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Active Hexose Correlated Compound (AHCC) Improves Immunological Parameters and Performance Status of Patients with Solid Tumors

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Introduction

In Japan, three conventional therapies, surgery, chemotherapy and radiation have shown some efficacy against malignant tumors. According to yearly reports, the number of patients who have died as a result of cancer has not decreased, although genetic, immunological research has revealed the micro-environments of cancers. Recently, more attention has focused on treatments, especially the mechanisms of cancer genesis.

We investigated several immunological parameters in advanced stage cancer patients and evaluated their immune response after the patients were administered the phyto-polysaccharide Active Hexose Correlated Compound (AHCC), which has shown biological response modifier (BRM)-like activity.

I. Subjects and Method

Subjects

38 cancer patients, diagnosed at our hospital from June 1999 to November 2000, and 117 healthy people, used as control, were investigated. Conventional therapies had little effect on the cancer patients, who were in stage 4 cancer. In order to eliminate the possible influence of cachexia or other side-effects from previous cancer treatments, the subjects were selected from the patients who have not had surgery, chemotherapy, radiation or any other therapies within a month before starting the investigation, and who showed a performance status (PS) grade under 3. The patients were consulted about this study through their own doctors, and once the patients signed the consent form, the investigation began. (Table 1)

Active Hexose Correlated Compound: AHCC™

AHCC is a food obtained from basidiomycetes mushrooms after long-term cultivation, several enzymtic reactions and its BRM activity has been reported. The subjects took 6 grams of AHCC daily, in three doses, after meals, for 6 months.

Investigated Parameters

The NK cell activity in the peripheral monocytes and Th1 related cytokine production (INF-γ, IL-12) were the immunological parameters investigated. PS and efficacy rate were the clinical parameters investagated. Each parameter was measured and evaluated 4 times, before AHCC and after the administration of AHCC at 2 months, 4 months and 6 months.

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Measurement

NK Cell Activity

As the effector cell for this study, the monocytes were isolated from the peripheral blood containing heparin, and the number of lymophocytes were prepared at 1×10^6/ml in a RPMI-1640 medium containing 10% FBS. CML originated tumor cell line (K-562, by Dainippon Pharmaceutical Co., Ltd.) was used as the target cell. 200 ml of ^{51}Cr-sodium chromate of 100 μCi was added to 3×10^6 K-562, and cultured for 1 hour at 37 degrees C. After washing, a number of cells was prepared at 1×10^6/ml, and investigated by γ-scintillator.

IFN-γ and IL-12 Production

The monocytes were prepared by the same method as 1). 20 mg/ml of phytohemagglutinin (PHA, by DIFCO) was added to the cell suspended liquid, and then cultured for 24 hours at 37 degrees C, under 5% CO_2. After the cultivation, the supernatant was collected for investigation. IL-12 was measured by a R & D SYSTEMS kit, and IFN-γ was measured by ELISA method using Biosource's kit.

Data Analysis

A Friedman test was used for the statistical analysis of the immunological parameters, and Dunnet's standard was used to judge the differences during multiple comparisons ($p<0.05$). Each value was illustrated by the box and spar in order of the median (P_{25}, P_{75}).

Results

Evaluation Progress

The number of subjects available from the immunological parameter evaluation was 36 cases at 2 months, 26 cases at 4 months, and 18 cases at 6 months. 2 cases died of cancer.

Improvement of NK Cell Activity, IFN-γ Production and IL-12 Production (figure 1)

NK cell activity

Before AHCC: median 44% (P_{25}: 36.8%, P_{75}: 53%)
At 2 months: median 47% (38%, 54% ($p=0.78$ vs before AHCC)
At 4 months: median 51% (43.2%, 59.2% ($p<0.05$))
At 6 months: median 57.5% (52%, 63.3% ($p<0.001$)).
Significant improvement was shown after taking AHCC.

INF-γ

Before AHCC: median 8.9IU/ml (P_{25}: 5.1IU/ml, P_{75}: 14.4IU/ml)
At 2 months: median 14.7IU/ml (9.3IU/ml, 25.1IU/ml ($p=0.07$))
At 4 months: median 25.3IU/ml (12.7IU/ml, 30.8IU/ml ($p<0.001$))
At 6 months: median 25.5IU/ml (16.2IU/ml, 38.9IU/ml ($p<0.001$)).
Significant improvement was shown after taking AHCC.

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IL-12
Before AHCC: median 7.8 pg/ml (P_{25}: 7.8 pg/ml, P_{75}: 8.8 pg/ml)
At 2 months: median 14.9 pg/ml (8.0 pg/ml, 23.6 pg/ml ($p<0.01$))
At 4 months: median 20.1 pg/ml (15.0 pg/ml, 28.5 pg/ml ($p<0.001$))
At 6 months: median 27.2 pg/ml (19.2 pg/ml, 37.1 pg/ml ($p<0.001$)).
Significant improvement was shown after taking AHCC.

PS and Therapeutic Effectiveness
In PS evaluation, significant improvement was shown after taking AHCC compared with before AHCC ($p<0.05$). Also in the therapeutic effectiveness evaluation, CR: 2 cases, PR: 11 cases, NC: 14 cases and PD: 11 cases were considered. (Table 2, Figure 2)

Changes of NK Cell Activity, IFN-γ Production and IL-12 Production based on PS

Evaluation
Immunological parameters were compared between the PS (0-1) group of positive clinical activity after AHCC, and the PS (2-4) group of negative clinical activity. The result found that the PS (0-1) positive group showed significant improvement in IFN-γ and IL-12, while the PS (2-4) negative group does not show significant difference. Namely, the significant difference of IFN-γ production and IL-12 production after taking AHCC as observed above in 2, mainly resulted from the positive group (PS (0-1)). This result indicates a positive correlation between immunological parameters and PS improvement.

Immunological Parameter in Healthy People
IL-12: median 27.0 pg/ml (P_{25}: 14.8pg/ml, P_{75}: 42.3pg/ml), INF-γ: median 29.5IU/ml (P_{25}: 19.5IU/ml, P_{75}: 44.8IU/ml) and NK cell activity: median 34% (P_{25}: 25%, P_{75}: 44.5%) were considered.

Toxic Response
A slight fever or feverish feeling within a month of the start of AHCC was found in 4 cases. (Fever diminished after 2 months, and the trial continued.) 1 case suffered from stiff joints in the hand as well as an elevation of Th1 related cytokine (INF-γ: 22.1IU/ml to 88.4IU/ml, IL-12: 32.2pg/ml to 90.8pg/ml). (This patient dropped out of the trial in order to prevent rheumatic symptoms). No abnormalities were found in blood, urine or feces during the trial.

Discussion
Several medicinal components obtained from phyto-polysaccharides are known to have BRM effects in order to enhance an anti-tumor immune system.

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Although there is a large amount of research that has been conducted in order to evaluate anti-tumor effects, the mechanisms have not yet been identified. In addition to medicinal components, foods containing phyto-polysaccharides are also attracting people's attention for their BRM-like effect and are being considered in a new category called, "Anti-tumor Health Food".

From the phyto-polysaccharide foods causing a BRM-like effect, AHCC was chosen for the advanced cancer patients in this trial and investigated several immunological parameters. As a result of the administration of AHCC, significant improvements were shown in NK cell activity and Th1 related cytokine production, as well as in the QOL marker, PS.

The significant increase in NK cell activity was shown in the AHCC group, compared with the group, before taking AHCC. NK cell shows cytotoxic activity without antigen sensitization. This activity is regulated by the receptors (KIR, KAR), which recognizes MHC class 1. Therefore, the enhancement of NK cell activity by AHCC suggests that AHCC has some efficacy on cytotoxic activity in tumor cells, which has less MHC class 1. However in this evaluation, the NK cell activity in cancer patients did not decrease as compared with healthy subjects. This result raised the question of whether the NK cell is active enough to fight against cancer cells in vivo although it is active in vitro. In addition, such NK cell activity might be shown in only the target cell line, K-562, which is not originated from the solid tumor.

To measure Th1 related cytokines, PHA, a T-cell stimulus factor, was added to peripheral monocytes and cultured. After taking AHCC, significant improvements in IFN-γ and IL-12 production was considerable even though the production was low before AHCC as compared with healthy subjects. IFN-γ is a key cytokine, which ripens and activates the effector cells of the tumor immune system as well as showing strong anti-tumor activity by itself. In the vitro experiments, significant improvement of IFN-γ was observed. This result suggests that AHCC activates a process within T-cell function and is related to PHA stimulation. IL-12 is a cytokine produced from APC by its interaction. While two channels are known to produce IL-12 – the macrophage/APC stimulation by Staphylococcus aureus cowan (SAC) or lipopolysaccharide (LPS), the contact with T-cell activated by T-cell-APC interaction, the increase of IL-12 production by PHA, during this trial, it was considered a result of T-cell-APC interaction. Further research is necessary to locate exactly which action within the cell during IL-12 production is affected. Niimi reported that there was no significant difference in IL-12 production between cancer patients and healthy subjects when the cancer patients' monocytes were cultured along with IFN-γ. He found that IL-12 production in cancer patients usually decreases when the monocyte stimulation is by LPS. This result suggested that there was a defect in T-cells, which supplies IFN-γ, and such a defect possibly obstructed the

macrophage's IL-12 production.

In the PS analysis, the significant increase of Th1 related cytokine production in the group showed positive PS improvement. This suggested that IFN-γ and IL-12, both of which are Th1 related cytokines, would be a proper marker for QOL. At the same time, there may be other possible factors which also inhibit the immune system and have an influence on PS. Therefore we intend to investigate humoral factors such as Th2 related cytokine, or the responder/non responder by MHC which have been reported in other studies on BRM. With regard to the evaluation of the efficacy rate, generally in chemotherapy research, it is difficult to identify the efficacy rate because the correlation between tumor size reduction and the survival term of the patient undergoing chemotherapy has not yet been proven. The same thing can be said about this research although the research was on BRM and not anti-cancer drugs. Also, more than one kind of the cancer was investigated in this trial making it difficult to accurately calculate efficacy rate. However, regarding the cases in this research showing improvement, the cases have not reported a relapse or their condition has not become worse, suggesting that the efficacy of AHCC is constant.

We have been indicating that cancer patient's IFN-γ and IL-12 response stimulated by PHA tend to decrease in early stage cancer as compared with healthy individuals. In this investigation, AHCC was found to produce 2 cytokines stimulated by PHA, IFN-γ and IL-12, which decreased in the cancer patients. This result strongly suggests the possibility of AHCC activating Th1 related cytokines and improving the immune system. Since Th1 related cytokines play an important role in the immune preparation system against cancer, it is possible to say that AHCC has anti-tumor effect on cancer cells. In clinical settings, patients tend to be diagnosed with "cancer" at the visible stage. However, seeing the possible decrease of PHA response in monocyte fraction, which is seen before the cancerous tumor grows, taking AHCC has the potential to delay or prevent cancer development by enhancing the immune preparation system.

Conclusion

1) It is possible that the administration of AHCC increases NK cell activity against K-562.
2) It was proved that the treatment of AHCC enhanced the potency of the monocytes in cancer patients to produce IFN-γ and IL-12.
3) It was found that AHCC intake will improve PS evaluation and QOL significantly.

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Table 1. Subject

	Cancer Patient (n=38)	Control (n=117)
Demography		
Sex (Male/Female)	17/21	70/47
Age (Median)	57	53
Age Range	27-74	27-78
Type of Cancer (Male/Female)		
Stomach	3/1	-
Colon	5/4	-
Liver	4/2	-
Pancreas	0/1	-
Lang	5/1	-
Breast	0/8	-
Ovary	0/4	-

Table 2. PS and Efficacy Rate before/after AHCC

Performance Status			After AHCC		
			PS (0-1)	PS (2-4)	
Before AHCC		1case			
		PS (2-4) 15cases 15cases			
PS (0-1)	7cases	Before AHCC 2month 4month 6month Friedman test	26.6(14.7-32)	27.4(19.8-38.5)	P<0.001
		Positive group NL cell activity 44(37-47) 50(38-61) 50(44-58) 58(54-63) P<0.001			
		INF-□ 9(3.8-13.8) 19(14.5-34.4)			

Table 3. Comparison between PS positive group and PS negative group

	IL-12	7.8(7.8-11.2)	17.7(16.5-28)	22.6(19.5-30.6)	29.8(24.4-41)	P<0.001
Negative group	NL cell activity	44(38-46)	44(35-49)	48(41-59)	37(29-49)	NS(p=0.615)
	INF-□	12.7(8.9-21)	11.7(9.1-31)	10.8(8.8-20)	10.9(8.8-24)	NS(p=0.532)
	IL-12	7.8(7.8-9.5)	9.8(7.8-13)	11(7.8-14)	9.8(7.8-9.8)	NS(p=0.392)

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Figure 1. Immunological Parameter

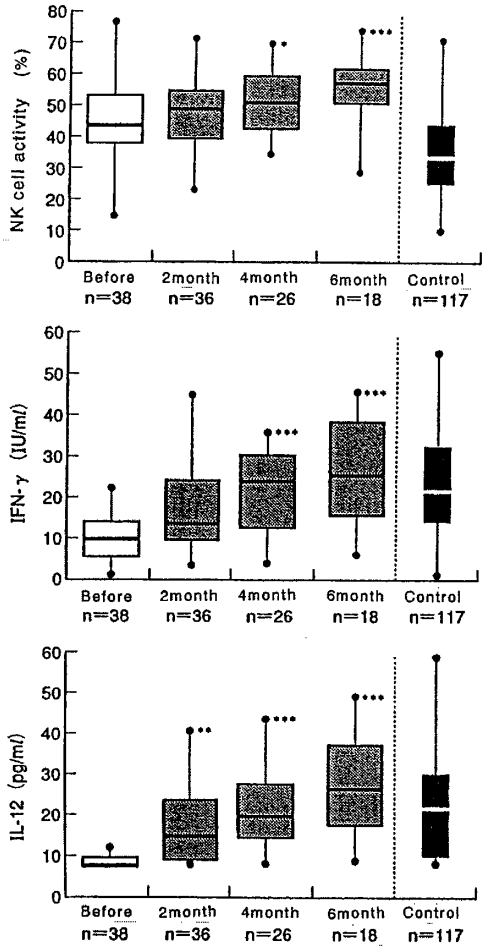
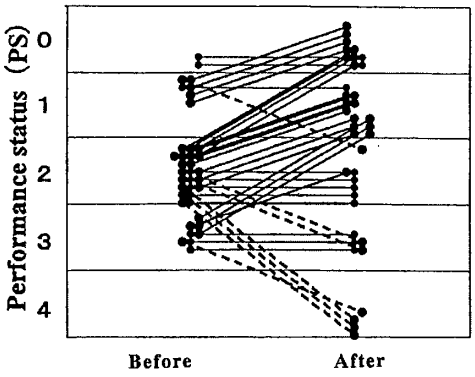


Figure 2. PS befoe/after AHCC



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