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Effect of *Astragalus Membranaceus* Extract against Improvement of Myelosuppression and Quality of Life in 5-Fluorouracil Treated Mice

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ABSTRACT

Objective : To evaluate the effect of *Astragalus Membranaceus Extract* (AME) on myelosuppression, activity and immune modulation in 5-fluorouracil (5-FU) treated mice.

Method : We carried out complete blood count, histological analysis of bone marrow, and cell colony forming assay for hematopoietic progenitor to evaluate the effect of AME on myelosuppression and conducted swimming test, survival rate, nitric oxide (NO) assay , 51Cr release assay in natural killer cell, mRNA expressions of IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, TGF-β and GM-CSF in spleen cells to evaluate the effect of AME on quality of life (QOL).

Results : AME improved 5-FU induced myelosuppression and peripheral blood count was recovered effectively, had significant efficacy to protect against chemotherapy induced marrow-destruction and on hematopoiesis compared with the control group, improved increase survival rate and the swimming time, had a stimulatory effect on macrophage activation and NK cell activity, and up-regulated cytokine gene transcription (IL-2, IL-6, IFN-γ) in murine immunologic system.

Conclusion : We can conclude that AM is an effective herbal agent for improvement of myelosuppression and QOL in 5-FU treated mice.

Key words : *Astragalus Membranaceus*, Myelosuppression, Quality of Life, 5-Fluorouracil

Ⅰ. Introduction

Most of the known anticancer drugs are toxic on the normal host cells as well as the cancer cells. Rapidly proliferating cells in normal tissues such as bone marrow, lymphatic system, epithelium of the gastrointestinal tract, hair follicles, and germinal epithelium are especially sensitive to cytostatic drugs. The common side-effects of these drugs are bone-marrow suppression, nausea, vomiting, diarrhea, and alopecia. Especially bone-marrow suppression is one of the most important factor to lower quality of life (QOL).

Astragalus membranaceus (AM) is the root of

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Astragalus membranaceus Bunge, which belongs to the leguminosae. AM is one of the oldest and most frequently used crude herb for traditional medicine in many asian countries and it is well known to strengthen the host defense system as a tonic[1,2]. It has been known that AM is composed of γ-amninobutyric acid (GABA), isorhamnetin, quercetin, kaempferol and polyamine. These flavonoids may have beneficial health effects because of their various biological effects, immunomodulating activity, antioxidant properties and their inhibitory role in various stages of tumor development in many studies[3-6]. AM restored the depression of macrophage function by renal carcinoma cells and enhanced the cytokine production on lymphocyte function depressed by anticancer agents or carcinogens[7]. Also, T cell blastogenic- and interleukin 2- producing activity in burned mice has been reported[8].

In our study, we investigated the effects of AME on myelosuppression by counting complete blood count, swimming test, survival rate, histological analysis of bone marrow (BM) and cell colony forming assay for hematopoietic progenitor. And we performed nitric oxide (NO) assay, ^{51}Cr release assay in NK cell and mRNA expressions of IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, TGF-β and GM-CSF in spleen cells to study the effect of AME on improvement of 5-FU induced lowered QOL.

II. Materials and Methods

1. Materials

The *Astragalus membranaceus* (AM) was provided from Deajeon University Oriental Hospital. AM was boiled in water for 2 h. Boiled solution was centrifuged for 15 min at 1,500 rpm. Supernatant were lyophilized and we prepared *Astragalus membranaceus* Extract (AME). 5-FU was obtained from Choongwae Pharmaceutical Co. (Korea). Other chemicals were obtained from Sigma (ST. Louise, USA).

2. Fingerprinting of Astragalus membranaceus by HPTLC

Powdered root of *Astragalus membranaceus* (5g) was soaked in 200 ml of 60% ethanol for 1 h with shaking. The mixture is filtered and dried out in under vacuum. Then, the residue was redissolved with 5 mL of DW and lipid was removed with 5 mL ethyl acetate (EtOAc) three times. The aqueous phase was fractionized three times with 5 mL of 1-butanol (BuOH). The combined BuOH fraction was washed 3 times with 2 mL water. The BuOH is evaporated, and the residue (78 mg) dissolved in 1 mL methanol For the HPTLC analysis. High-performance thin layer chromatography (HPTLC)-based fingerprint was produced by CAMAG application system (Switzerland). BuOH fraction of AME and astragaloside IV applied to the pre-washed silica gel 60 F254 HPTLC plates (size 10 × 10 cm; thickness of the silica gel 0.2 mm; Merck, Germany) with an automated applicator (Linomat IV, CAMAG, Merck KGaA, Germany). Then the samples were separated (migration distance 60 mm) using HPLC-grade Chloroform/Methanol/water (18:8:1). The migrated components were visualized at 366 with after derivatization with 10% sulfuric acid reagent.

3. Experimental Animals

Six-week-old male ICR, C57BL/6 mice and

Spragne Dawley(SD) rat were obtained from commercial animal breeder (Koatech, Korea) and used at 7-8 weeks of age. The mice were maintained at 22±2℃, relative humidity at 55±10% and 12 h light/dark cycles and fed with commercial pellets (Samyang Feed, Korea) and tap water *ad libitum*.

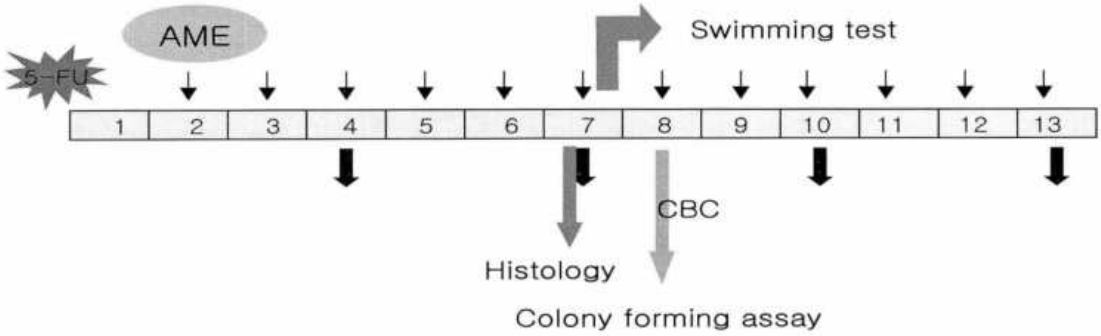

Fig. 1. Animal experimental design.

4. Myelosuppression Induction for Complete Blood Count

ICR mice were intraperitoneally injected with 300 mg/kg of 5-FU. Two days later, the mice were orally administered AME (50, 100 mg/kg) daily 15 consecutive days. Control mice were administered distilled water. Five mice per each group were sacrificed for blood count and histological examination of bone marrow on 0, 4th, 7th, 10th, 13th and 16th day after 5-FU treatment. Complete blood counts were determined by using blood cell counter (HEMAVET, CDC Technologies Inc., U.S.A.).

5. Histological Analysis of Bone Marrow(BM)

Histological analysis of BM was performed on 7th day after 5-FU injection in ICR mice. For the histomorphological evaluation, the femur was dissected and fixed in 10% neutral-buffered formalin. After decalcification, fixed samples were embedded in paraplast and sections of 4 ㎛ were prepared. The sections were stained with hematoxylin and eosin for histopathological examination.

6. Cell Colony Forming Assay for Hematopoietic Progenitor

Two C57BL/6 mice for each group were induced myelosupression by 5-FU injection (200 mg/kg). The mice were sacrificed at the 8th day after 5-FU injection and bone marrow from femur were collected. Bone Marrow cells were obtained by flushing femoral bones. Bone marrow cells ($2x10^4$) were cultured in methocult media (Stem cell technologies, USA) and the number of colonies was counted at 7th day by using an inverted microscope.

7. Swimming Test

ICR mice were tested their swimming ability in a cistern with 21℃ water for 5 minutes and all mice passed the test. One day later, ICR mice

were intraperitoneally injected with 300 mg/kg 5-FU. Two days later, the mice were orally administered with AME (50, 100 mg/kg) once a day for 6 consecutive days. Control mice were administered with distilled water. On the 7th day, their swimming time was tested in a cistern with 21℃ water.

8. Survival Rate of Mice Injected 5-FU (LD70 dosage)

For experiment, ICR mice were intraperitoneally injected with 5-FU (500 mg/kg). Two days later, the mice were orally administered AME (50, 100 mg/kg) daily for 13 consecutive days. Control mice were administered distilled water. Survival curve and body weight were measured.

9. Nitric Oxide (NO) Assay

Peritoneal macrophages (5×10^5) were isolated aseptically from SD rat and plated into 24 well plates (BD, NJ, USA) and treated with AME (0, 1, 10, 100 μg/ml) and LPS (0.1 μg/ml), and incubated at 37℃ with 5% CO_2. NO secretion was measured by analyzing its stable end product, nitrite (NO_2^-) in the culture supernatant with Griesse reagent. Briefly, an aliquot of culture supernatant (100 μl) was added to each well of 96 well plate and mixed with the same volume of Griesse reagent (1:1[v/v]: 0.1% N-[1-naphthyl] ethylenediamine dihydrochloride in H_2O, 1% sulfanilamide in 5% H_2PO_4), and then the A_{540} was read with microplate leader (Molecular Device, USA). Nitrite concentration was determined by using dilutions of sodium nitrite in culture medium as a standard. By adding AME to Griesse reagent, it was confirmed that AME did not interfere with the nitrite assay.

10. ^{51}Cr Release Assay in NK Cell

Spleen cell suspensions were prepared in RPMI 1640 from C57BL/6 mice. 100 ml of the splenocytes containing 4×10^6, 2×10^6 or 1×10^6 cells/well were plated onto the round bottom 96 well plate (3 well per group) with various concentration of AME (0, 1, 10, 100 μg/ml) and IL-2 (300 U/ml). These cells were incubated for 14 h at 37℃ with 5% CO_2 and prepared as effector cells.

On the other hand, YAC-1 cells (4×10^6) were cultured as target cells of NK cell. After labeling the target cells by incubating for 2 h (37℃, 5% CO_2) with ^{51}Cr (100 μCi), the labelled target cells were centrifuged for 5 min at 400 ×g, and adjusted to 4×10^5 cells/ml. Fifty microliter of cell suspension (2×10^4 cells) were added to the effector cells and incubated for additional 4 h. Maximum leased groups were added with 150 μl of 2% NP-40, and spontaneous leased group with 150 μl of complete medium. After 4 h, the cells were concentrated by centrifugation at 1000 ×g for 10 min, and cell-free supernatant was harvested from each well for assessment of radioactivity. Then gamma irradiation from each well was assessed in a scintillation counter (Packard Instruments). The percentage of specific lysis was calculated by the following equation :

$$\text{Specific killing activity (\%)} = \frac{\text{AME release} - \text{spont. release}}{\text{max. release} - \text{spont. release}} \times 100$$

11. mRNA Expressions of IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, TGF-β and GM-CSF in Spleen Cells

C57BL/6 mice were sacrificed and spleens were

subjected to cold phosphate buffered saline (PBS). After removement of RBC using lysing buffer, the spleen cells were washed twice with PBS and the prepared cells were plated on the 6 well plates. The splenocytes (4×10^7 cells) were treated with various concentration of AME (0, 1, 10, 100 ㎍/㎖) or LPS (0.1 ㎍/㎖) and incubated for 6 h at 37℃ with 5% CO_2. Total RNA was isolated by the Trireagent (MRC, USA). cDNAs synthesis and polymerase chain reaction (PCR) were done according to the manufacturer's instructions.

Briefly, PCR amplification was carried out in a thermal cycler using a protocol of initial denaturing step at 95℃ for 10 min; then 27 cycles for β-actin and 35 cycles for other genes at 95℃ for 1 min, 60℃ for 40 seconds and 72℃ for 40 seconds. The PCR products were run on 1% agarose gel in 0.5×TBE buffer. The used primers were described in Table 1.

Table 1. Oligonucleotide Sequences of Primers

Gene	Primer	Sequence	Product (bp)
IL-1β	Sense Antisense	5'-AAG CTC TCA CCT CAA TGG A-3' 5'-TGC TTG AGA GGT GCT GAT GT-3'	302
IL-2	Sense Antisense	5'-TGC TCC TTG TCA ACA GCG-3' 5'-TCA TCA TCG AAT TGG CAC TC-3'	391
IL-4	Sense Antisense	5'-TCA ACC CCC AGC TAG TTG TC-3' 5'-TGT TCT TCA AGC ACG GAG GT-3'	254
IL-6	Sense Antisense	5'-TGT GCA ATG GCA ATT CTG AT-3' 5'-TGG TCC TTA GCC ACT CCT TC-3'	357
IL-10	Sense Antisense	5'-TCC TTG GAA AAC CTC GTT TG-3' 5'-TCT CTT CCC AAG ACC CAT GA-3'	389
TNF-α	Sense Antisense	5'-CTC CCA GGT TCT CTT CAA GG-3' 5'-TGG AAG ACT CCT CCC AGG TA-3'	195
IFN-γ	Sense Antisense	5'-GGA TAT CTG GAG GAA CTG GC-3' 5'-GAG CTC ATT GAA TGC TTG GC-3'	250
TGF-β	Sense Antisense	5'-TGA GTG GCT GTC TTT TGA CG-3' 5'-TTC TCT GTG GAG CTG AAG CA-3'	310
GM-CSF	Sense Antisense	5'-TGG TCT ACA GCC TCT CAG CA-3' 5'-AGC TGG CTG TCA TGT TCA AG-3'	255
β-actin	Sense Antisense	5'-ACC GTG AAA AGA TGA CCC AG-3' 5'-TCT CAG CTG TGG TGG TGA AG-3'	285

12. Statistical Analysis

Results were expressed as the mean±standard deviation (S.D). Statistical analysis of the data was carried out by Student's *t*-test. A difference from the respective control data at the levels of $P<0.05$ and $P<0.01$ was regarded as statistically significant.

III. Results

1. Fingerprinting of *Astragalus membranaceus*

To identify AME and control the quality, we performed fingerprinting by HPTLC. As shown in Fig. 2, there were many components were detected in butanol fraction of *Astragalus membranaceus* at UV 366 nm after derivertization. Astragalosice IV, standard component of *Astragalus membranaceus* was detected Also our herbal sample has a component that also changed to the same color and achieved the same retention fraction (RF) value.

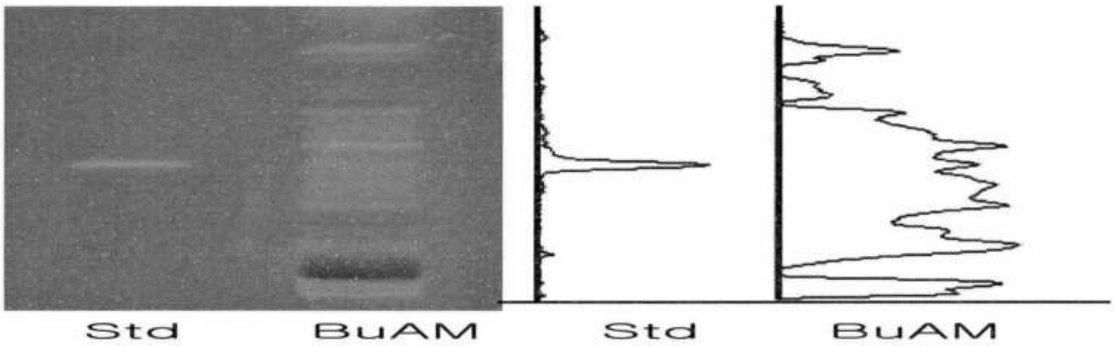

Fig. 2. Fingerprint of *Astragalus membranaceus* BuOH fraction of AME was separated (migration distance 60 mm) using HPLC-grade chloroform/methanol/water (18:8:1). The migrated components were visualized (left) after derivatization with 10% sulfuric acid reagent and analyzed with density and RF value (right). Std, Astragaloside IV; BuAM, BuOH fraction of *Astragalus membranaceus*.

2. Peripheral Blood Count

ICR mice were sacrificed and blood was extracted via posterior vena cava on 0, 4th, 7th 10th and 13th day after 5-FU (300 mg/kg) injection. Fig. 3. shows the peripheral leukocyte count of each group before and after 5-FU injection. After 5-FU injection the number of peripheral leukocyte promptly decreased and then recovered from 10th day. The groups administrated AME all showed enhancement of number of peripheral leukocyte except 4th day, as compared to the control group. In Red Blood Cell (RBC) counts, there is no difference between AME treatment group and control group. However, hemoglobin level was higher in AME treatment group than that of control group. AME (50 mg/kg) treated group increased platelets count but not significant.

3. Histological Analysis of BM

Histological data demonstrated that the control group had low density of BM cellularity with large vacuoles. On the other hand, AME treated groups had higher density of BM cellularity than the control group in dose-dependent manner (Fig. 4). These results imply that AME have significant effect on hematopoiesis compared with control group.

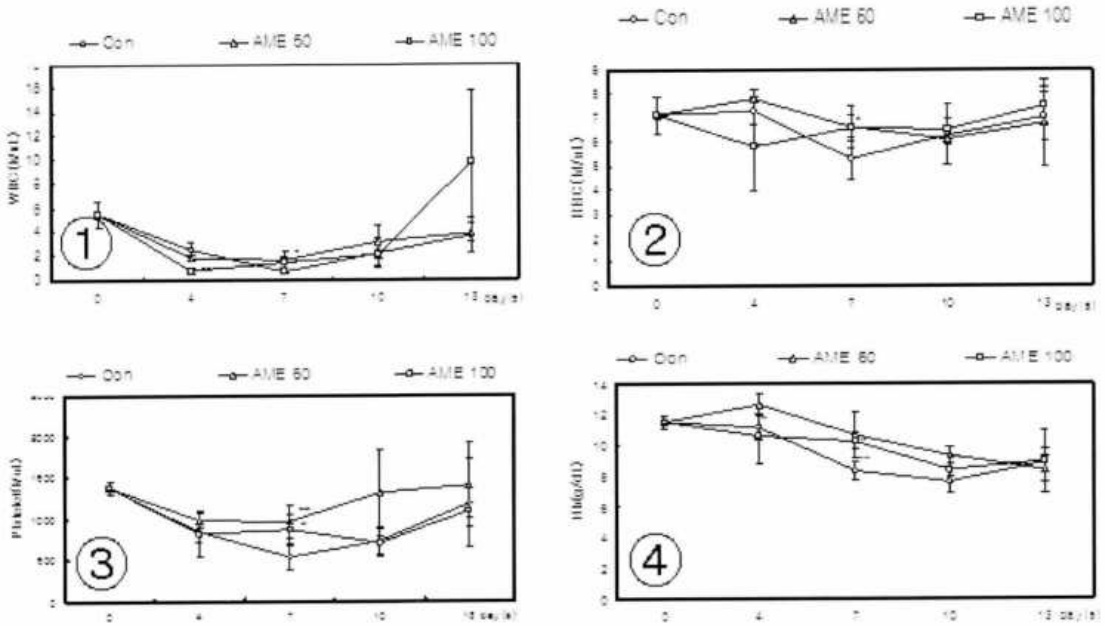

Fig. 3. ①-④ Changes of WBC ,RBC, Platelet and Hemoglobin. ICR mice were intraperitoneally injected with 5-FU (300 mg/kg). After two days, they were administered AME (50, 100 mg/kg). Hematologic parameters were monitored on 0, 4th, 7th, 10th and 13th day after 5-FU injection. Each data were represented as the mean±S.D. Statistical significance was tested by t-test. (*: $p<0.05$, **: $p<0.01$)

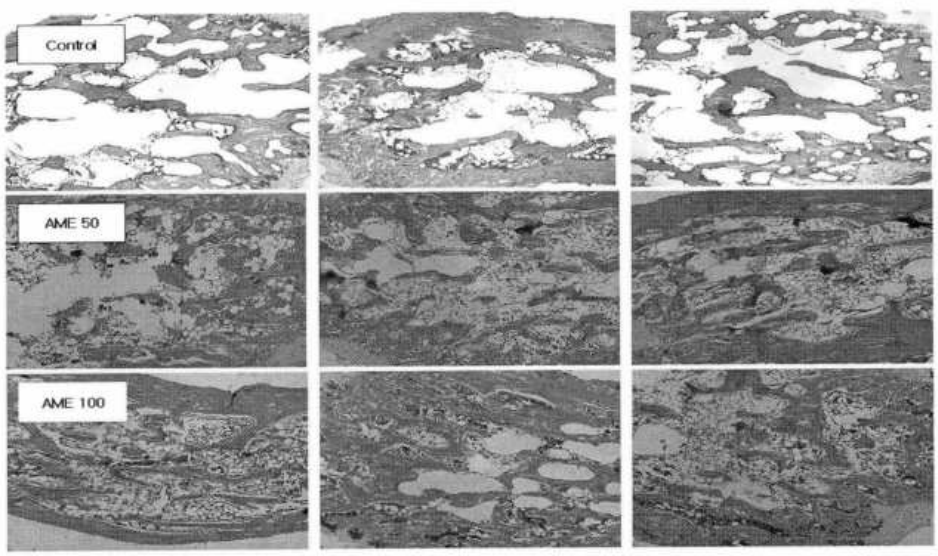

Fig. 4. Histological Analysis of Bone Marrow The bone marrow tissues were isolated from mice. They were fixed, dissected and stained with hematoxylin and eosin. The images were viewed at 100 X magnification.

4. Hematopoietic Progenitor Cell Colony Forming Assay

The effect of AME on bone marrow progenitor cells in 5-FU injected mice were summarized in Fig. 5 ①-④. Two types of progenitor cell, colony-forming unit granulo monocyte (CFU-GM) and burst-forming unit erythrocyte (BFU-E) were counted. The number of nuclear cells in control, AME 50 and AME 100 treated group decreased after 5-FU injection in comparison with naive group. However, number of two types colonies in AME treated groups increased significantly in dose dependent manner.

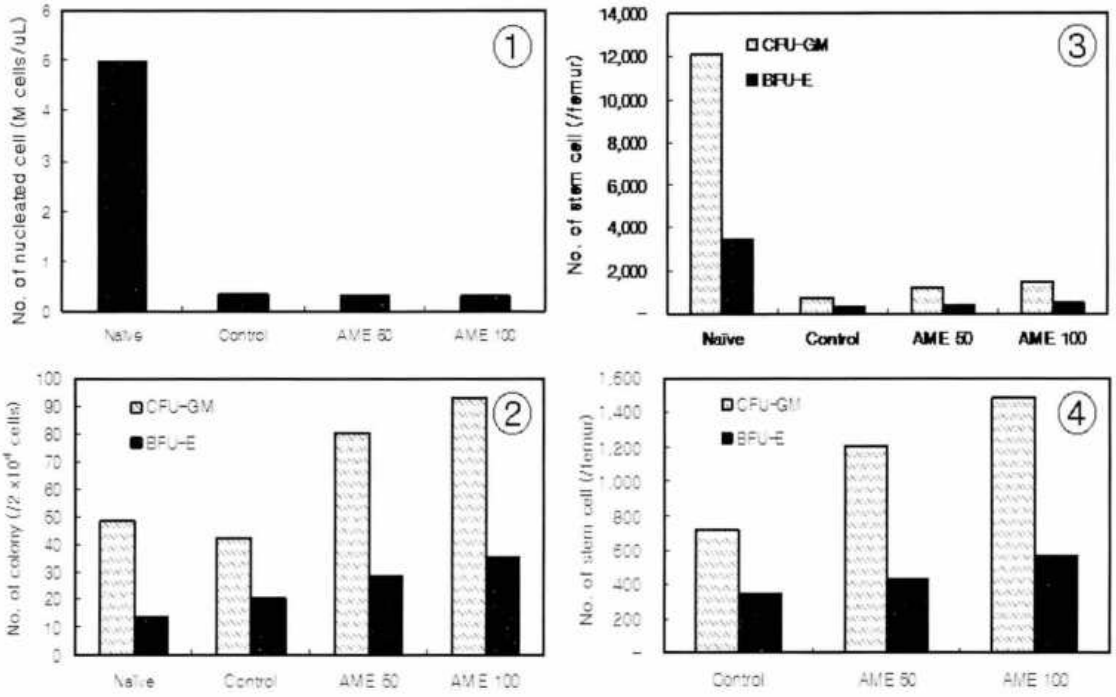

Fig. 5. ① Absolute Number of Nuclear Cells Two mice in each C57BL/6 group were sacrificed at the 8th day after 5-FU (200 mg/kg) injection and bone marrow from femur were collected. BM was obtained from flushing femoral bones. Nuclear cells were counted by using blood cell counter.
② Number of Colonies Two mice in each C57BL/6 group were sacrificed at the 8th day after 5-FU (200 mg/kg) injection and bone marrow from femur were collected. BM was obtained from flushing femoral bones. Nuclear cells were counted by using blood cell counter. Nuclear cells ($2x10^4$) were cultured in methocult media and the number of colonies was counted at 7th day by using an inverted microscope.
③,④ Absolute Number of Stem Cells Two mice in each C57BL/6 group were sacrificed at the 8th day after 5-FU (200 mg/kg) injection and bone marrow from femur were collected. BM was obtained from flushing femoral bones. Nuclear cells were counted by using blood cell counter. Nuclear cells ($2x10^4$) were cultured in methocult media and the number of colonies was counted at 7th day by using an inverted microscope. Absolute number of stem cell per femur was calculated with total nuclear cells and colony forming rate.

5. Survival Test

Survival rate of control group was 40%, whereas survival rate of AME treated groups were 60% (50 mg/kg) and 70% (100 mg/kg) respectively. As shown in fig.6. survival curve also shifts to right.

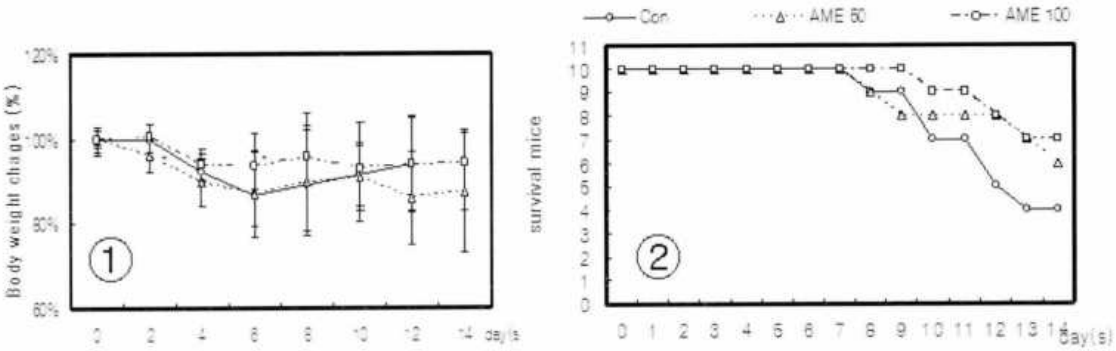

Fig. 6 ①,② Body weight changes and survival curves . ICR mice were intraperitoneally injected with 500 mg/kg 5-FU. Two days later, the mice were orally administered with AME (50 mg/kg, 100 mg/kg) daily for 13 consecutive days. Control mice were administered with distilled water. Survival rate was measured.

6. Swimming Test

ICR mice induced myelosupression by 5-FU injection (300 mg/kg) were administered with AME for 6 days. On 7th day, the mice were swum. Swimming test was checked to the mice. As shown in Fig. 7. The mice of AME 50 and 100 significantly increased the swimming time compared to the control group (*: $p<0.05$, **: $p<0.01$)

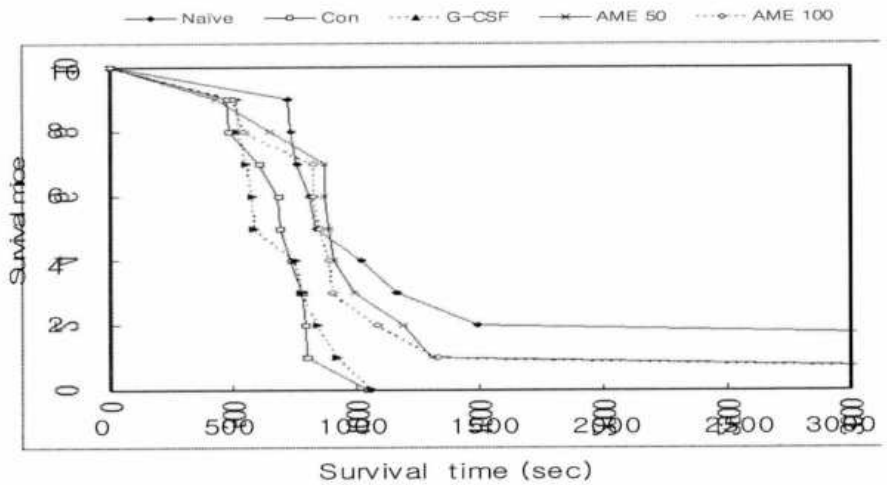

Fig. 7. Swimming Time ICR mice were intraperitoneally injected with 300 mg/kg 5-FU. Two days later, the mice were orally administered with AME (50, 100 mg/kg) once a day for 6 consecutive days. Control mice were administered with distilled water. On the 7th day, their swimming time was tested in a cistern with 21℃water. Each data were represented as the mean±S.D. Statistical significance was tested by t-test. (*: $p<0.05$, **:$p<0.01$)

7. NO Production

To investigate the ability of macrophage activation of AME, NO production was measured after treatment of various concentrations (0, 1, 10, 100 ㎍/㎖) in rat peritoneal macrophages. The accumulated nitrite, estimated by the Griesse method, in the culture medium was used as an index for NO synthesis from these cells. After treatment of LPS for 24 h, nitrite concentration markedly increased. When cells were treated with various concentrations of AME, nitrite production was significantly increased at concentrations of 10 and 100 ㎍/㎖ in a dose dependent manner(Fig. 8).

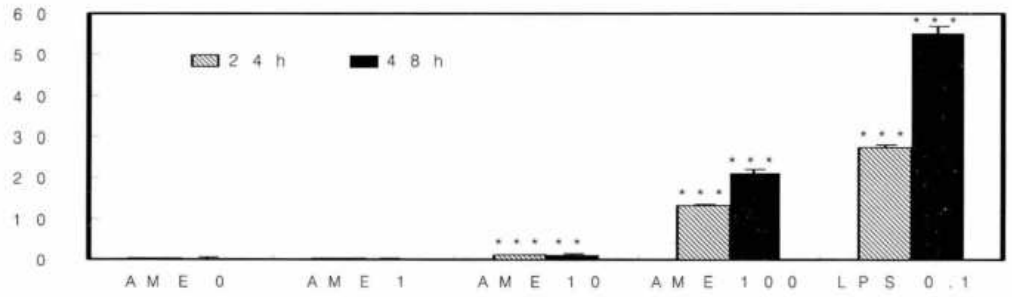

Fig. 8. Effect of AME on NO production Peritoneal macrophages were treated with AME (0, 1, 10, 100 ㎍/㎖) or LPS (0.1 ㎍/㎖). Accumulated nitrite in culture medium was determined by the Griesse reaction. Each data were represented as the mean±S.D. Statistical significance was tested by t-test. (**: $p<0.01$, ***: $p<0.001$).

8. NK Cell Activity

To investigate whether AME might be involved the modulation of NK cell activity, ^{51}Cr release assay were performed with spleen cells. As shown in Fig.9 ①②. At the concentration of AME (100 ㎍/㎖), NK cells had significant cytotoxic activity compared with control concentrations of AME, which indicating that AME had a stimulative effect on NK cell activity. Especially, the NK cytotoxic activity of AME and IL-2(300 U) treated case was more activated than AME single treated one. The increased ratio of effector and target cells (100 : 1) showed significantly increase with the ability of cell lysis by effector cells.

9. Changes of Gene Expression in Splenocytes

To investigate the role of AME on the regulation of cytokine gene transcription in murine immunologic system, splenocytes were isolated from C57BL/6 mice and treated with various concentrations of AME (0, 1, 10, 100 ㎍/㎖) or LPS (0.1 ㎍/㎖) for 6 h, and the mRNA expression of various inflammatory cytokines were assessed by RT-PCR.

When cells were treated with AME at the concentration of 1, 10 and 100 ㎍/㎖, they were not affected in mRNA expression of IL-1β, Il-4, IL-10 TNF-α, TGF-β and GM-CSF in splenocytes. However, the mRNA expression of IL-2 as well as IL-6, IFN-γ genes were significantly up-regulated after 6 h treatment at a dose dependant manner(Fig. 10).

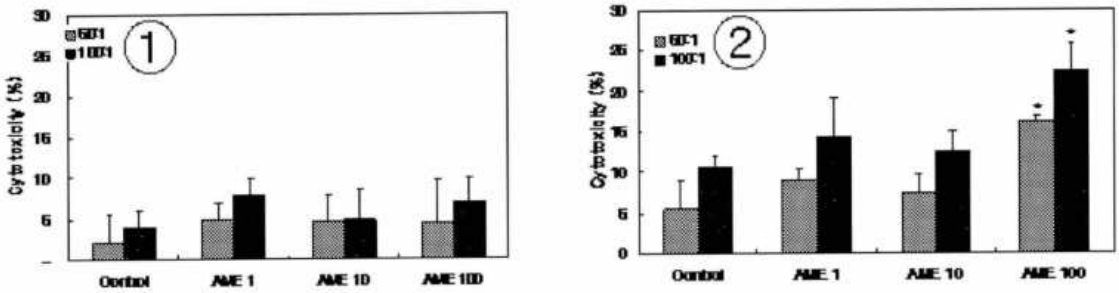

Fig. 9. ① Effect of AME on NK Cell Activity Spleen cells (effector cell) were treated with AME (0, 1, 10, 100 ㎍/㎖) for 18 h. YAC-1 cells (target cell) labeled with ^{51}Cr were mixed to effector cells for 4 h. Released ^{51}Cr was counted by using gamma scintillating counter. Each data were represented as the mean±S.D. Statistical significance was tested by *t*-test.
② Effect of AME on NK Cell Activity Spleen cells (effector cell) were treated with AME (0, 1, 10, 100 ㎍/㎖) with IL-2 (300 U/㎖) for 18 h. YAC-1 cells (target cell) labeled with ^{51}Cr were mixed to effector cells for 4 h. Released ^{51}Cr was counted by using gamma scintillating counter. Each data were represented as the mean±S.D. Statistical significance was tested by *t*-test.(*: $p<0.05$).

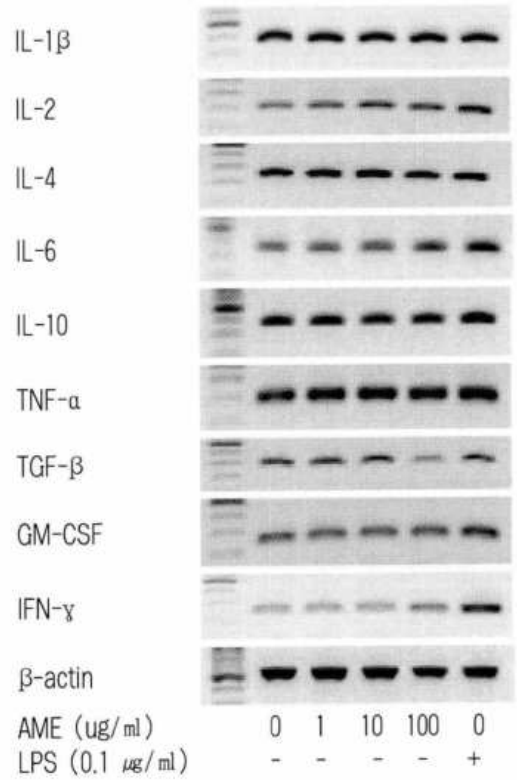

Fig. 10. IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, TGF-β, GM-CSF and IFN-γ gene expression in splenocytes. Splenocytes were treated with AME (0, 1, 10, 100 ㎍/㎖) or LPS (0.1 ㎍/㎖) for 6 h. Total RNA was isolated and RT-PCR was performed as described in Materials and Methods. The PCR products were separated on an 1% agarose gel and stained with ethidium bromide. β-actin was used as an internal control. The gene expression was shown as the percentage of IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, TGF-β, GM-CSF and IFN-γ to β-actin normalized by control group.

Ⅳ. Discussion

It is well known that conventional cancer therapeutics are toxic on the normal host cells as well as the cancer cells. Most of all, rapidly proliferating cells in bone marrow are especially sensitive to cytostatic chemotherapy or radiotherapy. Accordingly, bone-marrow suppression is one of the most common side-effects, thus, this myelosuppression has been sometimes accepted as an inevitable and life-threatening adverse effect in

process of cancer treatment[9-11]. Moreover, reduced number or function of immunocyte or platelet, and lowered level of blood hemoglobin are responsible for the high incidence of opportunistic bacterial infection, nonspecific bleeding and deficiency of energy. These therapeutics-derived poor condition can be main reasons disturbing effective treatment with optimal therapeutic dosage or for enough therapeutic period. Of course, those are the main cause to lower life of quality for cancer patients[12].

So, many alternative strategies have been tried, such as tumor-specific molecular targeting drug development, lower-dosage and highly focused irradiation, and management with CFU-GM or various colony stimulating factors (CSFs).

However, their lower efficiency and clinical limitation still require new drugs and therapeutics for myelosuppression and lowered QOL. Traditionally, a number of herbal drug were known to have effects on the hemopoietic system and immuno-augmentation, energy-restoration. And several prescriptions were reported to have therapeutic or protective properties to leukopenia or anemic conditions immuno-augmentation, reduction of chemotherapy induced side-effect[13,14].

AM is widely used for its "Qi tonifying" effects in Korea, Japan and China. In clinical setting, it is thought to act by boosting the body's general vitality and strengthening resistance to exogenous pathogens. Recently, AM in combination with other herbs, was used in treating allergic rhinitis, asthma and atopic dermatitis[15]. To date, AM is reported to have immunostimulant, epatoprotective, antidiabetic, diuretic and sedative effects[16]. Several studies focused on its immunomodulatory properties. It stimulated murine splenocytes to proliferate and macrophages to produce pro-inflammatory cytokines[17]. It also enhanced antibody responses and lymphokine-activated cell cytotoxicity in the immunosuppressed mice[18]. AM showed an effect on various immune responses such as T-lymphocyte blastogenesis, cytokine production of lymphocytes and macrophages and cytotoxicity of NK or lymphokine activated killer cells from the depression induced by carcinogen or anticancer agents[19-21]. It has also been reported that AM can enhance macrophage activation in the immune system[22], and the mixture formula incorporating the AME may affect the immune system[23].

So we investigated the effects of AME on myelosuppression and QOL in 5-FU treated mice. For this concern, we investigated the effects of AME on myelosuppression to examine complete blood count, swimming test, survival rate, histological analysis of BM, cell colony forming assay for hematopoietic progenitor, nitric oxide (NO) assay, ^{51}Cr release assay in NK cell, mRNA expressions of IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, TGF-β and GM-CSF in spleen cells in animal model treated with 5-FU.

Myelosuppression is one of the most common side-effects and has been sometimes accepted as an inevitable and life-threatening side effect to cancer patients. In this peripheral blood count study, the groups administrated AME all showed enhancement of number of peripheral leukocyte as compared to the control group. In RBC counts, there is no difference between AME treatment group and control group. However hemoglobin level was higer in AME tretment group than that of control group. AME (50 mg/kg) treated group increased platelets but not significant. Throughout these results, AM improved 5-FU induced

myelosuppression and peripheral blood count was recovered effectively. Histological pathology demonstrated that the control group had low density of BM cellularity with large vacuoles. On the other hand, AME treated groups had higher density of BM cellularity than the control group in dose-dependent manner. This result imply that AM has significant efficacy to protect chemotheraphy induced marrow-destruction.

By the hematopoietic progenitor cell colony forming assay, two types of progenitor cell, CFU-GM and BFU-E were counted. In AME treated groups, number of two types colonies increased significantly in dose dependent manner. These results imply that AME have significant effect on hematopoiesis compared with control group. Based on these results, We could conclude that AME has a therapeutic effect on myelosuppression induced by 5-FU in mice model.

AME is an effective remedy for the bone marrow failure and immune suppression induced by the chemotherapy. These results suggest that AM is a good candidate for new drugs or therapeuticson chemotherapy-associated myelosuppression. Survival rate of control group was 40%, whereas survival rate of AME treated groups were 60% (50 mg/kg) and 70% (100 mg/kg). This implies that AM improved 5-FU induced lowered general condition. Furthermore, it could alleviate chemotherapy side-effect and increase survival rate. Oral administration of AME 50 significantly increase the swimming time compared to control group. We could see that AM improve 5-FU induced lowered general condition through increasd swimming time. Poor condition induced by 5-FU can be main reasons disturbing effective treatment. Of course, those are the main cause to lower life of quality for cancer patients. Recently, cancer immunosurveillance have been thought as one of the most important factors for controlling both cancer. It is strongly assumed that non-specific effector cells like macrophages and NK cells play roles more critically[24,25].

Macrophages are known as major immune cells and synthesize a variety of immunomodulatory factors, including cytokine, leukocyte adhesion and nitric oxide (NO). It is widely acknowledged that cytokine plays an important role in the regulation of immune response[26,27]. Nitric oxide synthesized by nitric oxide synthase (NOS) also has an important role in the immune and nervous systems[28]. High level of NO production might cause host cell death and inflammatory tissue damage[29,30]. In this study, we observed the effects of ARE on macrophage activation. After treatment of LPS for 24h, nitrite concentration markedly increased. When cells were treated with various concentrations of AME, nitrite production was significantly increased at concentrations of 10 and 100 μg/ml in a dose dependent manner.

Next, to investigate whether AME might be involve the modulation of NK cell activity, ^{51}Cr release assay were performed with spleen cells. At the concentration of AME (100 μg/ml), NK cells had significant cytotoxic activity compared with control concentrations of AME, which indicating that AME had a stimulatory effect on NK cell activity. Especially, when AME was treated with IL-2 (300 U), the NK cytotoxic activity was increased more than when AME was treated only.

An increased ratio of effector and target cells (100 : 1) showed significant increase with the ability of cell lysis by effector cells. Ma:crophages and NK cslls are distributed throughout the body

and extravasate and migrate ti various tissue sites. They should be prepared to employ for elimination of cancer cells without previous notice. At this point, we surveied Changes in gene expression in splenocytes. To investigate the role of AME on the regulation of cytokine gene transcription in murine immunologic system, the mRNA expression of various inflammatory cytokines were assessed by RT-PCR. When cells were treated with AME at the concentration of 1, 10 and 100 ㎍/㎖, they were not affected in mRNA expression of IL-1β, Il-4, IL-10 TNF-α and GM-CSF in splenocytes. However, the mRNA expression of IL-2 as well as IL-6, IFN-γ genes were significantly up-regulated after 6 h treatment at a dose dependant manner. AM has significant properties to regulate cytokine gene transcription in murine immunologic system.

So AM present the anti-cancer effects by modulating immune response. We could see that AM increased survival rate, swimming time, activation of macrophage and NK cell, regulation of immune response. AM is useful to improve lowered QOL induced by 5-FU. In the future, further detail of the mechanism and compositional analysis of AM should be studied with controlled clinical evaluation.

V. Conclusion

This experimental study was carried out to evaluate the Effect of *Astragalus Membranaceus Extract* (AME) on myelosuppression, activity and immune modulation in 5-Fluorouracil (5-FU) treated mice.

1. AM improved 5-FU induced myelosuppression and peripheral blood count was recovered effectively.
2. AM has significant efficacy to protect chemotheraphy induced marrow-destruction.
3. AM has significant effect on hematopoiesis compared with control group.
4. AM improved increase survival rate and the swimming time.
5. AM has a stimulatory effect on macrophage activation and NK cell activity.
6. AM up-regulates cytokine gene transcription (IL-2, IL-6, IFN-γ) in murine immunologic system.

From these results, we can conclude that AM is an effective herbal agent for improvement of myelosuppression and Quality of Life in 5-FU treated mice.

황기 추출물이 5-Fluorouracil을 투여한 생쥐의 골수억제 및 삶의 질에 미치는 영향

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ABSTRACT

목 적 : 황기추출물이 생쥐의 5-FU로 유발된 골수억제와 삶의 질 저하의 개선에 미치는 효과를 연구하고자한다.

방 법 : 골수억제에 미치는 영향의 평가를 위해 Complete Blood Count, Histological Analysis of BM, Cell Colony Forming Assay for Hematopoietic Progenitor를 시행하고, 삶의 질에 미치는 영향을 평가하기 위해 Swimming Test, Survival Rate, Nitric Oxide (NO) Assay, ^{51}Cr Release Assay in NK Cell, mRNA Expressions of IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, TGF-β and GM-CSF in Spleen Cells를 시행하였다.

결 과 : 황기추출물은 골수억제를 호전시켜 말초혈액수치를 회복시키고, 골수파괴를 보호하는 효과를 보이며, 대조군에 비해 혈구생성을 촉진시키며, 대조군에 비해 생존율과 수영시간을 증가시키며, 대식세포와 자연살상세포의 활동성을 증가시키며, 종양면역과 관련된 싸이토카인(IL-2, IL-6, IFN-γ)의 발현을 증가시켰다.

결 론 : 5-FU로 유발된 골수억제와 삶의 질 저하의 개선에 미치는 황기추출물의 유용성이 기대되며 향후 이에 대한 지속적인 연구가 필요할 것으로 사료된다.

핵심단어 : 황기, 골수 억제, 삶의 질, 5-Fluorouracil

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