

Reduction of Adverse Effects by a Mushroom Product, Active Hexose Correlated Compound (AHCC) in Patients With Advanced Cancer During Chemotherapy—The Significance of the Levels of HHV-6 DNA in Saliva as a Surrogate Biomarker During Chemotherapy

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Chemotherapy improves the outcome of cancer treatment, but patients are sometimes forced to discontinue chemotherapy or drop out of a clinical trial due to adverse effects, such as gastrointestinal disturbances and suppression of bone marrow function. The objective of this study was to evaluate the safety and effectiveness of a mushroom product, active hexose correlated compound (AHCC), on chemotherapy-induced adverse effects and quality of life (QOL) in patients with cancer. Twenty-four patients with cancer received their first cycle of chemotherapy without AHCC and then received their second cycle with AHCC. During chemotherapy, we weekly evaluated adverse effects and QOL via a blood test, EORTC QLQ-C30 questionnaire, and DNA levels of herpes virus type 6 (HHV-6) in saliva. The DNA levels of HHV-6 were significantly increased after chemotherapy. Interestingly, administration of AHCC significantly decreased the levels of HHV-6 in saliva during chemotherapy and improved not only QOL scores in the EORTC QLQ-C30 questionnaire but also hematotoxicity and hepatotoxicity. These find-

ings suggest that salivary HHV-6 levels may be a good biomarker of QOL in patients during chemotherapy, and that AHCC may have a beneficial effect on chemotherapy-associated adverse effects and QOL in patients with cancer undergoing chemotherapy.

INTRODUCTION

With the recent advances in medicine and medical technology, disease structure has changed from acute to chronic disorders, most of which are lifestyle diseases including cancer, heart disease, stroke, diabetes, and obesity. In particular, cancer is the leading cause of death in Japan after 1981. Although the outcome of cancer therapy has improved, cancer takes on a serious aspect of systemic disease once it goes beyond a particular point. Complete recovery following a single treatment is difficult, and a multidisciplinary approach with a combination of surgery, chemotherapy, radiation, and immunotherapy is usually necessary.

Generally, surgery is performed, providing local treatment with curative intent. On the assumption of distant micrometastases, neoadjuvant or adjuvant chemotherapy is further considered with or without irradiation.

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Chemotherapy recently improves the outcome of cancer treatment. However, some patients are forced to discontinue chemotherapy or drop out of a clinical trial due to adverse effects, such as gastrointestinal disturbances and suppression of bone marrow function. Also, some patients discontinue therapy due to a decline in quality of life (QOL) and reduction in emotional strength during chemotherapy.

Some trials are aimed at reducing chemotherapy-induced adverse effects using complementary and alternative medicine (CAM), for which evidence of safety and efficacy is mostly not well established. However, mushroom preparations have a well-known, long history of immune support. One mushroom mycelium extract, active hexose correlated compound (AHCC), is a culture extract of mycelium of *Lentinula edodes* (Basidiomycetes family of fungi). According to basic and clinical studies, it has been reported that AHCC has various actions, such as anti-oxidant activity (1), antiinflammation (2), antitumor effects (3,4), antiinfectious effects (5), and immunoenhancing activity (6).

To evaluate the efficacy of such a functional food to reduce adverse effects during chemotherapy, an objective biomarker indicator is necessary. Recently, we considered human herpes viruses (HHV), which are reactivated by physical and psychological stresses, as possible biomarkers. We particularly focused on HHV-6, a causative virus for exanthema subitum (7), because most people (over 90%) are infected during childhood, and life-long latency is established. There is an interesting article that after cognitive task trials, moderate- to long-term fatigue was positively associated with the copy number of saliva HHV-6 DNA (8).

It is also reported that HHV-6 sometimes reactivates in the transplant recipients which can variably be associated with important outcome (9,10). Thus, we hypothesized that HHV-6 might be available as a possible biomarker of fatigue during chemotherapy.

The purpose of this study was to evaluate the safety and effectiveness of AHCC on chemotherapy-induced adverse effects and QOL in patients with cancer. We also examined whether HHV-6 can be an objective surrogate biomarker for QOL in patients undergoing chemotherapy.

METHODS

Patients

The characteristics of the patients are shown in Table 1. Twenty-four patients with pancreatic (9), ovarian (7), lung (5), and colorectal cancers (3) were enrolled in this study. These patients received different types of chemotherapy, such as paclitaxel (PTX), carboplatin (CBDCA), irinotecan, and docetaxel, as appropriate. Patients were in various clinical stages, including stage I (6), Stage II (2), Stage III (9), and Stage IV (7). This study (#051138) was approved by the ethical committee in Osaka University Hospital.

TABLE 1
Characteristics of cancer patients

	<i>n</i>	Age	Male:		Chemotherapy
			Female	Stage	
Total	24	59.3 (36–76)	9:15		
Colon	3	62.7 (60–65)	1:2	III: 2, IV: 1	UFT+CF; 2, CPT-11; 1
Pancreas	9	63.8 (52–76)	4:5	I: 1, III: 2, IV: 6	GEM: 8, S-1; 1
Lung	5	64.0 (57–70)	4:1	I: 3, II: 2	CBDCA+PTX; 5
Ovary	7	48.7 (36–67)	0:7	I: 2, III: 5	GEM; 3, PTX; 1, CBDCA+PTX; 2, CBDCA+DTX; 1

CBDCA = carboplatin; CF = calcium folinate; CPT-11 = irinotecan; DTX = docetaxel; GEM = gemcitabine; PTX = paclitaxel; S-1 = tegafur/gimeracil/oteracil potassium; UFT = tegafur/uracil. UFT+CF = UFT 300–600 mg + CF 75 mg (p.o., 3 times a day) for 28 days followed by 1-wk withdrawal period; CPT-11 = 40 mg/m² on Days 1, 2, 3, 8, 9, & 10 as a 1-h infusion followed by 2- to 3-wk withdrawal period; GEM = 1000 mg/m² on Days 1, 8, and 15 as a 30-min infusion followed by 1-wk withdrawal period; S-1 = 40–60 mg (p.o., twice a day) for 28 days followed by 1-wk withdrawal period; CBDCA+PTX = CBDCA 300–400 mg/m² +PTX 200 mg/m² on Day 1 as a 1-h infusion followed by 3-wk withdrawal period; CBDCA+DTX = CBDCA 300–400 mg/m² +DTX 60 mg/m² on Day 1 as a 1-h infusion followed by 3-wk withdrawal period.

Protocol Design and Characterization of AHCC

This clinical study involved 2 courses of chemotherapy: without AHCC and with AHCC treatment. AHCC, which was donated from Amino Up Chemical Co., Ltd. (Sapporo, Japan), was given to the patients at a dose of 3 g/day, p.o.

AHCC is primarily composed of carbohydrates (approximately 70%), protein (13%), ash contents (9%), fats (2%), and fiber (2%) (11,12). A significant portion of the carbohydrates (approximately 20%) is composed of α -1,4-glucans. The relatively high concentration of α -glucans (15.8/100 g) as compared with β -glucans (0.2/100 g) is considered to be secondary to the production process and is thought to be a major contributor to its pharmacologic effects (11,13).

AHCC is manufactured with a certification of ISO 9001:2008 as a quality control (Quality Management Systems), with that of ISO 22000:2005 (Food Safety Management Systems), and also with a GMP grade certified by Japan Health Food & Nutrition Association.

Evaluation of Adverse Effects

The adverse effects, especially hematotoxicity and hepatotoxicity, during chemotherapy were evaluated with the National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Event (CTCAE) v3.0. The numbers of leukocyte,

neutrophil, lymphocyte, and platelet were measured. Biochemical tests of blood, such as hepatic [alanine (ALT), aspartate aminotransferase (AST), etc.] and renal functions (serum creatinine, blood urea nitrogen), were also measured.

Measurement of Salivary HHV-6 DNA Levels by Quantitative Real-Time PCR

Viral DNA was purified from serum or liquor with an EZI Virus Mini Kit v2.0 (QIAGEN). PCR reactions were performed on an Applied Biosystems 7300 apparatus (Life Technologies, Carlsbad, CA, USA). The amplification mixture (50 μ l) contained 0.9 μ M primers, 0.25 μ M probe, and 1 \times Premix Ex Taq (Perfect Real Time; Takara Bio Inc., Ohtsu, Shiga, Japan). Primers were HHV-6 (forward): 5'-GACAATCACATGC CTGGATAATG-3', HHV-6 (reverse): 5'-TGTAAGCGTGTGGTAATGGACT A -3', And HHV-6 probe: FAM-AGCAGCTGGCGAAAAGTGCTGTGC-TAMRA.

The conditions for amplification were as follows: 1 cycle of 95°C for 30 s, followed by 50 cycles of 95°C for 5 s, and 60°C for 31 s. Reactions for each primer set were performed in triplicate, and the threshold cycle numbers were averaged.

Assessment of QOL

Patients' QOL was evaluated with the EORTC QLQ-C30 questionnaire (v3.0) (14), which was especially developed to assess the QOL of patients with cancer.

Statistical Analyses

The results are expressed as the means \pm SE. Statistical analyses were performed with a paired *t*-test. A *P* value < 0.05 was considered significant.

RESULTS

Hematological Findings and Blood Chemistry

Adverse effects during chemotherapy were evaluated using NCI-CTCAE v3.0. The changes in adverse effects regarding hematology and blood chemistry are shown during the chemotherapeutic courses with and without AHCC in Table 2.

Leukocytopenia was found in 15 cases including of 12 cases of Grade 1–2 and 3 cases of Grade 3–4 after the first course of chemotherapy. After the second course, however, all 14 cases were within Grade 1–2. Four cases showed neutropenia with Grade 3–4 after the first course. However, these 4 cases shifted to Grade 1–2 after the second course (Table 2). Also, the incidence of thrombocytopenia decreased from 7 cases after the first course to 3 cases after the second course. The number of neutrophils significantly increased from 1633.8 ± 220.7 after the first course to 2110.3 ± 197.6 after the second course (*P* = 0.003; Fig. 1). Also, the decreasing rate of neutrophils with AHCC significantly improved as compared to that without AHCC (*P* = 0.002).

Regarding hepatotoxicity (as measured by ALT), 7 cases were within Grade 1–2 after the first course, and this number

TABLE 2
Improvement of hematotoxicity and hepatotoxicity during chemotherapy by AHCC

	1st course		2nd course (AHCC)	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
WBC	12	3	14	0
Neu	8	4	12	0
Hb	19	0	20	0
PLT	7	0	3	0
ALT	7	0	1	0

The grade of adverse effects was determined by Common Terminology Criteria for Adverse Events v3.0. AHCC = active hexose correlated compound; WBC = white blood cell; Neu = neutrophil; Hb = hemoglobin; PLT = platelet; ALT = alanine aminotransferase.

decreased to only 1 case after the second course. The value of ALT significantly decreased from 32.6 ± 5.4 (7–108) U/L after the first course to 22.8 ± 2.6 (6–66) U/L after the second course (*P* < 0.05). A typical clinical case with hepatotoxicity is shown in Fig. 2. The patient was a 57-year-old female with lung cancer receiving CBDCA + PTX as adjuvant chemotherapy. She had an increased ALT level of 80 U/L 1 wk after the initiation of chemotherapy, and a higher level over 100 U/L at the third wk. However, ALT level remarkably decreased at the fourth wk after the intake of AHCC and reached a normal range by the fifth week.

Analysis of HHV-6 in Saliva

The DNA levels of HHV-6 in saliva significantly increased during the first course of chemotherapy, but administration of

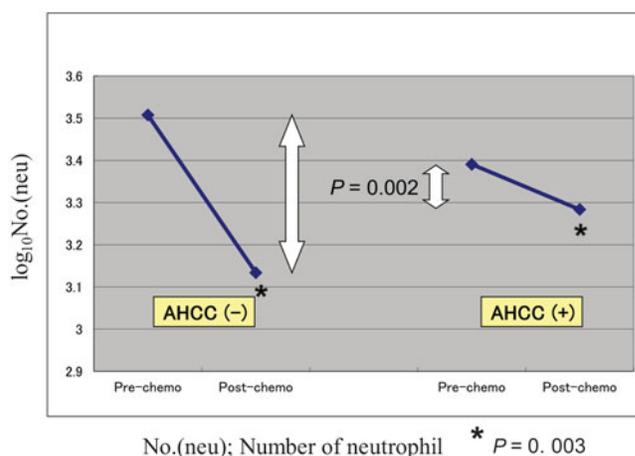


FIG. 1. Changes of neutrophil number during chemotherapy. The longitudinal axis is expressed as a common logarithm of neutrophil number. The number of neutrophils after the second course of chemotherapy significantly increased as compared to that after the first course (*P* = 0.003). The decreasing rate of neutrophils with active hexose correlated compound (AHCC) treatment significantly improved as compared to that without AHCC (*P* = 0.002). Statistical analyses were performed with a paired *t*-test. (Color figure available online.)

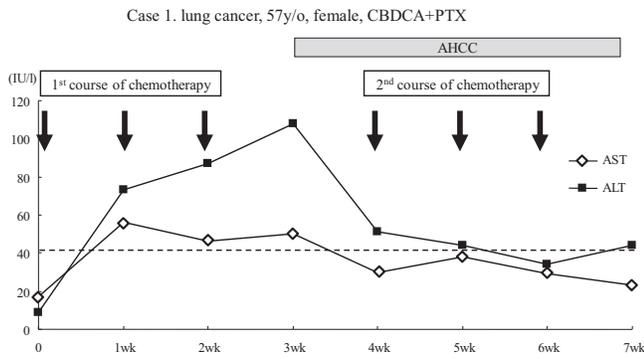


FIG. 2. A case with improvement of hepatotoxicity during chemotherapy by active hexose correlated compound (AHCC). Chemotherapy-induced hepatotoxicity improved in 6 out of 7 patients by AHCC. This figure displays the representative case.

AHCC significantly decreased the levels of HHV-6 in saliva from 1082.9 ± 333.6 to 253.7 ± 51.8 copies/ml (mean \pm SE; $P < 0.05$) (Fig. 3).

Analysis of QOL

Using the EORTC QLQ-C30 questionnaire, the score for the global health status/QOL after chemotherapy with AHCC significantly increased in female patients compared to that without AHCC (Fig. 4). Interestingly, AHCC significantly improved the symptoms of appetite loss in female patients (Fig. 5a) and dyspnea in all patients (Fig. 5b). Also, AHCC had a tendency to reduce the symptoms of fatigue in all patients (data not shown).

DISCUSSION

The term *complementary medicine*, which includes the entire spectrum of medicines that attempt to improve the QOL by adding them to current medicine, has recently been incorporated into a broad term, *complementary and alternative*

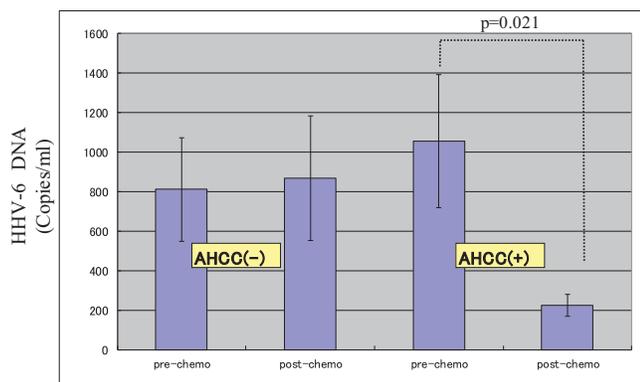


FIG. 3. The level of HHV-6 DNA in saliva during chemotherapy with or without active hexose correlated compound (AHCC) treatment. Salivary HHV-6 DNA levels significantly decreased in the second course of chemotherapy with AHCC ($P = 0.021$). Statistical analyses were performed with a paired t -test. (Color figure available online.)

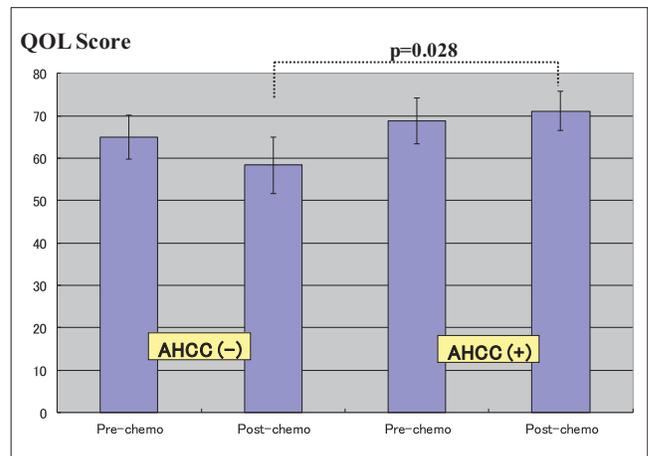


FIG. 4. EORTC QLQ-C30—quality of life (QOL) scale (female). The QOL scale significantly improved in female patients after the second course of chemotherapy with active hexose correlated compound (AHCC) ($P = 0.028$). Statistical analyses were performed with a paired t -test. (Color figure available online.)

medicine (CAM). An American study in 1993 reported that as many as 60 million U.S. adults (33.8%) had used some type of CAM modality (13). Several years later, this rate had surprisingly increased to 42.1% (83 million) when resurveillance regarding this issue was performed (16). Unfortunately, however, not enough evidence for the safety and efficacy of CAM has been generally reported. Therefore, many clinical studies of CAM have been performed at the National Center of CAM in the National Institutes of Health to assess safety as well as efficacy.

Changes in attitudes about treating the whole person, not just their disease, have recently been reported. Receiving information from the internet and other sources about alternative treatments, patients are now also asking for the treatments focused on improving QOL. Very recently, a new wave of medicine was introduced called *integrative medicine*, which is healing-oriented medicine that considers the whole person (body, mind, spirit) as well as aspects of lifestyle (17).

A surveillance study regarding CAM use in Japan was reported that 44.6% of patients with cancer used some type of CAM modality, almost all of which (96.2%) were dietary supplements mostly comprising of mushroom products (18).

Although the immunoenhanced mechanism of β -glucans including in mushrooms is well-known, that of α -glucans is less well studied. Recently, cytokine profiles in the culture supernatants of bone marrow derived cells (BMDC) from wild type, MyD88/TICAM-1 KO, and TLR2 KO mice demonstrated that IL-6 production was observed by ELISA in the culture supernatants of wild type-BMDC stimulated by the α -glucan fraction (1mg/ml) for 24 h, but not in those of BMDC derived from MyD88/TICAM-1 KO and TLR2 KO mice (T. Seya, personal communication).

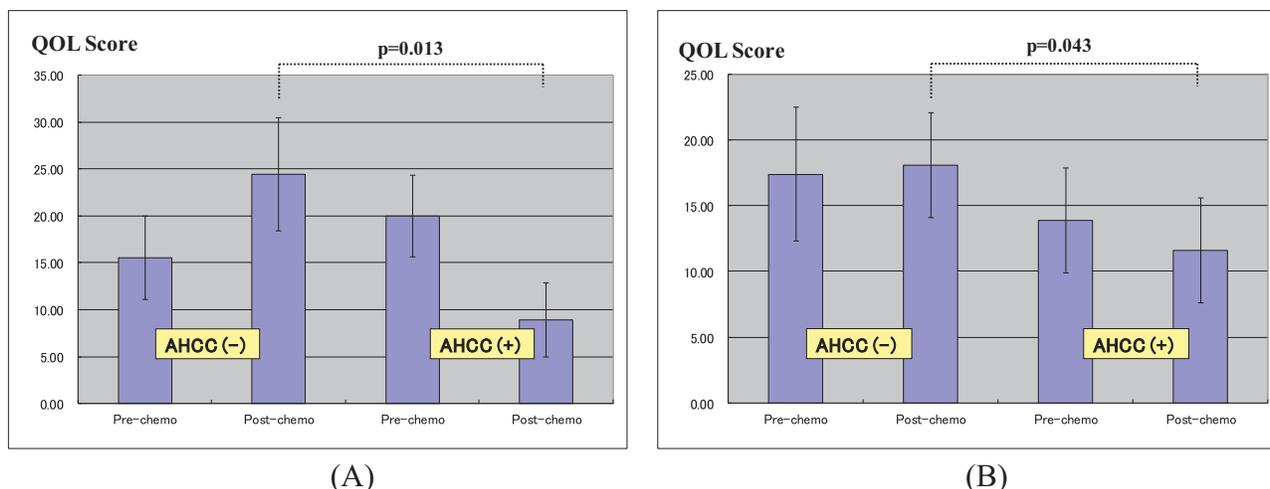


FIG. 5. A: EORTC QLQ-C30: Appetite loss (female). The quality of life (QOL) scale regarding appetite loss significantly improved in female patients after the second course of chemotherapy with active hexose correlated compound (AHCC) ($P = 0.013$). Statistical analyses were performed with a paired t -test. B: The QOL scale regarding dyspnea significantly improved after the second course of chemotherapy with AHCC ($P = 0.043$). Statistical analyses were performed with a paired t -test. (Color figure available online.)

Although various functions for AHCC have been reported mainly from basic research (1–6), no clinical reports regarding the reduction of adverse effects during chemotherapy with AHCC have been reported. Our current clinical study demonstrated that AHCC provided significant beneficial effects on hepatotoxicity and hematotoxicity. Because the antioxidative actions of AHCC are moderate, such a function is less likely to play a role in combination with chemotherapeutic agents. It remains unknown whether some of the beneficial effects of AHCC are due to mild antioxidant action or other functions. Further evaluation will be necessary to delineate the mechanism.

Some dietary supplements are known to interact with some chemotherapeutic agents via cytochrome P450 (CYP), a drug metabolizing enzyme in the liver. Because AHCC does not inhibit CYP2C8, 2C9, 2D6, or 3A4, a major drug metabolizing enzyme, AHCC is unlikely to interact with different drugs, including chemotherapeutic agents (19). Further, CYP induction metabolism assays indicate that AHCC induces CYP2D6 in the same way as doxorubicin. However, the overall data suggest that AHCC will be safe to administer with most other chemotherapeutic agents that are not metabolized via the CYP2D6 pathway.

In our current study, the scores for the global health status (female), appetite loss (female), and dyspnea (male and female) significantly increased after chemotherapy with AHCC by the EORTC QLQ-C30 questionnaire. This result is likely to reflect the reduction of adverse effects during chemotherapy.

HHV-6, a β herpes virus, generally remains latent in monocytes/macrophages (20) and the central nervous system (21) after a primary infection. Most of people (>90%) are infected with exanthema subitum during infancy until the age of 1 yr (22). Febrile convulsion during childhood are considered to be the reactivation of HHV-6 (21). There are 2 HHV-6 variants,

A & B. In our study, we used the common primers/probe for HHV-6A and HHV-6B. Although HHV-6B is present in almost 100% of the world's population, HHV-6A appears to be less frequent in Japan (23).

HHV-6 is frequently reactivated by physical and psychological stresses and is shed into saliva. Reactivation is also associated with serious diseases, such as chronic fatigue syndrome (24). The association between HHV-6 reactivation and work-induced fatigue was examined in healthy office workers (K. Kondo, personal communication). Reactivated HHV-6 was detected in the saliva of 80% of participants who worked overtime for 1 wk. However, HHV-6 was detected on only 23% of participants following a 1-wk holiday. Therefore, salivary HHV-6 DNA levels appear to be useful as an indicator of chronic fatigue (25). Therefore, we hypothesized that the reactivation of HHV-6 might occur also in the chemotherapy-induced fatigue. The precise mechanism of HHV-6 reactivation during chemotherapy remains unclear, but the reactivation might be explained by some immune suppressive mechanism due to the activation of macrophage where HHV-6 virus remains latent.

In our study, HHV-6 DNA levels increased during the first course of chemotherapy, but decreased remarkably at the end of the second course. These results suggested that AHCC may contribute some immune enhancing effects during chemotherapy, resulting in inhibiting reactivation of HHV-6. These immunologically activating effects of AHCC treatment may also explain the observed inhibition of leukopenia, neutropenia, thrombocytopenia, and impairment of liver function. A protective effect of AHCC on liver damage was reported in a clinical study (4) and an animal model (26).

Finally, in the present study, the patient number was small, further investigation will be necessary. We conclude that a

conventional dose of AHCC had no harmful effect on patients with cancer undergoing chemotherapy and may have some beneficial effects on chemotherapy-induced adverse effects. In addition, the level of HHV-6 DNA may be a good objective indicator of QOL in patients receiving chemotherapy.

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