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A Phase I Study of the Safety of the Nutritional Supplement, Active Hexose Correlated Compound, AHCC, in Healthy Volunteers

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Summary Active Hexose Correlated Compound (AHCC) is an extract of *Lentinula edodes* of the basidiomycete family of fungi rich in alpha glucans. AHCC has been used for many years as a dietary supplement to enhance the immune system and in clinical trials as an adjunctive treatment in Hepatocellular cancer. This multiple dose, Phase I trial, using FDA guidelines, directly investigates the clinical safety and tolerability of AHCC in healthy subjects. Its safety has been based previously on anecdotal reports and its use in clinical practice. Twenty-six healthy male or female subjects between the ages of 18 and 61 were recruited from the community and gave their consent to participate in the trial. The subjects were given 9 g of AHCC (150 mL of the currently available liquid AHCC) PO daily for 14 d. Laboratory data was obtained at baseline and after 14 d of exposure to AHCC and adverse events were monitored by a non-directed review of systems questionnaire three times during the trial. At each visit the vital signs and adverse events were recorded. Two subjects (7%) dropped out because of nausea and intolerance of the liquid. Adverse effects of nausea, diarrhea, bloating, headache, fatigue, and foot cramps occurred in a total of 6 subjects (20%) but were mild and transient. There were no laboratory abnormalities. When used in high dose in healthy subjects, AHCC causes no significant abnormality in laboratory parameters. The adverse effects of 9 g of liquid AHCC per day, a higher dose than used in routine clinical applications, are minimal and the dose was tolerated by 85% of the subjects.

Key Words safety, nutrition, supplements, AHCC, phase I

Plant sources have provided physiologically active substances for some of the most useful modern drugs. Active Hexose Correlated Compound (AHCC) is an extract of *Lentinula edodes* of the basidiomycete family of fungi. AHCC consists mainly of alpha glucans derived from processed mushroom. The main active component of AHCC is thought to be an oligosaccharide comprised of alpha (1-4) linked hexoses with about 30% of the hydroxyl groups acetylated. The glucans in AHCC are thought to provide a carbohydrate to stimulate the immune response.

Over the last 10 y research in animals and human beings has suggested that AHCC is a nutritional supplement with significant immunomodulator effect. In animal studies AHCC increased tumor necrosis factor (*1*) and it reduced the size of metastatic tumors in rats (*2*). AHCC treatment prevented Carbon Tetrachloride liver injury in mice (*3*) and protected against alopecia in rats treated with Cytosine Arabinoside (*4*). In human studies in Japan AHCC reduced the recurrence of hepatocellular carcinoma and reduced the side effects of anticancer drugs. The 5-y survival rate in 113 cases of hepatocellular carcinoma treated with AHCC was greater compared with 109 cases treated with surgery alone (5). Another widespread use of AHCC in Japan is to improve the feeling of well being of patients with liver disease or cancer.

Objective of the current study. Although there is anecdotal support for the safety of AHCC there is no direct phase I trial to assess laboratory and clinical effects of AHCC in humans. The current study was designed to assess the effect of AHCC on clinical laboratory parameters and to evaluate adverse events in healthy subjects. There was no attempt to assess efficacy in this trial. The data collected will be used to design future trials which address safety and efficacy (Phase 2 Trials) in specific populations.

Subjects ingested 50 mL of the currently available liquid AHCC supplement, equivalent to 3 g of AHCC powder, three times a day for 14 d. The 9 g dose is about three times the standard dose used by most patients; however some persons use over 6 g a day in routine clinical practice.

Materials. AHCC is produced under food grade GMP conditions by large scale tank culture of mycelia of *Lentinula edodes*. The process uses starter cultures and is processed in small and then larger batches. The culture

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medium is a proprietary mixture of malt extract, yeast extract, and rice bran. There are no salts, minerals, antibiotics, or growth factors added to the culture. The rice bran is monitored for aflatoxins and pesticide residue before it is used. The insoluble portion is removed in the manufacturing process. The soluble fraction which contains the polysaccharides, low molecular weight material, salts, and protein is separated by centrifugation, concentrated by evaporation, and sterilized by autoclaving. After filler is added the mixture is freeze dried to the AHCC powder used in other applications or sold as a raw material. The manufacturing process is rigidly controlled to prevent contamination, bacterial growth, and failure of purity.

Previous safety data. In a single oral dose toxicity study in Sprague Dawley rats 12,500 mg/kg caused no deaths and no changes at autopsy (*6*). Intra peritoneal toxicity in rats showed an LD_{50} of 8,490 mg/kg in male rats and 9,849 mg/kg in female Sprague Dawley rats (*7*). A study to investigate the potential of AHCC to induce genetic mutation in bacteria using *Salmonella typhimurium* TA102, TA1535 and TA1537 was completed in 2005. It was concluded that AHCC was not mutagenic to the tester strains under the conditions of the trial (*8*).

In human trials there have been no significant adverse events reported. There are currently about 25 to 50 thousand persons using AHCC every day in Japan and the rest of the world. The manufacturer estimates that over 300,000 persons have been exposed to AHCC in the 10 y of its production. In the routine use of AHCC in clinical settings no significant adverse effects have been reported (*9*, *10*) There have been no reports directly to the manufacturer about significant adverse events of AHCC.

Methods. This was an open label trial using the FDA guidelines for phase I trials. The trial was done at MedVadis Research Corporation, Wellesley, Massachusetts, USA. Twenty-six healthy male or female subjects between the ages of 18 and 60 were recruited from the community by advertisements in local media and from other sources between April and June 2005. The subjects were paid 55 dollars at each visit for their participation in the trial. The study was approved by an accredited IRB (Asentral IRB, 15 Main Street, Salisbury, MA 01952). All subjects signed an informed consent. The inclusion and exclusion criteria are listed in Tables 1 and 2. The subjects took 50 mL of liquid, containing 3 g of AHCC, three times a day for 14 d. The first dose was taken in the research center to assure the subject could tolerate the liquid. The subject received 7 d supply of the AHCC and returned to receive the second 7-d supply at an interim visit (Visit 2). Compliance, adverse events, and vital signs were assessed at Visit 2 and after 14 d at Visit 3. Laboratory data was collected at baseline (Visit 1) and at Visit 3. Compliance was monitored by bottle counts and dosing diaries at Visit 2 and Visit 3.

Table 1. Inclusion criteria.

Signed informed consent
Age 18 to 60
No previous use of AHCC
No history of significant illness
Willing to ingest the liquid AHCC in the quantity required in the study

Safety Evaluation was based on the laboratory data obtained at baseline and after 14 d exposure to the study compound. The laboratory assays were done by Quest Diagnostics, 415 Massachusetts Avenue, Cambridge, Massachusetts, a certified clinical laboratory. Adverse events were monitored by an non-directed review of systems questionnaire administered after the initial dose of the compound, at the 7-d interim and the 14-d final visit. The following laboratory parameters were measured: white blood count, hematocrit, hemoglobin, platelet count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, total protein, bilirubin, creatinine, calcium, magnesium, glucose, electrolytes, amylase, lipase, prothrombin time, partial thromboplastin time, thyroid stimulating hormone, urine analysis (including microscopic) and 12-lead electrocardiogram.

Adverse event definitions, causality and severity. Adverse events were defined by the subject's response to the non-directed questionnaire or to abnormal laboratory results.

An adverse event was defined as any undesirable event that occurred to a subject during the course of the study. The severity of each adverse event was characterized and then classified into one of three categories: Mild: The adverse event did not interfere in a significant manner with the patient's normal function; it is an annoyance. Moderate: The adverse event produced some impairment of function, but is not hazardous to health; it is uncomfortable. Severe: The adverse event

Table 2. Exclusion criteria.

Use of any prescribed medication currently or within the last 30 d except oral contraceptives
Use of any other supplements during the trial
Any diagnosed medical condition which might confound the evaluation of safety
History of clinically significant depression, anxiety or other psychiatric conditions
History of allergic reactions to drugs, bee sting or other common antigens
Pregnancy
Breast feeding
Abnormality on any screening lab tests done in this study
Cold, flu or any upper respiratory condition within the last month
Gastrointestinal condition (colic, diarrhea, viral or other) within the last month
Chronic fatigue, fibromyalgia, arthralgias, and other pain symptoms
Chronic low back pain
Other symptoms unexplained and untreated which are recurrent

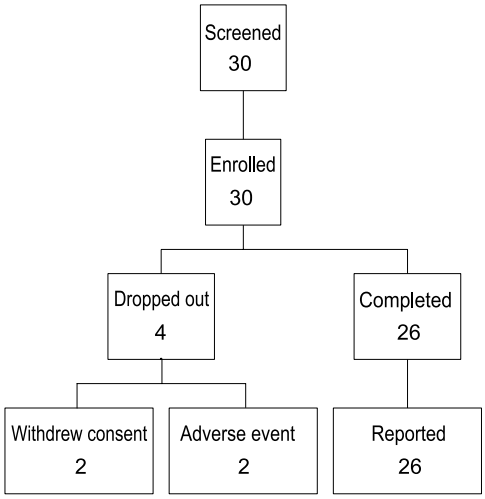

Fig. 1. Study Flow Diagram.

Table 3. Adverse events.

2 subjects dropped out of the trial while on AHCC (7%)
1 had nausea and vomiting during week one
1 could not tolerate the smell of the liquid supplement, felt nauseated, and stopped after 20 doses
4 completed subjects reported mild, transient adverse events (15%)
Headache at Visit 2 which was not reported at Visit 3
Cramps in the toes at Visit 2 not reported at Visit 3
Mild diarrhea and bloating at Visit 3 only
Fatigue at Visit 3 only
There were no consistent adverse events reported

Table 4. Laboratory results (mean values± standard deviation for 26 subjects).

Parameter	Baseline	Final
Systolic BP (mmHg)	112±14.8	114±15.8
Diastolic BP (mmHg)	71±9.2	72±13.5
Pulse (per min)	73±11.1	76±12.3
WBC (K/μL)	7±1.7	7±1.4
HCT (%)	41±4.1	42±4.0
HBG (g/dL)	14±1.4	14±1.4
Platelets (u/mL)	259±69.3	269±61.4
PT (s)	11±0.5	10±0.5
PTT (s)	29±1.5	28±1.4
TSH (mL IU/L)	2±0.8	2±1.0
BUN (mg/dL)	15±4.3	14±4.6
Creatinine (mg/dL)	1±0.1	1±0.1
Ca (mg/dL)	9±0.4	9±0.4
Mg (mg/dL)	2±0.1	2±0.1
Glucose (mg/dL)	5±13.5	84±10.3
Na (mmol/L)	139±1.7	139±1.9
K (mmol/L)	4±0.3	4±0.2
Cl (mmol/L)	104±1.9	103±1.6
CO_2 (mmol/L)	24±2.0	24±2.3
Amylase (U/L)	50±13.6	51±16.4
Lipase (U/dL)	38±10.5	37±10.6
ALT (U/L)	19±5.8	22±9.1
AST (U/L)	21±8.8	23±12.5
ALK PHOS (U/L)	64±20.3	64±18.0
Urine analysis	normal	normal
EKG	normal	normal

produced significant impairment of function or incapacitation; it is a hazard to the patient's health. These designations are based on the investigator's judgment of the patient's report.

An adverse event was considered "not related" to the study compound if any of the following existed in the judgement of the investigator: 1. An unreasonable temporal relationship between the study and the onset of the adverse event; 2. A causal relationship between the study and the adverse event is biologically implausible; 3. A more likely alternative explanation for the adverse event is present.

RESULTS

Demographics

Of the 30 subjects enrolled, 26 subjects completed the trial. There were 15 females and 11 males enrolled in the trial. The mean age was 34 with a range of 19 to 61. There were 20 whites, 3 blacks, 1 asian, 1 hispanic, and 1 who did not give an ethnicity. Compliance was 100% in 16 subjects, and the other 10 missed only one or two doses. The mean compliance for the 26 completed subjects was 99%.

Adverse events

Two enrolled subjects withdrew consent before the first dose. Two subjects dropped out before the study was completed (7%). Four subjects reported adverse events while on the AHCC (15%). The adverse events were mild and transient as shown in Table 3. There was no follow up after the subjects stopped the AHCC so we have no data about lingering effects in the two subjects who reported symptoms in the third week. Three subjects had transient complaints which stopped after week one while in the trial.

Laboratory data

There were no significant changes in the laboratory parameters measured between baseline and 14 d on the AHCC in the 26 subjects. There was no clinically significant change in any parameter in any of the 26 subjects who completed the trial, between baseline and the final visit. Table 4 shows the baseline and end of study mean laboratory values for the 26 completed cases. The urine analysis and the EKG were normal before and after exposure to AHCC. There was no final laboratory collected on the two subjects who dropped out after taking the AHCC.

DISCUSSION AND CONCLUSIONS

AHCC caused no significant laboratory abnormality in doses of 9 g a day in the 26 healthy subjects reported here. The mean changes in the baseline and the final visit laboratory parameters as reported here remained normal.

There were 6 subjects (6/28 or 20%) who reported adverse effects while taking the supplement and two (2/28 or 7%) dropped out because of nausea and inability

to tolerate the liquid supplement. The two subjects who dropped out may have had conflicting conditions which occurred after the baseline exam or may have been unable to tolerate the liquid vehicle rather than the AHCC. The subject who could not tolerate the smell and taste of the product was unique in the cohort; the high volume of the liquid rather than the AHCC itself may have contributed to the intolerance. The other subjects who complained of adverse effects did not report nausea or vomiting. The adverse events reported by the completed subjects were transient and mild.

This study shows that the liquid form of AHCC, even at a high dose, is well tolerated in most persons and that AHCC does not cause abnormalities in laboratory parameters in a dose of 9 g a day for 14 d. This trial supports the anecdotal evidence that AHCC is a safe supplement in clinical practice and that the side effects are generally mild and tolerable.

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