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Natural killer cell activity and quality of life were improved by consumption of a mushroom extract, *Agaricus blazei Murill* Kyowa, in gynecological cancer patients undergoing chemotherapy

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Abstract. Ahn W-S, Kim D-J, Chae G-T, Lee J-M, Bae S-M, Sin J-I, Kim Y-W, Namkoong S-E, Lee IP. Natural killer cell activity and quality of life were improved by consumption of a mushroom extract, *Agaricus blazei Murill* Kyowa, in gynecological cancer patients undergoing chemotherapy. *Int J Gynecol Cancer* 2004;**14**:589–594

A mushroom extract, *Agaricus blazei Murill* Kyowa (ABMK), has been reported to possess antimutagenic and antitumor effects. Here, we investigate the beneficial effects of ABMK consumption on immunological status and qualities of life in cancer patients undergoing chemotherapy. One hundred cervical, ovarian, and endometrial cancer patients were treated either with carboplatin (300 mg/m^2) plus VP16 (etoposide, 100 mg/m^2) or with carboplatin (300 mg/m^2) plus taxol (175 mg/m^2) every 3 weeks for at least three cycles with or without oral consumption of ABMK. We observed that natural killer cell activity was significantly higher in ABMK-treated group (ANOVA, $n = 39$, $P < 0.002$) as compared with nontreated placebo group ($n = 61$). However, no significant difference in lymphokine-activated killer and monocyte activities was observed in a manner similar to the count of specific immune cell populations between ABMK-treated and nontreated groups. However, chemotherapy-associated side effects such as appetite, alopecia, emotional stability, and general weakness were all improved by ABMK treatment. Taken together, this suggests that ABMK treatment might be beneficial for gynecological cancer patients undergoing chemotherapy.

KEYWORDS: alternative therapy, *Agaricus blazei Murill* Kyowa, gynecological cancer, quality of life.

Gynecological cancer is an important cause of death in women worldwide. In particular, cervical cancer is caused mostly by infection with a high-risk group of human papillomavirus (HPV)[1–3]. In particular, two HPV oncogenic proteins, E6 and E7, play a critical role in inducing cervical cancers by interacting with p53 and pRB for the inactivation of these cellular regulatory proteins, respectively[4,5]. Multimodal chemotherapy is one of the therapeutic modalities for the patients with cervical cancer. Ovarian cancer is also a

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highly lethal disease. Owing to an asymptomatic course of early disease stage, most ovarian cancers are diagnosed at an advanced stage. This is related with poor prognosis for ovarian cancers. Despite great progress in treating gynecological cancer patients in the last three decades, recurrent or persistent cancer has been problematic. Chemotherapy has made a significant advance in the treatment of cancer patients. However, chemotherapeutic agents cause a variety of severe and life-threatening side effects, such as severe immunosuppression and bone marrow depression, adding more importance to developing any regimens for reducing side effects of the chemotherapy.

A mushroom extract, *Agaricus blazei Murill* Kyowa (ABMK), has been reported to have antimutagenic effects[6]. Furthermore, ABMK possesses tumoricidal and immunopotentiating effects[7–11]. In one study, intratumoral injection of the *A. blazei* extract resulted in the infiltration of natural killer (NK) cells in the tumor sites and increased NK cell activity in animals[7,12]. Its major component, D-glucan, has been ascribed to these antitumor properties[7,8,10,11]. At present, ABMK is consumed as a food or tea worldwide due to its expected medicinal properties.

In this study, we evaluated whether ABMK consumption might have any beneficial effects in gynecological cancer patients undergoing chemotherapy with either carboplatin (300 mg/m^2) plus VP16 (etoposide, 100 mg/m^2) or carboplatin (300 mg/m^2) plus taxol (175 mg/m^2) in our clinic in the department of obstetrics and gynecology, Kangnam St. Mary's Hospital. We observed that ABMK exerted some positive effects on innate NK cell activity and, in general, on the quality of life in patients undergoing chemotherapy. This clinical finding suggests that ABMK consumption might be beneficial for maintaining immune activities as well as the quality of life in gynecological cancer patients undergoing chemotherapy.

Materials and methods

Cohorts

One hundred gynecologic patients who visited the Department of Obstetrics and Gynecology, Kangnam St. Mary's Hospital (Seoul, South Korea) in the 3-year period from 1999 to 2001 were recruited for this investigational trial. The patients were randomized for treatments with ABMK and placebo in a blinded fashion. The details of patients are summarized in Table 1.

Table 1. Details of patient information

Number of patients	100
Age (years)	
Median	52
Range	26–79
Diagnosis	**Number of patients**
Cervical cancer	61
Ia, b	18
IIa, b	32
IIIa, b	11
Ovarian cancer	35
Ia, b	3
IIa, b	8
IIIa, b	24
Endometrial cancer	4
Treatment	**Number of patients**
ABMK	39
Carbo-VP16	29
Carbo-taxol	10
Placebo	61
Carbo-VP16	39
Carbo-taxol	22

ABMK, *Agaricus blazei Murill* Kyowa.

Therapeutic regimen

Patients were treated with either carboplatin (300 mg/m^2) plus VP16 (etoposide, 100 mg/m^2) or carboplatin (300 mg/m^2) plus taxol (175 mg/m^2) every 3 weeks for at least three cycles with or without daily oral consumption of ABMK (three packs/day, one pack per time, obtained from Kyowa Engineering Co., Tokyo, Japan). Subsequently, the patients were bled 1 day before first chemotherapy, and 1 day before second chemotherapy. The patients tested were under primary-line therapy. The activities of NK and lymphokine-activated killer (LAK) cells and the counts of white blood cells (WBCs), lymphocytes, monocytes, $CD3^+$, $CD4^+$, $CD8^+$, $CD48^+$, and $CD56^+$ cells, as well as H_2O_2 production levels of monocytes were analyzed.

The number of immune cells

WBCs, lymphocytes, and monocytes were counted using an instrument, XE-2100 (Sysmex, Kobe, Japan). The counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD48^+$, and $CD56^+$ cells were analyzed by fluorescence-activated cell sorter (FACS) analysis. Fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies specific for human CD3, CD4, CD8, CD48, and CD56 cells were purchased from DiNona (Seoul, South Korea), Immunotech (Westbrook, ME), or Beckman Coulter (Fullerton, CA).

Isolation of peripheral blood mononuclear cells from blood

Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll–Paque density gradient centrifugation of leukocyte buffy coats, which in turn were obtained from patients at the Kangnam St. Mary's Hospital. The mononuclear cells were washed with phosphate-buffered saline twice which was then used to further purify selected cell population.

NK and LAK cell cytotoxicity assay

NK cells were purified from PBMCs using CD56 microbead (Miltenyi Biotec, GmBH, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The FACS analysis of the purified NK cells showed more than 95% purity. NK cells were then reacted with target cells, K562, at the relative cell count ratio of 20:1 for 4 h at 37 °C. In particular, for LAK activity studies, purified NK cells were first stimulated with recombinant human interleukin-2 (IL-2) (Sigma, St. Louis, MO) at the concentration of 400 U/ml for 24 h. LAK cells were then reacted with target cells, Daudi, at the relative cell count ratio of 3:1 for 3 h at 37 °C. After incubation, cell supernatant was collected and evaluated using cytotoxicity detection kit (BM, Manheim, Germany) according to the manufacturer's protocol. Subsequently, optical density was detected for lactate dehydrogenase activity at 490/630 nm, and then cytotoxicity was calculated as follows:

H_2O_2 assay of monocytes

Monocytes were purified from PBMCs using CD14 microbeads (Miltenyi Biotec) according to the manufacturer's protocol. The FACS analysis of the purified monocytes showed more than 95% purity. Monocytes were then reacted with 50-fold diluted 2′,7′-dichlorofluorescein diacetate (10 μg/ml) solution and incubated for 1 h at 37 °C. After incubation, H_2O_2 production levels in a form of relative fluorescence unit were evaluated by measuring optical density at the wavelength of 485/535 nm using Cytofluorometer (Millipore, Bedford, MA).

Questionnaires

At the time of completion of ABMK treatment, all participants completed a questionnaire that sought data on physical and emotional conditions of the patients. These conditions include insomnia, appetite, alopecia, body weight, nausea/vomiting, emotional conditions, discomfort, and general body strength. QLQ-30 Scoring Manual (2nd edition) of EORTC (European Organization for Research and Treatment of Cancer) was modified and then used in this study as a questionnaire. All questions were given in two parts under three answer conditions (physical status: better, worse, no change; emotional status: no help, helpful, very helpful). This was expressed in percentage.

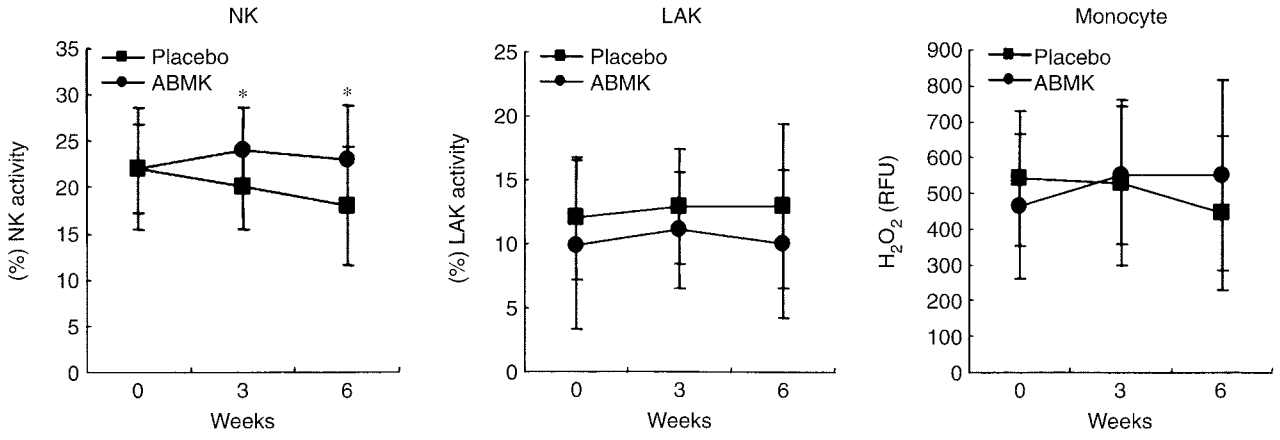

Fig. 1. Natural killer (NK), lymphokine-activated killer (LAK), and monocyte activities of patients undergoing chemotherapy with and without *Agaricus blazei Murill* Kyowa (ABMK) treatment. Gynecological cancer patients were treated with either carboplatin (300 mg/m2) plus VP16 (100 mg/m2) or carboplatin (300 mg/m2) plus taxol (175 mg/m2) at 0, 3, and 6 weeks. ABMK was orally administered every day (three packs/day). One day before each chemotherapy, blood was withdrawn, and the immune cells were tested as shown in Materials and methods. RFU, relative fluorescence unit. *Statistically significant using anova at $P < 0.05$ compared to placebo-treated group.

Results

Gynecological cancer patients were divided into two groups in a double-blind manner. These patients were administered systemically with carboplatin plus VP16 or with carboplatin plus taxol. Among these, one group of patients received ABMK, whereas the other group received placebo. Blood was withdrawn and evaluated for NK cell activity of these patients. As shown in Figure 1, NK cell cytotoxic activity was significantly higher (ANOVA, $P < 0.002$) in ABMK-treated group ($n = 39$) over 3- and 6-week periods, as compared with placebo control group ($n = 61$). However, no difference in NK cell activity was observed before ABMK treatment. This suggests that ABMK consumption might contribute to sustained NK cell cytotoxic activity in gynecological cancer patients undergoing chemotherapy. However, no significant difference was observed in LAK cell cytotoxicity activity between placebo- and ABMK-treated groups over time.

We were also interested in testing monocyte activities of gynecological cancer patients undergoing chemotherapy upon ABMK treatment. We evaluated H_2O_2 production levels of monocytes as an indicator of the monocyte activity. As shown in Figure 1, H_2O_2 production levels were maintained in a similar fashion over the time periods (0, 3, and 6 weeks) following

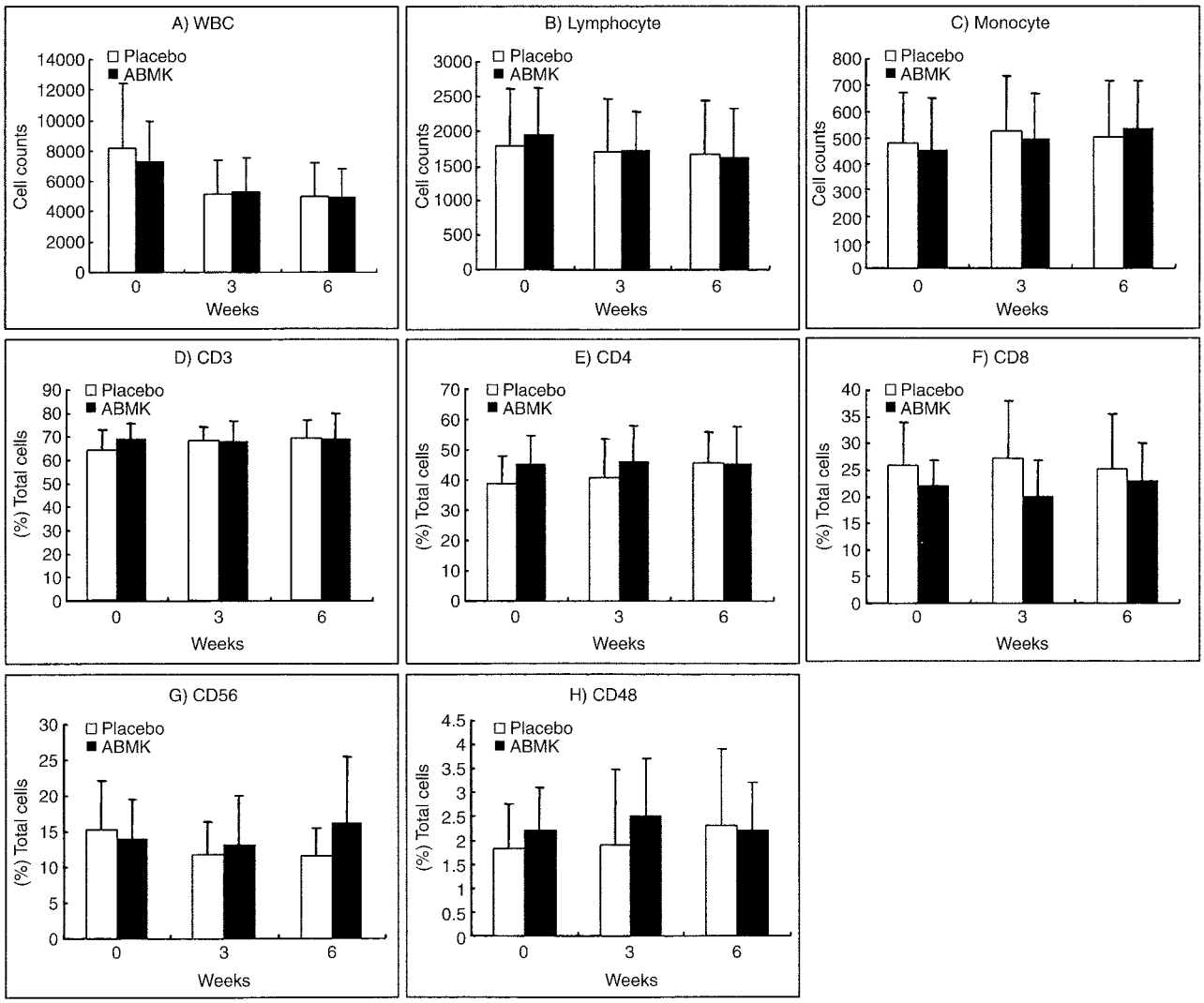

Fig. 2. The cell counts or percent of white blood cells (WBCs) (A), lymphocytes (B), monocytes (C), and specific cell population (D–H) in patients undergoing chemotherapy with and without *Agaricus blazei Murill* Kyowa (ABMK) treatment. Gynecological cancer patients were treated with either carboplatin (300 mg/m^2) plus VP16 (100 mg/m^2) or carboplatin (300 mg/m^2) plus taxol (175 mg/m^2) at 0, 3, and 6 weeks. ABMK was orally administered every day (three packs/day). One day before each chemotherapy, blood was withdrawn, and the immune cells were tested as shown in the Materials and methods.

chemotherapy with or without ABMK consumption. Furthermore, no significant difference in the monocyte activity was observed between placebo- and ABMK-treated groups.

To determine whether ABMK consumption might influence immune cell populations in patients undergoing chemotherapy, we next counted the specific immune cell populations, such as WBCs, lymphocytes, monocytes, $CD3^+$, $CD4^+$, $CD8^+$, $CD48^+$, and $CD56^+$ cells. As shown in Figure 2A, upon chemotherapy, a decrease in the WBC count was detected. However, no increase in the WBC count was detected after ABMK consumption. Furthermore, no significant difference in the counts of cells including lymphocytes, monocytes, T cells, $CD48^+$ cells, and $CD56^+$ cells was observed between placebo- and ABMK-treated cancer patient groups (Fig. 2B–H).

By questionnaires, we also evaluated physical and emotional conditions of patients undergoing chemotherapy with or without ABMK consumption. In this study, we focused solely on any benefits of ABMK treatments in contrast to placebo treatments in patients under chemotherapy. The data on insomnia, appetite, alopecia, body weight, nausea/vomiting, emotional conditions, discomfort, and general body strength were collected. As shown in Figure 3, these physical and mental conditions, in particular appetite, alopecia, nausea/vomiting, emotional conditions, and general body strength improved significantly after ABMK consumption, as compared with placebo consumption, suggesting a positive effect of ABMK consumption on the patients' overall conditions.

Discussion

Studies in many experimental animal models have demonstrated that a variety of natural fungal products have antitumor activities[13–15]. In particular, intratumoral injection or oral administration of *A. blazei* extracts results in tumor regression[16]. In the same study, the antitumor efficacy was improved in particular when *A. blazei* extracts were treated with acids, suggesting an importance of the chemical state of *A. blazei* extracts. We also observed that NK cell activity was maintained to a more significant level in the gynecological cancer patient groups undergoing chemotherapy when ABMK was orally consumed. This suggests that ABMK might have some beneficial effects on innate immunity in gynecological cancer patients undergoing chemotherapy. This is also in line with preclinical reports that treatment with *A. blazei* extracts results in more infiltration of NK cells in the tumor sites and more increased NK cell activity[7,12]. NK cells play an important role in the innate immunity by recognizing major histocompatibility class I-negative target cells, which can escape immune surveillance by cytotoxic T cells. NK cells display dramatic effects on the reduction of tumor growth as well as on the inhibition of metastatic tumors[17]. The mechanism(s) of controlling NK cell cytotoxicity are gradually being elucidated but still remain fragmentary. In addition to direct lysis of target cells, NK cells express CD16, which allows for their participation in antibody-dependent cell cytotoxicity of immunoglobulin-coated tumor cells. It has also been reported

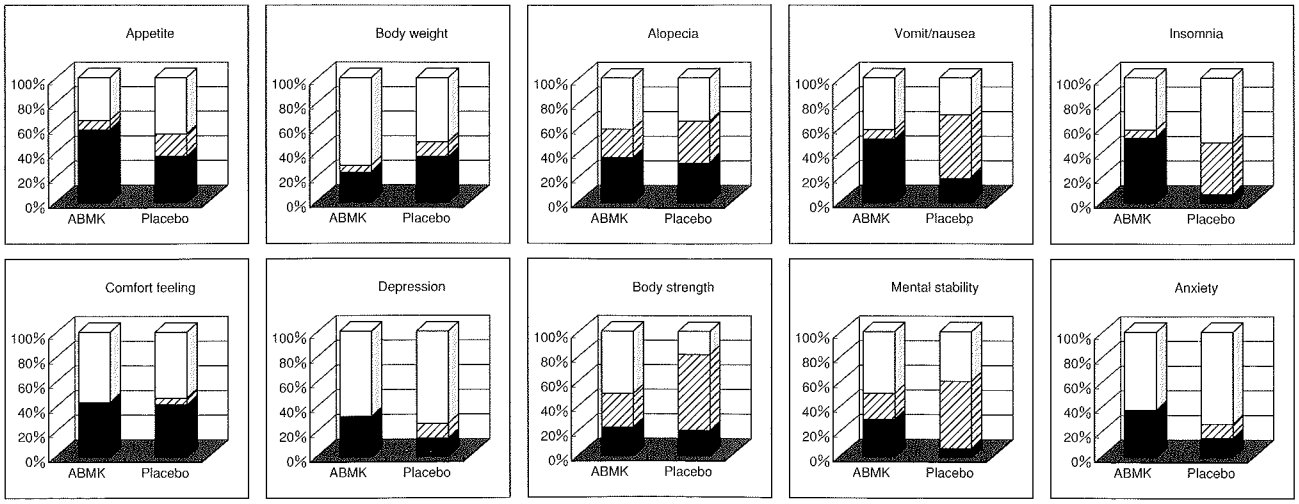

Fig. 3. Evaluation of qualities of life in patients undergoing chemotherapy with and without *Agaricus blazei Murill* Kyowa (ABMK) treatment. Gynecological cancer patients were treated with either carboplatin (300 mg/m^2) plus VP16 (100 mg/m^2) or carboplatin (300 mg/m^2) plus taxol (175 mg/m^2) at 0, 3, and 6 weeks. ABMK was orally administered every day (three packs/day). At the time of completion of treatment, the patients were asked to fill out questionnaires to evaluate their own physical and mental conditions. ■, improved; ▨, worsened; □, no change.

that direct intratumoral injection of ABMK can induce apoptosis and cell-cycle arrests of tumor cells[12]. Based on this, ABMK appears to be beneficial for cancer patients. Similarly, *A. blazei Murill* extracts can stimulate macrophages and then induce the secretion of tumor necrosis factor-α, IL-8, and nitric oxide[18]. This further supports the possible benefits of ABMK. We also investigated whether ABMK consumption might influence general physical and mental conditions of gynecological cancer patients undergoing chemotherapy. We observed that the ABMK consumption reduced some chemotherapy-related side effects in patients. In general, insomnia, appetite, alopecia, body weight, nausea/vomiting, emotional conditions, discomfort, and general body strength were all improved, indicating that ABMK consumption could be one effective approach to reduce some chemotherapy-associated side effects. It could be suspected that chemotherapeutic drugs might interact with ABMK extracts and then decrease the efficacy of chemotherapeutic drugs in the patients. However, it is unlikely that chemotherapeutic drugs administered intravenously can interact directly with any components of ABMK extracts fed orally. However, this needs further investigation for any possible complications.

Taken together, oral delivery of ABMK provides an additional alternative therapeutic modality to maintain innate NK cell activity and, in particular, reduce many severe side effects caused by chemotherapy in gynecological cancer patients.

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