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Effect of Active Hexose Correlated Compound (AHCC) in Alcohol-Induced Liver Enzyme Elevation

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Summary To investigate the effects of Active Hexose Correlated Compound (AHCC) supplementation and the mechanism action of AHCC in patients with alcohol-induced mildly elevated liver enzyme levels, participants were randomly allocated to the placebo, 1 g AHCC, or 3 g AHCC group and took the supplement for 12 wk. Subjects visited the hospital for clinical and biochemical measurements, for examination of adverse events, to return unused supplements, and to obtain their next supplements. Biochemical tests including liver enzymes, a questionnaire survey, and anthropometric measurements were collected at baseline and every 4 wk thereafter. Adherence and adverse events were evaluated. After 12 wk of supplementation, the percentage change in alanine aminotransferase (ALT) level was significantly different between the placebo ($4.02 \pm 59.07\%$) and both AHCC groups (1 g AHCC: $-23.89 \pm 20.59\%$, 3 g AHCC: $-24.09 \pm 30.73\%$) ($p=0.04$). Serum levels of tumor necrosis factor-α ($p<0.05$) and interleukin-1β ($p<0.01$) were significantly lower, while those of adiponectin were higher in both AHCC groups than in the placebo group ($p<0.01$). AHCC supplementation for 12 wk may improve the levels of liver enzymes and circulating pro-inflammatory and anti-inflammatory cytokines in patients with alcohol-induced liver enzyme elevation with mildly elevated liver enzyme levels.

Key Words alcoholic liver disease, alanine aminotransferase, active hexose correlated compound, adiponectin

Deaths attributable to alcohol comprise approximately 4% of all deaths worldwide (*1*). Alcoholic liver diseases, including fatty liver, hepatitis, cirrhosis, and cancer, account for a quarter of such deaths (*1*). Fatty liver, which is present in more than 90% of heavy drinkers, is asymptomatic and reversible with alcohol abstinence. However, with continued alcohol consumption, hepatic inflammation and injury, a condition known as alcoholic hepatitis, can occur. The prognosis of alcoholic hepatitis is variable, with nearly 100% survival in mild cases as compared to high short-term mortality in severe cases (*2*). Patients with continued excessive alcohol intake are at risk for the development of fibrosis and cirrhosis. Twenty to forty percent of patients with fatty liver will progress to fibrosis, of which 8–20% will develop cirrhosis (*3*). Despite the profound impact of alcoholic liver disease on public health, there are currently no effective treatments, and, other than abstinence, no preventive measures.

Although alcohol is considered a direct hepatotoxin, its resultant hepatic injury involves complex interactions with additional factors that remain incompletely defined (*4*). Oxidative stress and inflammatory cytokines have been investigated for their roles in the alcohol-induced liver inflammatory response (*5*, *6*). Several clinical trials examining the effects of antioxidants (*7*, *8*) or tumor necrosis factor (TNF)-α inhibitors (*9*, *10*) have been conducted in alcoholic hepatitis patients; however, evidence for these treatments in alcoholic liver disease is still lacking.

Active Hexose Correlated Compound (AHCC), extracted from several species of *Basidiomycetes* mushrooms, is a mixture of carbohydrates including oligosaccharides, amino acids, lipids, and minerals derived from fungi (*11*). The active ingredients of AHCC are α-glucan, acetylated α-glucan, and β-glucan of polysaccharides (*12*). It has been suggested that AHCC has anti-oxidative (*11*), anti-inflammatory (*12*, *13*) and immunostimulatory effects (*14*, *15*) as well as hepatoprotective properties in vitro (*16*, *17*) and in vivo (*12*).

As in other liver diseases, patients with cirrhosis are at risk for ascites, variceal bleeding, and encephalopathy from hepatic decompensation and hepatocellular carcinoma. Despite some advances in our understanding of the natural history, pathogenesis and clinical characteristics of alcoholic liver disease, relatively few advances have been made in the treatment field. Novel therapies are thus needed. In previous research, the effect of AHCC on liver disease was studied only in hepatocellular carcinoma patients (*18*). We intended to investigate the effects of AHCC supplementation and the mecha-

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Table 1. Inclusion and exclusion criteria.

Inclusion criteria
Sonographically diagnosed fatty liver
Elevated aminotransferase or gamma-glutamyl transferase (γ-GT) above the reference range (Reference range: aspartate aminotransferase=0–34 IU/L, alanine aminotransferase=0–40 IU/L and γ-GT=5–555 IU/L)
Alcohol consumption: ≥140 g/wk in women, ≥280 g/wk in men
Age between 18 and 75 y
Volunteers willing to participate in this clinical trial and to fill in a written informed consent
Exclusion criteria
>3 times the upper limit of the normal range in any one of the 3 liver enzymes
Viral or autoimmune hepatitis
Liver cirrhosis, decreased platelet count (<150,000/μL), prolonged prothrombin time (>12.5 s), or low albumin (3.3 g/dL)
Malignant tumor including hepatic cellular carcinoma or elevated α-fetoprotein (>7.0 ng/mL)
Uncontrolled diabetes (glycosylated hemoglobin, HbA1c >7.0%)
Decreased renal function (serum creatinine levels >1.5 mg/dL)
Cardiovascular diseases such as congestive heart failure, myocardial infarction, transient ischemic attack or cerebral infarction
Subjects with allergies, especially to mushrooms
Pulmonary diseases including pulmonary tuberculosis
Use of any medications that alter liver enzymes such as acetaminophen, non-steroidal anti-inflammatory drugs, anti-viral agents, HMG-CoA reductase inhibitors, or steroids within the past month
Use of agents for liver disease such as ursodeoxycholic acid, biphenyl-dimethyl-dicarboxylate, silymarin, Carduus marinanus ext., or any herbs within the past month
Pregnancy, lactation, or childbirth within the past 6 mo
Participation in any other clinical trial within the last 3 mo
Any other conditions deemed unsuitable for the trial as evaluated by the physician in charge

nism of AHCC in alcoholic liver disease patients with mildly elevated liver enzyme levels.

METHODS

This study was approved by the Institutional Review Board of Chung-Ang University Hospital, Seoul, Republic of Korea (C2009068(255)), and complied with the code of ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all participants.

Study design and participants. This study was a randomized, controlled, double-blind clinical trial conducted between May and September 2010 at the Chung-Ang University Healthcare System in Seoul. Eligible participants with elevated liver enzyme level associated with alcohol consumption were consecutively recruited. The inclusion and exclusion criteria are shown in detail in Table 1.

Preparation of AHCC. Capsules for the trial were supplied by Amino Up Chemical Co., Ltd. (Sapporo, Japan). Each capsule was a light brown color and contained 167 mg of AHCC with 333 mg of dextrin and malt extract or a placebo of dextrin and malt extract. AHCC is an enzyme-fermented extract of the *Basidiomycetes* mushroom that is available as a dietary supplement. The complex compound contains a mixture of polysaccharides, amino acids, lipids, and minerals (*12*). The predominant components are oligosaccharides, totaling approximately 71.2% of the total dry weight (*12*).

Interventions. The treatment duration was set at 12 wk since the effect of AHCC appeared after 3 mo in a previous study (*19*). Patients were treated 30 min before breakfast and dinner for 12 wk with 3 g/d AHCC (500 mg of AHCC/capsule×3 capsules×2 doses) for the 3 g AHCC group, 1 g/d of AHCC (167 mg of AHCC with 333 mg of dextrin and malt extract/capsule×3 capsules×2 doses) for the 1 g AHCC group, and capsules with dextrin and malt extract for the placebo group. Throughout the study, all participants were asked to maintain their regular diet, lifestyle, and routine oral medications, except any medications affecting liver enzymes such as acetaminophen, non-steroidal anti-inflammatory drugs, anti-viral agents, HMG-CoA reductase inhibitors, steroids, and agents for liver disease treatment such as ursodeoxycholic acid, biphenyl-dimethyl-dicarboxylate, silymarin, *Carduus marinanus* extract, or herbal supplements.

Outcome assessment. After the screening visit, each participant visited the hospital three times for clinical and biochemical measurements, examination of adverse events, return of unused supplements, and their next supply of supplements every 4 wk. When the value of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) in the study period increased 2-fold or more compared to the baseline or previous value, subjects were dropped from this study. The primary outcomes evaluated were the value and the percentage change of three liver enzymes (AST, ALT, and gamma-glutamyl transferase (γ-GT)) in each group. The percentage change was calculated using this formula: [(after supplementation−baseline)/baseline]×100. The secondary outcomes analyzed were the biochemical parameters and questionnaire scores in each group.

Biochemical tests were performed on blood samples

Table 2. Baseline characteristics.

	Placebo (n=19)	1 g AHCC (n=21)	3 g AHCC (n=22)	p-value
Demographic and clinical data				
Age (y)	41.68±12.01	39.81±9.64	37.68±8.54	0.45
Gender				0.20
Male	17 (89.47)	20 (95.24)	22 (100)	
Female	2 (10.53)	1 (4.76)	0	
Body mass index (kg/m^2)	25.71±2.68	26.51±2.45	26.67±3.04	0.50
Total body fat (%)	23.96±4.62	23.82±4.22	23.70±4.98	0.67
Total lean body mass (kg)	54.67±6.30	55.24±5.60	55.53±5.22	0.35
Systolic blood pressure (mmHg)	124.90±12.97	126.48±8.15	126.59±9.61	0.85
Diastolic blood pressure (mmHg)	81.32±10.69	78.24±6.00	79.00±6.46	0.45
Physical exercise	2 (10.53)	7 (33.33)	7 (31.82)	0.19
Current smoking	7 (38.89)	7 (35.50)	12 (54.55)	0.40
Brief fatigue inventory (score)	4.61±1.39	5.34±1.88	5.00±1.63	0.39
BEPSI-K[1] (score)	2.00±0.86	2.12±0.63	1.82±0.70	0.39
Biochemical data				
Aspartate aminotransferase (IU/L)	54.79±9.16	58.05±12.20	56.73±12.82	0.67
Alanine aminotransferase (IU/L)	46.53±26.44	52.81±23.58	63.05±34.38	0.18
γ-Glutamyl transferase (IU/L)	69.58±32.85	82.00±49.03	75.41±26.36	0.58
Bilirubin (mg/dL)	0.54±0.23	0.52±0.17	0.55±0.43	0.95
Albumin (g/dL)	4.75±0.36	4.84±0.38	4.77±0.38	0.72
Fasting glucose (mg/dL)	97.21±18.42	94.00±12.29	101.91±38.72	0.61
Fasting insulin (μIU/mL)	12.29 (7.74–22.81)	11.15 (9.51–14.58)	11.65 (6.69–19.84)	0.93
HOMA-IR[2]	2.82 (1.82–5.69)	2.89 (1.78–3.39)	2.77 (1.75–5.14)	0.94
Total cholesterol (mg/dL)	196.26±33.42	215.76±53.57	211.14±40.20	0.34
LDL-cholesterol (mg/dL)	108.19±29.87	125.95±59.90	124.17±37.24	0.39
HDL-cholesterol (mg/dL)	45.34±8.80	44.28±8.84	44.27±10.56	0.92
Triglycerides (mg/dL)	213.68±162.50	227.67±129.00	213.45±139.37	0.94
BUN[3] (mg/dL)	13.66±2.35	14.03±3.89	13.10±3.55	0.39
Creatinine (mg/dL)	0.91±0.09	0.99±0.08	0.98±0.11	0.10
Adiponectin (μg/mL)	3.53±1.98	3.79±1.37	3.09±1.35	0.43
TNF-α[4] (pg/mL)	1.03 (0.67–1.26)	1.18 (0.80–1.91)	1.44 (0.90–2.03)	0.22
IL-1β[5] (pg/mL)	0.08 (0.06–0.10)	0.12 (0.06–0.26)	0.14 (0.09–0.29)	0.02

Data are shown as mean±standard deviation, median (25–75%), or number (%).
p-values were calculated by ANOVA, Kruskal-Wallis test, or χ^2-test.
[1] Brief encounter psychosocial instrument-K.
[2] Homeostasis model assessment of insulin resistance.
[3] Blood urea nitrogen.
[4] Tumor necrosis factor-α.
[5] Interleukin-1β.

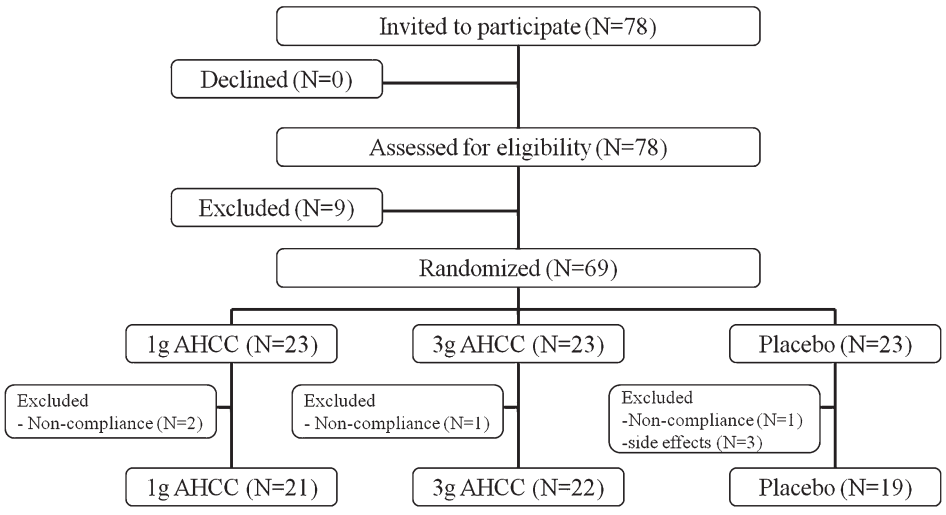

Fig. 1. Flow diagram of study patients.

Table 3. Clinical and biochemical characteristics after 12 wk of supplementation.

	Placebo (n=19)	1 g AHCC (n=21)	3 g AHCC (n=22)	p-value
Body mass index (m^2/kg)	25.51±1.89	26.31±2.21	26.28±2.84	0.62
Total body fat (%)	24.23±4.82	23.56±3.87	23.45±4.78	0.23
Total lean body mass (kg)	54.02±6.30	55.13±5.15	55.27±5.48	0.12
Systolic blood pressure (mmHg)	125.73±9.52	123.41±10.51	122.19±10.04	0.67
Diastolic blood pressure (mmHg)	82.18±6.79	78.46±5.77	76.69±4.83	0.03
Brief fatigue inventory (score)	4.10±1.59	4.95±1.44	4.73±1.45	0.34
BEPSI-K[1] (score)	1.82±0.64	2.06±0.79	1.76±0.43	0.39
Aspartate aminotransferase (IU/L)	45.73±13.43	40.41±12.12	40.88±11.05	0.48
Alanine aminotransferase (IU/L)	44.82±24.56	39.00±18.54	46.13±25.57	0.64
γ-Glutamyl transferase (IU/L)	78.45±43.76	61.29±36.74	62.81±23.58	0.40
Bilirubin (mg/dL)	0.65±0.14	0.60±0.16	0.72±0.42	0.47
Albumin (g/dL)	4.66±0.38	4.75±0.26	4.69±0.38	0.76
Fasting glucose (mg/dL)	103.45±22.06	94.82±28.76	103.06±25.30	0.41
Fasting insulin (μIU/mL)	12.57 (9.84–27.26)	14.50 (11.59–28.54)	14.46 (9.51–36.14)	0.94
HOMA-IR[2]	3.94 (2.13–7.54)	3.32 (2.81–5.92)	3.63 (2.13–8.46)	0.99
Total cholesterol (mg/dL)	191.82±37.74	206.76±46.28	194.25±32.86	0.55
LDL-cholesterol (mg/dL)	102.72±32.47	115.08±38.25	112.40±32.22	0.65
HDL-cholesterol (mg/dL)	45.19±6.57	45.07±9.62	39.94±6.79	0.13
Triglycerides (mg/dL)	219.55±152.84	233.06±143.21	209.56±73.07	0.86
BUN[3] (mg/dL)	12.38±2.13	14.22±3.12	13.34±2.32	0.20
Creatinine (mg/dL)	0.95±0.07	1.01±0.08	0.96±0.09	0.12
TNF-α[4] (pg/mL)	1.53 (0.62–1.95)	0.83 (0.65–1.13)	0.96 (0.53–1.29)	0.12
IL-1β[5] (pg/mL)	0.20 (0.10–0.73)	0.08 (0.06–0.09)	0.08 (0.06–0.11)	<0.01
Adiponectin (μg/mL)	2.96±1.38	4.95±1.58	3.63±1.90	<0.01

Data are shown as mean±standard deviation and median (25–75%),
p-values were calculated by ANOVA and Kruskal-Wallis test.
[1] Brief encounter psychosocial instrument-K.
[2] Homeostasis model assessment of insulin resistance.
[3] Blood urea nitrogen.
[4] Tumor necrosis factor-α.
[5] Interleukin-1β.

collected after overnight fasting (>12 h). White blood cells and hemoglobin levels were determined within 1 h after blood collection using the ADVIA 120 automated hematology analyzer (Siemens, Tarrytown, NY). Serum levels of AST, ALT, γ-GT, bilirubin, albumin, blood urea nitrogen, creatinine, fasting glucose, total cholesterol, HDL-cholesterol, and triglycerides were measured using an ADVIA 1650 Chemistry system (Siemens). LDL-cholesterol was calculated using Friedewald's formula [LDL-cholesterol=total cholesterol−HDL-cholesterol−(triglyceride/5)] if the serum triglyceride level was below 400 mg/dL. Fasting insulin levels were measured by electrochemiluminescence immunoassay (Roche, Indianapolis, IN), and insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) index [(insulin (μlU/mL)×fasting blood glucose (mg/dL)/18)/22.5]. Interleukin (IL)-1β and TNF-α levels were measured using a commercially-available enzyme-linked immunosorbent assay (R&D, Minneapolis, MN). The intra- and inter-assay coefficients of variation were 5.3±2.8% and 8.4±1.9% for TNF-α, and 6.7±3.1% and 5.6±4.5% for IL-1β. Plasma adiponectin levels, an anti-inflammatory adipokine, were measured using an enzyme immunoassay kit (AdipoGen, Seoul, Korea) with inter- and intra-assay variability of 4.63%±0.82% and 2.72%±0.52%, respectively.

Fatigue and mental stress was measured via questionnaire. The brief fatigue inventory (BFI) composed of simple numeric rating scales from 0 to 10 was used for assessing fatigue. Severe fatigue was defined as a worst fatigue score of 7 or greater. For mental stress assessment, the Korean brief encounter psychosocial instrument (BEPSI-K), a validated indicator of perceived stress, was used. BEPSI-K consists of 5 items on a 1–5 Likert scale.

Anthropometric measurements were performed in the morning after overnight fasting while subjects were wearing light clothing and no shoes by a single observer. A body composition analyzer (Inbody 3.0, Biospace, Seoul, Korea) was used to measure their heights and body weights. The body mass index (BMI) was calculated by dividing the measured weight (kg) by the square of height (m^2). Subjects sat on a chair in a stable and relaxed state, and their blood pressure was measured using a mercurial blood pressure tonometer.

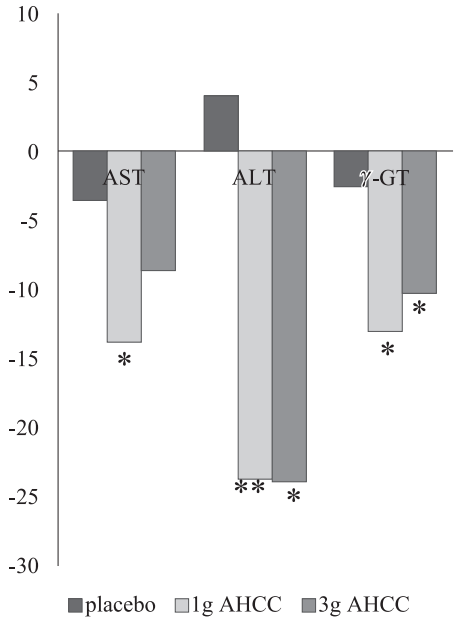

Fig. 2. Comparison of percentage changes by paired *t*-test of liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (γ-GT). Liver enzymes were significantly decreased in both AHCC groups.

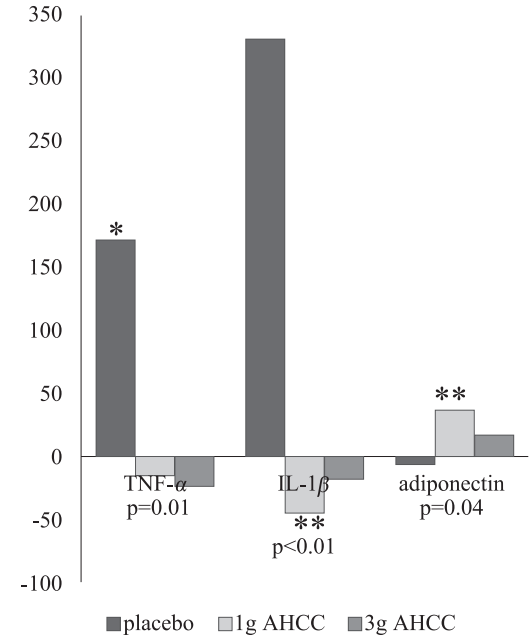

Fig. 3. Comparison of percentage changes by paired *t*-test and ANOVA of cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and adiponectin, after 12 wk of supplementation. In the 1 g AHCC group, IL-1β was significantly decreased while adiponectin increased. *p*-values calculated by ANOVA are shown below the name of the cytokine. * $p<0.05$, paired *t*-test, ** $p<0.001$, paired *t*-test.

Adherence was expressed as a percentage, calculated by taking the number of supplements ingested divided by the number the patient should have ingested and multiplying by 100. The number of supplements ingested was examined as the number of capsules given minus the number of capsules returned at the next visit. The incidence and type of adverse events were also included in the outcome parameters.

Randomization and concealment. The random allocation sequence was generated by a sponsor through SAS implementation with PROC PLAN. Subjects were included in one of three groups with equal probability. The sponsor provided a research pharmacist with supplements numbered according to the random allocation sequence. A clinical investigator received 69 sealed envelopes containing a sheet noting the assigned group from the sponsor. The investigator opened the relevant envelope only when an adverse event occurred and then announced the assigned group to the participant.

Blinding. Both study participants and all investigators, including a research nurse who contacted the participants, a research pharmacist, and a physician, were blinded after assignment to interventions.

Sample size. The estimated minimum number of required patients according to an anticipated difference in ALT value of 15 with a standard deviation of 13, a two-tailed $\alpha=0.05$, and a $\beta=0.2$ was 48 subjects (16 patients per each group). Taking into account an estimated 30% dropout rate, we enrolled total 69 participants.

Statistical analyses. Even after logarithmic transformation, variables such as IL-1β, TNF-α, fasting insulin and HOMA-IR did not have a normal distribution; thus non-parametric analysis was used. Clinical and biochemical baseline characteristics were compared among the three groups with ANOVA or the Kruskal-Wallis test for continuous variables and with the χ^2-test or Fisher's exact test for categorical variables. The mean values were compared with ANOVA and the Kruskal-Wallis test among the three groups. The percentage changes between time intervals within each group were analyzed by paired *t*-tests. Correlation between changes in liver enzymes and cytokines were calculated by Pearson's correlation or the Spearman/Kendall correlation. Significance was defined at the 0.05 level. The data was processed with an SAS 9.1 statistics package (SAS Institute Inc, Cary, NC). All values with normal distribution are expressed as the mean±SD, and the others as the median (25–75%).

RESULTS

Among 78 screened patients who were suspected of having alcoholic liver disease, 69 patients were eligible and were randomly assigned to one of the three groups. Demographic, clinical, and biochemical characteristics are given in Table 2. During the study, seven patients dropped out. Of those who dropped out, four patients discontinued due to disinclination (1 g AHCC group: $n=2$, 3 g AHCC group: $n=1$, placebo group: $n=1$), and in the placebo group, three participants experienced mild gastrointestinal distress. There were no adverse events in either AHCC group. A total of 62 participants completed the study (Fig. 1). Adherence of the study

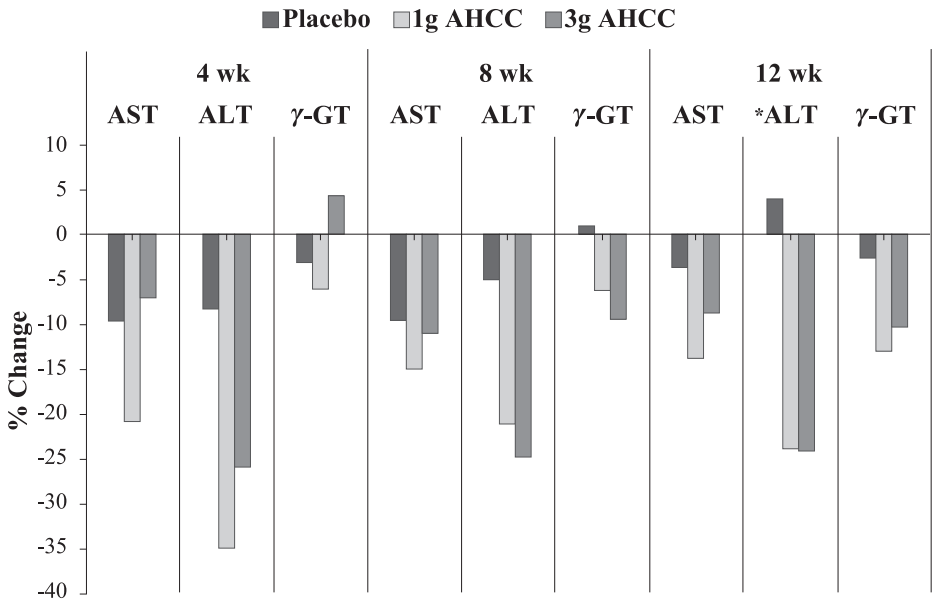

Fig. 4. Changes in liver enzymes within groups. We show mean changes in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (γ-GT) between baseline and 4, 8, or 12 wk after supplementation. p-values were calculated by ANOVA. * $p<0.05$.

participants was 92.4±6.8% with no significant difference between the groups ($p=0.88$).

The mean clinical and biochemical outcomes at 12 wk were analyzed with ANOVA (Table 3). The liver enzyme levels, lipid profiles, insulin resistance, and scores on fatigue and stress-related questionnaires did not show significant differences between the groups ($p>0.05$); however, cytokine levels did show significant differences (TNF-α, IL-1β and adiponectin, $p<0.05$).

Comparing the percentage changes at 12 wk as analyzed by a paired t-test, a decrease in liver enzymes in both AHCC groups (Fig. 2) and a change in IL-1β and adiponectin in the 1 g AHCC group (Fig. 3) were found to be statistically significant. When analyzed with ANOVA, the percentage changes of serum levels of inflammatory cytokines, such as TNF-α ($p<0.05$) and IL-1β ($p<0.01$), were lower in the AHCC supplementation groups than the placebo group (Fig. 3). Serum levels of adiponectin were higher in both AHCC groups than in the placebo group ($p<0.05$, Fig. 3).

Figure 4 depicts the percentage changes in three hepatic enzymes analyzed with ANOVA. The comparison of mean percentage changes was not found to be significantly different among the three groups except for ALT after 12-wk supplementation.

The association between changes in hepatic enzymes (AST, ALT, and γ-GT) and changes in cytokines (TNF-α, IL-1β, and adiponectin) was analyzed (Fig. 5). There were positive relationships among percentage changes in hepatic enzymes. The percentage change of IL-1β was positively correlated with that of ALT ($\tau=0.25$, $p=0.02$), whereas an inverse relationship was revealed between adiponectin and IL-1β ($\tau=-0.40$, $p<0.01$), and adiponectin and TNF-α ($\tau=-0.36$, $p<0.01$). Though not significant, a tendency toward a positive correlation of the percentage change between TNF-α and γ-GT ($\tau=0.19$, $p=0.07$) and an inverse correlation between adiponectin and AST ($r=-0.26$, $p=0.09$) was observed.

DISCUSSION

In this double-blind, randomized controlled trial, we examined whether AHCC is effective in liver injury caused by chronic and excessive alcohol ingestion and whether the effects of AHCC are dose-dependent. AHCC supplementation for 12 wk significantly improved ALT levels, decreased pro-inflammatory cytokines (TNF-α and IL-1β), and elevated anti-inflammatory cytokines (adiponectin) in both AHCC groups after 12 wk of supplementation without any adverse events. Hepatoprotective effects accompanied by striking anti-inflammatory effects were observed regardless of the dosage.

Ingestion of alcohol initiates a variety of metabolic responses that influence the final hepatotoxic response (*4*). Pro-inflammatory cytokines including TNF-α, IL-1β, and interferon-γ, are regarded as major contributors to the development of alcohol liver injury (*5*, *6*). In contrast, adiponectin, a fat-derived anti-inflammatory adipokine, is considered to play a protective role in fatty liver suppression by upregulation of AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor (PPAR)-α, and PPARγ coactivator (PGC)-1α, and reduction of lipopolysaccharide (LPS)-stimulated TNF-α production and the TNF-α mediated inflammatory response in alcoholic liver disease (*5*). Increased expression of iNOS is known to be associated with alcohol-induced hepatic dysfunction (*20*, *21*). Pro-inflammatory cytokines also induce expression of the iNOS gene (*22*, *23*). In iNOS-knockout mice, alcohol-induced liver injury with elevated serum ALT levels and fatty liver was prevented (*24*). The iNOS inhibitor, *N*-(3-aminomethyl) benzylacetamidine, showed similar protec-

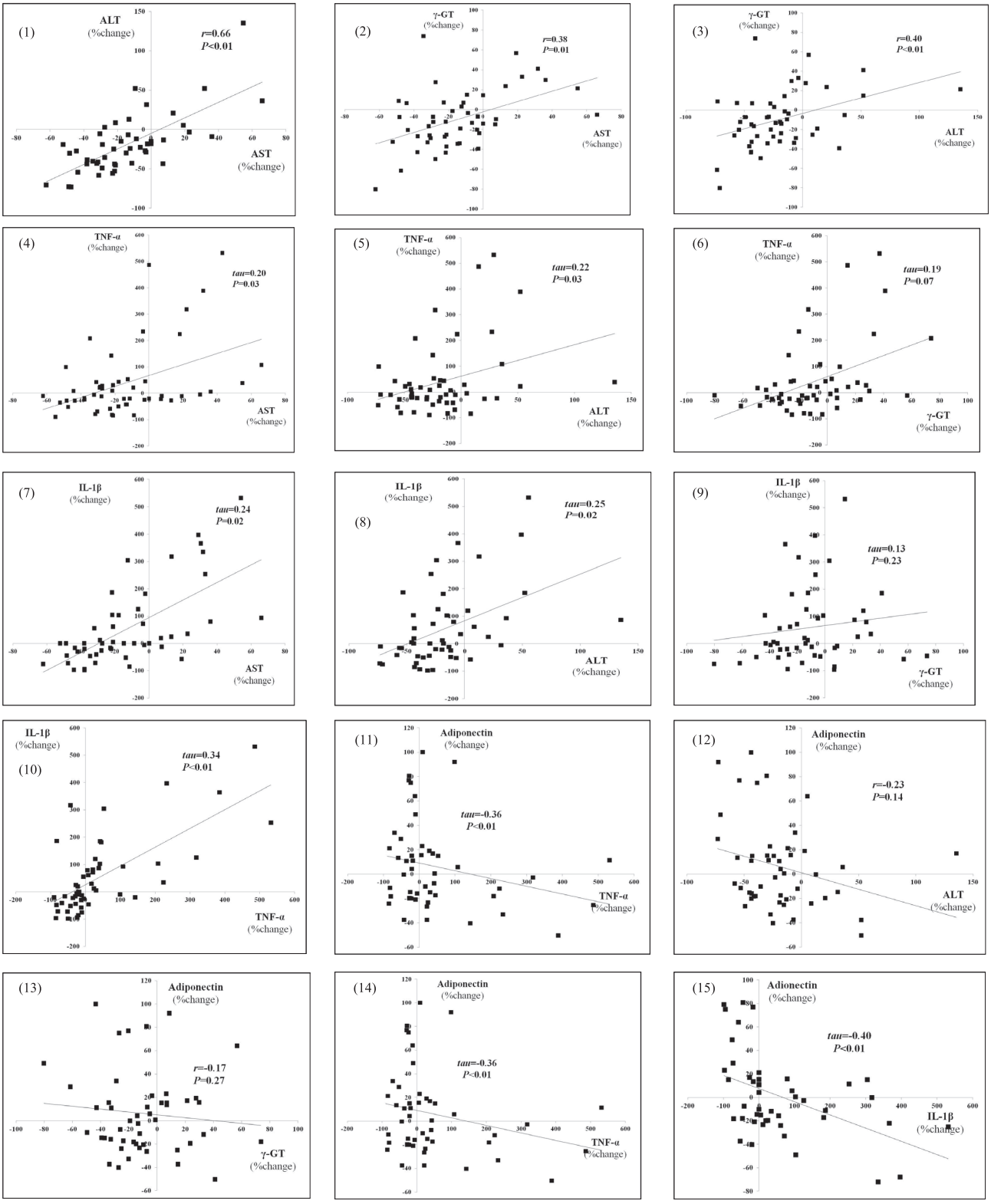

Fig. 5. Correlation between changes in liver enzymes and cytokines after 12 wk of supplementation. Correlation between changes in liver enzymes and cytokines were calculated by Pearson's correlation or Spearman/Kendall correlation. (1) Percentage change of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), (2) percentage change of gamma-glutamyl transferase (γ-GT) and AST, (3) percentage change of γ-GT and AST, (4) percentage change of tumor necrosis factor-α (TNF-α) and AST, (5) percentage change of TNF-α and ALT, (6) percentage change of TNF-α and γ-GT, (7) percentage change of interleukin-1β (IL-1β) and AST, (8) percentage change of IL-1β and ALT, (9) percentage change of IL-1β and γ-GT, (10) percentage change of IL-1β and TNF-α, (11) percentage change of adiponectin and AST, (12) percentage change of adiponectin and ALT, (13) percentage change of adiponectin and γ-GT, (14) percentage change of adiponectin and TNF-α, and (15) percentage change of adiponectin and IL-1β.

tive effects in alcohol-induced liver damage (*24*).

Endotoxins have been accepted as another pathophysiologic mechanism of alcoholic liver disease (*4*). Chronic alcohol ingestion promotes hepatic inflammation by increasing translocation of gut-derived endotoxins to the portal circulation and activating Kupper cells through the LPS/Toll-like receptor (TLR)-4 (*5*).

AHCC has been utilized for over a decade in Japan, and the typical daily recommended dose of AHCC-fine granules (60% AHCC-freeze dried preparation) is 3 g in clinical practice (*25*). The safety of AHCC as a dietary supplement has been reported in several studies evaluating genotoxicity or acute and subchronic oral toxicity (*14*, *25*, *26*).

In previous research, AHCC was shown to improve liver enzymes through the modulation of pro- and anti-inflammatory cytokines (*12*, *13*). In rats with hapten-induced colitis, AHCC administration reduced pro-inflammatory cytokines, including TNF and IL-1β, as well as colonic inflammation (*13*). AHCC increased detoxification enzymes in the liver and protected the liver from CCl_4^- (*27*) and ferric nitrilotriactate (Fe-NTA)-induced injury (*28*). AHCC administration normalized elevated liver enzyme levels in 6-mercaptopurine and methotrexate-induced liver injury in mice (*12*). In humans, AHCC intake improved the levels of AST and γ-GT in postoperative hepatocellular carcinoma patients (*18*). In accordance with previous research, our study showed improvement of the levels of liver enzymes and circulating pro-inflammatory and anti-inflammatory cytokines in patients with mildly elevated liver enzyme levels.

AHCC suppressed iNOS gene expression through inhibition of the Akt/type I IL-1 receptor (IL-1RI)-dependent pathway in hepatocytes (*17*). AHCC can decrease hepatic inflammation by reducing gut-derived endotoxins in alcoholic liver disease and increasing aerobes, lactic acid bacteria, and bifidobacteria in addition to decreasing clostridium in the feces of colitis rats through its role as a prebiotic (*13*). The colonic microflora of hapten-induced colitis rats treated with AHCC was different from that of not only non-treated hapten-induced colitis rats but also normal controls (*13*). Oligosaccharides, a main component including the active ingredient, may accelerate the growth of normal flora in the gut, inhibit the growth of pathogenic microorganisms (*29*, *30*), and prevent bacterial translocation through their interaction with bacteria (*31*, *32*).

The current study has limitations. First, the number of study participants was small and the study period was relatively short. In order to elucidate the beneficial action mechanism of AHCC for alcoholic liver disease patients, studies that include measurements of serum endotoxin levels and the identification of intestinal flora through fecal culture are necessary.

CONCLUSION

Our results demonstrated promising effects with a 12-wk course of AHCC supplementation for improving liver enzyme levels and circulating pro-inflammatory and anti-inflammatory cytokines in patients with alcohol-induced liver enzyme elevation.

Conflict of interest statement: none.

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