RESEARCH ARTICLE

The Effects of Active Hexose Correlated Compound (AHCC) on Levels of CD4+ and CD8+ in Patients with Epithelial Ovarian Cancer or Peritoneal Cancer Receiving Platinum Based Chemotherapy

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Abstract

Background: Adjuvant chemotherapy is a required treatment for most patients with epithelial ovarian cancer (EOC) or peritoneal cancer. However, it has many adverse events which may affect oncologic outcomes. Active hexose correlated compound (AHCC) has been reported to be an immunoenhancer to decrease adverse events of chemotherapy. **Materials and Methods:** Patients were randomized and allocated to receive either AHCC three grams/day (500mg/capsule) or placebo. These drugs were administrated as two capsules orally three times a day throughout six cycles of chemotherapy. The primary outcome was a change of CD4+ and CD8+ T cell lymphocytes in peripheral blood samples from baseline to completion of chemotherapy. Secondary outcomes were rate of bone marrow suppression, adverse events and quality of life (QOL) as assessed by Thai version of the Functional Assessment of Cancer Therapy-General (FACT-G). **Results:** Study outcomes were analyzed in 28 patients, 14 patients in each group. Changes in CD4+ and CD8+ T cell lymphocytes levels were not significantly different between AHCC and placebo group; 43.5/ul (-237.5, 143.3) versus -69.5 /ul (-223.8, 165) for CD4+ level, p=0.61 and 49.5.0 /ul (-80, 153.3) versus 4.0 /ul (-173, 62.5) for CD8+ level, p=0.19. However, CD8+ levels were significantly higher in the AHCC group at the sixth cycle of chemotherapy; 392.5.0 /ul (310.8, 598) versus 259.5 /ul (170.5, 462.3), p=0.03. There was no difference in bone marrow suppression and QOL between the two groups. Adverse events in terms of nausea and vomiting significantly decreased but muscle pain significantly increased in the AHCC group. **Conclusions:** Changes in CD4+ and CD8+ T cell lymphocytes from baseline were not significantly increased in AHCC group. However, CD8+T cell lymphocytes levels were significantly higher in the AHCC group at the sixth cycle of chemotherapy.

Keywords: Active hexose correlated compound- chemotherapy- epithelial ovarian cancer- immunoenhancer

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Introduction

Epithelial ovarian cancer (EOC) is the second most common gynecologic malignancy and has the highest mortality rate in North America and Europe (Ferlay et al., 2008; Siegel et al., 2012). Although there is lower incidence of primary peritoneal cancer than EOC, its incidence rate has increased more rapidly over the past 10 years. Spreading patterns of peritoneal cancer are similar to EOC (Goodman and Shvetsov, 2009). Principal treatment for EOC is primary cytoreductive surgery and adjuvant chemotherapy is given to all patients except those in stage IA, IB grade 1 and 2. Combination treatment of platinum and taxane is the standard regimen improves progression free survival and overall survival (McGuire et al., 1996; Ozoles et al., 2003). Unfortunately, chemotherapeutic agents are indiscriminate in their effects. Both malignant and normal tissue are affected and especially proliferate cell populations. Common adverse events are nausea, vomiting, hematologic toxicity, such as bone marrow suppression (anemia, leukopenia and thrombocytopenia), increased risk of spontaneous hemorrhage, infection, fatal sepsis and immunosuppression. These adverse events may delay treatment, decrease the dosage of chemotherapy or require a change to another regimen, which may affect oncologic outcomes.

Active hexose correlated compound (AHCC) is a nutritional supplement produced from the mycelia of shiitake mushrooms, containing a mixture of polysaccharides, amino acids and minerals. About 74% of AHCC is made up oligosaccharides and 20% of these contain α-1,4-glucans with a mean molecular weight under 5,000 Daltons that are believed to enhance AHCC biologic activities. AHCC therefore, acts as an immunoenhancer against pathogens and tumor cells (Lee et al., 2012; AHCC Thailand, 2013). A study in a healthy elderly population

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who took three grams of AHCC per day found that AHCC could enhance CD4+ and CD8+ immune responses which remained up to 30 days after AHCC discontinuation (Yin et al., 2010). The mechanism of AHCC action stimulates monocytes to promote T helper cell response and induce levels of IL-1β production that can promote cytokine (IL-17 and IFN-γ) production from CD4+ T cell lymphocytes (Lee et al., 2012). A study in mice showed that the group receiving gemcitabine 200 mg/m2 plus AHCC 1g/kg had higher white blood cells and hemoglobin than the group that received gemcitabine alone (Nakamoto et al., 2012). This was similar to a study in Japan which reported that AHCC can prevent anemia in pancreatic and biliary cancer patients who received gemcitabine. AHCC also had an antitumor effect in an animal model. AHCC significantly delayed tumor development in mice after inoculation of either melanoma or lymphoma and enhanced tumor immune surveillance through regulating both innate and adaptive immune responses (Gao et al., 2006). Another study showed that inoculating colon-26 tumor cells with cisplatin plus AHCC significantly enhanced antitumor effects in both size and weight of tumor, increased food intake, improved nephrotoxicity and modulated bone marrow suppression compared to placebo (Hirose et al., 2007). Moreover, cancer patients who received AHCC with chemotherapy and radiation had better quality of life (QOL) than patients who received chemotherapy and radiation alone (Kidd, 2000).

In this study, we aim to evaluate whether AHCC can improve immune response and decrease adverse events, especially bone marrow suppression in EOC or peritoneal cancer patients who received platinum based chemotherapy.

Materials and Methods

Study design

This study was a randomized trial, double blinded, controlled trial approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University. Randomization was done by block of four using a random number generator. Informed consent was obtained prior to study initiation.

Study population

Thirty-two EOC or peritoneal cancer patients were assigned to receive platinum based chemotherapy. Inclusion criteria were patients who were chemotherapy naive, aged between 20-70 years, no known immunodeficiency diseases or hematologic diseases, no recent use of immunosuppressive drugs and having normal liver and kidney function tests. Patients were excluded if they received prior radiation or concurrent chemotherapy, ECOG performance status of more than two or had bone marrow metastasis.

Study treatments

Patients were allocated into two groups to receive either AHCC three grams/day (500mg/capsules) or placebo identical in appearance. These drugs were administrated as two capsules orally three times a day throughout six cycles of chemotherapy. Patients were not allowed to receive other drugs or nutritional supplements that might affect the results of this study after enrollment.

Outcome measures

Baseline characteristics, ECOG performance status, stage, histopathology and surgical outcome were recorded. Peripheral blood was collected at baseline and every cycle of chemotherapy for complete blood count. CD4+, CD8+ T cell lymphocytes levels from peripheral blood were measured at baseline and after the first, third and sixth cycle of chemotherapy. CD4+ and CD8+ T cell lymphocytes levels were determined using flow cytometry where lymphocyte subsets were stained with specific monoclonal antibody (FITC for CD3+, and PE for CD4+ and CD8+) that were tagged with fluorescent substances and measured by Flowcytometer, version FACSCalibur. Molecules were flowed past a laser beam where they absorbed energy and fluoresced in different colors and then transferred to sensors for molecule evaluation.

Adverse events of chemotherapy were assessed by the record book of the South West Wales Cancer Network. Quality of life (QOL) was assessed by the Thai version of Functional Assessment of Cancer Therapy-General (FACT-G) that consists of four domains: physical, social and family, emotional and functional well-being. A higher score represents better QOL. Both adverse events and QOL were assessed at one week after every cycle of chemotherapy. Patient compliance was checked by the number of remaining capsules. Hemoglobin of less than 10 g/dl, white blood cells of less than 3,000/ul, absolute neutrophil of less than 1,500/ul, and platelets of less than 100,000/ul were defined as anemia, leukopenia, neutropenia and thrombocytopenia, respectively. The primary outcome was a change in CD4+ and CD8+ T cell lymphocytes levels from baseline to completion of chemotherapy. Secondary outcomes were rate of bone marrow suppression, adverse events and QOL scores. Outcomes were analyzed only in patients who completed the treatment that was originally allocated (per-protocol analysis).

Statistical analysis

Sample size calculations were based on a pilot study of eight patients (AHCC = 4, placebo = 4) by the change of CD8+ levels at the completion of chemotherapy [AHCC=105.0 /ul (45.8, 383.3), placebo=-34.0 /ul (-193, 4)]. To detect a significant difference between these two treatments with a power of 90% and type I error of 5%, the calculated number of patients was 13 patients per group. After factoring in a 20% drop out rate, the sample size required was 16 patients per group. Data were analyzed using statistical package SPSS Version 21. Normal distribution of data was tested with Kolmogorov-Smirnov test. Parametric descriptive data were described as mean ± SD or percentage and compared with Student t test and Chi-square test or Fisher Exact test, respectively. Nonparametric continuous data were shown as median (interquartile range; IQR) and were compared with Mann-Whitney U test. Statistical significance was considered if p values were less than 0.05.

Results

A total of 32 patients, 16 patients in AHCC group and 16 patients in placebo group, were included in the study. Baseline characteristics of patients including age, body mass index (BMI), menopausal status, parity, ECOG performance status, stage and histological characteristics and surgical outcomes were similar between the two groups (Table 1). Four patients dropped out: one had AHCC allergy presenting with rash and dizziness, one in placebo group had progressive disease and one patient from each group discontinued the study. Therefore, study outcomes were analyzed in 28 patients. AHCC administration did not significantly increase CD4+ and CD8+ T cell lymphocytes levels after the completion of six cycles of chemotherapy when compared with placebo, 43.5.0 /ul (-237.5, 143.3) versus -69.5 /ul (-227.8, 165) for CD4+ level, p=0.61 and 49.5 /ul (-80, 153.5) versus 4.0 /ul (-173, 62.5) for CD8+ level, p=0.19. However, the level of CD8+T cell lymphocytes in the AHCC group was significantly higher than placebo group at the sixth cycle of chemotherapy, 392.5.0 /ul (310.8, 598) versus 259.5 /

Table 1. Patient's Characteristics

Characteristic	AHCC (N=16)	Placebo (N=16)	P value
Mean age,years±SD	51.6±8.2	54.6±8.6	0.33
Mean BMI,kg/m2±SD	22.3±2.4	22.3±4.6	0.48
Parity (%)			
Nulliparous	4 (25.0)	9 (56.3)	0.15
Multiparous	12 (75.0)	7 (43.8)	
Menopause (%)			
Premenopause	7 (43.8)	4 (25.0)	0.46
Postmenopause	9 (56.3)	12 (75.0)	
ECOG performance status (%)			
0	5 (31.3)	3 (18.8)	
1	10 (56.3)	10 (62.4)	0.69
2	2 (12.4)	3 (18.8)	
Stage (%)			
I	5 (31.2)	8 (50.0)	
II	5 (31.2)	1 (6.3)	0.22
III	5 (31.2)	4 (25.0)	
IV	1 (6.4)	3 (18.8)	
Celltype (%)			
Serous	6 (37.4)	7 (45.4)	
Mucinous	1 (6.3)	0 (0)	
Endometrioid	3 (18.7)	3 (18.7)	0.82
Clear cell	5 (31.3)	4 (25.0)	
Round cell	0 (0)	1 (6.3)	
Surgical outcome (%)			
Optimal	14(87.5)	10 (62.5)	0.22
Suboptimal	2(12.5)	6(37.5)	
Neoadjuvant chemotherapy (%)	4(25.0)	5(31.3)	1
Baseline CBC			
Median hemoglobin,g/dl (IQR)	10.7(9.8,12.3)	11.1(10.5,12.7)	0.49
Mean hematocrit, % ±SD	34.2±4.4	34.9±4.5	0.64
Median white blood cell, /µl (IQR)	7,230.0(6,220,8,232.5)	6,945.0(5,042.5,9,350)	0.79
Median neutrophil, /µl (IQR)	4,645.0(4,020,6,245)	4,140.0(2,962.5,7,391.3)	0.57
Mean platelets, /µl ±SD	433,312.5±169,049.8	396,812.5±129,453.3	0.49
Treatment outcome			
Complete response (%)	14(87.4)	11(68.8)	
Partial response (%)	0(0)	2(12.5)	0.44
Stable disease (%)	1(6.3)	2(12.5)	
Progressive disease (%)	1(6.3)	1(6.2)	

SD, standarddeviation; BMI, bodymassindex; ECOG, TheEasternCooperativeOncologyGroup; CMT, chemotherapy; CBC, completebloodcount; IQR, interquartilerange

Table 2. CD4+ T-Cell Lymphocyte Level

CD4+ level (/ul)	AHCC (N=14) [median (IQR)]	Placebo (N=14) [median (IQR)]	P value
Baseline	579.5 (383.5, 950.5)	583.0 (311.0, 704.3)	0.42
After C1	651.0 (487.3, 814)	542.0 (361.0, 788)	0.29
After C3	674.0 (528.8, 810)	549.5 (461.8, 809.5)	0.87
After C6	623.0 (484.5, 771.5)	552.5 (311.8, 661.3)	0.17
C1-baseline	18.0 (-70, 134.5)	81.5 (-65, 123)	0.68
C3-baseline	46.0 (-144.3, 217.5)	52.0 (-33.3,143.5)	0.44
C6-baseline	43.5 (-237.5, 143.3)	-69.5 (-223.8, 165)	0.61

C, cycle of chemotherapy; IQR, interquartile range

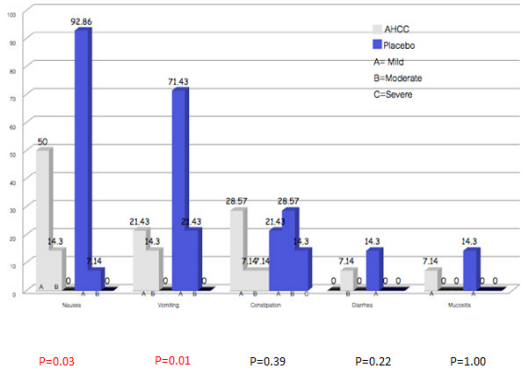


Figure 1. Adverse Events of Chemotherapy

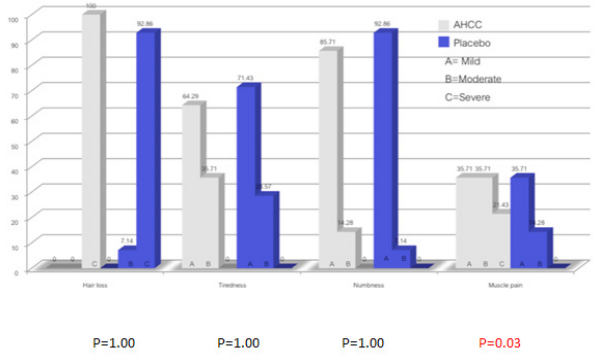


Figure 2. Adverse Events of Chemotherapy

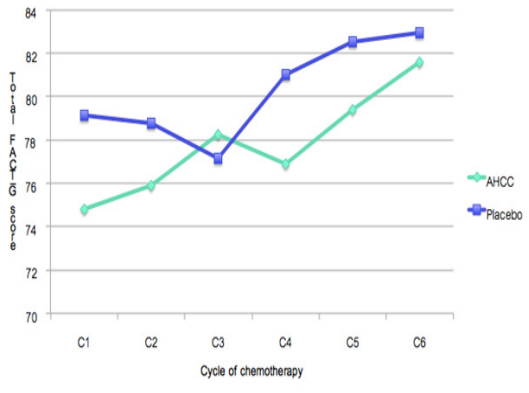
ul (170.5, 462.3), p=0.03. (Tables 2 and 3)

There was no significant difference in the rate of bone marrow suppression including anemia (57.1% versus 28.6%, p=0.25), leukopenia (28.6% versus 14.3%, p=0.65), neutropenia (42.9% versus 50.0%, p=1.00) and thrombocytopenia (7.1% versus 7.1%, p=1.00). Delayed chemotherapy for one week was used in patients with neutropenia and none of the patients received granulocyte colony-stimulating factor (G-CSF). Adverse events are shown in Figures 1 and 2. Severity of adverse events was graded as mild, moderate and severe. The placebo group had a significantly higher rate of mild to moderate nausea and vomiting. In contrast, the AHCC group had a significantly higher rate of moderate to severe muscle pain. QOL determined by FACT-G scores was not significantly different between the two groups in all domains (Figure 3).

There was no significant difference in the number of remaining tablets (61.1±41.7 versus 67.0±37.9, p=0.69). Results showed that 92.5% and 91.9% of total tablets were used in AHCC and placebo groups, respectively (p=0.75).

Discussion

T cell lymphocytes are an essential component of the immune system to fight against pathogens and tumor



Abbreviation: C = cycle of chemotherapy

Figure 3. Total FACT-G Score

Table 3. CD8+ T-cell Lymphocyte Level

CD8+ level(/u)	AHCC (N=9) [median (IQR)]	Placebo (N=9) [median (IQR)]	P value
Baseline	397.0 (311.5,518)	373.0 (192.8,487.8)	0.50
After C1	357.5 (303.5,583.8)	332.0 (194.3,536.8)	0.46
After C3	451.5 (261.0,666.8)	353.0 (256.5,548.3)	0.48
After C6	392.5 (310.8,598)	259.5 (170.5,462.3)	0.03
C1-baseline	50.0 (-42,122.5)	36.5 (-80.5,105.5)	0.72
C3-baseline	76.0 (-86.8,161.3)	37.0 (-55.3,100.3)	0.76
C6-baseline	49.5 (-80,153.3)	4.0 (-173,62.5)	0.19

C, cycle of chemotherapy; IQR, interquartile range

cells. They work together with NK cells, macrophages and antibody producing B cell lymphocytes to protect the body. Active hexose correlated compound (AHCC) was reported as an immunostimulator for both innate and adaptive immunity with anti-tumor antibodies (Gao et al., 2006; Hirose et al., 2007). Furthermore, AHCC decreases both bone marrow suppression (Nakamoto et al., 2012) and adverse events of chemotherapy (Hirose et al., 2007) while improving QOL (Kidd, 2000) in cancer patients. The mechanisms of AHCC for enhancing the immune system have remained unclear. High contents of carbohydrates may play a role in the regulation of the immune system. Carbohydrates can contribute T cells to recognize epitopes on the surface of an antigen presented by major histocompatibility complex (MHC) molecules (Acvi et al., 2013). AHCC can stimulate monocytes to promote a T helper cell response and induce IL-1β production, which can promote cytokine (IL-17 and IFN-γ) production from CD4+ T cell lymphocytes (Lee et al., 2012; AHCC Thailand, 2013). Moreover, glucans in AHCC contribute to improving the mechanism of antigen presentation by dendritic cells to induce tumor specific cytotoxic T cell lymphocytes (Mushiake et al., 2005). Therefore, CD4+ T lymphocytes promote an anti-tumor immune response by producing cytokines. In contrast, CD8+ T lymphocytes can directly mediate the lysis of tumor cells (Dorigo and Berek, 2015). CD4+ and CD8+ tumor infiltrating T cell lymphocytes, especially CD8+ tumor infiltrating T cell lymphocytes, are useful immunological biomarkers in predicting survival (Zhang et al., 2003). The presence of tumor infiltrating T cell lymphocytes correlates with progression-free and overall survival in advanced ovarian cancer patients (Hwang et al., 2012). In contrast to previous studies (Kidd, 2000; Hirose et al., 2007; Nakamoto et al., 2012), our study shows that AHCC administration cannot significantly increase CD4+ and CD8+ levels after completion of six cycles of platinum based chemotherapy. Moreover, bone marrow suppression and QOL were not significantly different between both groups. This might be due to a sample size with not enough power to reach statistical significance. Sample size calculations in this study were from a pilot study, which showed a higher change of CD8+ levels at the completion of chemotherapy than in the final results. Therefore, a larger sample size is required to reach statistical significance. However, our finding of the significantly higher CD8+ level at the sixth cycle of chemotherapy in the AHCC group was promising. The level of CD8+ T cell lymphocytes has been reported to be one significant prognostic factor. Although, there was statistical significance, clinical significance was unclear. Further study with a larger sample size and longer follow up period is needed.

There was only one patient in AHCC group who had a drug allergy presenting with rash and dizziness on the second day after AHCC administration. Symptomatic treatment with chlorpheniramine was given to the patient who then had complete recovery. No other serious adverse event was found in this study. However, AHCC can significantly decrease adverse events in terms of nausea and vomiting.

The strength of this study was its design as a randomized double blinded controlled trial. To our best knowledge, this was the first study that evaluated the effects of AHCC on levels of CD4+ and CD8+ in EOC or peritoneal cancer patients receiving platinum based chemotherapy. However, the limitation was the small sample size. Further studies should be conducted with a larger number of patients, longer follow up time and more robust evaluation of the survival outcomes in these patients.

The level of CD4+ and CD8+ T cell lymphocytes did not significantly change from the baseline in AHCC group compared with placebo. However, CD8+ T cell lymphocytes levels were significantly higher in the AHCC group at sixth cycle of chemotherapy. Although, bone marrow suppression and QOL were not significantly different, some adverse events, particularly nausea and vomiting, were found less frequent in the AHCC group.

Conflict of interest

The authors declare that AHCC Thailand Co., Ltd. supported AHCC and placebo but had no other involvement in study design, data collection or analysis.

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