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Chinese Herbal Formula, Bing De Ling, Enhances Antitumor Effects and Ameliorates Weight Loss Induced by 5-Fluorouracil in the Mouse CT26 Tumor Model

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ABSTRACT

The use of complementary and alternative medicines—including a variety of herbal therapies—by patients undergoing cancer chemotherapy has been well documented. Despite such widespread use, however, the benefits and potential mechanisms of such herbal medicines remain largely anecdotal. In this study we examined the effects of a Chinese herbal formula, Bing De Ling, when administered as an adjunct to chemotherapeutic agent 5-fluorouracil (5-FU) in the CT26 mouse colon cancer model. 5-FU and Bing De Ling were administered to both naïve and CT26 mouse colon cancer-bearing BALB/c mice. Our results indicate that although the herbal formula alone did not result in antitumor effects under experimental conditions, it significantly enhanced 5-FU–induced tumor growth inhibition. Oral administration of Bing De Ling also increased survival rates of both tumor-bearing and tumor-free mice treated with 5-FU. Furthermore, oral administration of Bing De Ling reduced weight loss in tumor-free mice receiving 5-FU when compared to tumor-free mice that received 5-FU alone. Our data further show that 5-FU upregulates serum levels of IL-6, known to contribute to weight loss, in tumor-free mice, and that this increase in IL-6 is significantly less in mice that received Bing De Ling in addition to 5-FU. These data show Bing De Ling both enhances the antitumor responses of 5-FU and ameliorates side effects.

INTRODUCTION

BECAUSE OF ITS SYSTEMIC DISTRIBUTION, chemotherapy is the most effective treatment for most cancer patients with clinically suspected or apparent metastatic disease. Despite the recent advances in the treatment of colon cancer, many patients do not respond to therapy and die of their disease. 5-Flourouracil (5-FU) is a fluorinated pyrimidine whose metabolites are believed to trigger apoptosis by depleting thymidine, partially through inhibition of thymidine synthase and partially through direct incorporation into RNA and DNA. It is commonly used in treatment of carcinomas of the colon, rectum, stomach, pancreas, breast, head and neck, anus, and gallbladder. As it has been for over 40 years, 5-FU has been the backbone for the treatment of colorectal carcinoma, either alone or in combination with newer chemotherapeutics such as irinotecan and oxaliplatin (Braun *et al*., 2004). Both these drugs are believed to increase efficacy but also cause more toxicity. The predominant side effects of 5-FU are diarrhea, anorexia, enteritis, hand–foot syndrome, and myelosuppression. While the addition of other drugs has increased the disease-free and overall survival in patients with gastrointestinal malignancies (Wilke and Van Cutsem, 2003), it has also been associated with more severe side effects. Modalities that decrease toxicity and at the same time maintain or enhance efficacy would greatly advance the treatment for patients with colorectal cancer and other malignancies.

Complementary and alternative medicines (CAM), including Chinese herbal medicines in both single and mixed forms, are very popular among patients undergoing chemotherapy

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(Richardson *et al*., 2000). Despite widespread use, the majority of these herbal therapies have no clear efficacy and their potential mechanisms of action remain unclear. Bing De Ling, a modern mixture of five Chinese medicinal herbs, has anecdotal success against common colds, influenza, chronic fatigue syndrome, herpes simplex, and chemotherapy-induced toxicities. We have previously demonstrated that systemic administering Bing De Ling stimulates IL-2, and IFN-γ production in splenocytes and enhances macrophage, natural killer cell, and lymphokine-activated killer cell activity in normal mice (Niu *et al*., 2000). We sought to build upon these results in this study by examining Bing De Ling's effect upon efficacy and toxicity in 5-FU therapy for CT26, a mouse colon cancer model.

MATERIALS AND METHODS

Agents

Bing De Ling solution consists of Astragalus root (*Astragalus membranaceus*), rhubarb root (*Rheum palmatum*), white atractylodes (*Atractylodes macrocephala*), isatis root (*Isatis tinctoria*), scutilliar root (*Scutellaria baicalensis*), dogberry

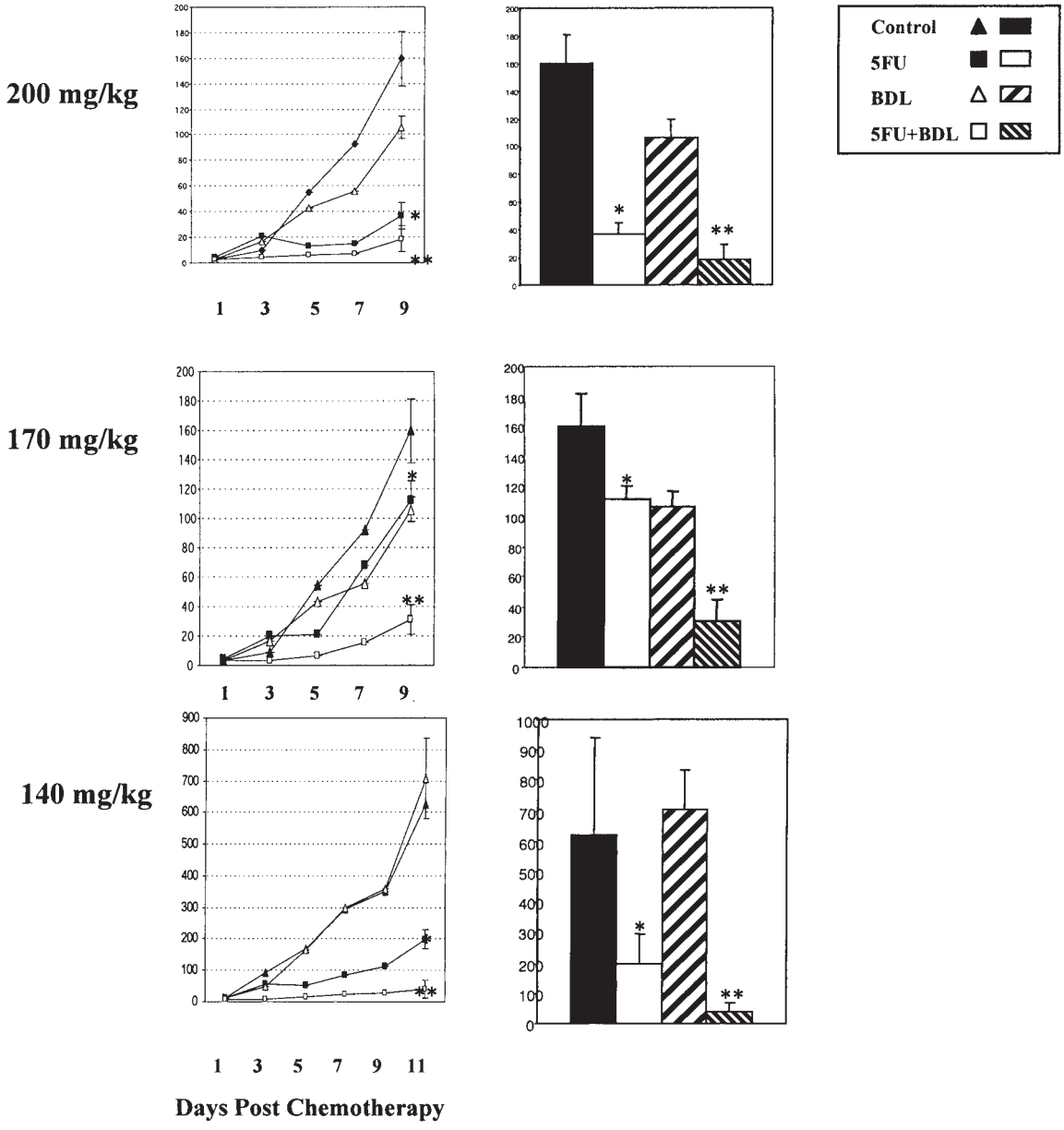

FIG. 1. Bing De Ling (BDL) increases tumor response to 5-FU. One-time doses of 5-FU were given at three different concentrations along with standardized dosages of saline control and BDL. Tumor size is expressed in days after administration of 5-FU dosage shown as means ± SD. For each dosage cohort, $n = 8$. For 200 mg/kg group, $P < 0.05$ for * compared with **. For 170 mg/kg group, $P < 0.01$ for * compared with **. For 140 mg/kg group, $P < 0.05$ for * compared with **.

(*Cormus officinalis*), and shield fern root (*Dryopnteris erassirhizoma*) at a concentration of 0.121 g/ml of water. A generic preparation manufactured for clinical use of 5-FU was obtained from Moffitt Cancer Center Pharmacy.

Mice and tumor formation in vivo

Experiments were conducted with female BALB/c mice, 6 to 8 weeks of age. Mice were purchased from the National Cancer Institute (Bethesda, MD) and housed in the animal facility at H. Lee Moffitt Cancer Center & Research Institute. Mice were maintained under pathogen-free conditions and all the *in vivo* experiments were performed in accordance with established institutional guidance and approved protocols. Cohorts of eight mice per group were used for these experiments. Mice were shaved on right flank and were given a subcutaneous injection of 1×10^6 CT26 cells in 100 μl saline to induce tumor.

In vivo *Treatment with Bing De Ling and 5-FU*

On the 10th day after tumor cell injection, both control fluid (saline) and Bing De Ling were administrated in 100 μl dosage twice daily via gastric lavage. Dosages were selected to correspond to those used in clinical practice at The Center for Traditional Chinese Medicine (Sarasota, FL). For chemotherapy, 5-FU was injected intraperitoneally at 140, 170, and 200 mg/kg. Tumor growth was monitored every 2 days by measuring two perpendicular tumor diameters with a caliper, and tumor volume was calculated according to the formula $V = 0.52 \times a \times b \times (a + b)/2$, where a is the smallest superficial diameter, and b is the largest superficial diameter.

Enzyme-linked immunosorbant assay

Seven days after 5-FU administration, mice sera were collected from 2 μl of tail blood diluted in 100 μl PBS and analyzed using OptEIA™ IL-6 ELISA sets (Pharmingen, San Diego, CA). Medium-bind EIA plates (Corning Corporation, Acton, MA) were coated with anti-IL-6 antibody overnight and blocked with PBS with 10% fetal bovine serum (FBS) for 30 min. Serial dilutions of IL-6 recombinant protein standards and samples were incubated for 2 h, followed by application of biotinylated anti-IL-6 antibody for 1hour and streptavidin–alkaline phosphotase (1:3000) for 30 min. After addition of TMB™ substrate (DAKO Corporation, Carpinteria, CA) plates were developed for 10 to 30 min, stopped by 2 N H_2SO_4, and read within 15 min at 450 nm.

RESULTS

Antitumor effect of 5-FU is augmented by Bing De Ling

Because the antitumor effects of 5-FU are limited by dosage-related toxicities in both animal and clinical studies, we sought to determine whether Bing De Ling could increase its antitumor efficacy. To determine whether Bing De Ling could improve the efficacy of 5-FU, BALB/C mice with tumors were treated with either saline, 5-FU (at 140, 170, and 200 mg/kg), Bing De Ling, or 5-FU and Bing De Ling. 5-FU was given in one dose on day 10 after tumor challenge. Bing De Ling was administered orally twice a day for the entire treatment period. A dose-dependent antitumor effect was noted following the administration of 5 FU. At 200 mg/kg, 5-FU treatment resulted in more substantial antitumor effects than 5-FU at 140 and 170 mg/kg. In both the 140 and 170 mg/kg 5-FU dosage groups, the addition of Bing De Ling significantly inhibited tumor growth (Fig. 1).

Bing De Ling increases survival rates in 5-FU-treated tumor-bearing mice

Although higher doses of chemotherapy can improve tumor reduction, its dosage is often limited by its severe side effects. In this study treating mice with one-time 200 mg/kg effectively reduced tumor volume, but the majority of the treated mice suc-

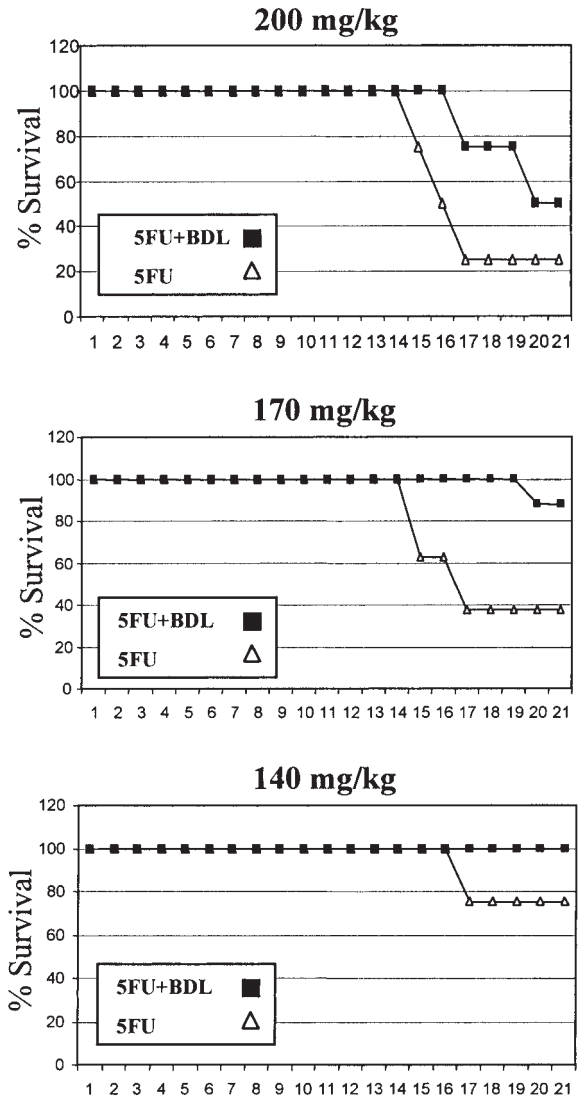

FIG. 2. Bing De Ling (BDL) improves survival during 5-FU treatment of tumor-bearing mice. Tumor-bearing mice were given one injection of 5-FU and twice a day of either Bing De Ling or saline. Mouse survival expressed in days after 5-FU treatment. For each dosage cohort, $n = 8$.

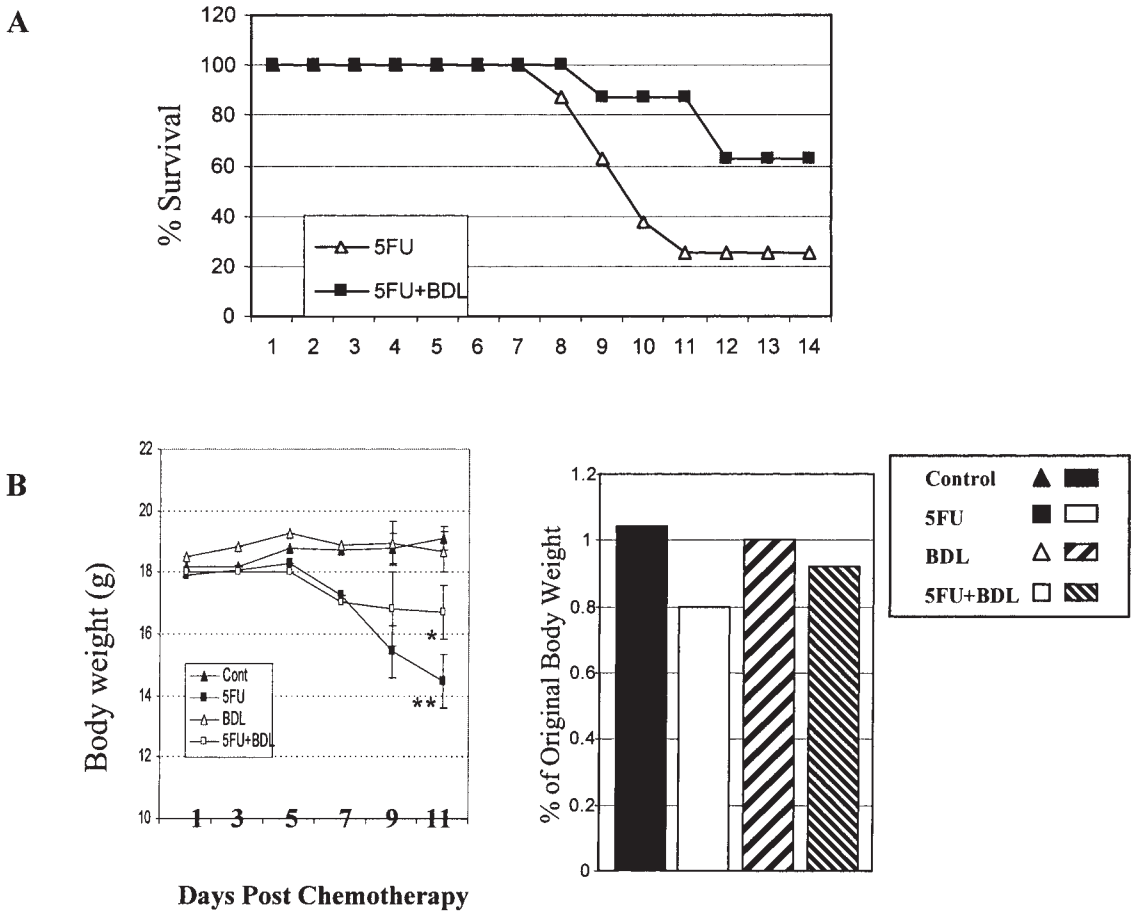

FIG. 3. Bing De Ling reduces 5-FU-induced toxicities in tumor-free mice. (**A**) Mouse survival expressed in days after 200 mg/kg 5-FU dosage. $n = 8$. (**B**) Mouse body weight (grams) measured in days after 200 mg/kg 5-FU dosage (left panel) and percentage of original body weight at final day of measurement (right panel). $n = 8$; $P < 0.05$ for * compared with **.

cumbed to treatment toxicity (Fig. 2). The addition of Bing De Ling to the 200 mg/kg 5-FU regimen significantly improved mouse survival (Fig. 2). Similar improvements in survival were seen with the addition of Bing De Ling to the 170 and 140 mg/kg 5-FU regimens (Fig. 2). Because survival is affected by tumor size, it is necessary to investigate if Bing De Ling can reduce 5-FU–associated death in normal animals.

Bing De Ling increases survival rates and decreases weight loss induced by 5-FU in tumor-free mice

It is known that patients with weight loss have a worse outcome when undergoing chemotherapy (Andreyev *et al*., 1998). To study Bing De Ling's effect upon 5-FU toxicity independent from any confounding variables caused by cancer, we fol-

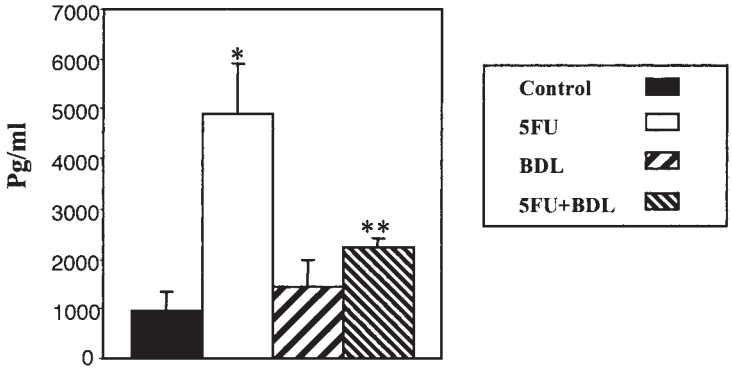

FIG. 4. Bing De Ling reduces 5-FU-induced IL-6 production in tumor-free mice. IL-6 production shown at 7 days after one 170 mg/kg 5-FU dosing, combined with twice a day oral administration of either Bing De Ling or saline. Data shown are means ± SD; $n = 8$. $P < 0.01$ for * compared with **.

lowed survival and weight loss in tumor-free mice treated with 5-FU at 200 mg/kg. Intraperitoneal administration of 5-FU in tumor-free mice at 200 mg/kg resulted in a considerable incidence of animal death (Fig. 3A). As seen in tumor-bearing mice, the addition of Bing De Ling to this dose of 5-FU in tumor-free mice resulted in significantly higher survival rates. In addition to affecting survival, administration of 5-FU at 200 mg/kg led to weight loss of up to 20% of original body weight (Fig. 3B). Addition of Bing De Ling to this regimen significantly reduced the severity of this weight loss ($P < 0.05$). These data demonstrate that Bing De Ling reduces 5-FU–related toxicity specifically in terms of survival and weight loss.

Bing De Ling inhibits 5-FU-induced IL-6 upregulation

Studies of many types of cancer have demonstrated that IL-6 is an important contributing factor in cachexia, and that elevated serum levels correlate with poor prognosis (Inadera *et al*., 2002; Kurzrock, 2001; Pfitzenmaier *et al*., 2003). Studies have also shown that several compounds can lower IL-6 levels in murine and human tumor models (Iizuka *et al*., 2002; Nukatsuka *et al*., 1994; Tamatani *et al*., 2004; Yamamoto *et al*., 2001) Lowering serum IL-6 in cancer patients by antibodies has also been shown to decrease the incidence of cancer-related cachexia (Trikha *et al*., 2003). Based on our demonstration that Bing De Ling ameliorates weight loss induced by 5-FU, we sought to determine if Bing De Ling would have measurable effect upon serum IL-6 levels in mice undergoing 5-FU treatment. Administration of 5-FU at 170 and 200 mg/kg to tumor-free mice led to increased serum IL-6 levels in both dosage groups (Fig. 4; 170 mg/kg data shown). Oral administration of Bing De Ling to mice receiving these doses of 5-FU reduced serum IL-6 levels, demonstrating that Bing De Ling significantly reduces 5-FU-induced IL-6 upregulation ($P < 0.05$).

DISCUSSION

In our previous study of Bing De Ling, we demonstrated that Bing De Ling stimulates immune activity in normal mice (Niu *et al*., 2000). In this study, our data show that the administration of Bing De Ling enhances the antitumor effect of 5-FU and prolongs survival of both tumor-bearing and tumor-free mice treated with 5-FU. Our experiments in tumor-free mice suggest that Bing De Ling can ameliorate 5-FU–induced weight loss, though it is difficult to determine the extent to which the weight loss experienced by tumor-bearing mice was due to tumor burden versus administration of 5-FU. Moreover, our data show that 5-FU–induced serum IL-6 upregulation is inhibited by oral administration of Bing De Ling. These data are particularly significant because they suggest at least one mechanism to explain Bing De Ling's reduction in 5-FU–induced weight loss and perhaps its previously noted immune stimulation as well. While it is difficult to ascertain whether the enhanced antitumor effect of 5-FU mediated by Bing De Ling is primary due to reduced weight loss, it is likely that preventing weight loss helps mice remain in a relatively balance condition of electrolytes and hydration, thereby increasing the efficacy of 5-FU. In support of this notion, some of the herbs, such as Astragalus root and dogberry, present in Bing De Ling, are known to increase the spleen and kidney energy and body's hydration.

The findings from this study are intriguing given the widespread use of herbal medicines among patients undergoing simultaneous conventional treatment for cancer. Previous studies have shown that between 66 and 83% of cancer patients have used some form of adjuvant Chinese Alternative Medicine (CAM), with 24 to 51% using herbal therapies in particular (Bernstein and Grasso, 2001), (Boon *et al*., 2000; Shen *et al*., 2002). Despite such widespread use, the majority of cancer patients do not inform their physicians of their use of CAM therapies (Boon *et al*., 2000; Shen *et al*., 2002). Correspondingly, most physicians desire more education when discussing CAM with their patients (Corbin Winslow and Shapiro, 2002). Together, these realities increase the likelihood that both toxic interactions and potential benefits of CAM therapies remain unrecognized by physicians and patients.

Studies such as ours are early efforts to bridge the gaps in knowledge and familiarity that currently relegate herbal medicines to the periphery of cancer care. In the case of Bing De Ling, further studies are needed to determine the role of the addition of this compound to the management of patients receiving traditional cancer therapies. Most importantly, the mechanisms of Bing De Ling's effects have yet to be elucidated. Bing De Ling's effect on IL-6 production also needs further study, as this effect may represent a common mechanism underlying both Bing De Ling's immunostimulatory effects and its potentiation of 5-FU (Chatterjee *et al*., 2002). Furthermore, the mechanisms of Bing De Ling's five individual components and their potential interaction with each other, which are likely a prerequisite to any understanding of the synergies that may exist within the formula, are unknown. Finally, and perhaps most importantly, formal clinical studies are needed to investigate the potential benefit of Chinese Alternative Medicines in a Western population either alone or in combination with chemotherapeutic agents.

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