Evaluation of Metabolism Pathways of AHCC and the Implications for Drug-drug Interactions

(paraprhrased titled: Evaluation of Metabolism Pathways of AHCC and the Implications for Drug-drug Interactions)

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Background

Liver metabolic pathways play an essential role in the active forms used by the body, and eventually the elimination, of a majority of medications and supplements people take. There are three primary pathways of liver metabolism for eliminating drugs and other chemicals and are known as phase I, phase II and phase III metabolic pathways. Studies have also been focused on what activates the phase II pathways to find out whether chemicals foreign to the body can speed up the detoxification of chemotherapy drugs, which leads to low drug effectiveness. Natural products can also do this, including active hexose correlated compound (AHCC) , and there has been little knowledge of the potential effects on drug efficacy and safety.

Summary

The primary objective of this study was to evaluate the potential phase II liver metabolism pathways associated with the effect of AHCC with drug interactions. Four primary liver metabolism pathways were tested, Preliminary results found AHCC is not an inhibitor of 2 of these pathways, but may have potential inhibition of one pathway. Evaluation of its activating effect on the phase II pathways is ongoing and finally results will be presented. In conclusion, additional studies are warranted to determine potential of any phase II liver interactions with AHCC when administered with other medications or supplement substrates of these pathways.

Clinical Effects of AHCC on Refractory Non-tuberculous Mycobacterium Infection

(Paraphrased: Clinical Effects of AHCC on a type of lung bacteria infection related to tuberculosis known as mycobacterium)

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Background

Despite treatment, non-tuberculous mycobacterium infection can become a incurable disease in some patients. They are constant carriers and the infected areas become worsened over time. As a result, many patients die of lung failure eventually. The patients suffering from this infection show the suppression of cellular immunity and natural killer cell (white blood cells) activity although the number of these cells with low activity is increased. Natural killer cells (NK cells) are important types of white blood cells that help the body get rid of cancer cells. AHCC has been found to enhance cellular immunity and natural killer cell activity, and its efficacy has also been reported in cancer patients receiving it as a functional food.

Summary

The study was to test the impact of AHCC inhuman subjects in the clinic of immune function and enhancement of NK cell activity in infection patients having non-tuberculosis mycobacteria lung infection. In the hospital setting where the study took place 10 subjects showing visible lung damage by X-ray and/or exhaling the mycobacteria were enrolled. All 7 patients were females at the mean age of 63.0 ± 10.5. Lung hole formation was observed in 1 case, and other 6 had damage to the airway bronchial tubes. Although AHCC increased NK cell activity in 1 subject, the chest CT image showed no improvement. On the other hand, 2 of 7 subjects were classified into the group that did not have any worsening of symptoms. However, there were no increased NK cell activity and no increased immune factors in the 2 patients. No side effect of AHCC was observed during the AHCC administration.

[Conclusion] Though AHCC showed no relationship between clinical efficacy and NK cell activity in this trial, there were 2 cases that did not worsen in 7 subjects, suggesting that AHCC might work through other mechanisms except for NK cell activity in these bacterial infection patients. Further studies are needed to confirm the mechanism. In addition, it is beneficial for future clinical trials that AHCC had no side effect in all subjects.

Decisive AHCC Action in a Severe Malnutrition Patient

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Background

In this present case study, it is reported on the situation of an elderly lady 84 years old, who was about 80 lbs in weight and about 5 feet in height at and complained of weakness, loss of appetite, fatigue, bad digestion, and depression. She had one episode of fainting two years ago and has been progressively losing weight and more depressed with insomnia, dry mouth, no appetite, swallowing difficulty, bad digestion, weakness, slimness, and slowed ability to move for last eight months.

At the physical examination, the presence of signals of malnutrition, tissue below the skin almost absent, and bones prominent) was observed. Her body mass index was 14.06 (normal is 20 or above). The presence of malnutrition was evidently promoted mainly by hypothyroidism and Sjogren disease that were confirmed by complementary exams.

She had no improvement with the treatments including the use of special neutrino through tube feeding. Clinical treatment was started on November 22, 2012 taking in account all her daily needs, adding a thyroid hormone and AHCC 1 g three times a day.

She had full recovery in less than one month. She recovered normal saliva levels on her tongue, and gradually had food taste, appetite, and a better mood. This year, she was weighing 50 kg, and her BMI was 19.5.

Effect of AHCC on the Autonomic Nervous Function

(Paraphrased: **Effect of AHCC on Nervous Function)**

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Background

The aim of this study was to examine the effectiveness of active hexose correlated compound (AHCC) on nervous function that is related to the involuntary part (autonomic) of the nervous system and how stress stimulation affects it.

Summary

Experiment 1: The randomized, double-blind, placebo-controlled, cross-over trial was undertaken with healthy volunteers (n=10). Intake of AHCC (3 g/day) or placebo was scheduled, each of 5 days, with a 9-day period in between with no treatment. The autonomic nervous function was measured by the heart rate variability. The measurement data when subjects changed posturel position from sitting to standing was analyzed in with heart rate variation. As a result, the stimulating (adrenaline related) activity of the AHCC group in standing up increased significantly, compared to the placebo group.

Experiment 2: AHCC was given to the responders (n=5) of the experiment 1 for 7 days. On Day 1 and Day 7, the questionnaire survey of condition was carried out, and the heart rate and the autonomic nervous function in sitting position were measured for 10 minutes. Furthermore, moods were estimated after sitting position using a specific criteria. The AHCC group showed how effective how effective the results were in the questionnaire survey with ratings of "fatigue" and "cannot sleep well" and POMS ("depression-falling", "anger-hostility", "fatigue" and "confusion"). The AHCC intake for 7 days significantly increased the calm nervous system activity related to basic body function, and significantly decreased the heart rate.

Experiment 3: The randomized, double-blind, placebo-controlled, cross-over trial was undertaken with healthy volunteers (n=17). Supplementation with AHCC or placebo was scheduled, each of 7 days, with a 14-day non-use period. The subjects performed mental arithmetic as a psychological stress. Before and during the

mental arithmetic, the autonomic nervous function and the level of immune factors in saliva were measured. In the AHCC group during mental arithmetic, stimulated nervous system activity increased, while calmer basic bodily nerve function tended to decrease. In addition, saliva immune factor which have a protective effect levels tended to increase.

These data suggested that AHCC intake might have beneficial effects on basic nerve function relating to the calmer body nerve functions and moods was increased during rest, and the more stimulated activity was increased in case of standing and psychological stress.

Effect of AHCC on $CD4^+CD62L^+$ T Cell-transfer Induced Colitis in RAG Mice

(paraphrased: Effect of AHCC on Immune Cells and How they Affect Colitis in Mice That Are Bred to be Susceptible)

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Background

A major advancement in our understanding of the causes of inflammatory bowel diseases (IBD) has been the development of mouse models of chronic gut inflammation. The immune based model shares many pathological features with human IBD and has the advantage of being a strictly chronic model of colitis. We tested the anti-inflammatory effects of active hexose correlated compound (AHCC), a nutritional supplement prepared from the mycelium of edible shiitake mushrooms that contains 74% active compounds.

A colitis model was used to produce a disruption of immune cells, resulting in large and small bowel inflammation at 5-8 weeks. Treatment was started in animals with diarrhea and significant body weight loss. Experimental groups: control with colitis model and AHCC (714 mg/kg).

Summary

AHCC treated mice displayed a lower body weight loss (–20.2 ± 2.5 vs. –6.6 ± 2.2%*)* and intestine weight/length ratio. AHCC has beneficial effects in the immune related model of colitis.

Effects of AHCC on Tumor Growth and Development

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Background

Bioactive substances and white blood cells activated by these compounds inhibit radiation- and chemical-induced birth defect malformations and tumors in mice, probably by eliminating cells that lead to birth defects and precursor tumor cells and replacing with normal cells (Nomura T, et al. *J. Exp. Med.* 1990),. First, we found active hexose correlated compound (AHCC) treatment given to pregnant N5 mice can significantly inhibit gamma-ray induced birth defect malformations, and continuous oral intake of AHCC for 2 years significantly suppressed the incidence of radiation induced leukemia in mice. A more important study is the examination of AHCC effects on very low dose radiation-induced or spontaneously developed cancer and various disorders.

Summary

Such studies are going on in mice which develop common types of cancer, *e.g.*, lung cancer, liver cancer, breast cancer, etc. as in humans. Preliminary results show that continuous oral intake of AHCC for 2 years daily or 2 days per week delay cancer development and suppresses the incidence of animals having tumors. Inhibitory effects of AHCC on the human mesothelioma and prostate cancer in a specific strain of mice are also studied.

AHCC as Nutritional Supplement in Patients with a History of Cancer: Preliminary Results

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Background

Plant sources have provided physiologically active substances for some of the most useful modern drugs. Active hexose correlated compound (AHCC) is an extract of shiitake mushroom. AHCC consists mainly of alpha glucans derived from processed mushroom. The main active component of AHCC is thought to be a unique polysaccharide known as an alpha glucan. The glucans in AHCC are thought to provide a carbohydrate to stimulate the immune response.

Summary

In the last 12 months, we observed 43 patients at the median age of 66.3 (range 35-83 years) with a history of cancer (gastrointestinal tract, lung and breast). These patients took a dose from 3 to 6 g per day of AHCC for a period of at least 3 months or more. We evaluated the following parameters at 0, 1, 2 and 3 month; blood counts, liver functions, inflammatory states, ferritin, LDH, lipid profile, immunoglobulins, DHEA-s and white blood cell types. In four patients, we observed grade 2 skin toxicity and it was necessary to reduce the doses to improve the symptom. No other toxicity was observed.

The results we observed are the following: Almost all patients (about 80%) reported a general improvement of their condition. There were no laboratory abnormalities. 22% of the patients reported mild difficulty in taking the capsules, especially those who assumed 6 g per day. 82% of patients showed an increase of 2 types of white blood cells (immune cells). 82% of patients showed an increase of some immune parameters and a decrease in others related to suppression, normalization or increasing of natural killer cells important for killing cancer cells, and an improvement of quality of life. Three patients (7%) in particular showed a further reduction in size of lesions after two months of integrative treatment. These data, even preliminary, show the safety of AHCC in our population and confirm its role in immune stimulation. Further studies are necessary to evaluate its role in contrast to cancer growth.

AHCC's Impact on the Immunological System in Patients with Brain-Endocrine Tumors within Three Months after Treatment with a radioactive substance－An Initial Report－ (paraphrased)

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Background

The aim of this study is an assessment of the efficacy of AHCC in patients on the immunological system three months after the last dose of a radioactive treatment. The main purpose of our study in the future will be the evaluation of the influence of AHCC on immunological profile, time to progression of cancer, progression-free survival, overall survival and response rate.

Summary

We evaluated three patients with confirmed brain-endocrine tumors, who were included in the study. These patients were treated with four doses of radioactive treatments in 8-10 week intervals. Patients took AHCC at the doses of 6 g per day (2 g at 3 times), 40 minutes before meals. The local ethics committee approval was obtained before the study commenced. All results are presented as medium results. Three months after the last

dose of a radioactive element treatment was given, the following was found: white blood cells of several different types decreased by 4.91%, 36.27%, 14.48% and 28.2%, respectively; other white blood cells increased by 60.43% and 18.84%, respectively. The results showed that AHCC is a promising extract, which increases the amount of some white blood cells while decreasing others in patients with brain-endocrine tumors. In the further study, we want to find out if these changes in white blood cells are important for brain-endocrine patients and check whether these results will be confirmed in others patients.

AHCC Compared with Placebo in Decreasing Chemo-radiation Treatment-related Side Effects for Head and Neck Cancers Therapy (paraphrased)

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Background

Head and neck cancers (such as nasal throat, tongue, mouth, etc.) are usually treated with chemo and radiation therapy (CRT). Side effects such as painful inflammation and sores of the mouth, dry eyes, difficulty swallowing, and suppression of bone marrow occur in 60 to 90% of patients, which are mostly grade 2-3 in toxicity, and the concurrent use of chemo-radiation leads to an increase of side effects. They start after 10 Gy irradiation or 1 week from CRT and progress during treatment. These lead to a decrease in nutritional intake, weight loss, impairment of the immune system, leading to impaired quality of life, poor tolerance to treatment and poor treatment outcome. Various interventions have been used to alleviate these side effects (medicines, virgin coconut oil, RT techniques) but they are not consistently effective.

Active hexose correlated compound (AHCC) is a nutritional product from fermented mushrooms. It is believed to have various therapeutic properties as well as stimulates the body's own immune system. It has been studied as an adjunctive treatment for cancer, and other studies have shown its amelioration of chemotherapeutic side effects. However, no study has yet been done on AHCC with concurrent chemo-radiation for head and neck malignancies.

Summary

This study is a double-blinded, multi-center, randomized, controlled trial on AHCC vs. placebo for head and neck cancer undergoing concurrent chemo-radiation. The main objective is to decrease the occurrence and severity of expected side effects for these patients. Specifically, we aim to compare the incidence of Grade 2 and above chemo-radiation side effects, severity, impacts on quality of life, adverse events and acceptability.

Efficacy of Oral AHCC Supplementation in Female Breast Cancer Patients Undergoing Chemotherapy
Randomized, Double-blind, Placebo Controlled, Phase-II Study: A Study Protocol

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Background

Anthracycline and taxane are adjuvant types of chemotherapy for treating breast cancer patients. Adjuvants are additional *cancer* treatment given after the primary treatment to lower the risk that the *cancer* will come back. However, significant toxicities and side effects can occur in patients during these treatments. Our previous retrospective studies demonstrated that AHCC has the potential to reduce the severity of a specific type of low white blood cell counts induced by breast cancer chemotherapy.

Summary

The objective of the study is to investigate the beneficial effects of AHCC on adverse events in female patients administered before receiving adjuvant chemotherapy for breast cancer.

A double blind, randomized, controlled trial will be conducted using AHCC in breast cancer female patients who are going to undergo chemotherapy. A total of 1.0 g of AHCC will be self-administered by each patient orally after each meal (3 g per day) for 24 weeks with 12 weeks follow-up. Twenty patients will be recruited at two medical institutions, which are specialized in breast cancer treatment, with the registration period of one year. The primary endpoint will be the occurrence of adverse events (grade 2 or higher) associated with blood chemistries, including those for the liver, kidneys, white blood cells according to the National Cancer Institute criteria. The secondary endpoints will be quality of life scores, adherence, the use of bone marrow stimulating agents if required, and treatment success rate. A central monitoring will be carried out by NPO JORTC data center and the independent data monitoring committee during the trial.

Effects of AHCC in an Animal Model of Inflammation-induced Free Radicals (paraphrased)

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Background

High blood pressure, inflammation, and cancer are primarily causes of mortality worldwide. Although conventional medicine has been used to treat inflammation and free radical damage, our preliminary studies provide evidence that active hexose correlated compound (AHCC), alpha glucan rich nutritional supplement produced from the mycelia of shiitake and developed by Professor Toshihiko Okamoto as a therapeutic aid for life-style diseases will directly inhibit free radical damage by changing the nitric oxide (NO) pathway. We hypothesize that AHCC could be an effective therapy in diseases involving blood vessel and artery dysfunction, e.g. high blood pressure, inflammation, and cancer, especially when a specific biochemical pathway (NO-sGC-cGMP) is impaired.

Summary

Six rats were operated under anesthesia to implant catheters into the artery in the leg and jugular vein to record mean artery blood pressure (MAP) and heart rate (HR) and for drug administrations, respectively. Following recovery, conscious animals were pretreated with AHCC in drinking water and/or by stomach tube (6%) prior to being subjected to an inflammation inducing compound (lipopolysaccharide (LPS; 20 mg/kg) administered by *i.v.*. MAP and HR were continuously recorded for 6 hrs following LPS administration. In parallel with blood pressure and flow measurements, blood samples were collected for NO and production of biochemical factors. Animals were sacrificed at 6 hrs and lungs were harvested for further determination of markers of free radical damage, protein of the nitric oxide signaling pathway, and cell study.

The results of our preliminary data indicate that AHCC administered at 6% in drinking water for 24 hours (n=3) or by stomach tube (3 test animals) did not induce significant changes in the blood flow values. In contrast, our data demonstrate that AHCC (6%) diminished the effect of the inflammation inducing compound and decreases MAP by 20%, suggesting that AHCC changes the vascular tone in animal studies. Although in the early stage, our data show potential for AHCC as a therapeutic aid, additional experiments are being conducted to assess the level of free radical damage in LPS-administered and in animals who develop spontaneous high blood pressure pre-treated with AHCC.

AHCC Has Specific Immune Effects on White Blood Cells Known as T Cells in Stressed Environments Related to the Immune System and Physiology (paraphrased)

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Background

AHCC (active hexose correlated compound) might potentially play a nutritional role for the metabolism role in regulating physical stress in the body.

Summary

Experiments to assess the effects of AHCC in physiological (physical) and immune stress were performed in one type of human white blood cells (peripheral blood mononuclear cells) under the effect of regular gravity ('1G': normal physiological model) [+/-AHCC] and reduced gravity conditions ('MMG': physiological stress model) [+/-AHCC]. The cells were treated with 5μM, 10μM and 20μM AHCC powder. Both cells and supernatants were saved at the end of 24, 48 and 72 hour time points in triplicates. They were analyzed by how much cell proliferation took place and by its response to antibodies (ELISA cell function assays), and immune proteins which were compared across groups. Preliminary analysis has been done and detailed results will be presented.

From previous studies (Dr. Kulkarni's laboratory; manuscript in preparation) in hind limb unloaded mice treated with AHCC, trends indicated that AHCC modulated white blood cell development (T helper cell differentiation) and the production of an important type of white blood cells for killing cancer (natural killer cells proliferation). Hence in the first phase, our '*in vitro*' analysis will be focused on these aspects.

In preliminary genetic analysis of these white blood cells (human peripheral blood mononuclear lymphocytes) in 1G and MMG, regulation of chemical messengers in these types of white blood cells (human lymphocytes) reveals opposing expression patterns of the pro-inflammatory immune factors, and increases and decreases in other immune factors and cell receptors. One type of immune related gene (IL-1α) was significantly down-regulated in MMG compared with 1G and is consistent with Maier et al's finding in blood vessel cells cultured in MMG. Investigations on these three genes in AHCC treated samples are ongoing and will be presented.
Genetic response studies in human lymphocytes in response to microgravity, high altitude stress etc., are

important to identify and further study in order to augment human physiological adaptation to novel environments. These genes may prove to be therapeutically valuable as new targets for protective measures, or as predictive biomarkers of response to these new environments.

Activation of Skin by AHCC

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Background

We successfully developed a rapid 1 hour, high sensitive, label-free and non-invasive cell-based sensing technology for reliable prediction of the effectiveness of bioactive compounds at concentrations normally found in the body and involving their various types of activity.

Conventional technology is based on the endpoint of an assay using 3 dimensional cell culture with the surrounding areas outside of the cells having being important in the analysis. This requires a time-consuming experimental process and labeling agents causing interference with the target compound that provides low reliability. This will not allow the simultaneous prediction of efficacy and toxicity. An alternative method using specialized devices such as impedance monitoring depends on the pharmaceutical mode of action, and gives no relation to the conventional method.

New technology is brought by a custom-made instrument and method to monitor early stage of the cells energy metabolic function in response to stimulation utilizing a high-precision instrument (surface plasmon resonance as a measurement principle). And we have found the energy structures change in electrical charge (mitochondrial polarization) as helping to determine a specific endpoint a test result. From these, we have overcome all problems with testing for toxicity and efficacy while we have achieved prediction of endpoint cell status related to target compound(s) correlating to conventional cell-based chemical sensitivity tests.

Expected applications for compound screening are anti-cancer, anti-Alzheimer disease, anti-aging (skin care), anti-diabetes, pro-fat burning, pro-metabolism, pro-hair restoration and pro-body temperature, even suitable in case of DDS.

We report the application of our new technology for skin activation by AHCC.

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Effects of AHCC on the Lifespan Extension and Tolerance to Heat of the Nematode *C. elegans* (paraphrased)

○Tetsuya Okuyama [1], Emi Yoshigai [2], Yukinobu Ikeya [3], Mikio Nishizawa [2]

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Background

Active hexose correlated compound (AHCC) is the extract from the cultured mycelia of the mushroom shiitake (*Lentinula edodes*). AHCC contains various polysaccharides compounds known for immune strengthening effects. AHCC has anti-inflammatory effects (reducing nitric oxide production in liver cells). AHCC might affect resistance to environmental stress, which is assumed to play a pivotal role in the longevity of many organisms. To investigate the effect of AHCC on longevity, we measured the lifespan of the nematode (*Caenorhabditis elegans*), a model of an insect that is widely used to assess longevity, and examined the effect of AHCC on resistance to heat stress, i.e., thermotolerance.

Summary

The lifespan of *C. elegans* animals grown on media in the absence or presence of AHCC at 20°C was evaluated. Thermotolerance assays were performed at 35°C, the temperature that affects the physiology of the animals. The effects of AHCC on lifespan and tolerance to heat were analyzed with organisms that live longer due to genetic mutations. Expression levels of stress-related genes, including heat shock genes, were measured by PCR.

Wild-type *C. elegans* animals exhibited a longer mean lifespan by up to 10% in the presence of AHCC in the growth media than animals in the absence of AHCC. Furthermore, AHCC markedly increased heat tolerance at

35℃. Lifespan extension by AHCC at least partly required the involvement of two longevity-promoting genes (DAF-16 and HSF-1). After heat shock, AHCC activated the transcription of the heat shock genes, which are the targets of HSF-1 and help to protect the organism from stresses.

AHCC conferred lifespan extension and heat tolerance to *C. elegans*. Our analyses suggest that the beneficial effects of AHCC on longevity are involved in the activation of at least two transcription factors, DAF-16 and HSF-1.

Protective Activity of Combination of AHCC and UREX Against Urinary Tract Infection in Mice (paraphrased)

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Background

We have already reported that oral administration of the combination of AHCC and UREX, mixture of probiotics *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, may have protective activity against mouse vaginal candida. Here, we also examined the protective effect of this combination against urinary tract bacterial infections using a mouse model.

In recent years, it was suggested that AHCC may display a significant protective role against microbial infection in immune-suppressed hosts, and that *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 can decrease the risk of urinary tract infection (UTI) disease development clinically. So, we examined the protective roles of the combination against experimental UTI disease.

Summary

The mice had been given food with or without 2.5% AHCC and 0.4% UREX, or with 0.4% UREX only with access to unlimited amounts for a week before *E. coli* infection in urinary tracts, and during infectious period. At 15 days after the infection, viable *E. coli* cells in kidney and bladder tissues were counted. The viable bacterial cells in the bladder of the mice treated with AHCC + UREX appeared to be decreased. On the base of the results in details, we wish to discuss about the protective roles of the combination against these types of microbial infection.

Effects of AHCC Supplementation on Chemotherapy-induced Impairment of Gastrointestinal Immunity in Mice

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Background

Continuation of cancer chemotherapy is often difficult for patients due to multiple side effects. Active hexose correlated compound (AHCC) has been used as complementary and alternative medicine to alleviate chemotherapy-induced side effects. Previous studies demonstrated that AHCC prevented liver toxicity, kidney toxicity, or bone marrow suppression during various types of chemotherapy treatments (paclitaxel, cisplatin, or cyclophosphamide). However, influences of AHCC on immune tissue in the gut (GALT), an important immune organ for systemic mucous membrane immunity, were not clear. Our pilot study suggested that AHCC would partly restore chemotherapy induced GALT atrophy. In this study, we examined effects of AHCC on gut immunity during treatment by evaluating GALT cell number, its physiological expression, and mucosal immunity (IgA levels).

Summary

Male mice were randomized to the control (n=9), chemotherapy or chemotherapy + AHCC (n=9) group. The control and chemotherapy groups received normal diets, while chemotherapy + AHCC group received a 0.4% AHCC-supplemented diet. After 5 days of respective diets, jugular vein catheters were inserted. Chemotherapy was continuously infused for 5 days. During intravenous administration, mice continued to receive their respective diets. The mice were then killed by cardiac puncture and whole small intestines were harvested. Gut immune cells (lymphocytes) were isolated from the intestines. White blood cells from each site were counted, and the types of functions of each were determined. Protective antibody immunity in the mucous membrane of the small intestine, nasal, and respiratory tract washings were also measured with antibody testing.

There were no significant differences in pre- or post-experimental body weight among the 3 groups. Chemo reduced lymphocyte numbers as compared with the control group. AHCC supplementation reversed lymphocyte numbers to the control level ($p<0.05$). Chemo increased one type of white blood cell. The mucous membrane antibodies did not show changes for the most part, however, nasal immunoglobulin type antibody immunity tended to be decreased in the Chemo group as compared with the control, and to be restored in the Chemo + AHCC group ($p=0.07$). AHCC supplementation may prevent chemo-induced impairment of gut immunity.

Are There Any Immunological Changes in Healthy Subjects after Three Months Intake of 6 g/day of AHCC? －A Pilot Study in the First Two Patients－

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Background

To evaluate the efficacy of AHCC on the immunological system in healthy subjects after three months intake we evaluated two healthy subjects.

Summary

Patients have taken AHCC at the doses of 6 g per day (2 g × 3), 40 minutes before meals for three months. The changes of cell messengers affecting immune function (interleukin) will be presented in a few months.

All of results are presented as medium results. After three months, the following was revealed: s cells decreased, while others increased. During intake of AHCC (from December 2012 to February 2013), QOL (quality of life) was improved and they had no infection.

After three months intake of AHCC, presented parameters did not change, but QOL was improved.

First Report on Efficacy of AHCC in Patients with Late Stage Liver Cancer in Mongolia: A Randomized, Double-blind, Placebo-control Study (paraphrased)

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Background

From the statistics of 2012 National cancer center, liver cancer is the leading cancer in Mongolia with 72.1 cases per 100,000 people. Nearly 1,600 new liver cancer cases are diagnosed annually and almost 80% of all cancer cases are diagnosed in late stage. Therefore treatment strategy for late stage cancer patients in Mongolia is critical issue. Active hexose correlated compound (AHCC) is widely used supplement in Japan for its immune-modulator and anti-tumor properties. We seek to determine whether AHCC can improve quality of life (QOL) in patients with late stage liver cancer in Mongolia.

Summary

To assess the efficacy and safety of AHCC in patients with late stage liver cancer, our first report indicates a randomized, double-blind, placebo-control study of 12 patients (group 1: 7 subjects, group 2: 5 subjects) with late stage liver cancer. All patients were randomly chosen from the outpatient department of National cancer center, with the criteria of non-surgical treatment. All patients received 9 tablets (AHCC or placebo) daily, and quality of life and liver functions were respectively tested by a questionnaire (QLQ-C30) and laboratory tests for the liver (including AST, ALT, total bilirubin (TB) and platelets (PLT).

The results in the QOL questionnaire show an average QOL score for group 1 was 58.3 before sample intake and for group 2 was 49.9. After 4 weeks of sample intake, the QOL score for group 1 was changed to 63 and for group 2 to 61.7. Liver function test: No significant correlation was seen in laboratory tests such as AST, ALT, TB and PLT in both groups.

Present result shows QOL increased significantly in group 2. This is the first clinical data of this study and present laboratory data of liver function shows no significant changes in both groups.

Alleviating Effect of AHCC for Chemotherapy-related Side Effects in Pancreas Cancer Patients where Surgery is not of help (paraphrased)

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Background

Advancements in cancer chemotherapy have contributed to a high response rate. On the other hand, it is possible to lower the QOL of patients because of its severe and frequent side effects. AHCC is a functional food extracted from cultured shiitake mushroom, and has been revealed to boost immunity of healthy subjects and improve outcomes of liver cancer patients (Matsui et al., *Nutr. Cancer*, 2008; *J. Hepatol.*, 2002). However, there have been few studies on the alleviating effect of AHCC against side effects caused by cancer chemotherapy. The present study was conducted to determine whether AHCC possesses the potential to reduce side effects in inoperable pancreatic cancer patients receiving chemotherapy.

Summary

Inoperable pancreatic cancer patients undergoing the first chemotherapy treatment with gemcitabine (GEM) were divided into two groups with and without AHCC supplementation. The subjects of AHCC group ingested 6.0 g of AHCC between meals every day for 2 months. Blood cells toxicity and non-blood cells toxicity were

measured before and after starting the supplementation. The data was represented as median values (ranges in parentheses). In addition, the therapeutic efficiency was evaluated in terms of response rate and survival duration.

The subject number of AHCC group and no supplementation (control) group was 35 and 40, respectively. There was no subject who discontinued the AHCC consumption due to its side effects. No significant difference was observed in blood tests and backgrounds between both groups before GEM treatment. All subjects significantly suffered bone marrow suppression, liver impairment, and increases in inflammation (C-reactive protein (CRP) elevation). In comparison of both groups, increased inflammatiuon (CRP elevation) of the AHCC group was less than that of control group following GEM administration (AHCC group: 0.66 mg/dL (0.59-6.11) vs. control group: 3.22 mg/dL (0.98-20.2), $p<0.05$), while there was no difference in white blood cell and platelet reductions and liver damage.

The taste disorder caused by GEM treatment was improved in AHCC group (16% vs. 56%, $p<0.05$). In addition, AHCC induced the significant increase in a blood protein (serum albumin) value (3.7 g/dL (2.6-4.7) vs. 3.45 g/dL (2.3-4.7), $p<0.05$). The efficiency of the therapy showed no alteration in the response rate (29% vs. 25%), but the disease control rate (DCR) was significantly higher in AHCC group, compared to control group (88% vs. 56%, $p<0.05$). AHCC group tended to show good result in the survival duration although there was no significant difference ($p=0.075$).

AHCC is effective to reduce the side effects associated with chemotherapy, suggesting that AHCC might contribute to maintaining the quality of life of patients.

The Effect of AHCC on Hepatitis B Antigegn Positive Patients on Long Term Oral Anti-viral Treatment (paraphrased)

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Background

Chronic hepatitis Be antigen (CHBeAg) - positive patients who cannot spontaneously clear hepatitis B and have the more pathogenic variant which has the potential risk for the development of cirrhosis and liver cancer. The current oral antiviral agents (OAA) can suppress hepatitis B virus (HBV) multiplication with the annual e seroconversion rate of about 10%. However the clearance of HBeAg requires not only viral suppression but also immune modulation. Numerous AHCC studies clearly demonstrate both cellular and humoral immune stimulation, which may facilitate HBeAg clearance.

Summary

The goal of this study was to study the twelve months effect of AHCC on HBeAg level in CHBeAg positive patients who were on long term OAA treatment with persistence of HBV DNA that was undetectable.

The prospective and observational study of CHBeAg positive patients who were treated with OAA toward undetectable HBV DNA for more than two years but remain BeAg positive was conducted. AHCC was given 1,000 mg three times a day before meal. The BeAg level was measured by an immune assay every three months.

From September 2011 to May 2013, ten patients were enrolled in the study; three males and seven females with mean age of 48.10. Three were treated with Entecavir, four with Tenofovir, two with Entecavir and Tenofovir, and one with Larmivudine and Tenofovir. Five cases (two males and three females) showed increase of BeAg level after receiving AHCC treatment. Two Cases (Case #3 and #10) showed faster decline of eAg level but the was less than during control period (-5.4 to -2.4, -2.9 to -2.6).

One case (Case #2) showed slow decline of eAg level but better than control period. Two of female cases (Case #1 and #4) showed rapid decline of eAg level after taking AHCC and subsequently eAg was lost in one year of treatment. All patients had good compliance and did not develop any side effects from AHCC.

During the one year course of the additional AHCC in CHBeAg positive patients on OAA treatment, 20% displayed faster decline of eAg level compared to OAA alone, and 10% showed delay response to AHCC treatment. Further prospective and extended duration study should be conducted to confirm this result and find out the prognostic factor of AHCC responder in order to shorten the treatment period and discriminate patients who may not get benefit from AHCC.

Absorption of Adenosine into Blood after Ingestion

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Background

Shiitake mushroom culture extract of AHCC (active hexose correlated compound) has been demonstrated to suppress progression of cancer and viral infections through immune modulating activity. On the other hand, AHCC also suppresses IL-1β-induced excess inflammatory response of normal primary cultured liver cells.

Recently, adenosine has been identified as one of the anti-inflammatory compounds in AHCC.

Summary

The objective of the present study was to confirm absorption of orally administered adenosine in rat.

Blood was collected from portal vein and lower aorta from Wister strain rats after ingestion of adenosine (20 mg/kg body weight). Liver and small intestine (duodenum, jejunum and ileum) were also collected. Extracts were prepared from blood and organs as reported by Kiyono et al.

Contents of adenosine, adenine, inosine and hypoxanthine were determined by LC-MS/MS in positive mode.

Ingestion of adenosine increased adenosine level in peripheral blood and liver. Intestines and portal blood contained normal levels of adenosine 60 min after ingestion of adenosine. On the other hand, contents of metabolites of adenosine; adenine, inosine and hypoxanthine did not change after ingestion of adenosine. These results indicate that adenosine is rapidly absorbed into blood stream and delivered to liver. Adenosine in AHCC might play a significant role in protection of liver against excess inflammation induced by chemotherapy and enhanced immune response induced by other compounds in AHCC.

Effect of AHCC on Immune Parametrs Related to Interferon
A Molecular Analysis of Action (paraphrased)

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Background

We recently reported the critical role of a natural antisense (AS) RNA (a form of RNA that inactivates a key regular RNA) in the synthesis of the immune activator interferon-α1 (IFN-α1). Following infection with influenza virus A/PR/8/34 (PR8), expression of IFN-α1 AS RNA becomes elevated in infected human B cells. The antisense RNA increased the interferon RNA stability by interacting with a part of the regular RNA. In order to confirm this finding and to evaluate its regulatory effect on the innate antiviral response in an *in vivo* condition, we selected guinea pigs as a model animal. They express the functional Mx GTPase, a decisive antiviral factor induced during the type I IFN response, whereas standard laboratory mice carry defective alleles of the *Mx1* gene.

Summary

Using a virus-infected guinea pig model, we reported at the ICNIM meeting last year that active hexose

correlated compound (AHCC) reduced antisense interferon and RNA levels in the respiratory tract of the model animals. AHCC was previously found to destabilize specific types of RNA. To further clarify the mechanism of action of AHCC on guinea pig RNA expression *in vivo*, we aimed to determine the recognition site of the AS RNA in the mRNA. Using a program we found the presence of one form showing the most stable predictions. Based on this genetic analysis and RNA testing, the molecular analysis of AHCC effect on various types of interferon and its activity related to RNA is currently under way and the results will be presented at the meeting.