagonist activity in relation to an anticancer drug. (Chavez *et al.*, 2006).

The US Food and Drug Administration define herbal medicines, which have been widely used in Asia and have become more popular worldwide, as dietary supplements. A dietary supplement is considered a "food" and not a "drug" and is a product that is administered orally to supplement the diet and regulate physiological functions in the human body (Bin and Kiat, 2011). Herbal medicines have been used in cancer therapy to ameliorate therapy-induced side effects because of their perceived benefits (e.g., boosting the immune system) (Schonekaes *et al.*, 2002; Wong *et al.*, 2005; Yoshimura *et al.*, 2005; Corner *et al.*, 2009).

The natural herbal medicines *Panax ginseng* (*PGS*, or ginseng) and *Salvia miltiorrhiza* Bunge (*SMB*, or Danshen) are ingested by people worldwide (Gardiner *et al.*, 2007; Rosecrans and Dohnal, 2009; Yang *et al.*, 2010). *PGS* is traditionally used for the development of physical strength, especially to reduce weakness and fatigue (Wang *et al.*, 2010). In western countries, *PGS* has a very high sales volume and is one of top 10 selling individual herbs. *PGS* reportedly plays many roles in the treatment of cancer, such as anti-angiogenesis, anti-proliferation, and apoptosis (Varjas *et al.*, 2009; Hwang *et al.*, 2012). *PGS* also improves the function of the central nervous system (CNS) and the immune system and alleviates climacteric symptoms (Braz *et al.*, 2009). Ginsenosides Rg1, Rb1, and Rd, which are considered to be the major components of *PGS*, account for its pharmacological effects (Varjas *et al.*, 2009; Peng and Xie, 2009).

SMB is another herb that is commonly used in Asia. It has been used in the clinic for the treatment of coronary heart disease, cerebrovascular disease, hypertension, chronic renal failure, and dysmenorrhea (Zhou *et al.*, 2005). The major constituents of *SMB* are lipophilic compounds, such as tanshinone IIA, and hydrophilic phenolics, such as salvianolic acid B (Ng *et al.*, 2000).

5-Fluorouracil (5-FU) is one of the most widely used chemotherapeutic agents that inhibit the growth of cancer cells. Many types of cancers have been treated with 5-FU, including cancer of the colon, rectum, breast, stomach, head, and neck (Malet-Martino and Martino, 2002; Navolanic and McCubrey, 2005; Sakaeda *et al.*, 2009). However, serious

side effects, including nausea, fatigue, and pancytopenia, are common (Delval and Klastersky, 2002).

The importance of herbal medicines with chemotherapy agents is becoming a more pressing issue. *PGS* or *SMB* is an extensively used herb medicine (Martinez *et al.*, 2008; Ye *et al.*, 2008; Xiong *et al.*, 2009; Wong *et al.*, 2010; Zhao *et al.*, 2010; Chan *et al.*, 2011). Therefore, it is important to evaluate potential consequences of *PGS* or *SMB* with co-administered anticancer drugs. The information obtained from this study will provide insights to enable the understanding of herb-drug interactions for patients and clinicians.

The aim of the present study was to investigate the effects of the interaction of *PGS* or *SMB* with 5-FU on *in vivo* pharmacokinetics and *in vitro* pharmacodynamics. In the *in vivo* experiment, rats were orally pretreated with extracts of *PGS* or *SMB*, and 5-FU was administered by intraperitoneal injection. The pharmacokinetics of 5-FU were determined with high performance liquid chromatography (HPLC). Meanwhile, the pharmaceutical effects of *PGS* or *SMB* on anticancer activity, alone or in combination with 5-FU, were studied *in vitro* with cytology methods. A normal gastric cell line comparable to a gastric cancer cell line and three other cancer cell lines were used to investigate the cytotoxicity of *PGS*, *SMB* and 5-FU alone and in combination.

Materials and Methods

Reagents and Cell Lines

5-FU (99.9% purity) was purchased from Shanghai Bangcheng Chemical Co., LTD. (Shanghai, China). Dried raw materials for *PGS* and *SMB* were purchased from Beijing Tongrentang Pharmacy (Beijing, China), and their authenticity was verified by Professor Wensheng Zhang (Beijing Normal University, Beijing China). Salvianolic acid B, tanshinone IIA, ginsenoside Rb1 and ginsenoside Re standards were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 2,4,5-Trichlorophenoxyacetic acid (the internal standard) was obtained from Sigma Chemical Co. (St Louis, USA). HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), and formic acid was purchased from J.T. Baker (Phillipsburg, NJ, USA).

Animals

Male Sprague-Dawley rats that were six weeks old and weighed 150–200 g were obtained from Vital River Laboratory Animal Co., Ltd. (Beijing, China). The rats were maintained in a clean room at temperatures of 20–23°C with an alternating 12:12 h light:dark cycle and 50% relative humidity. Filtered tap water and a standard animal diet were available *ad libitum*.

All experimental procedures involving animals were approved by the Ethics Committee of Beijing Normal University (approved case number: BNU/EC/01/2011, date: 07/07/2011).

Preparation of PGS and SMB Extract

PGS was pulverized to a powder (approximately 40-mesh). The powder (10.8 g) was extracted with 1000 ml of water at 80°C for 40 min, and this extraction was performed twice. The extracts were combined, concentrated to 200 ml and stored in the refrigerator.

SMB (8.1 g) was chopped into small pieces (1 cm × 1 cm) and sequentially extracted with boiling 95% ethanol (60 ml) and boiling water (100 ml) for 1 h. The ethanol extract and the water extract were combined, concentrated to 100 ml and stored in the refrigerator. The preparation method was performed according to Official Chinese Pharmacopoeia (National Pharmacopoeia Committee, 2005).

The dose of *PGS* or *SMB* for rats was converted according to the recommended daily dose of Official Chinese Pharmacopoeia (approximately 9 g of *PGS* or 15 g of *SMB* in raw material) in humans (National Pharmacopoeia Committee, 2005). The preparation method was designed to achieve the desired concentration of *PGS* extract (0.81 g/kg) or *SMB* extract (1.35 g/kg) administrated to rats.

Quality Control of SMB and PGS Extracts

Based on Official Chinese Pharmacopoeia, HPLC methods were applied to evaluate the quality of the *PGS* or *SMB* extracts (National Pharmacopoeia Committee, 2005). Ginsenosides Rb1 and Re were detected with HPLC to evaluate the quality of the *PGS*. Tanshinone IIA and salvianolic acid B were chosen to be the markers for the *SMB* quality evaluation.

Administration of Drugs to Rats

The *PGS* extract (0.81 g/kg) or the *SMB* extract (1.35 g/kg) was administered orally twice daily for ten consecutive days. The control rats received an equal volume of saline. The body weight of each rat was measured on the fifth and tenth days of the experimental period. After the ten-day treatment period, the rats were cannulated with polyethylene tubing in the right jugular veins under light ether anesthesia. 5-FU (48 mg/kg) was administered by intraperitoneal injection after a 12-h fast (Wu *et al.*, 2006). Blood samples (0.5 ml) were collected into heparinized tubes at 0, 1, 3, 5, 10, 20, 30, 60, 90, 120 and 240 min after dosing. The plasma was immediately separated by centrifugation and stored at −20°C until drug analysis.

Pharmacokinetic Studies

A volume of 0.2 ml of the internal standard (10 ng/ml) was added to the plasma (0.2 ml) before the addition of 3 ml of ethyl acetate/isopropyl alcohol (v:v = 9:1). After oscillating for 1 min and centrifuging at 3000 rpm for 5 min, the organic phase was dried at 40°C under nitrogen. The residue was reconstituted in 200 μl of the mobile phase and 10 μl of each sample was introduced into the HPLC system.

The 5-FU concentrations in plasma were determined with an HPLC system equipped with a 1525 pump and a 2487 UV detector (Waters, Milford, MA, USA) using an Atlantis

DC C-18 column (3 μm 30 mm $\times$ 2.1 mm, Waters, Milford, USA). The analytes were separated using 2 μm organic films. The mobile-phase consisted of 10 mM ammonium acetate in water (A) and acetonitrile (B) pumped at 0.2 ml/min. The proportion of B was linearly increased from 10% to 80% in the first 2 min, held for 2 min, and then returned to 10% B. The detection wavelength was performed at 254 nm, and the analytical run time was 10 min.

The pharmacokinetic parameters were estimated using 3p87 software (Chinese Pharmacological Society, Beijing, China) (Xu *et al.*, 2003; Zhou *et al.*, 2011).

Cell Culture

Human cell lines (A549, SW480, BIU-87, BGC823 and GES-1) were kindly provided by the Department of Pathology, Peking University Health Science Center and the Key Laboratory of Cell Proliferation and Regulation Biology, Beijing Normal University.

The following cell lines were cultured in Dulbecco's Modified Eagle Media/Nutrient Mixture F-12 (DMEM/F12) culture medium supplemented with 10% fetal bovine serum and 100 UI penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in a humidified atmosphere containing 5% CO_2: the human bladder cancer cell line BIU-87, the human lung cancer cell line A549, the human colon cancer cell line SW480, the human gastric cancer cell line BGC823 and the human normal gastric epithelial cell line GES-1.

Cell Viability Measurement

The cells were seeded on 384-well micro plates at 1×10^3 cells/well. After seeding for 24 h, the cells were treated with 5-FU, *PGS*, *SMB*, *PGS*+ 5-FU or *SMB*+ 5-FU at various concentrations and the plates were incubated at 37°C for 48 h. Each group was set up in quadruplicate. Cell viability was measured with the CellTiter-Glo Luminescent Cell Viability Assay kit (Promega, Madison, WI, USA). A volume of 10 μl of the ATP kit working solution was added to each well. The luminescence of each well was measured using a Victor^3 V Multilabel reader (PerkinElmer, Waltham, MA, USA).

Statistical Analysis

The significance of the differences was estimated using a one-way ANOVA; $p < 0.05$ was the accepted level for statistical significance. All values were expressed as the mean $\pm$ S.D.

Results

Quality Control of PGS and SMB Extracts

Quality assurance of an herbal medicine is very important both in research and in daily intake. To investigate an herbal medicine, quality control is absolutely necessary. According to HPLC results, the linearity of each standard curve was confirmed by plotting

Table 1. Calibration Curves of 4 Standard Chemicals

Standard Chemicals	Equations	R^2	Linear Range (μg/ml)
Ginsenosides Rb1	$y = 3.49e + 003x - 4.41e + 004$	0.9995	10–1000
Ginsenosides Re	$y = 3.46e + 003x - 3.93e + 004$	0.9983	10–1000
Tanshinone IIA	$y = 6.19e + 004x - 2.83e + 004$	0.9999	2–100
Salvianolic acid B	$y = 7.87e + 003x - 2.17e + 004$	0.9994	7–140

the peak area (y) against the corresponding concentration (x, μg/ml) of the analytes. The regression equations are described in Table 1, and the contents of active compounds were calculated using the standard curve (ginsenosides Rb1 and Re, 611.9 μg/ml and 977.2 μg/ml, respectively, corresponding to 12.2 mg/g and 19.5 mg/g *PGS*, respectively; tanshinone IIA and salvianolic acid B, 34.6 μg/ml and 11.3 μg/ml, respectively, corresponding to 42.7 μg/g and 1.75 μg/g *SMB*, respectively). The results showed that the herbs used in the experiments qualified as *PGS* or *SMB* according to the Official Chinese Pharmacopoeia (National Pharmacopoeia Committee, 2005).

Pharmacokinetics of 5-FU

To study the effect of *PGS* or *SMB* on the plasma concentration of 5-FU, a detection method was first established. Low (0.1 μg/ml) and high (50 μg/ml) concentrations were tested under the HPLC conditions and the limit of quantification (LOQ) for 5-FU in the plasma was 0.05 μg/ml. The typical equation describing the standard line was as follows: $y = 0.0536x + 0.1627$, where y is the ratio of peak areas between the drug and the internal standard, and x is the plasma concentration (μg/ml) of 5-FU. An average correlation of $R^2 = 0.99955$ was obtained. The interday and intraday relative standard deviations were all less than 5%. Figure 1 shows a chromatographic separation of an extract prepared from blank plasma spiked with 5-FU and the internal standard. The approximate retention times for 5-FU and the internal standard were 0.54 min and 4.93 min, respectively. Both 5-FU and the internal standard eluted as symmetrical peaks, and no significant endogenous peaks that could interfere with the analysis were observed.

According to the detection method established above, the plasma concentration-time curves after a single intraperitoneal injection of 5-FU are presented in Fig. 2. The time to reach the maximum concentration (T_{max}) was 14.56 min and 14.34 min in the *PGS* and *SMB* pretreated groups and the control group, respectively, and 10.37 min in the control group.

The pharmacokinetic parameters are summarized in Table 2. After pretreatment with *PGS*, the elimination half-life ($t_{1/2}(k_e)$) of 5-FU was significantly increased from 79.17 to 125.72, which was an increase of approximately 58.8% compared with the control. In the *SMB* extract pretreated group, the area under the curve (AUC) of 5-FU was remarkably increased from 2486.34 to 3399.92, which was an increase of approximately 36.7%. However, the statistical analysis indicated that other parameters were not significantly different. The results indicate that *PGS* may affect the elimination of 5-FU and that *SMB* may change the overall absorption of 5-FU.

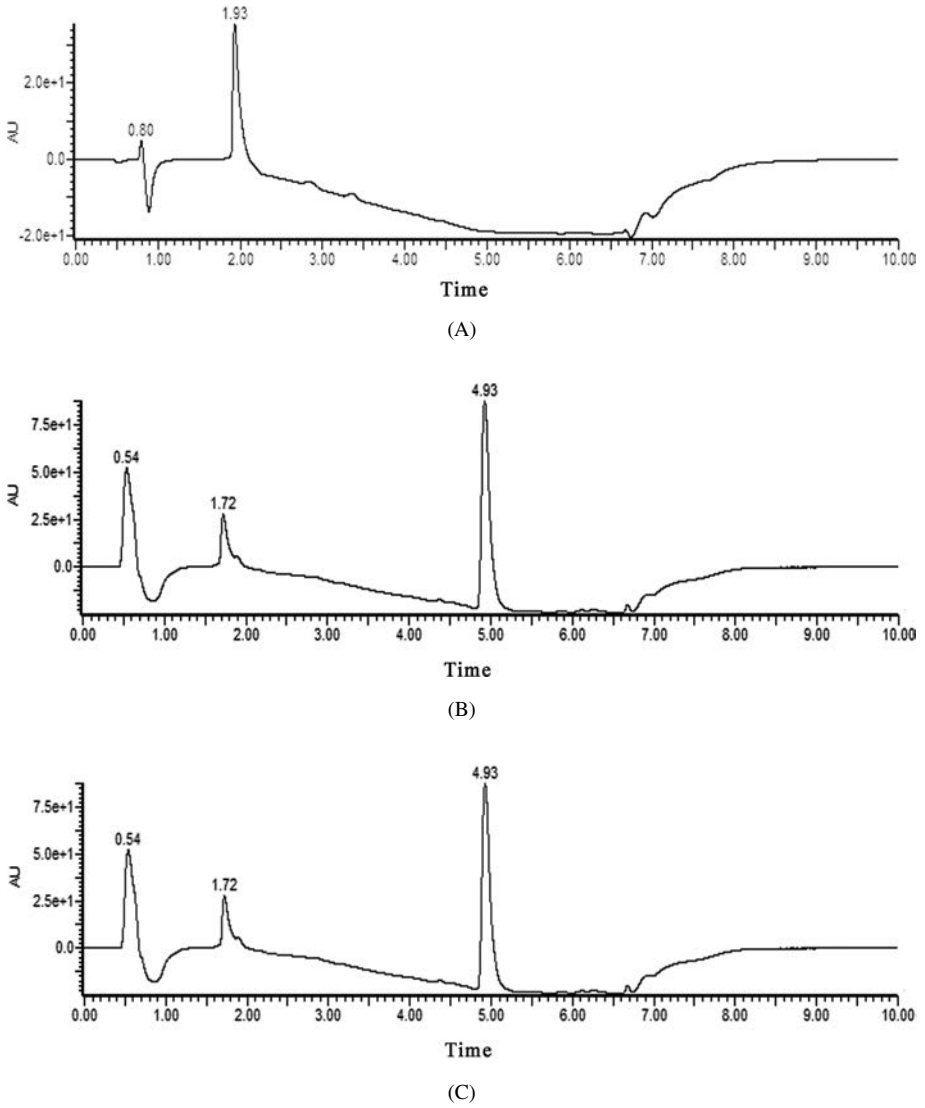
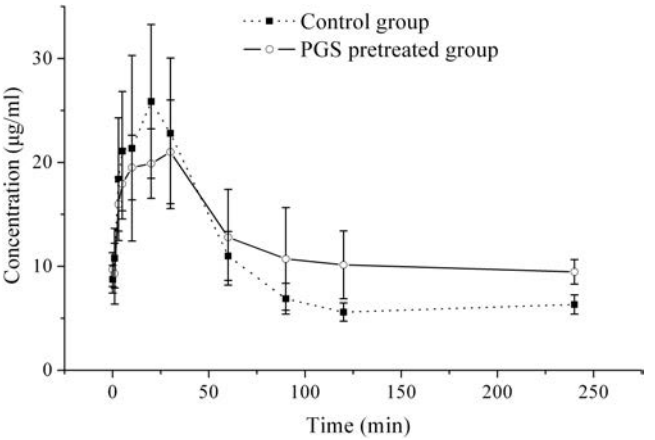


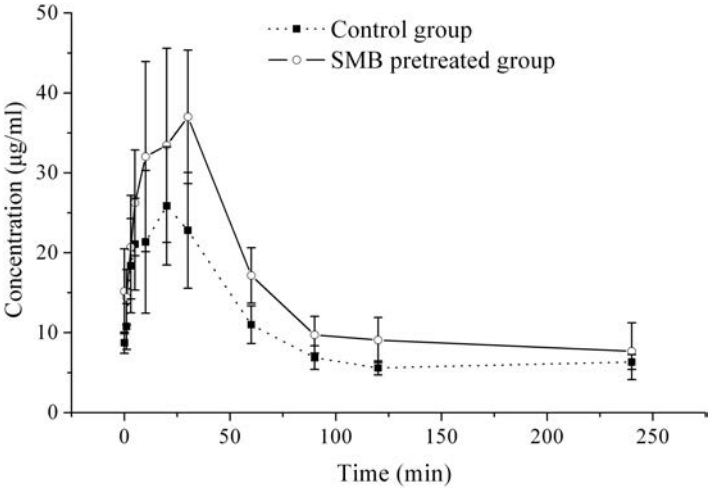
Figure 1. Chromatographic separation of 5-FU and the internal standard. No endogenous interfering peaks were observed in blank plasma (A), the standard sample with 5-FU and the internal standard (B) and the tested plasma samples (C).

Changes in the Body Weights of Rats

The changes in the body weights of the rats were fully investigated before the 5-FU injection (Fig. 3); the rats that were pretreated with *PGS* extract weighed more on the fifth and tenth days than those of the control group that were pretreated with saline. In contrast, the rats that were pretreated with *SMB* weighed less than those of the control group on the



(A)



(B)

Figure 2. Plasma concentration-time curves of 5-FU after a single intraperitoneal injection dose of 48 mg/kg 5-FU to control rats ($n = 5$; ■), to rats pretreated with PGS extract (A) (3 mg/kg for ten days, p.o., $n = 5$; •), and rats pretreated with SMB extract (B) (1.5 mg/kg for ten days, p.o., $n = 5$; •) Each value represents the mean ± S.D. of five animals.

tenth day. These data imply that *PGS* could increase body weight. The body weight change suggests that *PGS* may be a safer choice, whereas *SMB* may have side effects in rats.

Cytotoxicity in Human Cancer Cell Lines

To investigate whether *PGS* or *SMB* affects 5-FU cytotoxicity, cell viability was measured with an ATP assay in three different cancer cell lines (A549, BIU-87 and SW480). Because

Table 2. Pharmacokinetic Parameters of 5-FU (48 mg/kg) after a Single Intraperitoneal Injection to Control Rats ($n = 5$), to Rats Pretreated with 1.5 mg/kg *SMB* Extract ($n = 5$) and Rats Pretreated with 3.0 mg/kg *PGS* Extract ($n = 5$).

Parameter	Control Group	*PGS*-Extract Pretreated Group	*SMB*-Extract Pretreated Group
AUC (μg/ml)×min	2486.34 ± 435.86	3252.45 ± 786.56	3399.92 ± 315.07*
T_{max} (min)	10.37 ± 4.68	14.56 ± 4.36	14.34 ± 4.37
C_{max} (μg/ml)	22.11 ± 9.50	19.45 ± 2.04	30.31 ± 6.68
CL mg/kg/min/(μg/ml)	0.020 ± 0.0041	0.018 ± 0.0078	0.031 ± 0.032
V/F (mg/kg)/(μg/ml)	2.21 ± 0.84	2.22 ± 0.28	1.39 ± 0.39
$t_{1/2}(k_e)$ (min)	79.17 ± 36.25	125.72 ± 8.43*	62.88 ± 17.49
$t_{1/2}(k_a)$ (min)	2.08 ± 1.29	2.78 ± 0.53	3.41 ± 1.65

Note: AUC, area under the curve; T_{max}, time to reach the maximum concentration; C_{max}, maximum concentration; CL, plasma clearance; V/F, apparent volume of distribution; $t_{1/2}(k_e)$, elimination half-life; $t_{1/2}(k_a)$, absorption half-life. Values are mean ± S.D. of five animals. *$p < 0.05$ compared with the control group.

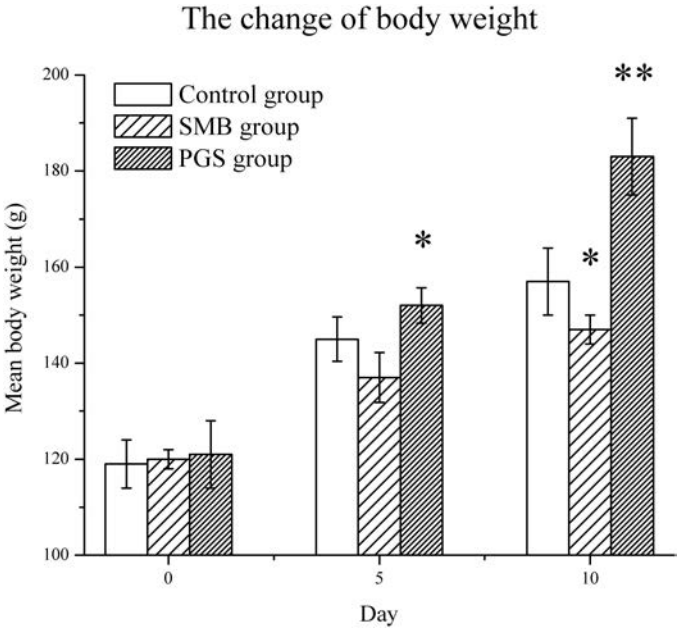
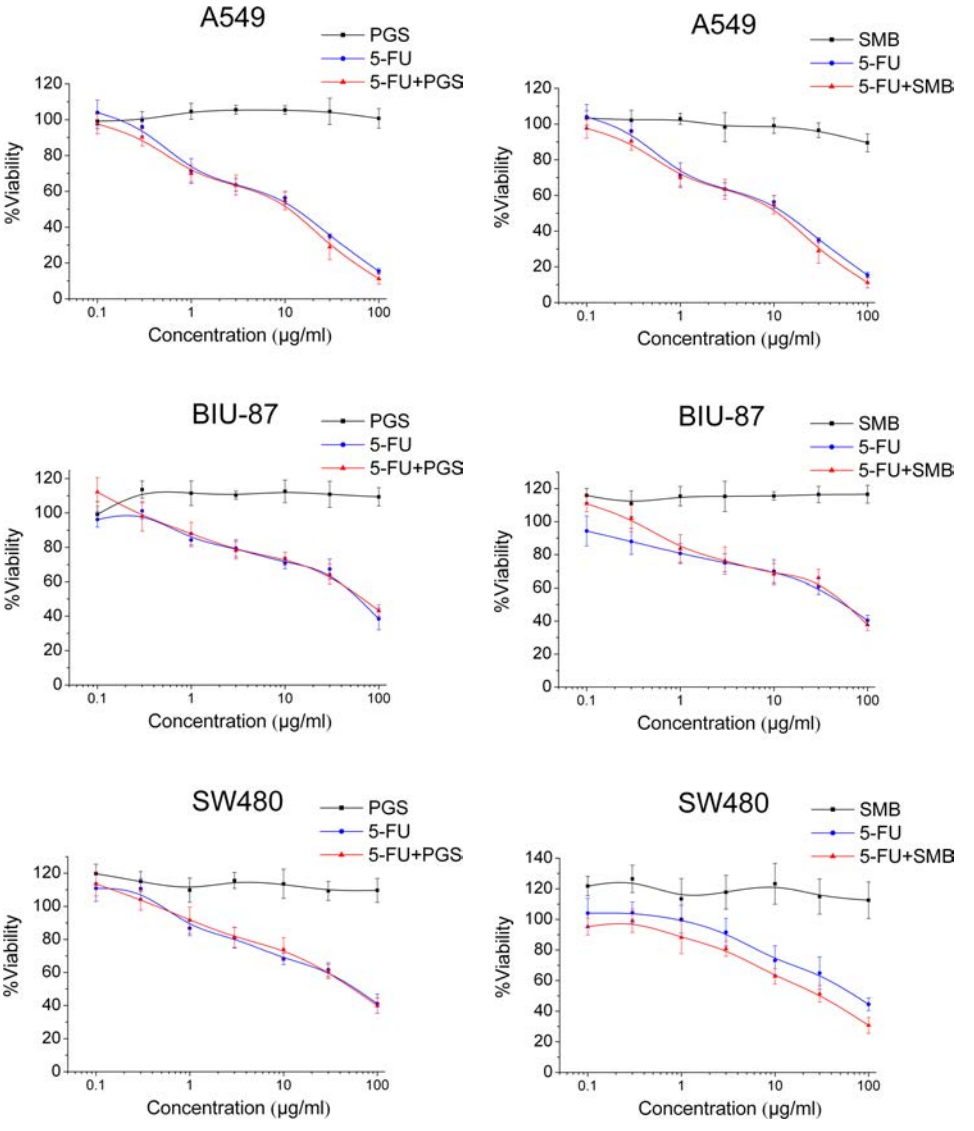


Figure 3. During the ten-day treatment period, the mean body weights of control rats with saline ($n = 5$), rats pretreated with PGS extract ($n = 5$) and rats pretreated with SMB extract ($n = 5$) were measured at zero, five, and ten days. The values are presented as means ± S.D. *$p < 0.05$, **$p < 0.01$ compared with the no-treatment group.

5-FU is a common anticancer drug for colon, lung and bladder cancers, BIU-87, A549 and SW480 were used. The inhibitory effect of 5-FU, *PGS* or *SMB* individually on cell growth and the various combined inhibitory effects of 5-FU and *PGS* or *SMB* are shown in Fig. 4. The IC50 of 5-FU and its combination with *PGS* or *SMB* indicated that there were not



(A) 5-FU + PGS (B) 5-FU + SMB

Figure 4. The cell viability of three different cancer cell lines (A549, BIU-87 and SW480) treated with 5-FU, PGS, SMB and 5-FU combined with PGS or SMB. Each value represents the mean ± S.D. ($n = 4$). (A) Cells were treated with 5-FU, PGS and 5-FU combined with PGS. (B) Cells were treated with 5-FU, SMB and 5-FU combined with SMB.

significantly different in A549, BIU-87 and SW480 (Table 3). The results indicate that 5-FU has significant inhibitory effects and that neither *PGS* nor *SMB*, when used alone, show an inhibitory effect on cell growth at concentrations of 0.1-100 μg/ml. Moreover, compared with the inhibitory effect of 5-FU alone on cell growth, the combination of 5-FU and *PGS*

Table 3. The IC50 of 5-FU and Its Combination with *PGS* or *SMB* in Different Cell Lines

Cell Line	5-FU (μg/ml)	5-FU+PGS (μg/ml)	5-FU+SMB (μg/ml)
A549	9.92	7.43	6.75
BIU-87	38.25	47.79	41.77
SW480	57.82	53.99	27.99
BGC823	123.02	14.56**	74.64
GES-1	39.34	30.85	40.68

Note: $^{**}p < 0.01$ compared with the control group.

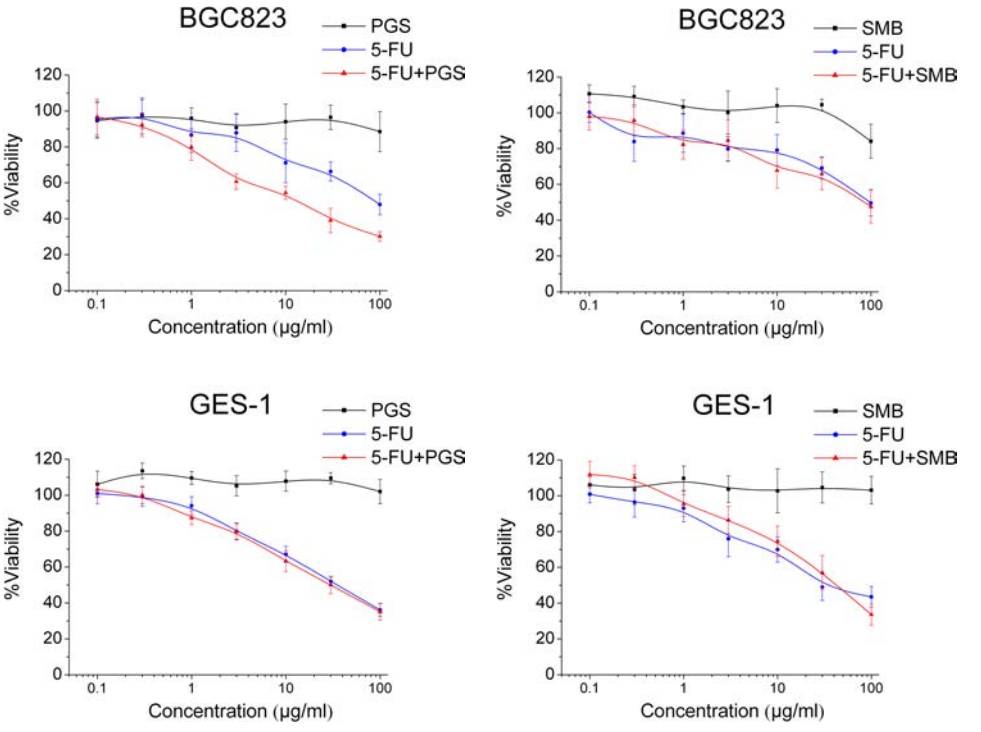
or *SMB* did not show a synergistic inhibitory effect on A549, BIU-87 or SW480. No significant differences were detected between the growth of cells treated with 5-FU combined with *PGS* or *SMB* and cells treated with 5-FU alone. The results indicate that the observed cytotoxicity was primarily generated by 5-FU alone.

Comparison of the Cytotoxicity in BGC823 and GES-1

Gastric cancer is one of the most commonly diagnosed cancers worldwide. 5-FU is a first-line drug for the treatment of gastric cancer and is associated with serious gastrointestinal side effects. To compare the combined cytotoxicity of 5-FU and *PGS* extract or *SMB* extract and to compare the different effects between gastric cancer cells and normal gastric cells, the human gastric cancer cell line BGC823 and human normal gastric epithelial cell line GES-1 were chosen to measure cell viability. The individual inhibitory effect of 5-FU, *PGS* and *SMB* on cell growth and the combined inhibitory effect of 5-FU and *PGS* or *SMB* are shown in Fig. 5. The results indicate that 5-FU, but not *PGS* or *SMB*, significantly inhibits cell growth. Compared with the inhibitory effect of 5-FU alone on cell growth, the BGC823 cell viability was significantly decreased from 87.83% to 60.65% at 3 μg/ml, 71.14% to 54.47% at 10 μg/ml, 66.28% to 39.03% at 10 μg/ml and 47.94% to 30.14% at 100 μg/ml after pretreatment with *PGS*. The IC50 of combination of 5-FU with PGS is 14.56 μg/ml, while the IC50 of 5-FU is 123.02 μg/ml. These data indicated that the combination of 5-FU and *PGS* showed a synergistic inhibitory effect on BGC823 but not on GES-1. The combination of 5-FU and *SMB* did not show a synergistic inhibitory effect in either BGC823 or GES-1.

Discussion

Our study found that a herbal-drug interaction does occur; both *PGS* and *SMB* may change the pharmacokinetics of 5-FU when rats are pretreated withthese two herbs. In addition, the two extracts display significant differences in certain parameters. Clinically, drug combinations should be avoided in principle. In this study, *SMB* did not have a direct cytotoxic effect. Combined with 5-FU, no synergistic cytotoxicity effect was observed, but lower body weight of rats was observed ten days after *SMB* pretreatment. However, in individual



(A) 5-FU + PGS (B) 5-FU + SMB

Figure 5. The cell viability of gastric cancer cells (BGC823) and normal gastric cells (GES-1) treated with 5-FU, PGS, SMB and 5-FU combined with PGS or SMB. Each value represents the mean ± S.D. ($n = 4$). (A) Cells were treated with 5-FU, PGS and 5-FU combined with PGS. (B) Cells were treated with 5-FU, SMB and 5-FU combined with SMB.

cases, herbal-drug interactions may have advantages. *PGS* also did not inhibit cell growth. The combination of 5-FU with *PGS* showed a synergistic inhibitory effect in gastric cancer cells (BGC823) but not in normal gastric cells (GES-1). In addition, the body weight of rats was increased ten days after *PGS* pretreatment. With cytotoxic effects on BGC823 and minimal side effects, the combination of *PGS* with 5-FU may have the potential to become a novel combination therapy.

Our findings have several clinical implications. First, the administration of *PGS* prior to 5-FU might reduce the rate of elimination and prolong drug effect time with the added benefit of weight gain. In contrast, the weight loss of *SMB* administration prior to 5-FU should be noted. Second, *PGS* showed a synergistic inhibitory effect in human gastric cancer cells (BGC823) but not in normal human gastric cells (GES-1).

Gastric cancer is one of the most common malignant tumors worldwide with a high morbidity and mortality rate. Based on the GLOBOCAN 2008 estimates, a total of 989,600 new stomach cancer cases and 738,000 deaths occurred in 2008 (Jemal *et al.*, 2011). 5-FU is a first-line drug for the treatment of gastric cancer, and gastrointestinal side effects of

5-FU are well known. Therefore, studying the interaction of herbs with 5-FU is of great importance for the treatment of gastrointestinal cancer. In the present study, *PGS* combined with 5-FU showed a synergistic cytotoxicity effect in BGC823 without affecting the cytotoxicity of 5-FU in GES-1. The increase in $t_{1/2}(k_e)$ caused by *PGS* indicates that the rate of elimination of the drug from the body was reduced and 5-FU remained at low concentration for a longer period of time, which may lead to a longer drug effect time and longer-lasting efficacy of 5-FU. Moreover, *PGS* may increase body weight, which could decrease the side effect of weight loss by 5-FU. It was reported that ginsenosides can increase the protein and RNA contents of muscles and liver in rats (Wang *et al.*, 1982). Therefore, we suggest that *PGS* may have influence on RNA and protein synthesis and thereby promote the growth of rats. Overall, *PGS* may be beneficial to the anticancer effects of 5-FU. The results suggest that the interaction of *PGS* with 5-FU may have the potential to be a novel treatment.

Chun-Su Yuan and his colleagues have found that ginseng enhances the anti-proliferative effect of 5-FU in human colorectal cancer (Fishbein *et al.*, 2009; Wang *et al.*, 2012). Panaxadiol, a purified ginseng component, has also been reported to enhance the anti-cancer effects of 5-FU in human colorectal cancer cells (Li *et al.*, 2009). In this paper, colorectal cancer cells were tested, but *PGS* did not show an enhanced inhibitory effect. The lack of inhibition may be due to differences in the preparation methods. Different methods of preparation greatly affect Rg3 content, which has been shown to contribute to the potent anti-proliferation effect (Fishbein *et al.*, 2009). Nitric oxide (NO) might play a role in the mechanism by which *PGS* shows a synergistic inhibitory effect with 5-FU in human gastric cancer cells. NO has been reported to be involved in several steps of carcinogenesis (Cheng *et al.*, 2010; Masini *et al.*, 2010). It has been found that NO directly suppresses the growth of BGC823 cells by inducing G0/G1 phase arrest through the regulation of Akt signaling (Sang *et al.*, 2011). It has also been found that ginsenosides may increase NO production (Hien *et al.*, 2010). Ginsenoside Rg3-induced endothelial nitric oxide synthase (eNOS) phosphorylation requires the ER-mediated PI3-kinase/Akt pathway (Hien *et al.*, 2010). Therefore, it is possible that *PGS* increases NO production through the PI3-kinase/Akt pathway to achieve the synergism observed in our study.

The results of pharmacokinetic experiments showed that *SMB* can increase the concentration of 5-FU. The increase in the AUC indicates an increase in the overall effect of 5-FU, which may lead to increased side effects. Considering the weight loss phenomenon, the risk of potential side effects cannot be ignored. Moreover, *SMB* did not affect cell growth in various cancer cells and normal gastric cells. A significantly beneficial effect of *SMB* combined with 5-FU was not observed.

Tanshinone IIA, an ingredient of *SMB*, has been widely found to inhibit cancer cell growth, including human premyelocytic leukemia cells, breast cancer cells, and HeLa cells (Yoon *et al.*, 1999; Chow *et al.*, 2004; Zhou *et al.*, 2008). However, the effect of whole *SMB* on anticancer drugs has seldom been studied. One possible reason for the lack of a direct anticancer effect of *PGS* or *SMB* may be the use of an *in vitro* study rather than a study in a living system. Many experiments have shown that various herbs, including *PGS*, exert their anticancer activities through effects on immune system components

(Ernst, 2010). Ginsenosides are the immunologically active components in ginseng, indicating that ginseng may be useful to increase bodily resistance (Block and Mead, 2003).

The overall observations suggest that *PGS* might increase the anticancer effect of 5-FU without increasing gastrointestinal side effects and that *SMB* may cause side effects in rats. We consider *PGS* to be a better candidate for further study. However, this impression must be confirmed in a clinical setting before a conclusion can be reached.

Our study is a preliminary investigation with some limitations. In our pharmacokinetic experiment, only normal healthy rats (not a disease-specific model) were used. The generation of a rat tumor model will be necessary to fully understand the *in vivo* effects of *PGS* or *SMB* on 5-FU. Additional research is also needed to gain an understanding of mechanism of action.

Conclusion

In our study, both *PGS* and *SMB* could affect the pharmacokinetics of 5-FU, and the extracts displayed significant differences with respect to certain parameters. The *PGS* pretreated group displayed greater weight increases, while weight loss was observed in the *SMB* pretreated group. Moreover, *PGS* had a synergistic inhibitory effect with 5-FU in human gastric cancer cells but not in normal human gastric cells. Therefore, *PGS* may be a better candidate for further study as a beneficial herbal medicine when patients are treated with 5-FU. Additional clinical research is required before a definitive conclusion can be reached.

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