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Original Article

Anti-Myelosuppression Effects of Korean Red Ginseng in SD Rat Injected with 5-fluorouracil

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Objectives: This study aimed to investigate the preventive effect of red ginseng (RG) on 5-fluorouracil (5-FU)-induced side effects focusing on myelosuppression.
Methods: Rats (n = 50) were divided into five groups, nave, control (*ip*, 5-FU injection of 150 mg/kg), and RG pre-treatment (*po*, 25, 50 and 100 mg/kg for 5 days before 5-FU injection). On the 7th day after 5-FU injection, we evaluated the effects using peripheral hematological parameters, colony-forming assay, cytokine levels and histopathological finding.
Results: The peripheral white blood cell and the differential count were dramatically suppressed by 5-FU, while RG (50 and 100 mg/kg) treatment significantly improved total white blood cell, neutrophil, lymphocyte and platelet counts. Also, RG (100 mg/kg) pre-treatment significantly increased the number of CFU-GM colony compared with the control group. RG pre-treatment also ameliorated the histopathological damage in bone marrow, spleen, stomach and small intestine tissue.
Conclusions: These results demonstratethat Korean RG has preventive effects against 5-FU-induced myelotoxicity and gastrointestinal damage.

Key Words : Red ginseng, 5-fluorouracil, Myelosuppression, Side effect, Chemotherapy

Introduction

Chemotherapy can extend survival rate in patients with cancer, alone or combined with other therapies like radiotherapy or surgery. However, its clinical therapeutic efficacy is limited due to the undesirable side effects in the patients including nausea, vomiting, myelosuppression, gastrointestinal, liver and renal toxicity[1-3]. These side effects affect not only their therapeutic options but also overall health-related quality of life and disruption of treatment in cancer patients[4].

5-fluorouracil (5-FU) is a chemotherapeutic drug widely used in cancer treatment, such as breast, head and neck, gastric, and colorectal carcinomas[5], and it commonly causes hematopoietic and gastrointestinal (GI) toxicity[6]. Sometimes, 5-FU-induced side effects lead to discontinuation of treatment[7]. A clinical trial study reported that 47% of patients with protracted venous infusion of 5-FU for two years discontinued treatment due to toxicity, such as handfoot syndrome, diarrhea, stomatitis, fatigue and a decrease in the

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number of blood and bone marrow cells[8].

Red ginseng (RG, originated from *Panax ginseng* C.A. Meyer.) is a well-known traditional herbal medicine or supplement widely used for various diseases and health issues. Several earlier studies have confirmed the protective effects of red ginseng against cancer, gastric ulcer, and fatigue[9-12]. Recently, RG and/or its components have become known as potential agents to reduce the side effects of anti-cancer drugs[13,14]. However, there is a lack of evidence whether RG is effective in prevention of side effects of 5-FU.

In the present study, we investigated the protective properties of RG on 5-FU-induced myelotoxicity in a SD rat model.

Materials and Methods

1. Korean red ginseng and chemical agents

Red ginseng product was obtained from KT&G (Seoul, Korea) as a form of semi-colloid. Twenty grams of red ginseng product were dissolved in 500 ml distilled water (DW), then lyophilized using a vacuum-freeze drying system. The extract yield was 58.98%, and the final extract was stored at -20℃ for future experiments.

5-FU was purchased from Choongwae Pharmaceutical Co. (Seoul, Korea), and MetoCult methylcellulose medium was purchased from Stem Cell Technologies (Seattle, WA, USA). All the other reagents were obtained from Sigma (St Louis, MO, USA).

2. Animals and experimental design

Male Sprague-Dawley rats (6-week-old) were purchased from Koatek (Gyeongido, Korea). Those weighing between 160 and 180 g were used in this study. They were acclimated under an environment of 22 ± 2℃ 12 h light/dark cycle, and fed commercial standard chow and tap water ad libitum for seven days. Animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Daejeon University (DJUARB2011-030).

The SD rats (n = 50) were randomly divided into five groups of 10 rats each: nave group, control group(pretreated with DW followed by 5-FU injection), and RG groups (pre-treated with 25, 50, or 100 mg/kg of RG followed by 5-FU injection). Except for the nave group, the other four groups were orally administered with DW or RG (once a day) for five days, and then they were intraperitoneally injected with 5-FU (150 mg/kg). Five rats of each group were sacrificed at two time points, 7 and 14 days after 5-FU injection.

3. Determination of body weight and food intake

Rat body weight was measured every 3 or 4 days for 14 days. The total food consumption was calculated by measuring the remainder from the previous day.

4. Hematological and histopathological analysis

The hematological parameters were monitored every 3 or 4 days for 14 days after 5-FU injection, including white blood cell (WBC), hemoglobin, red blood cell (RBC), platelet and lymphocyte counts. They were determined using a HEMA VET 850 automatic analyzer (CDC Technologies, CT, USA) by 60-100 ㎕ of retro-orbital sinus blood.

At two experimental points on the 7th and 14th days, the femur, stomach, small intestine and spleen tissue were removed and stored separately in 10% formalin for histopathological examination. The femur was decalcified by 8% hydrochloric acid and 8% formic acid mixed solution. Fixed tissues were then embedded in paraffin and sectioned (4 ㎛ hickness). The slides were stained with hematoxylin and eosin (H&E), and the representative microphotographs were obtained under a light microscope (×200 magnifications; Leica, Wetzlar, Germany).

5. Isolation of bone marrow cells and colony-forming assay

Bone marrow cells were harvested from one side femurs, and nucleated cells were collected using a gradient method (Histopaque®-1077, Sigma, St. Louis, MO, USA). The collected nucleated cells were counted by hemocytometer. After thoroughly mixing the nucleated cells (2×10^5/300 $\mu\ell$ with 3 ml MetoCult methylcellulose-based medium (Stem Cell Technologies, Seattle, WA, USA), the media (1 ml per dish in duplicate) were cultured in a 5% CO_2 incubator for 7 days. According to the morphological characteristics, the number of colonies assessed by colony-forming unit-granulocyte and macrophage (CFU-GM) or colony-forming unit-erythrocyte (CFU-E) was counted (>30 cells per colony) under a light microscope.

6. Measurement of cytokine levels

Serum was prepared from total blood collected via the abdominal aorta under ether anesthesia and stored at -70℃ until analysis. The serum concentration of cytokines was measured with enzyme-linked immune sorbent assay (ELISA) kits for IL-3 (R&D system, Minneapolis, MN) and granulocyte macrophage-colony stimulating factor (GM-CSF; MyBiosource, San Diego, CA) respectively.

7. Statistical analysis

All results are expressed as mean standard deviation (S.D.). Significant differences between the groups were statistically analyzed using Student's t-test. $P < 0.05$ was regarded as statistically significant.

Results

1. Effects on changes in body weight and food intake

5-FU injection induced loss of body weight most on day 3, and gradually recovered. The body weight of the control group ($P < 0.05$). Pre-treatment with RG (50 and 100 mg/kg) significantly ameliorated the reduction of body weight compared with the control group on especially 7th and 10th days ($P < 0.05$, Fig. 1A).

5-FU injection slightly inhibited the intake of food by rats, and RG pre-treatment didn't significantly affect food consumption (Fig. 1B).

2. Effect on hematologicalparameters

Total WBC number including five differential counts of neutrophil, lymphocyte, monocyte, and eosinophil was drastically decreased by 5-FU injection most severely on day 7, and they gradually recovered by day 14. Meanwhile, pre-treatment with

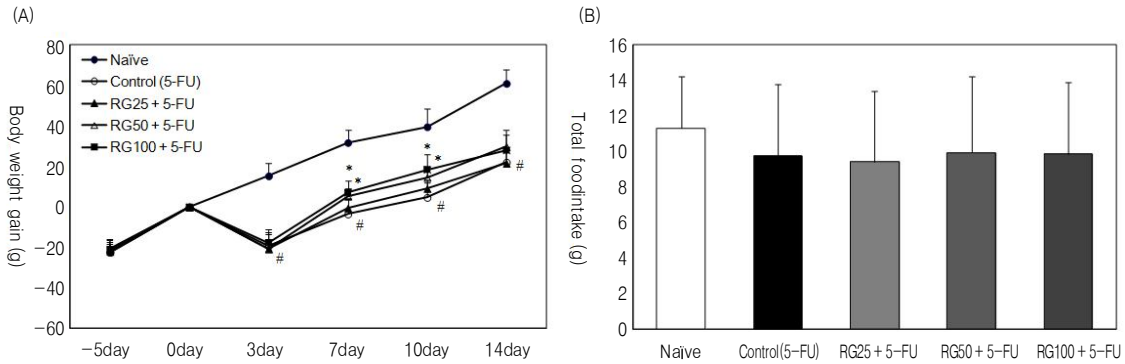

Fig. 1. Changes in body weight and food intake. Rats were pre-treated with RG for 5 days (*po*, once a day) before 5-FU injection (*ip*, 150 mg/kg). The change of body weight (A) and total food consumption (B) were measured for 14 days. $^{\#}P < 0.05$ compared with the naive group; $^{*}P < 0.05$ compared with the control group.

RG (50 and 100 mg/kg) significantly attenuated the leucopenia including total WBC, neutrophil, and lymphocyte compared with the control group ($P < 0.05$). The RBC count, hemoglobin and plateletnumber were significantly reduced by 5-FU injection compared with the naïve group ($P < 0.05$). Pre-treatment with RG (50 and 100 mg/kg) significantly attenuated the reduction of platelet number compared with the control group ($P < 0.05$), however it was not effective for RBC count and hemoglobin level (Fig. 2, Table 2).

3. Effect on the colony formation and serum level of GM-CSF and IL-3

The colony formation number of CFU-GM and CFU-E were drastically decreased in the control group injected with 5-FU, to approximately half of the naïve group. However, RG (25, 50 and 100 mg/kg) pre-treatment showed an increased pattern of the colony numbers of both CFU-GM and CFU-E. The statistical significance was shown in only the CFU-GM of 100 mg/kg RG group compared with the control group ($P < 0.05$, Fig. 3A and B).

5-FU injection markedly decreased serum levels of

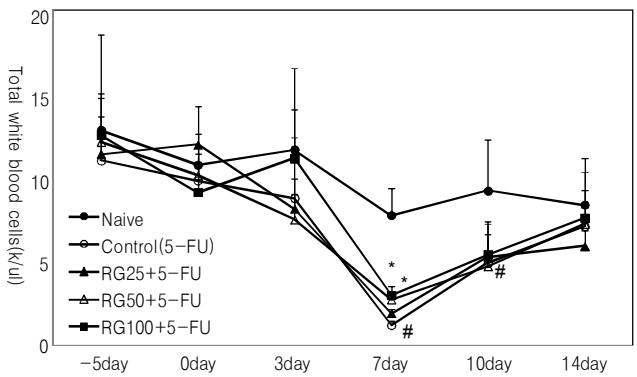

Fig. 2. Change of WBC count in peripheral blood.
Rats were pre-treated with RG for 5 days (po, once a day) before 5-FU injection (ip, 150 mg/kg), and then WBC count were chased periodically. #P < 0.05 compared with the naïve group *P < 0.05 compared with the control group.

Table 2. The Change of Hematologic Parameters at 7 Day

Parameters	Normal	5-FU (150 mg/kg)	Pre-treatment (mg/kg)		
			RG25	RG50	RG100
WBC (k/㎕)	7.76 ± 1.62	1.84 ± 0.31#	1.91 ± 0.25	2.74 ± 0.49*	2.86 ± 0.54*
RBC (m/㎕)	7.80 ± 0.41	6.94 ± 0.10#	7.23 ± 0.44	7.23 ± 0.34	6.70 ± 0.84
Hemoglobin (g/dl)	12.68± 0.70	10.58 ± 0.33#	10.86 ± 0.65	10.78± 0.36	9.92 ± 1.55
Platelet (k/㎕)	908.2 ± 337.9	316.3 ± 55.1#	402.3 ± 78.4	447.4± 61.8*	437.3 ± 14.9*
Neutrophils (k/㎕)	2.15 ± 0.77	0.05 ± 0.03#	0.08 ± 0.03	0.17 ± 0.09*	0.11 ± 0.07
Lymphocytes (k/㎕)	5.32 ± 0.88	1.73 ± 0.28#	1.78 ± 0.21	2.48 ± 0.43*	2.68 ± 0.54*
Monocytes (k/㎕)	0.26 ± 0.09	0.05 ± 0.02#	0.05 ± 0.01	0.08 ± 0.03	0.08 ± 0.03
Eosinophil (k/㎕)	0.03 ± 0.02	0.00 ± 0.00#	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00
Basophil (k/㎕)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00

The hematologic parameters were presented for the results on day 7. Data was expressed as mean ± SD (n = 5). #$P < 0.05$ compared with the naïve group *$P < 0.05$ compared with the control group.

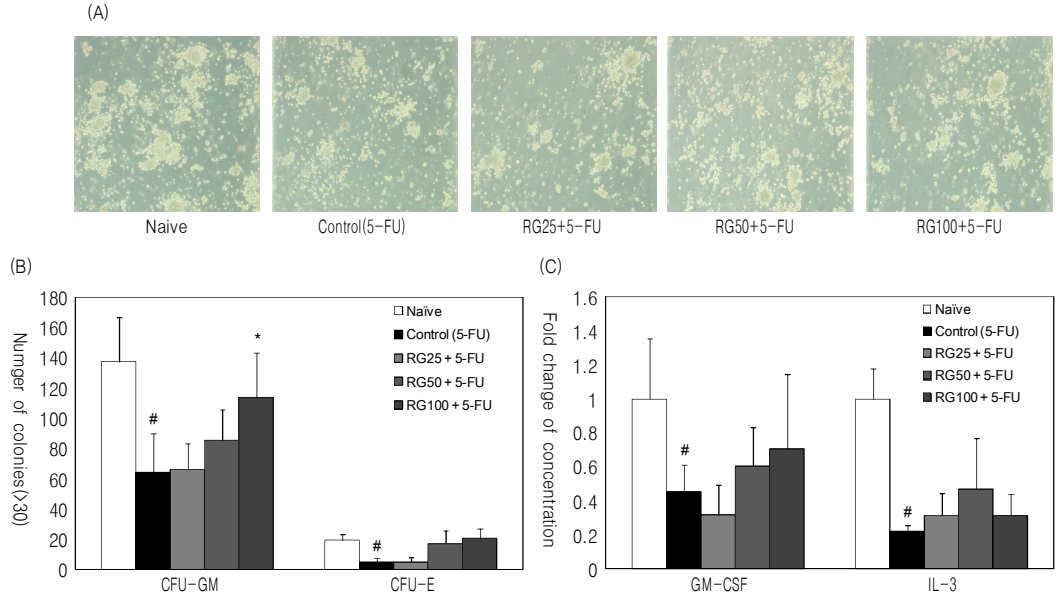

Fig. 3. Effects on the colony formation and serum levels of GM-CSF and IL-3.
After 7 days of 5-FU (150 mg/kg) injection, bone marrow stem cells were cultured in methylcellulose for colony formation of CFU-GM and CFU-E (A and B). The serum levels of GM-CSF and IL-3 were measured (C). #$P < 0.05$ compared with the naïve group *$P < 0.05$ compared with the control group.

GM-CSF and IL-3 as compared with the naïve group ($P < 0.05$). Pre-treatment with RG (50 and 100 mg/kg) increased in serum levels of GM-CSF and IL-3 but there was no significant change as compared with the control group (Fig. 3C).

4. Histopathological findings

The 5-FU injection leaded to drastic reduction of the cellular component in femoral bone marrow, while pre-treatment with RG (25, 50 and 100 mg/kg) notably attenuated the alteration of the cellular component (Fig. 4A). The white pulp region of spleen tissue was drastically decreased by 5-FU injection, while this alteration was moderately protected by RG (50 and 100 mg/kg) pre-treatment (Fig. 4B). In addition, 5-FU injection considerably injured the gastric epithelium in stomach and small intestine tissue displayed as dilated villi and inflammatory infiltration. However, pre-treatment RG (25, 50 and 100 mg/kg) enormously protected the disruption of epithelial architecture (Fig. 4C, D).

Discussion

Although there have been rapid advances in cancer treatment, chemotherapy remains a central therapy in management of cancerous diseases. However, unwanted effects inevitably happen in most cases with chemotherapy-based treatments. Among those side-effects, myelotoxicity is a typically serious problem leading to impairment of the quality of life and sometimes shortening lifespan[15,16]. 5-FU, pyrimidine analogs, is a chemotherapeutic reagent widely used for solid carcinomas[17], but myelotoxicity and mucosal damage frequently limit its clinical applications. 5-FU involves resistance to hematopoietic progenitor cells in bone marrow and decreases in both the number of mucus cells and the mucin content[18].

On the other hand, herbal medicines have been investigated as preventive candidates against chemo-

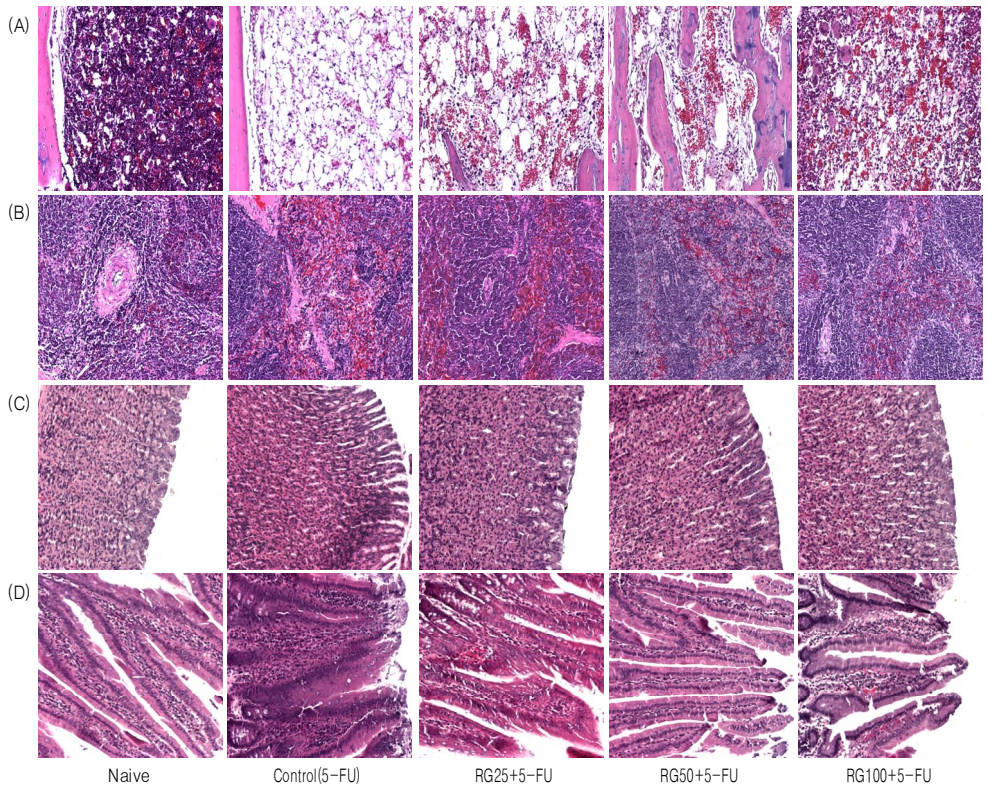

Fig. 4. Effect on histopathological changes.
Rats were pre-treated with RG for 5 days(*po*, once a day) before 5-FU injection(*ip*, 150mg/kg). On 7 day, bone marrow (A), spleen (B), stomach (C) and small intestine (D) were removed and processed for hematoxylin and eosin(H&E). The stained tissues were examined under a light microscope (×200 magnifications).

therapy-induced myelosuppression. *Panax ginseng* is a potential agent showing anti-cancer effects as well as anti-adverse effects of chemotherapy[19,20]. Korean RG is steam-processed *Panax ginseng* at 100℃ and it believed that Korean RG has better therapeutic properties qualitatively and quantitatively[21,22]. There are experimental data presenting anti-myelotoxicity effects of Panax ginseng[13,14,23], however no study for RG on the preventive effect on chemotherapy-induced myelosuppression *in vivo* study. So, the present study investigated the efficacy of Korean RG against myelotoxicity induced by 5-FU in a rat model.

The 5-FU injection drastic decreased peripheral hematological parameters including WBC, RBC, hemoglobin, platelet, and the differential count in rats. These severe alterations of hematological parameters was very similar with previous reports of using 5-FU treatment[24-26]. The time point showing the most severe leucopenia was day 7, presenting around 20% of total WBC and 30% of platelet count compared with the nave group. Pre-treatment with RG significantly prevented myelosuppression in aspect of total WBC, platelet, neutrophil and lymphocyte counts. However, the absolute counts of WBC and platelets were much lower than the normal range. This result was in accordance with others of histopathology of bone marrow[27-29] and colony-forming assay using bone marrow stem cells. The 5-FU injection drastically reduced the cellular component

in femoral bone marrow while RG pre-treatment moderately prevented this alteration. In the process of isolation of bone marrow stem cells, the cell number was radically rare in the control group (data not shown). The colony formation of the control group also significantly decreased compared with the nave group. The number of bone marrow stem cells means the quantity of the cellular component in bone marrow while colony formation ability indicates the quality of stem cells. RG pre-treatment notably increased the number of colony formation, especially in leukocyte-lineage.

Hematopoiesis is regulated by various hematopoietic growth factors including IL-3, erythropoietin, thrombopoietin, granulocyte colony-stimulating factor (G-CSF), or GM-CSF[30]. Both IL-3 and GM-CSF have pleiotropic effects on hematopoietic cells and exhibit overlapping activities[31]. IL-3 roles as earliest progenitors to induce their proliferation and differentiation of hematopoietic stem cells, and GM-CSF is known as a hematopoietic cytokine with the ability to stimulate proliferation and maturation of granulocyte and macrophage myeloid cells[32]. Pre-treatment with RG showed an ameliorative pattern in serum levels of GM-CSF and IL-3; this examination tends to overlap the above results. The above results indicate that RG has a pharmaceutical role in enhancing hematopoiesis. Hematotoxicity is a representative parameter for distortion of microenvironment and immune system in patients with cancer. The histopathological data showed the protective effect of RG on cellular distortion in the spleen, a classic immune organ. Therefore, Korean RG could be a potential remedy valuable for cancer treatment itself as well as patients suffering from cancer-associated complications includeing leukemia.

5-FU treatment also shows a high incidence of GI toxicity in patients with cancer, which is related to potent cytotoxic action against all rapidly growing cells, including those of the GI mucosa[33]. This GI damage can seriously affect the quality of life and limit the therapy[34,35]. 5-FU injection considerably injures gastric epithelium in the stomach and small intestine tissue displayed as dilated villi and infiltration of inflammatory cells. These alterations in GI track are associated with loss of body weight and food intake. Pre-treatment with RG enormously protected the epithelial disruption and the body weight loss, but food intake gain was not different in both RG pre-treatment and control groups.

Taken together, these results provide experimental evidence that Korean red ginseng has preventive effects against chemotherapy-induced side effects, especially of hematotoxicity.

Acknowledgements

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