

Alleviating effect of active hexose correlated compound (AHCC) for anticancer drug-induced side effects in non-tumor-bearing mice

Kota Shigama, Akihiro Nakaya, Koji Wakame, Hiroshi Nishioka* and Hajime Fujii

Amino Up Chemical Co., Ltd., 363-32 Shin-ei, Kiyota, Sapporo 004-0839, Japan

*Correspondence to: Hiroshi Nishioka, Amino Up Chemical Co., Ltd., 363-32 Shin-ei, Kiyota, Sapporo 004-0839, Japan.
Telephone: +81-11-889-2233; Fax: +81-11-889-2375. E-mail: nishioka@aminoup.co.jp

(Received March 2, 2009; accepted March 19, 2009)

Active hexose correlated compound (AHCC) is an extract of a basidiomycete mushroom that is used as a supplement by some cancer patients undergoing chemotherapy; it is thought to enhance the therapeutic effects and reduce the side effects of select anticarcinogenic agents. AHCC has been reported to strengthen the anticancer effects of cisplatin (CDDP) and ameliorate its side effects in female BALB/cA mice inoculated with Colon-26 tumor cells. In this study, the role of AHCC in alleviating the side effects induced by several other anticancer drugs was explored in non-tumor-bearing mice receiving monotherapy with paclitaxel (TAX), or multi-drug chemotherapy with TAX plus CDDP, 5-fluorouracil (5FU) plus irinotecan, CDDP plus 5FU, or doxorubicin plus cyclophosphamide. Outcomes from the drug treatment groups with and without AHCC supplementation were compared to controls that received vehicle alone. The multi-drug treatments significantly reduced bone marrow cell viability in all groups and leukocyte count in all groups except for TAX+CDDP; these myelosuppressive effects were generally alleviated by AHCC. Hepatotoxicity and nephrotoxicity caused by the treatments that included TAX and CDDP were also significantly improved by AHCC. The death rate was 20 to 30 percent in all treatment groups except TAX+CDDP, and supplementation with AHCC greatly reduced or eliminated mortality. These results support the concept that AHCC can be beneficial for cancer patients receiving chemotherapy.

Key words: Active hexose correlated compound (AHCC); Mushroom extract; Mice; Anticancer drug-side effects; Hepatotoxicity; Nephrotoxicity; Myelosuppression

INTRODUCTION

The incidence of neoplasm has been reported to be increasing in numerous countries including Japan (1,2). Chemotherapy plays an important role in the treatment of various kinds of cancer such as hematological malignancies. However, chemotherapy is often difficult for patients, in part due to multiple side effects including hair loss, hematological and gastrointestinal toxicities, hepatotoxicity, nephrotoxicity, and neurotoxicity. These side effects lower the quality of life in cancer patients, and they often trigger reductions in the dosage, frequency and duration of chemotherapy, even when it is not therapeutically optimal. Furthermore, chemotherapy inflicts considerable distress, anxiety and depression in almost all cancer patients (3). While the current strategy of anticancer drugs is focused on molecular-targeted agents with high selectivity, such as gefitinib (4,5) and erlotinib (6) that are epidermal growth factor receptor inhibitors, these newer drugs continue to be associated with problems, including dermatologic (7,8) and ocular side effects (9).

One approach to alleviate chemotherapy-induced side effects is the use of complementary and alternative medicine (CAM) that has received great attention. Many cancer patients use CAM with the hope of reducing the side effects of anticancer drugs, and to obtain additional anticancer effects through the boosting of the immune system (10-13). A survey in Japan reported that the prevalence of CAM use was 44.6 percent in cancer patients, with the most frequently used CAM

therapy being dietary supplements of mushrooms such as agaricus (*Agaricus blazei Murill*) and active hexose correlated compound (AHCC) (14).

AHCC is a mixture of polysaccharides, amino acids, lipids and minerals derived from cultured broth of the basidiomycete mushroom, *Lentinula edodes* (shiitake). The predominant component of AHCC is oligosaccharides, which contain α -1, 4 glucans and partially acetylated α -1, 4 glucans with a mean molecular weight of 5,000 Daltons. Investigators have reported that AHCC can increase the number and function of dendritic cells and NK cell activity in adult human (15,16), and enhance both the activation and proliferation of CD4⁺ and CD8⁺ T cells in mice (17). In tumor-bearing rodent models, AHCC strengthened the chemotherapeutic effects of UFT and cisplatin for mammary adenocarcinoma SST-2 cells and Colon-26 tumor cells, respectively (18,19). AHCC has been reported to be safe for human consumption, based on the results from several pre-clinical studies and a Phase I study using healthy volunteers (20). No inhibition of CYP450 activity was observed in presence of AHCC, however, AHCC was a substrate of CYP450 2D6. The CYP450 induction metabolism assays indicate that AHCC is an inducer of CYP450 2D6. AHCC does have the potential for drug-drug interactions involving CYP450 2D6 such as ondansetron, however overall data suggests that AHCC would be safe to administer with most other chemotherapy agents that are not metabolized via the CYP450 2D6 pathway (21). Furthermore, two clinical studies among liver cancer patients showed a significant increase in survival rate among those taking AHCC (22,23).

While the immunomodulating actions and anti-tumor effects of AHCC have been demonstrated, the effect of AHCC on anticancer drug-induced toxicities is not well characterized. In the present study, we investigated the influence of AHCC on some of the side effects caused by monotherapy with paclitaxel, or multi-drug chemotherapy in non-tumor-bearing mice, but not tumor-bearing mice because of estimating intrinsic body weight change and mortality rate associated with anticancer agents.

MATERIALS AND METHODS

Reagents

AHCC, manufactured by Amino Up Chemical Co., Ltd. (Japan), was produced from cultured broth of the basidiomycete mushroom, *Lentinula edodes*, in a manufacturing process according to Good Manufacturing Practice (GMP) standards for dietary supplements, and ISO9001 and ISO22000 standards. Following pre-cultivation in flasks, the basidiomycete was cultured

in large (15 tons) tanks for 45 days, and then AHCC was obtained through filtration, sterilization, concentration and freeze-drying. Paclitaxel (TAX), doxorubicin (DXR) and cyclophosphamide (CY) were purchased from Sigma-Aldrich Japan (Japan). Cisplatin (CDDP), 5-fluorouracil (5FU) and irinotecan (CPT) are commercially available drugs as Randa Inj. (NIPPON KAY-AKU CO., LTD., Japan), 5-FU Injection (Kyowa Hakko Kirin Co., Ltd., Japan) and CAMPTO Inj. (YAKULT HONSHA CO., LTD., Japan), respectively, and these drugs all were obtained from JUNSEI CHEMICAL CO., LTD. (Japan).

Animals

SPF male ddY mice were purchased from Japan SLC, Inc. (Japan) and studied at five weeks of age. The animals were maintained in a temperature- and humidity-controlled room at $23 \pm 1^\circ\text{C}$ and 55-60%, respectively, under a 12-hour light-dark cycle (lights on 08:00 to 20:00), and were fed a standard pelleted rodent chow (CE-2; CLEA Japan Inc., Japan) and water *ad libitum*. Mice in each experiment were divided into three groups: control, anticancer drug(s) alone, and AHCC plus anticancer drug(s).

Treatments

The anticancer drugs used in this study were TAX, CDDP, 5FU, CPT, DXR and CY. TAX or CY was dissolved in DMSO and mixed with saline prior to administration. The commercialized CDDP, 5FU or CPT solution was directly injected into mice. DXR was dissolved in saline followed by the treatment. AHCC was dissolved in saline just before the supplementation. The anticancer agents and AHCC were given to mice intraperitoneally and by gavage, respectively. The study consisted of five experiments (Exp. 1 to Exp. 5). The experimental designs are briefly outlined in Table 1. All experiments had a control group that was treated with same regimen of anticancer-treated groups with and without AHCC, except for administration of vehicle instead of anticancer drugs and AHCC. In Exp. 1, 15 mg/kg of TAX was administered into mice at days 8, 11, 15, 18 and 22 (a total of 5 injections). AHCC was given daily at a dose of 500 mg/kg from day 0 to day 25. In Exp. 2, mice were co-treated with 20 mg/kg of TAX and 8 mg/kg of CDDP once a week for 2 weeks (days 7 and 14). Supplementation with AHCC (1 g/kg) was commenced 7 days prior to the first treatment with anticancer drugs and was continued until day 18. Two experiments (Exp. 3; 100 mg/kg of 5FU plus 50 mg/kg of CPT, and Exp. 4; 8 mg/kg of CDDP plus 100 mg/kg

Table 1. Experimental designs

Experiment #	Treatment	Drug dosing schedule (dose and days)	AHCC dosing schedule (dose and days)	Number of mice*
Exp. 1	TAX ^{a)}	^{a)} 15 mg/kg Day 8, 11, 15, 18 and 22	None	8
		^{a)} 15 mg/kg Day 8, 11, 15, 18 and 22	500 mg/kg Day 0 to 25	8
Exp. 2	TAX ^{a)} + CDDP ^{b)}	^{a)} 20 mg/kg + ^{b)} 8 mg/kg Day 7 and 14	None	9
		^{a)} 20 mg/kg + ^{b)} 8 mg/kg Day 7 and 14	1 g/kg Day 0 to 18	9
Exp. 3	5FU ^{c)} + CPT ^{d)}	^{c)} 100 mg/kg + ^{d)} 50 mg/kg Day 7 and 14	None	10
		^{c)} 100 mg/kg + ^{d)} 50 mg/kg Day 7 and 14	1 g/kg Day 0 to 21	10
Exp. 4	CDDP ^{b)} + 5FU ^{c)}	^{b)} 8 mg/kg + ^{c)} 100 mg/kg Day 7 and 14	None	10
		^{b)} 8 mg/kg + ^{c)} 100 mg/kg Day 7 and 14	1 g/kg Day 0 to 21	11
Exp. 5	DXR ^{e)} + CY ^{f)}	^{e)} 8 mg/kg + ^{f)} 120 mg/kg Day 7	None	10
		^{e)} 8 mg/kg + ^{f)} 120 mg/kg Day 7	360 mg/kg Day 0 to 21	10

^{a)} TAX: paclitaxel, ^{b)} CDDP: cisplatin, ^{c)} 5FU: 5-fluorouracil, ^{d)} CPT: irinotecan, ^{e)} DXR: doxorubicin,

^{f)} CY: cyclophosphamide.

* Number of mice in control group was as same as that of anticancer drug-treated group without AHCC.

of 5FU) were conducted in accordance with a similar schedule, where dual drugs were co-injected on days 7 and 14, and 1 g/kg of AHCC was successively administered from day 0 to day 21. In Exp. 5, mice received a single administration of DXR (8 mg/kg) and CY (120 mg/kg) at day 7, and daily supplementation with 360 mg/kg of AHCC from day 0 to day 21. In each experiment, animals were killed under anesthesia to collect blood and bone marrow cells on the final day of AHCC administration.

In past studies, the effect of AHCC was assessed at a dosage range from 100 mg/kg to 1 g/kg (17-19,24). Hence, the working dose of AHCC in this study was chosen within this range. The dose and schedule of anticancer drugs used in this study were based on previous investigations (25-29) with some modifications. All

experiments were approved by the Animal Care Committee of Amino Up Chemical Co., Ltd.

Evaluation of Parameters

The following parameters were assessed: body weight, liver function (serum AST and ALT), kidney function (blood urea nitrogen (BUN) and serum creatinine), bone marrow suppression (total white blood cell count and bone marrow cell viability), and mortality rate. Body weight was measured twice a week. AST (GOT) and ALT (GPT), BUN, and serum creatinine were measured using Transaminase CII-test WAKO, Urea Nitrogen B-test WAKO, and Creatinine-test WAKO assay kits (Wako Pure Chemical Industries Limited, Japan), respectively.

Blood samples collected from the heart were diluted to 1:10 with Turk solution (Wako Pure Chemical Industries Limited, Japan) to determine the number of white blood cells in accordance with the Nageotte chamber counting procedure (30). Bone marrow cells collected from mouse femora were suspended in 0.83% NH_4Cl solution to hemolyze red blood cells and incubated at 37°C for 10 min. After centrifugation, the cells were prepared at a concentration of 1×10^7 cells/ml in DMEM supplemented with 10% FBS. A 100- μl aliquot of the suspension was cultured in a 96-well plate for 3 days, and viability (percent of control group) of bone marrow cells was estimated using the MTT assay. Mortality data were collected daily.

Statistical Analysis

Experimental data except for mortality rate are shown as mean \pm SEM. Data were analyzed by one-way analysis of variance (ANOVA). Fisher's Protected Least Significance Difference (PLSD) was used as a post hoc test, and values of p less than 0.05 were determined to be statistically significant.

RESULTS

Change of Body Weight

The change in body weight was calculated the ratio of body weight gain in the treated groups compared to the gain in their respective control (non-treatment) groups on the final day of each experiment (Figure 1). Interestingly, treatment with TAX alone significantly increased body weight compared to either the control group or the AHCC plus TAX group. The increase in body weight was likely due to dyschezia since the large bowel was visually swollen with feces when mice were dissected at day 25. AHCC administration suppressed TAX-induced body weight elevation, suggesting that AHCC might improve this disorder. All multi-drug therapies predictably decreased body weight gain compared to the respective control group, with the most pronounced decrease in body weight gain noted for the combinations with CDDP (TAX plus CDDP, and CDDP plus 5FU). Supplementation with AHCC tended to prevent body weight loss although the effect was not statistically significant. Though TAX alone (five-time repeated dose of 15 mg/kg; Exp. 1) resulted in weight gain, the multi-drug treatment with TAX (twice weekly dose of 20 mg/kg) plus CDDP (Exp. 2) decreased body weight and no dyschezia was noted when the animals were dissected; it is possible that this difference in find-

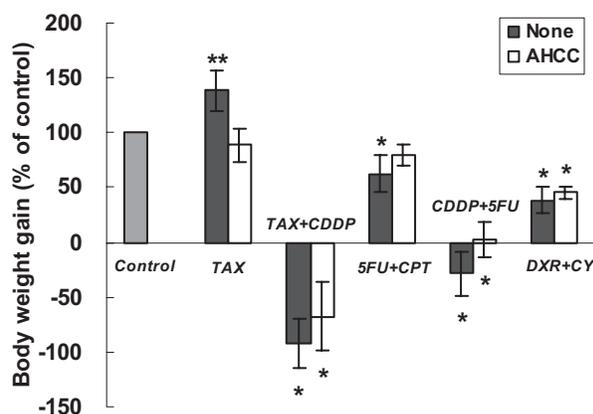


Figure 1. Body weight change on the final day of each experiment.

Body weight of mice was measured twice a week through the experiment period, and this figure shows the body weight gain on the final day of each experiment. The values (% of control) are represented as the ratio of treated groups with anticancer drug(s) alone or with AHCC plus anticancer drug(s) to the control (non-treatment) group. * $p < 0.01$ vs control, ** $p < 0.05$ vs control, AHCC plus TAX.

ings was due to the lower dosage of TAX used in the second experiment.

Hepatotoxicity and Nephrotoxicity

Serum AST and ALT levels were significantly increased in the TAX alone group ($p < 0.05$ vs control; Table 2). AHCC administration reduced both levels, with the reduction in ALT being statistically significant ($p < 0.05$; Table 2). In contrast, TAX plus CDDP treatment did not change serum AST and ALT concentrations, and all values were in the normal range (data not shown). As noted above, the differences between TAX alone and TAX+CDDP may be due to the different doses of TAX used in Exp. 1 and 2.

In humans, CDDP treatment can result in renal dysfunction, which is a dose limiting factor. In this study,

Table 2. Levels of serum AST and ALT at the end of Exp. 1

Group	AST (IU/L)	ALT (IU/L)
Control	30.8 \pm 2.2	17.5 \pm 0.7
TAX	75.3 \pm 19.7 ^{a)}	35.2 \pm 7.0 ^{a)}
AHCC+TAX	50.6 \pm 9.2	22.3 \pm 4.1 ^{b)}

All values represent the mean \pm SEM. ^{a)} $p < 0.05$ vs control, ^{b)} $p < 0.05$ vs TAX.

the kidney function parameters of BUN and serum creatinine were evaluated in the two combination groups with CDDP at the end of Exp. 2 and Exp. 4. The concentrations of BUN and serum creatinine were significantly increased in both CDDP-treated groups compared to the control group ($p < 0.01$; Table 3). AHCC administration attenuated the levels of BUN and serum creatinine, and a significant difference in both the BUN (Exp. 2 and Exp. 4) and creatinine (Exp. 2) level was measured.

Bone Marrow Suppression and Mortality Rate

All treatments with dual anticancer drugs caused significant bone marrow suppression as measured by leukocyte count and bone marrow cell viability ($p < 0.01$ vs control; Figures 2A and 2B). Supplementation with AHCC significantly improved the reduction of leuko-

cytes in all groups except for TAX+CDDP, though the levels did not completely return to control values for any of the treatments. Bone marrow cell viability was also depressed by single and all multiple treatments, and AHCC supplementation significantly reversed this trend ($p < 0.01$), though the amelioration did not show complete recovery.

The drugs were lethal to 20 to 30 percent of the animals given the anticancer drug(s) alone, except for the TAX+CDDP group, and addition of AHCC resulted in either reduction or elimination of mortality (Table 4).

DISCUSSION

Cancer treatment including mono- and combination chemotherapies reliably improves the disease-free

Table 3. BUN, serum creatinine and the ratio on the final day of Exp. 2 and 4

Group	BUN (mg/dL)	Creatinine (mg/dL)	BUN/Creatinine
Control	24.5 ± 0.9	0.59 ± 0.02	36.2 ± 1.6
TAX+CDDP	34.9 ± 1.7 ^{a)}	0.93 ± 0.03 ^{b)}	37.9 ± 2.1
AHCC+TAX+CDDP	30.5 ± 1.5 ^{c)}	0.78 ± 0.03 ^{c)}	39.5 ± 2.6
Control	23.8 ± 1.3	0.59 ± 0.02	41.6 ± 2.5
CDDP+5FU	35.3 ± 6.3 ^{d)}	0.77 ± 0.08 ^{e)}	45.9 ± 6.2
AHCC+CDDP+5FU	25.4 ± 1.1	0.66 ± 0.03	38.5 ± 1.3

All values are expressed as mean ± SEM. ^{a)} $p < 0.01$ vs control, $p < 0.05$ vs AHCC+TAX+CDDP, ^{b)} $p < 0.01$ vs control, AHCC+TAX+CDDP, ^{c)} $p < 0.05$ vs control, ^{d)} $p < 0.01$ vs control, AHCC+CDDP+5FU, ^{e)} $p < 0.01$ vs control.

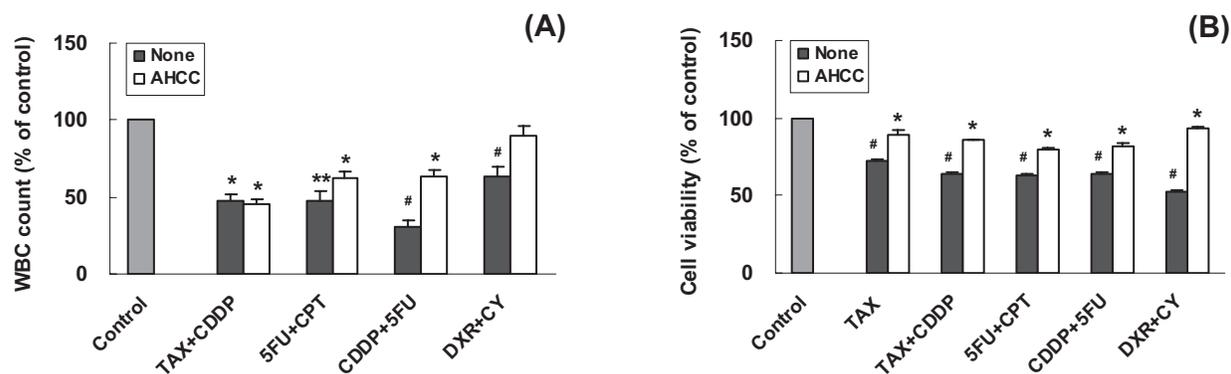


Figure 2. Ameliorative effect of AHCC for anticancer drug(s)-induced myelosuppression.

Bone marrow suppression was determined using two parameters that were total white blood cell (WBC) count (A) and bone marrow cell viability (B). Both assessments were carried out when mice were sacrificed at the end of each experiment, and the evaluation methods are briefly described in the section, Materials and Methods. The values are expressed as the ratio to control (% of control). * $p < 0.01$ vs control, ** $p < 0.01$ vs control, $p < 0.05$ vs AHCC supplemented group, # $p < 0.01$ vs control, AHCC supplemented group.

Table 4. Mortality rate in the treatment groups without or with AHCC

Treatment	None		+ AHCC	
TAX	25%	(2/8)	0%	(0/8)
TAX+CDDP	0%	(0/9)	0%	(0/9)
5FU+CPT	30%	(3/10)	0%	(0/10)
CDDP+5FU	30%	(3/10)	9%	(1/11)
DXR+CY	20%	(2/10)	0%	(0/10)

The values in parenthesis represent dead mice/total mice.

and overall survival in cancer patients, but the clinical usefulness is frequently limited by side effects. This study was designed to investigate the impact of AHCC in terms of side effects induced by anticancer drugs in animal models.

In humans, major TAX-related side effects include peripheral neuropathy, myelotoxicity, granulocytopenia, bradycardia, hypotension, arthralgia, myalgia and hypersensitivity (31,32). These side effects were not duplicated in Exp. 1, but observable side effects included dyschezia, hepatotoxicity and bone marrow suppression. Supplementation with AHCC significantly alleviated the hepatotoxicity and myelosuppression and showed a tendency to reduce the severity of dyschezia. In addition to monotherapy, current clinical oncology practice employs a strategy to use multiple anticancer agents with distinct molecular mechanisms, anticipating higher chemotherapeutic efficacy and/or lower toxicity. In the present study, we also assessed the action of AHCC on four combination treatments, which were selected because the multi-drug therapies tested here are commonly used for treatment of non-small-cell cancer of the lung (33,34), and cancer of the ovary (35), colon (36), gastrointestinal tract (37), liver (38), cervix (39) and breast (40) as a first- or second-line treatment. The most noteworthy effect of AHCC was an improvement of leukocyte levels (though not for TAX plus CDDP) and bone marrow cell viability. Mortality was also markedly improved by AHCC supplementation in most experiments, suggesting that AHCC might systematically attenuate anticancer drug-related toxicity.

One of the most serious side effects in cancer chemotherapy is leucopenia including neutropenia, which often induces infectious complications, and is subsequently dose-limiting, which may compromise treatment efficacy. Opportunistic infections are a major cause of morbidity and mortality in cancer patients receiving myelotoxic chemotherapy, resulting from invasive fungal infections, particularly invasive aspergillosis, and an increasing spread of Gram-positive pathogens such

as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (41). In current clinical practice, colony-stimulating factors such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are increasingly used to recover white blood cell counts or increase dose-density (42-44). Although G-CSF and GM-CSF are generally safe and well tolerated and have a favorable outcome, several reports on G-CSF and GM-CSF associated side effects exist (45-49). Recently, it has been reported that G-CSF enhances bone tumor growth in mice in an osteoclast-dependent manner (50). The results of the present study suggest that AHCC may have the potential to alleviate myelosuppression, and that AHCC might be useful to complement the properties of G-CSF and GM-CSF. However, the mechanism(s) of action that AHCC attenuates myelosuppression is unclear. Several studies demonstrated that polysaccharides such as β -glucans reduce myelosuppression and enhance hematopoiesis *in vitro* and the mobilization of stem cells in animal models (51-53). Maitake β -glucans (MBG) was found to promote bone marrow cell viability and protect the bone marrow stem cell colony formation unit from DXR-induced hematopoietic toxicity (54). The recent study has reported that MBG induces hematopoietic stem cell proliferation and differentiation and acts to replace and induce G-CSF (55). It is speculated that the effect of AHCC on alleviating myelosuppression might be mediated by the mechanism similar to MBG although the predominant polysaccharide component of AHCC is partially acetylated α -glucans but not β -glucans. In future, further studies are needed to elucidate the precise mechanism(s) of action on improving bone marrow suppression as well as hepatotoxicity and nephrotoxicity.

With increasing use of CAM, it is important to address safety issues and interactions between CAM products and conventional treatments (56,57) including chemotherapy, surgical resection, radiotherapy, and increasingly, targeted molecular therapies. The safety of AHCC in cancer patients and healthy volunteers has been previously reported (15, 16, 20, 22, 23). The current study assessing the role of AHCC in reducing chemotherapy-related side effects in animal models suggests that AHCC may be safe to administer with the drugs tested, and perhaps other chemotherapy agents that are not metabolized via the CYP450 2D6 pathway (21). For many cancer patients, CAM approaches are pursued in an attempt to maximize the efficacy of conventional modalities, as well as to reduce treatment-related symptoms and other side effects that diminish their quality of life (10-14). Cancer patients also use CAM products such as AHCC for strengthening their overall function to recover from the debility of cancer treatment and supporting their ability to fight against cancer (58-60).

CONCLUSION

The present study was conducted to assess whether AHCC reduces anticancer drug-induced toxicities including body weight loss, liver and kidney damages, myelosuppression, and mortality in non-tumor-bearing mice. As a result, AHCC significantly alleviated hepatotoxicity, nephrotoxicity, bone marrow suppression and overall mortality, and showed the possibility to reduce the severity of dyschezia. If these results are extended to humans, AHCC might contribute to improved quality of life and well-being of cancer patients undergoing chemotherapy.

ACKNOWLEDGMENT

We thank Dr. Robert M. Hackman and Dr. Carl L. Keen, University of California-Davis, and Dr. Judith A. Smith, University of Texas, MD Anderson Cancer Center, for providing greatly appropriate suggestions and comments.

REFERENCES

- Ajiki W, Tsukuma H, Oshima A. (2004). Cancer incidence and incidence rates in Japan in 1999: estimates based on data from 11 population-based cancer registries. *Jpn J Clin Oncol*, 34, 352–356.
- Matsuda T, Marugame T, Kamo K, Katanoda K, Ajiki W, Sobue T. (2008). Cancer incidence and incidence rates in Japan in 2002: based on data from 11 population-based cancer registries. *Jpn J Clin Oncol*, 38, 641–648.
- Pandey M, Sarita GP, Devi N, Thomas BC, Hussain BM, Krishnan R. (2006). Distress, anxiety, and depression in cancer patients undergoing chemotherapy. *World J Surg Oncol*, 4, 68–72.
- Ciardello F. (2000). Epidermal growth factor receptor tyrosine kinase inhibitors as anticancer agents. *Drugs*, 60, 25–32.
- Baselga J, Averbuch SD. (2000). ZD1839 ('Iressa') as an anticancer agent. *Drugs*, 60, 33–40.
- Pollack VA, Savage DM, Baker DA, Tsaparikos KE, Sloan DE, Moyer JD, Barbacci EG, Pustilnik LR, Smolarek TA, Davis JA, Vaidya MP, Arnold LD, Doty JL, Iwata KK, Morin MJ. (1999). Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition *in situ* and antitumor effects in athymic mice. *J Pharmacol Exp Ther*, 291, 739–748.
- Agero AL, Dusza SW, Benvenuto-Andrade C, Busam KJ, Myskowski P, Halpern AC. (2006). Dermatological side effects associated with the epidermal growth factor receptor inhibitors. *J Am Acad Dermatol*, 55, 657–670.
- Sipples R. (2006). Common side effects of anti-EGFR therapy: acneiform rash. *Semin Oncol Nurs*, 22, 28–34.
- Methvin AB, Gausas RE. (2007). Newly recognized ocular side effects of erlotinib. *Ophthalm Plast Reconstr Surg*, 23, 63–65.
- Astin JA. (1998). Why patients use alternative medicine: results of a national study. *JAMA*, 279, 1548–1553.
- Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, Kessler RC. (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA*, 280, 1569–1575.
- Boon H, Stewart M, Kennard MA, Gray R, Sawka C, Brown JB, McWilliam C, Gavin A, Baron RA, Aaron D, Haines-Kamka T. (2000). Use of complementary/alternative medicine by breast cancer survivors in Ontario: prevalence and perceptions. *J Clin Oncol*, 18, 2515–2521.
- Mansky PJ, Wallerstedt DB. (2006). Complementary medicine in palliative care and cancer symptom management. *Cancer J*, 12, 425–431.
- Hyodo I, Amano N, Eguchi K, Narabayashi M, Imanishi J, Hirai M, Nakano T, Takashima S. (2005). Nationwide survey on complementary and alternative medicine in cancer patients in Japan. *J Clin Oncol*, 23, 2645–54.
- Terakawa N, Matsui Y, Sato S, Yanagimoto H, Takahashi K, Yamamoto T, Yamano J, Takai S, Kwon AH, Kamiyama Y. (2008). Immunological effect of active hexose correlated compound (AHCC) in healthy volunteers: a double-blind, placebo-controlled trial. *Nutr Cancer*, 60, 643–651.
- Belanger J. (2005). An in-office evaluation of four dietary supplements on natural killer cell activity. *TOWNSEND LETTER, February/March*, 88–92.
- Gao Y, Zhang D, Sun B, Fujii H, Kosuna K, Yin Z. (2005). Active hexose correlated compound enhances tumor surveillance through regulating both innate and adaptive immune responses. *Cancer Immunol Immunother*, 55, 1258–1266.
- Matsushita K, Kuramitsu Y, Ohiro Y, Obata M, Kobayashi M, Li YQ, Hosokawa M. (1998). Combination therapy of active hexose correlated compound plus UFT significantly reduces the metastasis of rat mammary adenocarcinoma. *Anti-cancer Drugs*, 9, 343–350.
- Hirose A, Sato E, Fujii H, Sun B, Nishioka H, Aruoma OI. (2007). The influence of active hexose correlated compound (AHCC) on cisplatin-evoked chemotherapeutic and side effects in tumor-bearing mice. *Toxicol Appl Pharmacol*, 222, 152–158.
- Spierings ELH, Fujii H, Sun B, Walshe T. (2007). A phase I study of the safety of the nutritional supplement, active hexose correlated compound, AHCC, in healthy volunteers. *J Nutr Sci Vitaminol*, 53, 536–539.
- Mach CM, Fujii H, Wakame K, Smith J. (2008). Evaluation of active hexose correlated compound hepatic metabolism and potential for drug interactions with chemotherapy agents. *J Soci Integr Oncol*, 6, 105–109.
- Matsui Y, Uhara J, Sato S, Kaibori M, Yamada H, Kitada H, Imamura A, Takai S, Kawaguchi Y, Kwon AH, Kamiyama Y. (2002). Improved prognosis of postoperative hepatocellular carcinoma patients when treated with functional foods: a prospective cohort study. *J Hepatol*, 37, 78–86.
- Cowawintaweewat S, Manoromana S, Sriplung H, Khuhaprema T, Tongtawe P, Tapchaisri P, Chaicumpa W. (2006). Prognostic improvement of patients with advanced liver cancer after active

- hexose correlated compound (AHCC) treatment. *Asian Pac J Allergy Immunol*, 24, 33–45.
24. Ritz BW. (2008). Supplementation with active hexose correlated compound increases survival following infectious challenge in mice. *Nutr Rev*, 66, 526–531.
 25. Nicoletti MI, Lucchini V, D'Incalci M, Giavazzi R. (1994). Comparison of paclitaxel and docetaxel activity on human ovarian carcinoma xenografts. *Eur J Cancer*, 30A, 691–696.
 26. Fujimoto S, Chikazawa H. (1998). Schedule-dependent and -independent antitumor activity of paclitaxel-based combination chemotherapy against M-109 murine lung carcinoma in vivo. *Jpn J Cancer Res*, 89, 1343–1351.
 27. Azrak RG, Cao S, Slocum HK, Tóth K, Durrani FA, Yin M, Pendyala L, Zhang W, McLeod HL, Rustum YM. (2004). Therapeutic synergy between irinotecan and 5-fluorouracil against human tumor xenografts. *Clin Cancer Res*, 10, 1121–1129.
 28. Fujimoto S. (2006). Schedule-dependent antitumor activity and toxicity of combination of 5-fluorouracil and cisplatin/carboplatin against L1210 leukemia-bearing mice. *Biol. Pharm. Bull.*, 29, 2260–2266.
 29. Braunschweiger PG, Schiffer LM. (1980). Cell kinetic-directed sequential chemotherapy with cyclophosphamide and adriamycin in T1699 mammary tumors. *Cancer Res*, 40, 737–743.
 30. Masse M, Naegelen C, Pellegrini N, Segier JM, Marpaux N, Beaujean F. (1992). Validation of a simple method to count very low white cell concentrations in filtered red cells or platelets. *Transfusion*, 32, 565–571.
 31. Sekine I, Nishiwaki Y, Watanabe K, Yoneda S, Saijo N. (1996). Phase II study of 3-hour infusion of paclitaxel in previously untreated non-small cell lung cancer. *Clin Cancer Res*, 2, 941–945.
 32. Furuse K, Naka N, Takada M, Kinuwaki E, Kudo S, Takada Y, Yamakido M, Yamamoto H, Fukuoka M. (1997). Phase II study of 3-hour infusion of paclitaxel in patients with previously untreated stage III and IV non-small cell lung cancer. *Oncology*, 54, 298–303.
 33. Bunn PA Jr. (1996). Combination paclitaxel and platinum in the treatment of lung cancer: US experience. *Semin Oncol*, 23, 9–15.
 34. Chen CH, Chang JW, Lee CH, Tsao TC. (2005). Dose-finding and phase 2 study of weekly paclitaxel (taxol) and cisplatin combination in treating Chinese patients with advanced non-small cell lung cancer. *Am J Clin Oncol*, 28, 508–512.
 35. Sandercock J, Parmar MK, Torri V. (1998). First-line chemotherapy for advanced ovarian cancer: paclitaxel, cisplatin and the evidence. *Br J Cancer*, 78, 1471–1478.
 36. Cunningham D. (1996). Current status of colorectal cancer: CPT-11 (irinotecan), a therapeutic innovation. *Eur J Cancer*, 32A, S1–S8.
 37. Rougier P, Ducreux M, Mahjoubi M, Pignon JP, Bellefqih S, Oliveira J, Bognel C, Lasser P, Ychou M, Elias D. (1994). Efficacy of combined 5-fluorouracil and cisplatin in advanced gastric carcinomas. A phase II trial with prognostic factor analysis. *Eur J Cancer*, 30A, 1263–1269.
 38. Itamoto T, Nakahara H, Tashiro H, Haruta N, Asahara T, Naito A, Ito K. (2002). Hepatic arterial infusion of 5-fluorouracil and cisplatin for unresectable or recurrent hepatocellular carcinoma with tumor thrombus of portal vein. *J Surg Oncol*, 80, 143–148.
 39. Kaern J, Tropé C, Sundfoer K, Kristensen GB. (1996). Cisplatin/5-fluorouracil treatment of recurrent cervical carcinoma: a phase II study with long-term follow-up. *Gynecol Oncol*, 60, 387–392.
 40. A'Hern RP, Smith IE, Ebbs SR. (1993). Chemotherapy and survival in advanced breast cancer: the inclusion of doxorubicin in Cooper type regimens. *Br J Cancer*, 67, 801–805.
 41. Maschmeyer G, Haas A. (2008). The epidemiology and treatment of infections in cancer patients. *Int J Antimicrob Agents*, 31, 193–197.
 42. Groopman JE. (1990). Status of colony-stimulating factors in cancer and AIDS. *Semin Oncol*, 17, 31–37.
 43. Dale DC. (2002). Colony-stimulating factors for the management of neutropenia in cancer patients. *Drugs*, 62, 1–15.
 44. Heuser M, Ganser A, Bokemeyer C. (2007). Use of colony-stimulating factors for chemotherapy-associated neutropenia: review of current guidelines. *Semin Hematol*, 44, 148–156.
 45. Morstyn G, Campbell L, Souza LM, Alton NK, Keech J, Green M, Sheridan W, Metcalf D, Fox R. (1988). Effect of granulocyte colony stimulating factor on neutropenia induced by cytotoxic chemotherapy. *Lancet*, 1, 667–672.
 46. Kehoe S, Poole CJ, Stanley A, Earl HM, Blackledge GR. (1994). A phase I/II trial of recombinant human granulocyte-macrophage colony-stimulating factor in the intensification of cisplatin and cyclophosphamide chemotherapy for advanced ovarian cancer. *Br J Cancer*, 69, 537–540.
 47. Merlano M, Benasso M, Numico GM, Danova M, Santelli A, Ameli F, Blengo F, Ricci I, Rosso M. (1998). 5-Fluorouracil dose intensification and granulocyte-macrophage colony-stimulating factor in cisplatin-based chemotherapy for relapsed squamous cell carcinoma of the head and neck: a phase II study. *Am J Clin Oncol*, 21, 313–316.
 48. Azoulay E, Attalah H, Harf A, Schlemmer B, Delclaux C. (2001). Granulocyte colony-stimulating factor or neutrophil-induced pulmonary toxicity: myth or reality? *Chest*, 120, 1695–1701.
 49. Takatsuka H, Takemoto Y, Mori A, Okamoto T, Kanamaru A, Kakishita E. (2002). Common features in the onset of ARDS after administration of granulocyte colony-stimulating factor. *Chest*, 121, 1716–1720.
 50. Hirbe AC, Uluçkan O, Morgan EA, Eagleton MC, Prior JL, Piwnica-Worms D, Trinkaus K, Apicelli A, Weibaecher K. (2007). Granulocyte colony-stimulating factor enhances bone tumor growth in mice in an osteoclast-dependent manner. *Blood*, 109, 3424–3431.
 51. Hashimoto K, Suzuki I, Ohsawa M, Oikawa S, Yadomae T. (1990). Enhancement of hematopoietic response of mice by intraperitoneal administration of a beta-glucan, SSG, obtained from *Sclerotinia sclerotiorum*. *J Pharmacobiodyn*, 13, 512–517.
 52. Patchen ML, Liang J, Vaudrain T, Martin T, Melican D, Zhong S, Stewart M, Quesenberry PJ. (1998). Mobilization of peripheral blood progenitor cells by betafectin PGG-glucan alone and in combination with granulocyte colony-stimulating factor. *Stem Cells*, 16, 208–217.

53. Patchen ML, Vaudrain T, Correira H, Martin T, Reese D. (1998). In vitro and in vivo hematopoietic activities of betalectin PGG-glucan. *Exp. Hematol*, 26, 1247–1254.
54. Lin H, She YH, Cassileth BR, Sirotiak F, Cunningham-Rundles S. (2004). Maitake beta-glucan MD-fraction enhances bone marrow colony formation and reduces doxorubicin toxicity in vitro. *Int Immunopharmacol*, 4, 91–99.
55. Lin H, Cheung SWY, Nesin M, Cassileth BR, Cunningham-Rundles S. (2007). Enhancement of umbilical cord blood cell hematopoiesis by Maitake beta-glucan is mediated granulocyte colony-stimulating factor production. *Clin Vaccine Immunol*, 14, 21–27.
56. Tascilar M, de Jong FA, Verweij J, Mathijssen RH. (2006). Complementary and alternative medicine during cancer treatment: beyond innocence. *Oncologist*, 11, 732–741.
57. Seely D, Oneschuk D. (2008). Interactions of natural health products with biomedical cancer treatments. *Curr Oncol*, 15, S81–S86.
58. Richardson MA, Sanders T, Palmer JL, Greisinger A, Singletary SE. (2000). Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. *J Clin Oncol*, 18, 2505–2514.
59. Jazieh AR, Kopp M, Foraida M, Ghouse M, Khalil M, Savidge M, Sethuraman G. (2004). The use of dietary supplements by veterans with cancer. *J Altern Complement Med*, 10, 560–564.
60. Correa-Velez I, Clavarino A, Eastwood H. (2005). Surviving, relieving, repairing, and boosting up: reasons for using complementary/alternative medicine among patients with advanced cancer: a thematic analysis. *J Palliat Med*, 8, 953–961.