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A Pilot Open-Label Study Assessing the Effects of AHCC® Supplementation on Lyme Disease Patients

John Salerno[1], Manami Misu[2], Eriko Ogawa[2], Jun Takanari[2]

[1]The Salerno Center for Complementary Medicine, New York, NY, 10022, USA; [2]Amino Up Co., Ltd., Sapporo, Hokkaido, 004-0839, Japan

***Corresponding author:** Manami Misu, PhD, Amino Up Co., Ltd., Sapporo, Hokkaido, 004-0839, Japan

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ABSTRACT

Background: Lyme disease is the most commonly reported vector-borne illness in the United States. The disease is caused by the bacterium *Borrelia burgdorferi* and is transmitted to humans through the bite of infected ticks. Typical symptoms include fever, headache, fatigue, a skin rash, and joint pain. If left untreated, infection can spread to the joints, heart, and nervous system, resulting in inflammation and long-term symptoms that include arthritis and/or intermittent pain in joints and muscles, facial palsy, cardiovascular abnormalities and cognitive disturbances. Recent research suggests that late-stage Lyme disease may be a result of malfunctioning immune function. AHCC® is a natural immune modulating compound derived from a unique fraction of special cultured medicinal mushroom mycelia. Previous studies have also suggested that AHCC® exhibits preventive effect against a wide range of infections caused by MRSA, influenza and West Nile virus. The aim of this study is to investigate the effects of AHCC® supplementation on Lyme disease patients on the reduction of symptoms, improvement of immune parameters and the quality of life of patients.

***Methods*:** In a pilot open-label study, 12 Lyme disease patients were administered 3 grams of AHCC® daily for 8 weeks. Before commencement of the administration and after 4 and 8 weeks, the effects of AHCC® were evaluated clinically based on symptoms, serum antibodies to pathogens, inflammatory activity and serum immunological parameters. Patients completed questionnaires pertaining to Qualify of Life parameters.

***Results*:** After 8 weeks of AHCC® administration, AHCC® ameliorated flu-like symptoms and manifestations in the eye, joint, muscle, nervous system and cardiovascular system. In addition, erythrocyte sedimentation rate, an index as inflammation and serum interleukin-8 was significantly decreased.

Conclusion: This study provides preliminary evidence that AHCC® may be effective in treating patients with Lyme disease.

Background

Lyme disease (LD) is a tick-borne zoonotic disease, which is caused by certain spirochete *Borrelia* spp. parasitizing in field rats and birds. According to the data of the Centers for Disease Control and Prevention, 300,000 people are diagnosed with LD annually in the United States [1]. LD occurs in multiple stages, manifesting in a range of clinical symptoms, many of which result from inflammation generated by the body's immune responses [2].

Early stage LD initially presents erythema migrans (EM) and flu-like symptoms, such as fever, malaise, headache and joint pains, for several weeks. In many cases, treatment with antibiotics is effective for treating early stage LD. Delay of initial treatments can lead to a number of neurological abnormalities. After several months to years of infection, systemic infection with the pathogen can cause severe tissue inflammation of skin, joint, muscle, heart and central nervous system [2]. Persistent and reoccurring symptoms are seen in 10-20% of patients. The term post-treatment Lyme disease syndrome (PTLDS) is used to describe the pattern of symptoms that persist 6 months after infection. Symptoms include fatigue, sleep disorders, widespread joint pain and/or Lyme arthritis, and complaints of cognitive difficulties [3,4]. PTLDS, which is also referred to as chronic LD, has not shown any clinical benefits from long-term antibiotic treatment [2,5].

The etiology of PTLDS is not well understood, and a debate has existed whether PTLDS is due to persistent infection or a compromised immune system resulting from the initial infection. However, recent research supports the hypothesis that PLDS most likely is a result of a malfunctioning immune system rather than continued infection. Using xenodiagnostic testing [6], *B. burgdorferi* DNA was found in only 2 out of 26 subjects with diagnosed LD. Inflammation has also been established to play a causal role in the neurological changes associated with LD [7] and it is also linked to the development of PTLDS [4]. In addition, a recent animal study provides evidence that persistent LD may be a result of an overactive immune response, resulting in unnecessary inflammation [8]. Combined with the fact that antibiotics have no effect on PTLDS, the hypothesis that PTLDS appears to be a post-infectious autoimmune response is biologically plausible.

AHCC® is a dietary supplement and an extract of shitake mushroom, *Lentinula edodes*, of the basidiomycete family of fungi. The main active component of AHCC® is thought to be an oligosaccharide comprised of alpha (1-4) linked hexoses with partially-acylated hydroxyl groups. Polysaccharides from *Lentinula edodes* and AHCC® have been shown to influence the immune system as an immunomodulatory agent [9] through multiple mechanisms including natural killer

cell enhancement, up-regulation of cytokines, anti-inflammatory properties and anti-tumor activity [10,11,12,13,14]. In clinical studies, AHCC® has been shown to improve immune function in immunocompromised cancer patients [15,16,17] as well as healthy subjects [18,19,20,21].

This study is the first clinical study for LD of AHCC®, so that we positioned this study as a preliminary study. This study is a pilot open-label study without control group.

MATERIALS AND METHODS

An 8-weeks intervention pilot study was conducted between May 2016 and August 2016 to determine whether AHCC® can reduce symptoms of LD and improve quality of life.

Subject recruitment

Twelve patients (20-80 years) who received a definitive diagnosis with stage I (early infection) to Stage III (PTLDS) were recruited for this study. Patients using steroid or other immune suppressors and patients with brain and/or nerve dysfunction from complications were excluded from the study. The study procedures were carried out in accordance with the Declaration of Helsinki. The Institutional Review Board of Nonprofit Organization TACTICS approved the study as approval no. 2015-93 (Sapporo, Japan). All subjects were informed about the study and all provided informed consent.

Study Product

AHCC® is trademarked and manufactured by Amino Up Co, Ltd (Sapporo, Japan). *Lentinula edodes* mycelia were cultured in liquid medium. The culture was separated, concentrated, sterilized, and freeze-dried to produce the fine granular powder of AHCC®. AHCC® consists of carbohydrates, amino acids, lipids, minerals, and oligosaccharides, which are assumed to confer the biological activities of AHCC®. AHCC® contains approximately 20% (w/w) alpha glucan. This manufacturing process was carried out in accordance with Good Manufacturing Practice for dietary supplements, ISO9001:2008, and ISO22000:2005.

Study Design

This study was an 8-week, open-label study. The patients received AHCC® in the form of capsules and were instructed to take 3 capsules (500 mg each) after breakfast and the evening meal, for a total daily dose of 3 grams. Inspection was held 3 times, before commencement of the administration (visit 1), after 4 (visit 2) and 8 weeks (visit 3). All patients were assessed for clinical symptoms by physicians, and underwent blood test and the questionnaire about their health-related quality of life.

Clinical Symptoms

Physicians assessed clinical symptoms such as skin manifestation/EM, flu-like symptoms, eye manifestation, joint manifestation, muscle manifestation, neurological manifestation, cardiovascular manifestation, neck stiffness, and lymph node swelling.

Blood Testing

At each visit, physicians assessed clinical symptoms and took blood samples. The blood samples were collected using collection tubes, and sent to Empire City Laboratories (New York, United States) for measurement of hematological and clinical chemistry, erythrocyte sedimentation rate (ESR, an index for inflammation), detection of *Borrelia* pathogen, serum immunoglobulin (immunoglobulin(Ig)G, IgM, IgA) levels, serum inflammatory cytokine levels (Interferon gamma, tumor necrosis factor alpha, Interleukin (IL)-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-1beta), C-Reactive Protein (CRP) and natural killer (NK) cell activity.

Questionnaire

At each visit, participants completed a questionnaire. Patients were asked to rate appetite, sleep, fatigue, mood and 26 symptoms on a scale from 1 to 5, where 1 was the patient's worse state, 3 was the normal state and 5 was the better state.

Statistical Methods

The qualitative variables including mean, median, standard error, the number of cases and percentages, were found using descriptive statistics. The 2-tailed paired Student's t test was used to determine whether the difference between before treatment and after 4 weeks or 8 weeks was significant. A *P* value less than .01 was considered significant and a *P* value less than .05 but larger than .01 was considered to have tendency.

RESULTS

The data of eleven patients was analyzed, excluding one patient with an inadequate data.

Clinical Symptoms

The flu-like symptoms such as fever, chills, weakness, muscle and joint pain, and headache were fully or partially resolved in 100% of the patients after 8 weeks of supplementation. The eye floaters found in two patients at the first visit were also resolved by the third visit. Joint manifestations experiences in seven patients at first visit were either completely resolved or reduced in severity. Muscle manifestations experiences in two patients, neurological manifestation in three, and cardiovascular manifestation and neck stiffness in each one at first visit were either completely resolved. There were no clinical findings of EM or skin manifestations or lymph node swelling in any patient before or after treatment.

Blood Testing

After 8 weeks of supplementation, patients also experienced a significant reduction in inflammation as measured by ESR ($p<.001$) and IL-8 ($p<.001$) compared to baseline (Figure 1 and 2). Although all patients were positive for serum IgM antibodies to pathogens before AHCC® administration, all of them were negative after 8 weeks. There were no changes in hematological parameters or clinical chemistry.

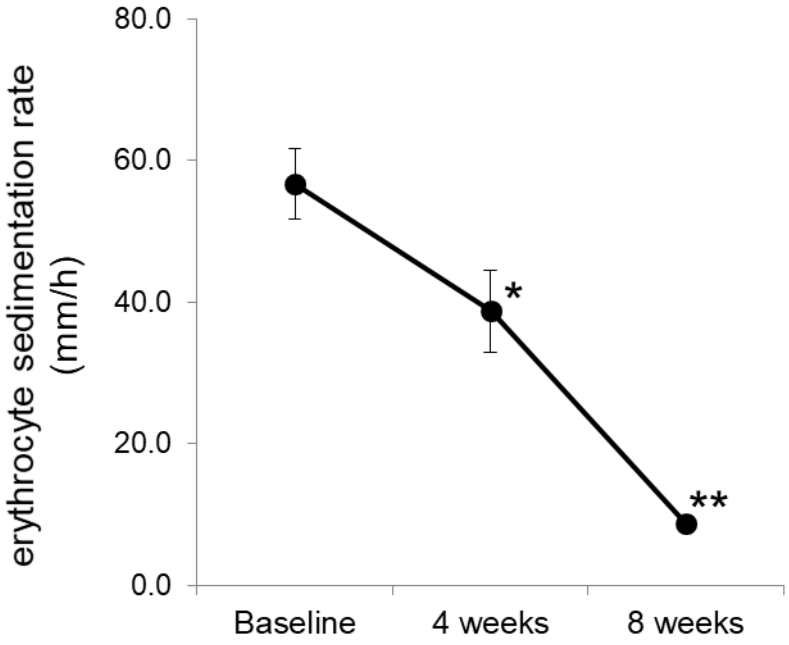

Figure 1. Erythrocyte sedimentation rate

Erythrocyte sedimentation rate which is an index for inflammation was assessed before treatment, after 4 weeks and after 8 weeks. The value represents mean ± standard error (SE) (n=11). **P* <.05, ***P* <.01 versus baseline.

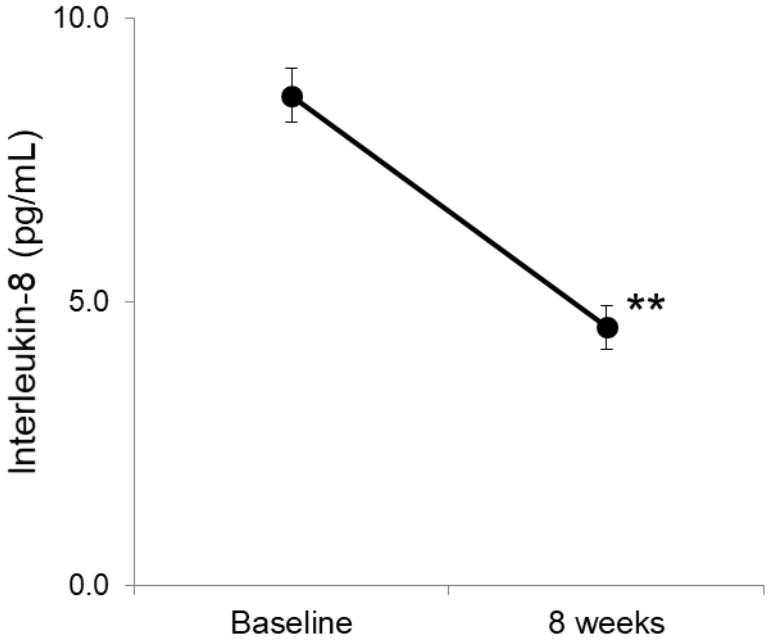

Figure 2. The level of interleukin-8

The level of interleukin-8 which is a serum inflammatory cytokine was assessed before treatment and after 8 weeks. The value represents mean ± standard error (SE) (n=11). ***P*<.01 versus baseline.

DISCUSSION

In this study we evaluated the effects of AHCC® supplementation on LD patients on the reduction of symptoms, improvement of immune parameters and the quality of life of patients.

Previous studies, in a diverse number of animal and human models, have also suggested that AHCC® exhibits other immune enhancement, particularly increasing the number of T cells, dendritic cells, and cytokines, all of which enable the body to effectively respond to viral infections and cancer cells [19,20,21,22,23,24,25], and under various conditions in which the immune system is compromised, suppressed or overactive [15,16,17,18,26]. In this study, the significant decrease in IL-8 is noteworthy since elevated levels of IL-8 have been seen in *Borrelia*-infected animals [27,28] and in the cerebrospinal fluid in *Borrelia*-infected humans [29,30], which *Borrelia* are pathogens of LD. In particular, IL-8 levels were significantly higher in patients infected with the *Borrelia. burgdorferi* genotype RST1, which *Borrelia. Burgdorferi* are main pathogens of LD in North America [31]. In addition, it was shown that ESR, which is an index for inflammation, was decreased after treatment of AHCC®. Thus, in this study, two indexes of inflammation suggested that AHCC® suppressed the inflammation caused by LD. Furthermore, AHCC® completely or partially resolved one or more LD symptoms in 100% of the patients as monitored by the physicians, including flu-like symptoms, eye manifestations, joint manifestations, muscle manifestation, neurological manifestation, cardiovascular manifestation and neck stiffness. It appeared that the improvement of LD symptoms was induced by suppression of inflammation with AHCC®. The wide variety of the symptoms, which is one of LD characteristics, severely troubled the patients. Therefore, it is worth noting that AHCC may improve LD symptoms.

AHCC® has also shown preventive effects against a wide range of viral and bacterial agents including influenza [32,33,34], *Leishmania infantum* [35,36], West Nile [37], *Chlamydia trachomatis* [38], avian influenza virus, *Klebsiella pneumoniae*, *Candida albicans*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* [39]. Another point of interest is the effect of AHCC® on pathogen clearance. In animal models, AHCC® supplementation resulted in bacterial clearance (*Klebsiella pneumonia*) of mice [22,40,41] and rapid viral clearance of influenza virus in the lungs of mice [32,33]. In this study, pathogen clearance was not able to be analyzed due to inadequate data. However, based on previous studies, AHCC® may also be able to clear LD pathogens. Further studies on the clearance of LD pathogens are expected.

CONCLUSION

This pilot study provides preliminary evidence that AHCC® may be effective in reducing symptoms and improving immune function in LD patients. While LD is a complex and its etiology is not completely understood, the immunomodulatory activity of AHCC® appears to enhance the body's own response to the bacteria infection, as well as help counteract immune dysfunction that results in inflammation and symptoms associated with PTLDS. Additional studies are now needed to determine the mechanism of action of AHCC® as an immunomodulatory agent in LD. To clarify the effects of AHCC® on LD, placebo-controlled clinical studies in more subjects and further studies to elucidate the mechanism of action of AHCC® as an immunomodulator are needed.

List of Abbreviations used: LD, Lyme disease; EM, erythema migrans; PTLDS, post-treatment Lyme disease syndrome; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin; IL, Interleukin; NK, natural killer.

Competing interests: This study was conducted at the research expense of Amino Up Co., Ltd.

Author's Contributions: All authors contributed to this study.

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