Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2008 April 21; 14(15): 2323-2328
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Myelophil, a mixture of Astragali Radix and Salviae Radix extract, moderates toxic side effects of fluorouracil in mice

Jang Woo Shin, Myong Min Lee, Jin Young Son, Nam Heon Lee, Chong Kwan Cho, Won Kyu Chung, Jung Hyo Cho, Chang Gue Son

Jang Woo Shin, Myong Min Lee, Jin Young Son, Nam Heon Lee, Chong Kwan Cho, Jung Hyo Cho, Chang Gue Son, East-West Cancer Center, Dunsan Oriental Hospital of Daejeon University, 1136 Dunsan-dong, Seo-gu, Daejeon 302122, Korea
Won Kyu Chung, Department of Radiotherapy, Hospital of Konyang University, 865 Gasuwon-dong, Seo-gu, Daejeon 302718, Korea
Author contributions: Shin JW, Cho CK, Cho JH, Chung WK and Son CG designed the research; Shin JW, Lee MM, Son JY and Lee NH performed the research; Shin JW, Son CG and Lee MM contributed analytic tools; and Shin JW, Lee MM and Son CG wrote the paper.
Supported by The Oriental Medicine R&D Project, B050018; Ministry of Health and Welfare, Korea
Correspondence to: Professor Chang Gue Son, East-West Cancer Center, Dunsan Oriental Hospital of Daejeon University, 1136 Dunsan-dong, Seo-gu, Daejeon 302122, Korea. ckson@dju.ac.kr
Telephone: +82-42-4709422 Fax: +82-42-4709581
Received: November 16, 2007 Revised: February 21, 2008

Abstract

AIM: To evaluate the efficacy of Myelophil, an extract containing Astragali Radix and Salviae Radix, for reducing complications induced by 5-fluorouracil (5-FU) in a gastrointestinal cancer model.

METHODS: We injected 5-FU into mice and then administered Myelophil to examine the ability of the drug to treat the side effects of 5-FU in mice. Peripheral blood counts, histological examinations, and colony-forming assays of bone marrow were conducted, followed by swimming tests and assessment of survival times.

RESULTS: Myelophil restored red and white blood cells and platelets in blood, and recovered cell density in bone marrow to levels comparable to those observed within the control group. In addition, Myelophil significantly increased colony-forming unit granulocyte-macrophage (CFU-GM) and CFU-erythroid (CFU-E) compared to the control group. We confirmed that interleukin-3 gene expression was upregulated by Myelophil in spleen cells. Myelophil administration also doubled the survival rate of mice that were severely myelosuppressed as a result of 5-FU injection at a lethal dose of 70%. Finally, the swimming performance of mice significantly improved as a result of Myelophil treatment.

CONCLUSION: These results provide experimental evidence in support of clinical applications of Myelophil to minimize 5-FU-induced myelosuppression and improve general post-chemotherapy health.

Key words: Astragalus membranaceus; Salvia miltiorrhizae; Myelosuppression; Immunosuppression; Chemotherapy

Peer reviewer: Paul Y Kwo, Professor, Gastroenterology and Hepatology Division, Indiana University School of Medicine, 975 West Walnut, IB 327, Indianapolis, Indiana 46202-5121, United States

Shin JW, Lee MM, Son JY, Lee NH, Cho CK, Chung WK, Cho JH, Son CG. Myelophil, a mixture of Astragali Radix and Salviae Radix extract, moderates toxic side effects of fluorouracil in mice. *World J Gastroenterol* 2008; 14(15): 2323-2328 Available from: URL: http://www.wjgnet.com/1007-9327/14/2323.asp DOI: http://dx.doi.org/10.3748/wjg.14.2323

INTRODUCTION

The occurrence of undesired effects that result from conventional chemotherapy or irradiation for cancer is inevitable. Nevertheless, reducing adverse effects is a critical issue for patients and doctors, given the importance of quality of life, as well as survival gains[1-3]. Accordingly, mitigation of chemotherapy-induced side effects and the development of novel chemotherapeutic agents with fewer toxic effects have been major focuses of recent medical investigations[4,5]. In particular, cancer-therapy-related fatigue, diarrhea or myelosuppression-related symptoms are closely associated with failure of the therapy itself. Therefore, many therapeutic developments, including herb-derived remedies, have focused on treating these side effects[6-9].

Fluorouracil is one of the most commonly used drugs to treat gastrointestinal cancers, including those in the stomach, colon and liver, and it commonly causes fatigue, diarrhea and sometimes myelosuppression[10,11]. Myelophil is a mixture of Astragali Radix and Salviae Radix extract representing Qi and blood, respectively, which support the liver and gastrointestinal system according to theories of Oriental medicine. Astragali Radix displays immunomodulating, hematopoietic, and anti-fibrotic properties[12-14]. Salviae Radix exhibits antioxidant, antiatherosclerosis, and antiplatelet aggregation pharmaceutical effects[15-17]. We have used this

drug to treat mainly gastrointestinal cancer patients with post-therapeutic complications such as leukopenia, anemia or severe fatigue since 2002.

Here, we evaluated the therapeutic efficacy of an extract mixture that contained Astragali Radix and Salviae Radix for reducing complications from cancer chemotherapy, using a 5-fluorouracil (5-FU)-induced myelosuppression mouse model.

MATERIALS AND METHODS

Manufacturing and fingerprinting of Myelophil

Astragalus membranaceus (Leguminosae, VS No: AM-2006-02-Ra) and *Salvia miltiorrhizae* (Labiatae, VS No: SM-2006-01-Ra) were provided by Daejeon Oriental Medical College, Dunsan Oriental Hospital, of Daejeon University, identified by Professor SI Yim of Daejeon University and stored at our laboratory for future use. Samik Pharmaceutical Company (Seoul, Korea) manufactured a lyophilized aqueous extract of Myelophil (mixture of Astragali Radix and Salviae Radix; 1:1) according to over-the-counter Korean monographs. A final product with 20.52% (w/w) yield was stored for future use (VS No: MP-2006-01-WE). 5-FU was purchased from Choongwae Pharma Corporation (Seoul, Korea). Other chemicals were obtained from Sigma (St. Louis, MO, USA).

For the fingerprinting of Myelophil, the water extract of Astragali radix and Salviae radix, and their standard components, formononetin (Sigma) and rosmarinic acid (Carl Roth, Karlsruhe, Germany) were prepared for the high performance thin layer chromatography (HPTLC) system (CAMAG, Muttenz, Switzerland). They were dissolved in HPLC-grade methanol and applied to pre-washed HPTLC plate silica gel 60 F_{254} (Merck, Darmstadt, Germany) with an automated applicator (Linomat Ⅳ; CAMAG). The samples were then separated and the migrated components were visualized prior to or after derivatization under UV radiation at 366 nm or white light using Reprostar 3 with a digital camera (CAMAG, Figure 1).

Induction of myelosuppression: Hematological and histopathological analysis

To examine the therapeutic effects of Myelophil on 5-FU-induced myelosuppression, 60 6-wk-old male ICR mice (Koatech, Gyeonggi-do, Korea) were divided into three groups (20 control, and 20 with low- and 20 with high-concentration Myelophil treatment). Another five mice were sacrificed to record physiological standards for hematological parameters at time 0. All three groups were injected intraperitoneally with 0.3 g/kg 5-FU on d 0. Beginning on d 2, Myelophil (0.05 g/kg or 0.1 g/kg) or distilled water (control group) was administered orally once daily for 10 consecutive days. Five mice per group were serially sacrificed on d 0, 4, 7, 10 and 13, and complete blood counts were analyzed using a blood cell counter (HEMAVET; CDC Technologies, CT, USA). In addition, all left-side femoral bones from the mice on d 7 were prepared for general histopathological evaluation, including fixation, decalcification, sectioning (4 μm thickness), as well as hematoxylin and eosin (HE) staining.

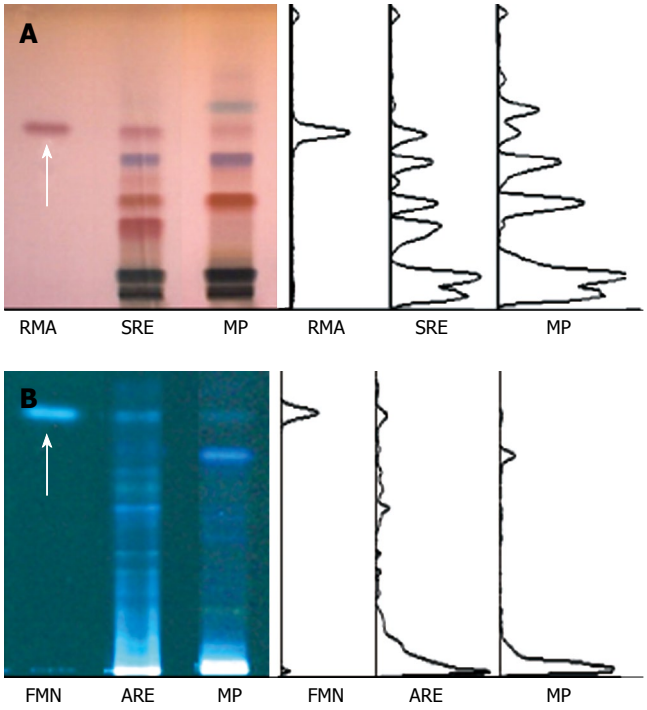

Figure 1 Fingerprinting of Myelophil. **A**: Rosmarinic acid (RMA, 2 μg), Salviae Radix water extract (SRE, 200 μg) and Myelophil (MP, 400 μg) were separated using chloroform/ethyl acetate/benzene/formic acid/methanol (15:10:10:10:1), and then visualized by white light after derivatization with p-anisaldehyde sulfuric acid; **B**: Formononetin (FMN, 0.1 μg), Astragali Radix water extract (ARE, 0.8 mg) and Myelophil (MP, 1.6 mg) were separated using dichloromethane/methanol/water (45:10:1), and then visualized under UV at 366 nm.

Isolation of bone marrow cells and colony-forming assay

To directly examine the effects of Myelophil on bone marrow stem cells, C57BL/6 mice (three for each of the control, and low- and high-concentration Myelophil groups) were injected intraperitoneally with 5-FU (0.2 g/kg). Mice were given Myelophil (0.05 g/kg or 0.1 g/kg) or distilled water (for naive and control groups) for 5 consecutive days beginning 2 d after 5-FU injection. Bone marrow cells were isolated from femurs, and nucleated cells were counted using a blood cell counter (HEMAVET; CDC Technologies). After thoroughly mixing the nucleated cells (400 μL 2×10^5) with 4 mL MethoCult methylcellulose-based medium (Stem Cell Technologies, Seattle, WA, USA), media (1 mL per dish in triplicate) were cultured in a 5% CO_2 incubator for 7 d. According to the morphological characteristics, the number of colonies assessed by CFU-GM or CFU-E was counted under an inverted microscope.

Interleukin-3 (IL-3) gene expression analysis using real-time PCR

Splenocytes isolated from BALB/c male mice were seeded in a six-well culture plate (2.8×10^7 cells per well), and treated with or without Myelophil (0.001, 0.01 or 0.1 g/L) for 18 h. After purification of total RNA using an RNA mini kit (Qiagen, Valencia, CA, USA) and cDNA synthesis, quantitative real-time PCR was performed using SYBR Green Supermix reagent (Bio-Rad, CA, USA) according to the manufacturer's protocol. The primer sequences (forward and reverse, respectively) were

Table 1 Hematological effect of Myelophil on 5-FU-induced myelosuppression

Cells/groups		d 0	d 4	d 7	d 10	d 13
WBC (10^3 cells/μL)	Control	5.5 ± 1.2	2.3 ± 0.3	0.7 ± 0.2	2.0 ± 1.0	3.7 ± 1.5
	MP 50	5.5 ± 1.2	2.3 ± 0.9	1.2 ± 0.4[a]	2.5 ± 0.9	6.4 ± 1.6[a]
	MP100	5.5 ± 1.2	2.4 ± 0.3	1.6 ± 0.4[b]	2.9 ± 0.6	4.2 ± 1.0[a]
RBC (10^6 cells/μL)	Control	7.1 ± 0.8	7.3 ± 0.6	5.3 ± 0.2	6.3 ± 1.2	7.0 ± 1.0
	MP 50	7.1 ± 0.8	6.3 ± 0.6[a]	5.9 ± 0.4[b]	6.9 ± 0.7	6.9 ± 1.2
	MP 100	7.1 ± 0.8	6.6 ± 0.5	6.6 ± 0.4[b]	6.7 ± 0.5	7.2 ± 1.0
Platelets (10^5 cells/μL)	Control	13.8 ± 0.8	8.5 ± 1.3	5.3 ± 1.5	7.3 ± 1.7	12.0 ± 5.3
	MP 50	13.8 ± 0.8	8.8 ± 1.3	8.2 ± 1.4[a]	9.1 ± 3.2	17.7 ± 6.6
	MP 100	13.8 ± 0.8	9.8 ± 1.8	9.0 ± 2.2[a]	10.9 ± 4.5	16.2 ± 4.9

From 2 d after 5-FU injection, Myelophil (0.05 g/kg or 0.1 g/kg) or distilled water (for the control group) was given to mice for 10 d. On day 0, 4, 7, 10 and 13, complete blood counts were analyzed using a blood cell counter. Data are expressed as mean ± SD (n = 5). [a]$P < 0.05$, [b]$P < 0.01$ *vs* control group. MP: Myelophil.

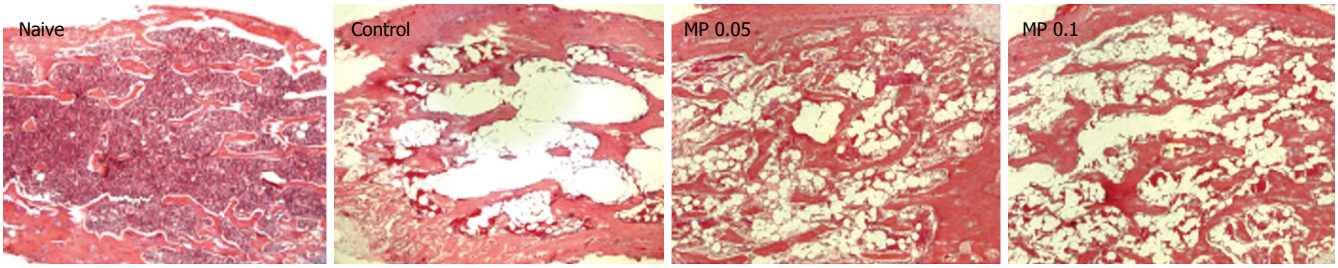

Figure 2 Histopathological analysis of bone marrow. After 7 d 5-FU injection (0.3 g/kg, intraperitoneally) and Myelophil treatment for 5 d, the femoral bone was dissected and fixed in 10% neutral-buffered formalin, followed by decalcification, embedding, and micro-sectioning (4 μm). Histopathological examination was performed under a microscope (× 200) after HE staining.

as follows: β-actin, GTGGGGCGCCCCAGGCACCA and CTCCTTAATGTCACGCACGATTTC; IL-3, TACATCTGCGAATGACTCTGC and GGCTGAGGTGGTCTAGAGGTT.

Monitoring survival rates with or without swimming-forced stress

To examine whether Myelophil affected the survival time of mice with severe or moderate myelosuppression induced by 5-FU, we conducted two tests. First, severe myelosuppression was induced in 30 male ICR mice (10 in each of the control, and low- and high-concentration Myelophil groups) by 5-FU treatment (0.5 g/kg, intraperitoneally). Two days later, Myelophil (0.05 or 0.1 g/kg) or distilled water (induced group) was administered orally once daily for 10 consecutive days. The number of surviving mice was monitored for the next 20 d.

For the second test, moderate myelosuppression was induced in 30 male ICR mice (10 in each of the control, and low- and high-concentration Myelophil groups) by 5-FU treatment (0.3 g/kg, intraperitoneally). Beginning 2 d following 5-FU injection, Myelophil (0.05 or 0.1 g/kg) or distilled water (for the naïve and control groups) was given orally once daily for five consecutive days. On the final day, all mice were forced to swim in a pool with 22℃ water for 30 min. Mice were monitored for survival time and swimming performance.

Statistical analysis

The results were expressed as mean ± SD. Statistical analysis of the data was conducted using Student's t test with significance levels of $P < 0.05$.

RESULTS

Hematological parameters

First, we examined changes in hematological parameters (leukocyte, erythrocyte and platelet counts) in 5-FU-induced (0.3 g/kg) myelosuppressed mice every 3 d. As shown in Table 1, peripheral blood white blood cell, platelet, and red blood cell levels drastically decreased, with the lowest numbers recorded on d 7. However, the observed pancytopenia was ameliorated by Myelophil administration, and the number of leukocytes rapidly recovered compared to that in untreated control mice (0.05 g/kg Myeolophil, $P = 0.0387$; 0.1 g/kg Myelophil, $P = 0.0014$).

Histological examination of bone marrow

We examined the cellular density of femoral bone marrow from d 7 mice. Similar to the peripheral blood counts, 5-FU injection radically reduced the cellular component in bone marrow by vacuolation, and this was moderately improved by Myelophil treatment (Figure 2).

CFUs in bone marrow

To investigate how Myelophil affected the hematopoietic stem cells, leukocyte or erythrocyte-lineage colonies were determined using colony forming assay. Myelophil treatment significantly increased the colony numbers of both leukocyte (0.05 g/kg Myeolophil, $P = 0.0087$; 0.1 g/kg Myelophil, $P = 0.0029$) and erythrocyte lineages (0.05 g/kg Myeolophil,

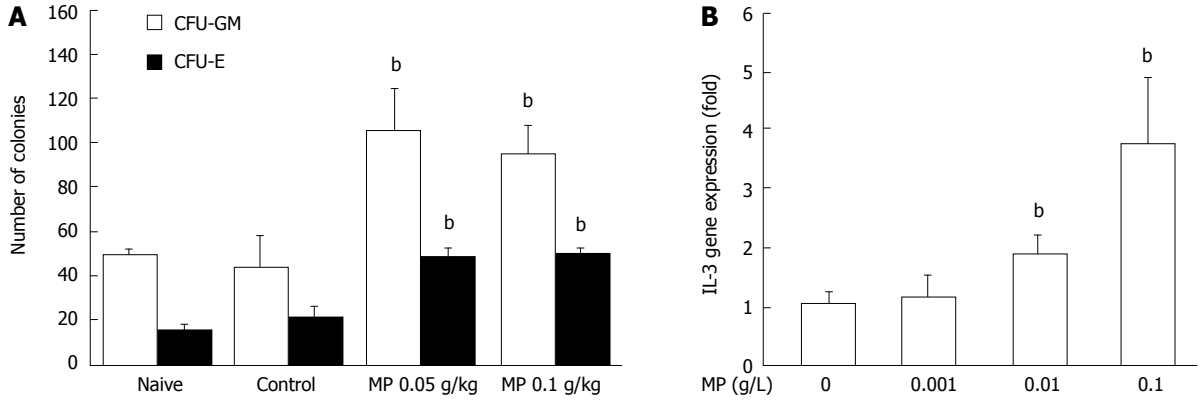

Figure 3 Colony-forming assay and IL-3 gene expression. **A**: After 7 d 5-FU injection (0.2 g/kg, intraperitoneally) and Myelophil treatment for 5 d, purified bone marrow cells were cultured for 7 d for colony counts of CFU-GM and CFU-E; **B**: Spleen cells were treated with Myelophil for 18 h, and then IL-3 gene expression was analyzed using real-time-PCR. Data are expressed as mean ± SD (n = 3). $^bP < 0.01$ *vs* control group.

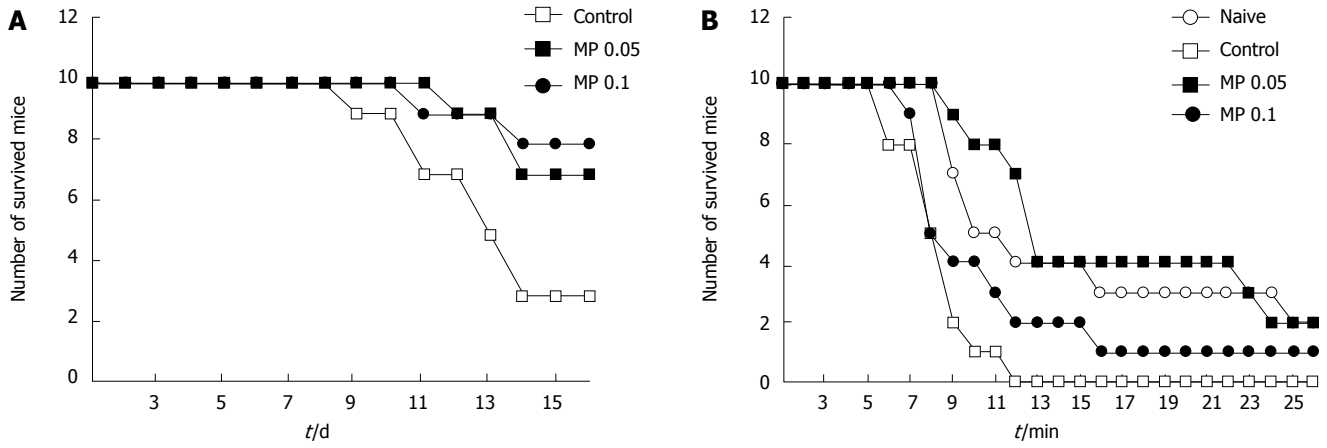

Figure 4 Survival of myelosuppressed mice. **A**: Beginning 2 d after 5-FU injection (0.5 g/kg, intraperitoneally), Myelophil (0.05 or 0.1 g/kg) or distilled water (control) was administered for 10 d. The number of surviving mice was monitored for 20 experimental days; **B**: Beginning 2 d after 5-FU injection (0.3 g/kg, intraperitoneally), Myelophil (0.05 or 0.1 g/kg) or distilled water (for naïve and control groups) was administered orally for 5 d. All mice were then forced to swim in a pool to monitor their survival time for 30 min.

$P = 0.0018$; 0.1 g/kg Myeolophil, $P = 0.0021$), as shown in Figure 3A.

IL-3 gene expression in vitro

We examined changes in IL-3 gene expression in splenocytes following co-culturing with Myelophil using RT-PCR. IL-3 expression was increased in a dose-dependent manner. At a concentration of 0.1 g/L Myelophil, this gene was up-regulated four-fold (Figure 3B).

Survival rate of myelosuppressed mice

Following injection with 0.5 g/kg 5-FU (LD70, determined in our experiments), the survival rate of the control group was 30% by 14 d, whereas in the Myelophil-treated groups it was 70%-80% (Figure 4A). Myelophil treatment significantly protected mice from loss of body weight after 5-FU injection (data not shown). In addition, Myelophil treatment extended the survival time of mice that were forced to swim in a pool after injection with 0.3 g/kg of 5-FU (Figure 4B).

DISCUSSION

We used a 5-FU-induced myelosuppression mouse model to evaluate the experimental efficacy of Myelophil, with relevance to the clinical application of reducing chemotherapy-induced side effects. 5-FU is one of the most commonly used anti-metabolitic chemotherapeutic drugs for colon, stomach, liver, and head and neck cancer. It has also been applied in anti-myelosuppressive studies due to its observed toxicity toward bone marrow[18-20]. We observed that a single injection of 0.5 g/kg 5-FU caused > 70% mortality within 20 d, whereas 0.3 g/kg 5-FU induced mild myelosuppression with no incidence of death.

Peripheral white blood cell, platelet and red blood cell levels drastically decreased in 5-FU-induced (0.3 g/kg) myelosuppressed mice. On d 7, these levels were the lowest and were in accordance with those of severe leukopenia, moderate thrombocytopenia and mild anemia. However, the observed pancytopenia was ameliorated by Myelophil administration, and the number of leukocytes rapidly recovered compared to that in untreated control mice. Next, we examined the cellular density of femoral bone marrow from d 7 mice. Similar to the peripheral blood results, 5-FU injection radically reduced the cellular component in bone marrow induced large vacuole formation, and this was moderately improved

by Myelophil treatment (Figure 2). These results suggest that Myelophil might be beneficial for the alleviation of chemotherapy-associated high susceptibility to pathogenic microorganisms, which is a major problem post-treatment[21,22].

Consequently, we investigated how Myelophil affected the hematopoietic stem cells *via* differential examination of leukocyte- or erythrocyte-lineage colonies. The number and lineage of a colony was decided mainly by quantity and quality of stem cells in different groups. Our results showed Myelophil treatment significantly increased the colony numbers of both leukocyte and erythrocyte lineages (Figure 3A). Processes of hematopoiesis are under the control of various hematopoietic growth factors, such as IL-3, erythropoietin (EPO), thrombopoietin (TPO), granulocyte colony-stimulating factor (G-CSF), or granulocyte/macrophage CSF (GM-CSF)[23]. These growth factors have lineage-specific hematopoietic functions and different cellular excretion sources[24]. Specifically, IL-3 supports proliferation and differentiation of hematopoietic stem cells, as well as various cell lineages in hematopoiesis[25], and is secreted mainly from natural killer T cells[26]. Therefore, we examined changes in IL-3 gene expression in splenocytes following co-culturing with Myelophil. The result showed that IL-3 expression was increased four-fold (Figure 3B).

Given the above results, we observed how Myelophil could restore myelosuppression via IL-3 up-regulation. Generally, myelosuppression is linked strongly to other common side effects caused by conventional cancer therapy, such as fatigue or low energy, as well as low immunity.

Myelophil-treatment significantly protected mice from loss of body weight after 5-FU (0.5 g/kg) injection (data not shown). In addition, Myelophil treatment extended the survival times of mice that were forced to swim in a pool after injection of 0.3 g/kg 5-FU (Figure 4B).

5-FU-induced myelosuppression is the dose-limiting toxicity associated with substantial life-threatening risk and life span of cancer patients[27,28]. Many herbal medicines are currently being investigated as good candidates for improving quality of life and reducing toxic side effects such as myelosuppression[29-32]. One group has reported the efficacy of one of the two medicinal plant extracts in Myelophil on hematopoiesis induction in mice[14]. We found that a mixture of Astragali Radix and Salviae Radix was more effective than a single herb administered alone in our model system (data not shown). We have prescribed Myelophil to treat post-therapeutic complications such as anemia, leukopenia or severe fatigue, mainly for gastrointestinal cancer patients, according to its oriental pharmaceutical theory since 2002.

Herein, we have provided experimental evidence relevant to clinical applications of Myelophil for minimizing cancer chemotherapy-induced side-effects, using a fluorouracil-induced myelosuppression mouse model.

COMMENTS

Background

Anticancer-therapy-induced side effects are closely associated with life span and quality of life in cancer patients, so reducing or preventing these has been a major issue in cancer treatment. Astragali Radix and Salviae Radix extract, Myelophil, has been used to treat mainly gastrointestinal cancer patients with post-therapeutic complications such as leukopenia, anemia or severe fatigue since 2002. This study demonstrated the efficacy of Myelophil for moderating the toxic side effects of 5-FU.

Research frontiers

Fluorouracil is one of the most commonly used drugs to treat gastrointestinal cancers, including stomach, colon and liver, but it commonly causes fatigue, diarrhea and sometimes myelosuppression. Myelophil significantly ameliorated the toxic side effects of 5-FU, such as leukopenia, anemia and thrombocytopenia and improved survival rate in myelosuppressed mice.

Innovations and breakthroughs

Myelophil showed dramatic activity against the side effects of 5-FU, a very widely used drug. This study showed evidence that a herbal remedy can be a good candidate for improving quality of life in cancer patients.

Applications

Myelophil may be of benefit to cancer patients suffering from chemotherapy-induced side effects.

Peer review

This manuscript examines two herbal extract mixtures that reduce complications induced by the chemotherapeutic agent, 5-FU in a gastrointestinal cancer model. The bone marrow data, colony-forming assay, and interleukin gene expression all support the moderating effects of Myelophil on toxic side effects of 5-FU, and show the possibilities for clinical applications.

REFERENCES

1 **Carelle N**, Piotto E, Bellanger A, Germanaud J, Thuillier A, Khayat D. Changing patient perceptions of the side effects of cancer chemotherapy. *Cancer* 2002; **95**: 155-163

2 **Schuell B**, Gruenberger T, Kornek GV, Dworan N, Depisch D, Lang F, Schneeweiss B, Scheithauer W. Side effects during chemotherapy predict tumour response in advanced colorectal cancer. *Br J Cancer* 2005; **93**: 744-748

3 **Hassett MJ**, O'Malley AJ, Pakes JR, Newhouse JP, Earle CC. Frequency and cost of chemotherapy-related serious adverse effects in a population sample of women with breast cancer. *J Natl Cancer Inst* 2006; **98**: 1108-1117

4 **Friberg LE**, Henningsson A, Maas H, Nguyen L, Karlsson MO. Model of chemotherapy-induced myelosuppression with parameter consistency across drugs. *J Clin Oncol* 2002; **20**: 4713-4721

5 **Senecal FM**, Yee L, Gabrail N, Charu V, Tomita D, Rossi G, Schwartzberg L. Treatment of chemotherapy-induced anemia in breast cancer: results of a randomized controlled trial of darbepoetin alfa 200 microg every 2 weeks versus epoetin alfa 40,000 U weekly. *Clin Breast Cancer* 2005; **6**: 446-454

6 **Ludwig H**, Strasser K. Symptomatology of anemia. *Semin Oncol* 2001; **28**: 7-14

7 **Mayers C**, Panzarella T, Tannock IF. Analysis of the prognostic effects of inclusion in a clinical trial and of myelosuppression on survival after adjuvant chemotherapy for breast carcinoma. *Cancer* 2001; **91**: 2246-2257

8 **Steele TA**. Chemotherapy-induced immunosuppression and reconstitution of immune function. *Leuk Res* 2002; **26**: 411-414

9 **Son CG**, Han SH, Cho JH, Shin JW, Cho CH, Lee YW, Cho CK. Induction of hemopoiesis by saenghyuldan, a mixture of Ginseng radix, Paeoniae radix alba, and Hominis placenta extracts. *Acta Pharmacol Sin* 2003; **24**: 120-126

10 **Wilke HJ**, Van Cutsem E. Current treatments and future perspectives in colorectal and gastric cancer. *Ann Oncol* 2003; **14** Suppl 2: ii49-ii55

11 **Louvet C**, Andre T, Tigaud JM, Gamelin E, Douillard JY, Brunet R, Francois E, Jacob JH, Levoir D, Taamma A, Rougier P, Cvitkovic E, de Gramont A. Phase II study of oxaliplatin, fluorouracil, and folinic acid in locally advanced or metastatic

gastric cancer patients. *J Clin Oncol* 2002; **20**: 4543-4548
12 **Cho WC**, Leung KN. In vitro and in vivo immunomodulating and immunorestorative effects of Astragalus membranaceus. *J Ethnopharmacol* 2007; **113**: 132-141
13 **Sun WY**, Wei W, Wu L, Gui SY, Wang H. Effects and mechanisms of extract from Paeonia lactiflora and Astragalus membranaceus on liver fibrosis induced by carbon tetrachloride in rats. *J Ethnopharmacol* 2007; **112**: 514-523
14 **Zhu XL**, Zhu BD. Mechanisms by which Astragalus membranaceus injection regulates hematopoiesis in myelosuppressed mice. *Phytother Res* 2007; **21**: 663-667
15 **Tang MK**, Ren DC, Zhang JT, Du GH. Effect of salvianolic acids from Radix Salviae miltiorrhizae on regional cerebral blood flow and platelet aggregation in rats. *Phytomedicine* 2002; **9**: 405-409
16 **Koo BS**, Kwon TS, Kim CH. Salviae miltiorrhizae radix inhibits superoxide generation by activated rat microglias and mimics the action of amphetamine on in vitro rat striatal dopamine release. *Neurochem Res* 2004; **29**: 1837-1845
17 **Li S**, Wan L. Experimental study on the preventive mechanism of salviae miltiorrhizae against atherosclerosis in rabbits models. *J Huazhong Univ Sci Technolog Med Sci* 2004; **24**: 233-235
18 **Kojima S**, Takaba K, Kimoto N, Takeda T, Kakuni M, Mizutani M, Suzuki K, Sato H, Hara T. Protective effects of glutathione on 5-fluorouracil-induced myelosuppression in mice. *Arch Toxicol* 2003; **77**: 285-290
19 **Takano F**, Tanaka T, Aoi J, Yahagi N, Fushiya S. Protective effect of (+)-catechin against 5-fluorouracil-induced myelosuppression in mice. *Toxicology* 2004; **201**: 133-142
20 **Vento S**, Cainelli F. Infections in patients with cancer undergoing chemotherapy: aetiology, prevention, and treatment. *Lancet Oncol* 2003; **4**: 595-604
21 **Rolston KV**. Challenges in the treatment of infections caused by gram-positive and gram-negative bacteria in patients with cancer and neutropenia. *Clin Infect Dis* 2005; **40** Suppl 4: S246-S252
22 **Savino W**, Smaniotto S, Dardenne M. Hematopoiesis. *Adv Exp Med Biol* 2005; **567**: 167-185
23 **Kaushansky K**. Lineage-specific hematopoietic growth factors. *N Engl J Med* 2006; **354**: 2034-2045
24 **Mangi MH**, Newland AC. Interleukin-3 in hematology and oncology: current state of knowledge and future directions. *Cytokines Cell Mol Ther* 1999; **5**: 87-95
25 **Leite-de-Moraes MC**, Lisbonne M, Arnould A, Machavoine F, Herbelin A, Dy M, Schneider E. Ligand-activated natural killer T lymphocytes promptly produce IL-3 and GM-CSF *in vivo*: relevance to peripheral myeloid recruitment. *Eur J Immunol* 2002; **32**: 1897-1904
26 **Cella D**. Factors influencing quality of life in cancer patients: anemia and fatigue. *Semin Oncol* 1998; **25**: 43-46
27 **Groopman JE**, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. *J Natl Cancer Inst* 1999; **91**: 1616-1634
28 **Biran H**, Sulkes A, Biran S. 5-Fluorouracil, doxorubicin (adriamycin) and mitomycin-C (FAM) in advanced gastric cancer: observations on response, patient characteristics, myelosuppression and delivered dosage. *Oncology* 1989; **46**: 83-87
29 **Zhang M**, Liu X, Li J, He L, Tripathy D. Chinese medicinal herbs to treat the side-effects of chemotherapy in breast cancer patients. *Cochrane Database Syst Rev* 2007; CD004921
30 **Liao HF**, Chen YJ, Yang YC. A novel polysaccharide of black soybean promotes myelopoiesis and reconstitutes bone marrow after 5-flurouracil- and irradiation-induced myelosuppression. *Life Sci* 2005; **77**: 400-413
31 **Sugiyama K**, Ueda H, Ichio Y. Protective effect of juzen-taiho-to against carboplatin-induced toxic side effects in mice. *Biol Pharm Bull* 1995; **18**: 544-548
32 **Molassiotis A**, Fernadez-Ortega P, Pud D, Ozden G, Scott JA, Panteli V, Margulies A, Browall M, Magri M, Selvekerova S, Madsen E, Milovics L, Bruyns I, Gudmundsdottir G, Hummerston S, Ahmad AM, Platin N, Kearney N, Patiraki E. Use of complementary and alternative medicine in cancer patients: a European survey. *Ann Oncol* 2005; **16**: 655-663

S- Editor Zhong XY **L- Editor** Kerr C **E- Editor** Ma WH