Efficacy and Safety of Human Placental Extract for Alcoholic and Nonalcoholic Steatohepatitis: An Open-Label, Randomized, Comparative Study

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Human placental extract (HPE) is a traditional medicine that has been used for the symptomatic treatment of liver disease without any verifying clinical evidence. This study aimed to evaluate the efficacy and safety of HPE in patients with alcoholic or nonalcoholic steatohepatitis (ASH or NASH). We designed this clinical trial as a multicenter, open-label, randomized, comparative noninferiority study to improve the reliability of analyses. The enrollment criteria were limited to ASH or NASH patients with serum alanine aminotransferase (ALT) 1.5-fold higher than the normal level. Patients in the control group were treated with a commercially available mixture of liver extract and flavin adenine dinucleotide (LE–FAD). Intention-to-treat (ITT) analysis was applied to 194 patients, and per-protocol (PP) analysis was available for 154 patients. The rate of primary goal achievement of treatment efficacy was arbitrarily defined as 20% or greater improvement in ALT level compared with the pretreatment level and did not differ significantly between the HPE and control groups [62.9% (44/70) *vs.* 48.8% (41/84); $p=0.0772$]. ITT and modified ITT analysis showed results similar to those of PP analysis. Adverse drug reactions (ADRs) of minimal to moderate degree occurred in 3.1% of patients. The ADR and treatment compliance rates were similar in both groups. In conclusion, the clinical value of HPE in the treatment of ASH and NASH is equivalent to that of LE–FAD.

Key words human placental extract; alcoholic steatohepatitis; nonalcoholic steatohepatitis

Alcoholic and non-alcoholic steatohepatitis (ASH and NASH) are two major non-viral chronic liver diseases worldwide. In Korea, the prevalence of NASH increases during the last four years, whereas that of ASH decreases.[1] NASH reflects not only the derangement in life style, but it has the potential for metabolic syndrome such as hypertension, Type 2 diabetes mellitus, hyperlipidemia and obesity.[2] Alcohol abuse and obesity reciprocally affect each condition of alcoholic and non-alcoholic steatohepatitis as compounding factors.[3] Concurrent management of alcohol abstinence and weight control by the lifestyle correction is the key recommended treatment for those patients.[4,5] However, the non-medicinal management is frequently not successful to maintain wellness because of yo-yo dieting and habitual alcoholism. Consequently, some of those steatohepatitis cases may progress further to the fibrosis or cirrhosis stage. These imply that the combination of drug therapy in addition to the lifestyle management is necessary to reduce the severity of the disease, together with the promotion of hepatic regeneration.[6]

Human placental extract (HPE) has been used for the first time in 1950s in Japan as a drug to treat hepatic diseases. Reportedly, the constituents of HPE are quite diverse and include the followings: uracil, tyrosine, phenylalanine, L-tryptophan and collagen derived peptides[7–9]; certain steroid hormones[10]; cytokines such as hepatocyte growth factor (HGF).[11,12] Because of its strong antioxidant substances, anti-inflammatory mediators and growth factors, HPE may play a potential role in liver cell protection from such kind of hepatic injury. Therefore, in this study, we planned to evaluate the clinical value of HPE in terms of efficacy and safety for the treatment of liver diseases, especially ASH and NASH, and designed as a multicenter controlled clinical trial with an open-labeled randomized comparative non-inferiority study to improve the reliability.

MATERIALS AND METHODS

Study Design This study was performed as a prospective, randomized, open-labeled, multicenter, and active treatment controlled clinical trial between September 28, 2007 and July 07, 2009. The clinical protocol was approved by the Institutional Boards of Review in all nine participating university medical centers and general hospital in Korea, including Bundang Jesaeng General Hospital, Seoul Medical Center, Bundang Cha General Hospital, St. Mary's Hospital, Kosin University Gospel Hospital, Keimyung University Dongsan Medical Center, Gachon University Gil Hospital, Yonsei University Severance Hospital, Ulsan University Gangnueng Asan

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Hospital. All relevant circumstances of the clinical trial were explained to the voluntary participants with plain language and informed consents were received from them before enrolment in this study. All assignment of patients, randomization, record keeping, data collection, and data analysis were conducted by a third party. The entire study followed the ethical accordance with the principles of the Declaration of Helsinki.

The eligibility criteria required the demonstration of non-viral chronic hepatitis (including ASH and NASH) persisting at least 6 months. The criteria included elevation of at least two consecutive liver tests, persisting at least 6 months.[13] The candidates based on diagnostic criteria were evaluated with screening assessments within 2 weeks before randomization. The screening included: body weight, vital sign, physical examination, pregnancy test for women of child bearing age, liver function tests including alanine transaminase (ALT), aspartate transaminase (AST) and total bilirubin (TB), viral hepatitis test with HBs Ag, anti-HBV and anti-HCV antibodies, and laboratory tests, including complete blood count with differential count, urine analysis, and blood biochemistry profile. The abdominal sonogram was performed only for those patients it deemed necessary. Patients who met the eligibility criteria were enrolled in this study. Test results obtained at the screening were used as a baseline. All patients were then randomized to each group. Follow-up liver tests (ALT, AST, and TB) were taken at each visit every 2 weeks for three times to assess the efficacy of study. For each patient, the time point of treatment ending was defined as either ALT reaching normal level at 10–40 international units per liter (IU/L), or when scheduled dosing was finished.

If patients had been taking other medications for liver disease, they had one week of a washout period before the screening assessments. Patients were not permitted to receive any associated concomitant medications for hepatic diseases such as cholagogues, any known or suspected drug with risk of affecting liver function, and the over-the counter/prescription medications known to be used for liver function improvement or for the treatment of liver disease. However, conventional or temporary medications used for the treatment of other diseases that would not affect the result of this trial were allowed by physician's judgment.

Participants Patients were evaluated for their eligibility by examining the inclusion and exclusion criteria. Inclusion criteria were as follows: 1) age between 18 and 74, 2) alcoholic hepatitis or chronic nonalcoholic hepatitis, lasting more than 6 months, 3) baseline ALT levels 1.5 times over the upper normal level or 60 IU/L. Patients with abnormal platelet counts at the baseline or coarse parenchymal texture by ultrasonogram were not included in this study, because those parameters may complicate the analyses more than is necessary. Exclusion criteria were as follows: 1) abnormal liver function due to the following disorders: viral hepatitis, biliary atresia, autoimmune hepatitis, Wilson's disease, hemochromatosis, galactosemia, and congenital tyrosinosis, 2) history of hypersensitivity to the product of human placental extract, 3) systemic infection such as tuberculosis, 4) patients with a severe debilitating condition including liver cirrhosis with child class C, metabolic disease, renal disease, pulmonary disease, cardiovascular disease, and neurologic and psychiatric disease, 5) history of malignant neoplasm, 6) history of allergic reaction to the liver extracts compound or Vitamin B2, 7) participation in another clinical trial within last 3 months before enrollment, 8) history of taking the product of human extract within 6 months, 9) pregnancy, nursing mothers, or women of child bearing age not using adequate contraceptive methods, 10) uncooperative patients.

Drugs In this study, the human placenta extract, HPE (Laennec®, GCJBP Corporation, Seoul, Korea) was used for the treatment in the study group. In the control group, the treatment with an active drug, liver extract and flavin adenine dinucleotide (LE–FAD) (Adelavin-9®, manufactured by Choongwae in Seoul, Korea) was chosen rather than the treatment with placebo. Both drugs, HPE and LE–FAD were approved for use to improve liver functions in chronic hepatic diseases mainly in Japan and Korea. HPE is extracted from human placenta including umbilical cord through heating and adding acid for hydrolysis, and composed of proteins of molecular weight (MW) 10–100 kDa, minerals, amino acids, and steroid hormones. A hundred milligram of placenta extract is dissolved in a 2 mL mixture of distilled water and benzoyl alcohol. Each ampule of LE–FAD contains 15 μL of mammalian liver extract and 10 mg of flavin adenine dinucleotide mixture. Patients in each group were injected for 5 consecutive days per week, by either a subcutaneous or intramuscular route. Patients whose ALT level became normal were no longer treated. Patients whose ALT did not improved by more than 10% and those with increased ALT were treated by doubling the dose of ampule per day. The total duration of treatment was 6 weeks.

Evaluation Methods Patients who received at least one dose of study medications consist of safety analyses population. Modified intention-to-treat (mITT) population was defined as all randomized patients who received at least one dose and had at least one efficacy evaluation result. Per-protocol (PP) population was defined as the randomized patients who completed the study without any major violation of the protocol and whose treatment compliance was 80% or more.

Since the histological assessment of a liver-biopsy specimen is not practical on outpatients and the serial change of liver enzymes reflects the biochemical and histological responses to inflammatory liver injury, only the ALT level was used to analyze the clinical outcome in this study.

Primary goal achievement of treatment efficacy was arbitrarily defined as the 20% and above improvement of ALT when compared to the pre-treatment level. The primary efficacy of HPE was evaluated by comparing the number of patients with primary goal achievement after 6 weeks of HPE treatment *versus* number of patients with similar results in LE–FAD control group.

Secondary efficacy endpoints included assessment of: number of patient whose ALT level decreased to less than 40 IU/L; number of patients whose ALT level was 1.2 times or less and 1.5 times or less of the upper limit of normal value (40 IU/L); number of patients whose AST level was normalized; changes in ALT, AST and TB levels at 2 weeks, 4 weeks, and 6 weeks from the baseline; and the time to reach normal ALT level.

For safety evaluation, investigators recorded ADRs according to the WHO Adverse Reaction Terminology (WHO-ART) and judged severity of each adverse drug reaction (ADR).

Statistics All statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, U.S.A.). In the analyses of primary and secondary efficacy, a

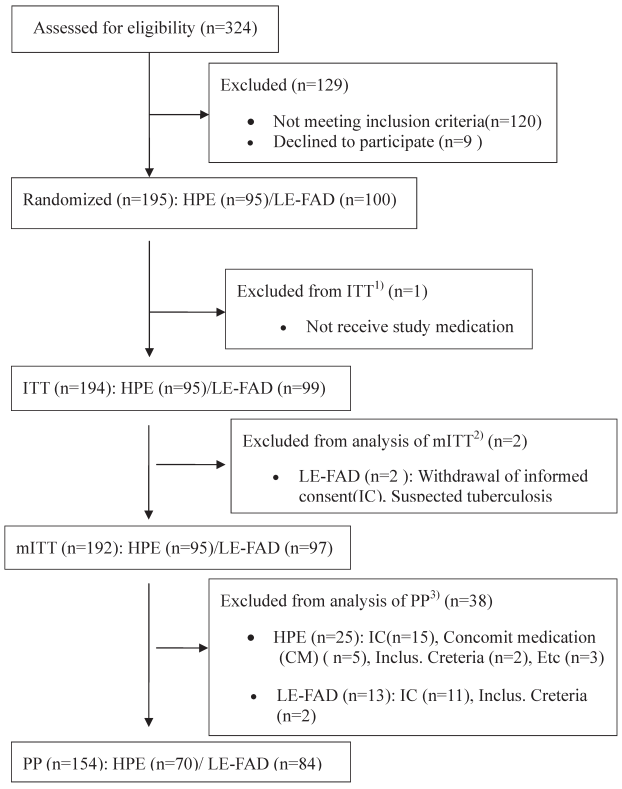

Fig. 1. Study Flow Diagram

HPE, human placental extract; LE–FAD, the mixture of liver extract and flavin adenine dinucleotide[1)] ITT, Intention To Treat[2)]; mITT, modified Intention To Treat[3)]; PP, Per Protocol.

logistic regression analysis was used (one-tailed test, significance level 0.025), and the imputation using last observation carried forward (LOCF) was used for missing data. The disease category (ASH or NASH) and medication history (presence or absence) were used as the covariates of the analysis (ANCOVA). Time to reach normal ALT and AST was estimated by Kaplan–Meier method and analyzed using log-rank test.

Compliance was calculated as following equation: Compliance (%)=(Total numbers of days actually dosed/The total numbers of days should be dosed)×100 and analyzed by using Wilcoxon rank sum test. The frequencies of adverse events were analyzed using chi-square test or Fisher's exact test. The changes of findings in physical examination at visits and changes in laboratory values between the groups were analyzed using paired *t*-test, ANCOVA, generalized estimating equation (GEE), McNemar's test, or Cochran–Mantel–Haenszel test. Demographic and other baseline characteristics were analyzed using chi-square analysis and Fisher's exact test or Wilcoxon rank sum test, accordingly. Two-tailed *p* values of <0.05 were considered significant.

RESULTS

Flow Chart of the Study A total of 324 patients were screened for this trial (Fig. 1). One hundred ninety four patients were enrolled following the initial-treatment-intent criteria (group for ITT analysis), 95 patients were assigned to the HPE (Laennec) group and 99 patients to the LE–FAD (Adelavin-9) group. After two patients were excluded from mITT population due to suspected pulmonary tuberculosis, 192 patients were included for efficacy evaluation. thirty eight patients were excluded from the PP analysis because of withdrawal of their consent, violations of eligibility criteria or concomitant medication, and other major protocol violations. At the end, PP analysis group consisted of one hundred fifty four patients who completed the entire clinical trial without any major violation of the protocol. Seventy patients belonged to the HPE injection group, and 84 patients belonged to the LE–FAD injection group.

Demographic Information and Subject Characteristics There was no statistical difference between the two groups in liver function parameters, platelet counts, age, gender, height, weight, smoking history, alcohol consumption, period of alcohol drinking, or medical history (all *p* values >0.05) (Table 1). Mean platelet counts in the treatment group and in the control group were 248.13±68.52 ($\times10^9$/L) and 251.90±62.15 ($\times10^9$/L), respectively (normal range 150×10^9–450×10^9/L). Number of patients consuming alcohol was 49 (70.0%) in the test group and 54 (64.3%) in the control group, respectively. The average period of alcohol consumption in each group was 21.00±11.22 years and 21.17±10.22 years with an average frequency per month at 11.15±10.06 and 10.37±10.34, respectively. The amounts and types of alcohol consumed per drinking occurrence were difficult to determine, due to drinking habits of combining different types of alcohol in Korea. Medical conditions which subjects have experienced during the study were compared between the test group and control group: some disorders with cardiovascular system (29 patients *vs.* 41 patients), gastrointestinal and hepatobiliary system (20 patients *vs.* 26 patients), and metabolic and endocrine system (13 patients *vs.* 20 patients) were common but insignificant differences between both groups ($p>0.05$). The use of concomitant medications in the control group was more frequent (83.3%) than in the test group (64.3%) with *p* value<0.05. It is accorded with slightly higher number of medical diseases in the control group *vs.* the test group, 81.0% *vs.* 72.9%, respectively, which was not statistically different ($p=.2326$).

Efficacy The difference in the achievement rate of the primary study endpoint 20% or more improvement of ALT level, was 14.1% on per-protocol population between the test and control group, but it was not significant (62.9% (44/70) *vs.* 48.8% (41/84), respectively; $p=.0772$) (Table 2). The reduction of ALT level was 53.7% (51/95) in the test group and 46.4% (45/97) in the control group for the modified intention-to-treat population ($p=.2848$). The lower limits of the 95% confidence intervals in the per-protocol set (−1.52%) and the modified intention-to-treat set (−6.82%) did not exceed the pre-specified non-inferiority margin (−10.0%), showing that the reduction of ALT levels in the HPE group was non-inferior to that of the LE–FAD group.

Moreover, there was a significant difference among the patients with ALT reduction within 1.2 times of the upper normal limit. The percentage of such patients was higher in the test group than in the control group (40.0% ($n=28$) *vs.* 22.6% ($n=19$), $p=0.0181$; 97.5% one-tailed, lower confidence limit, 2.8).

The baseline levels of liver function parameters in both groups were similar: 1) ALT, 99.5±40.7 (IU/L) *vs.* 98.5±50.7 (IU/L); 2) AST, 69.0±67.2 (IU/L) *vs.* 74.6±73.0 (IU/L); 3) gamma-glutamyl transpeptidase (γ-GTP), 146.1±195.1 (IL/U) *vs.* 179.5±313.1 (IL/U); 4) alkaline phosphatase (ALP),

Table 1. Demographic Information of Per Protocol Population

Demographic information		HPE injection group (n=70)	LE–FAD injection group (n=84)	p-Value
Type of steatohepatitis	ASH n (%)	26 (37.1)	31 (36.9)	0.45[1)]
	NASH n (%)	49 (70.0)	54 (64.0)	
Liver function parameters,[#] baseline				
	Alanine transaminase (IU/L)	99.5±40.7	98.5±50.7	0.89[2)]
	Aspartate transaminase (IU/L)	69.0±67.2	74.6±73.0	0.63[2)]
	Gamma-glutamyl transpeptidase (IU/L)	146.1±195.1	179.5±313.1	0.26[2)]
	Alkaline phosphatase (IU/L)	162.7±121.3	183.3±150.0	0.36[2)]
	Total bilirubin (mg/dL)	1.4±3.9	1.0±1.0	0.36[2)]
Platelet counts ($\times 10^9$/L), baseline		248.1± 68.5	251.9± 62.2	0.82[2)]
Sex	Men n (%)	55 (78.6)	65 (77.4)	0.86[1)]
	Women n (%)	15(21.4)	19 (22.6)	
Age (year)	Mean±S.D.[♦]	44.7±10.8	46.5±11.3	0.34[1)]
Height (cm)	Mean±S.D.[♦]	168.4±7.5	166.9±9.2	0.42[3)]
Body weight (kg)	Mean±S.D.[♦]	76.2±13.9	77.5±15.7	0.59[3)]
Smoking	Non-smoker n (%)	23 (32.9)	36 (42.9)	0.38[1)]
	Ex-smoker n (%)	11(15.7)	9 (10.7)	
	Smoker n (%)	36 (51.4)	39 (46.4)	
Alcohol consumption	Non-drinker n (%)	19 (27.1)	26 (31.0)	0.72[4)]
	Ex-drinker n (%)	2 (2.9)	4 (4.8)	
	Drinker n (%)	49 (70.0)	54 (64.3)	
Duration of alcohol consumption (year)	n	49	54	
	Mean±S.D.[♦]	21.0±11.2	21.2±10.2	0.74[3)]
Frequency of alcohol consumption per month	Mean±S.D.[♦]	11.2±10.1	10.4±10.3	0.52[3)]
Medical history[5)]	(+) n (%)	51 (72.9)	68 (81.0)	0.23[1)]
	(−) n (%)	19 (27.1)	16 (19.1)	
Concomitant medications	(+) n (%)	45 (64.3)	70 (83.3)	0.01*[1)]
	(−) n (%)	25 (35.7)	14 (16.7)	

[#]Reference ranges in liver function parameters were used as follows; The range of alarnine transaminase is 10 to 40 IU/L, the range of aspartate transaminase is 6 to 40 IU/L. The range of gamma-glutamyl transpeptidase is 15 to 85 IU/L for men and 5 to 55 IU/L for women, the range of alkaline phosphatase is 20 to 140 IU/L, and the range of total bilirubin is less than 1.2 mg/dL. ASH; alcoholic steatohepatitis, NASH; non-alcoholic steatohepatitis, [♦]S.D.; standard deviation, HPE; human placental extract, LE–FAD; the mixture of liver extract and flavin adenine dinucleotide. 1) Chi-square test. 2) t-Test. 3) Wilcoxon rank sum test. 4) Fisher's exact test. 5) Other underlying disease was diagnosed within 1 year. *Statistically significant difference.

Table 2. Comparison of ALT Changes between HPE Injection Group and LE–FAD Injection Group in Per Protocol Analysis

	HPE injection group (n=70)	LE–FAD injection group (n=84)	p-Value
Improvement[$] n (%)	44 (62.9)	41 (48.8)	0.08[1)]
Differences between groups (97.5% CI[§])	14.1 (−1.52, ∞)		
Recovering to normal ALT[&] n (%)	21 (30.0)	17 (20.2)	0.11[1)]
Patients with ALT improvement within 1.2 times of the upper normal limit[&] n (%)	28 (40.0)	19 (22.6)	0.02*[1)]
Differences between groups (97.5% CI[§])	17.4 (2.8, ∞)		
Patients with ALT improvement within 1.5 times of the upper normal limit[&] n (%)	38 (54.3)	35 (41.7)	0.08[1)]
Differences between groups (97.5% CI[§])	12.6 (−3.11, ∞)		
The period of getting normal level of ALT[&] (day), Mean±standard error	38.3±1.4	45.7±1.1	0.15[2)]

1) Logistic regression analysis (covariate: alcoholic chronic hepatitis/nonalcoholic chronic hepatitis, medication history). 2) Log rank test. HPE; human placental extract, LE–FAD; the mixture of liver extract and flavin adenine dinucleotide, ALT; alanine transaminase. [$]"Improvemen" means at least 20% decrease in the level of ALT at the end point of study compared with the baseline ALT level. [&]Normal range of ALT was defined as 10–40 international units per liter (IU/L). [§]One-sided 97.5% confidence interval. *Statistically significant difference.

162.7±121.3 (IL/U) *vs.* 183.3±150.0 (IL/U); 5) TB, 1.4±3.9 (mg/dl) *vs.* 1.0±1.0 (mg/dl), respectively. After the treatment, the levels of AST, γ-GTP, ALP, and TB decreased in both groups without any significant differences (all p>0.05) (Fig. 2). Those of post-treatment 6 weeks were also similar in both groups: −22.8±65.2 *vs.* −21.6±65.8 for AST, −51.3±141.0 *vs.* −75.6±187.3 for γ-GTP, −11.5±44.6 *vs.* −21.5±72.7 for ALP, and −0.5±3.1 and −0.2±0.8 for TB, respectively.

Figure 3 shows individual ALT changes at 6 weeks of treatment. The decrease of ALT level from the baseline was not significantly different between the test (n=70) and the control groups (n=84) (−27.8±38.1 and −17.3±68.5, respectively, p>0.05) (Fig. 2). PP analysis also showed no significant difference between the two groups, although both HPE and LE–FAD decreased ALT levels throughout the study. As shown in the PP summary, patients' demographics, alcohol consump-

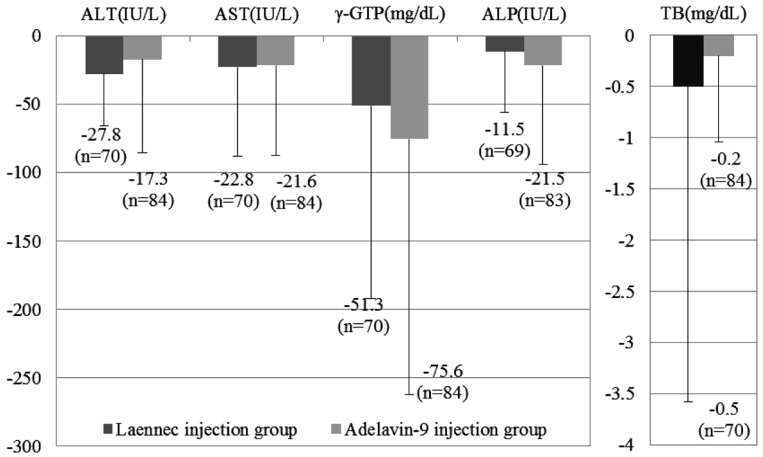

Fig. 2. The Comparing Average Difference from Baseline Level of Liver Function Parameters at the End Time Point between HPE (Laennec®) and LE–FAD (Adelavin-9®) Injection Groups in Per Protocol Analysis

HPE, human placental extract; LE–FAD, the mixture of liver extract and flavin adenine dinucleotide; ALT, alanine transaminase; AST, aspartate transaminase; TB, total bilirubin; γ-GTP, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; Error bar represents standard deviation (S.D.).

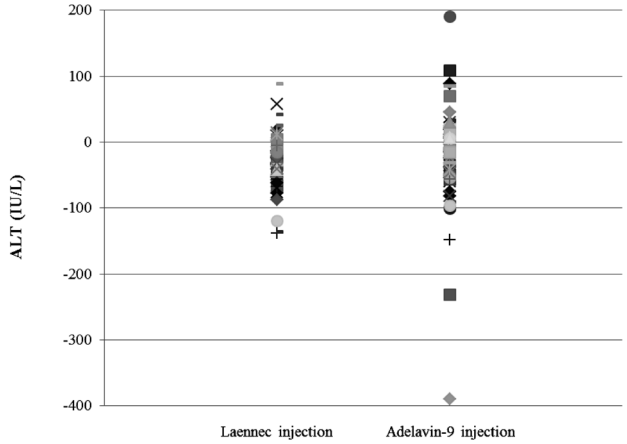

Fig. 3. ALT Changes of Individual Subjects from the Baseline at the End Time Point of the Treatment

tion, dose escalation, and basic liver functions parameters were similar between the test and control groups (Table 3). To evaluate the primary efficacy, we divided the patients into two subgroups: responders and non-responders. Responders were arbitrarily defined the as patients who achieved 20% or more reduction in the ALT level at the end of the treatment in comparison with its baseline level. When compared to the non-responders, responders of the test group were significantly higher in the baseline levels of AST, γ-guanosine 5′-triphosphate (GTP), and ALP, while responders of the control group were significantly higher in the baseline ALT level only. The weight of responders was smaller than the non-responders in the control group (p=0.0246). In the group without dose escalation, there was a significant difference between the responders who received HPE *vs.* control drug, 86.8% *vs.* 65.9%, respectively (p=0.0292). However, there was no significant difference between responders in escalated dose group (p=0.8689). Overall dosage was 1.24±0.29 ampules per dosing in the study group and 1.27±0.30 ampules per dosing in the control group (p=0.4905).

Safety Safety was analyzed in 194 patients who received at least one dose during the study. The causality of adverse events was determined by physicians participating in the study. The frequency of treatment of non-related adverse events was not significantly different between the test and control groups (21 events in 19 patients (20%) *vs.* 42 events in 22 patients (22.2%), respectively) (Table 4). Yet, serious adverse events did not occur during this study. One patient in the test group showed skin eruption, whereas 5 patients (8 cases) in the control group had adverse events related to the control medication, including vomiting (n=2), gastritis (n=1), arthralgia (n=1), anxiety (n=1), chilling sensation (n=1), rash (n=1), and itching (n=1) (Table 4). The majority of the adverse events were mild to moderate. There were no treatment-associated adverse events in the laboratory tests, including hematological, blood chemistry, and urine tests. The median values of overall compliance of two groups were 98.1% and 98.3%, respectively (p>0.05).

DISCUSSION

This study is the first multicenter controlled clinical trial with an open-labeled randomized comparative design to verify the clinical utility of HPE for the treatment of two candidate hepatic diseases, ASH and NASH. Though not remarkable, therapeutic efficacy and safety of HPE were not less than those of LE–FAD. ASH and NASH have similar pathogenesis with different causes. It is well known that the over-deposition of lipids in hepatocytes leads to an oxidative stress that induce cytokines such as tumor necrosis factor alpha (TNF-α). Pathological findings in both diseases, there are inflammatory infiltrations in hepatocytes, conseqently.[14,15] The treatment principles of ASH and NASH are abstinence of alcohol and control of abnormal metabolic consequences. To maximize the effects, a lot of effort has been put to find more effective medications. Corticosteroids, anti-TNF antibody agents, anti-oxidative agents, and Pentoxifylline have been introduced as medical agents for ASH.[5] Although corticosteroids and Pentoxifylline are used in the management of alcoholic liver disease, their standardization to current use has been limited to severe disease state by undetermined conclusive effects and side effects.[16]

NASH has direct correlation with obesity, type 2 DM, hypertension, and high level of cholesterol.[17] Insulin-sensitizing

Table 3. Comparison of Patient Demographics, Alcohol Consumption, Liver Function and Dosing between Responder[ψ] and Non-responder in HPE Injection Group and LE–FAD Injection Group (Evaluated with Per-Protocol Analysis)

		HPE injection group (n=70)			LE–FAD injection group (n=84)		
		Responder[ψ] (n=44)	Non-responder (n=26)	p-Value	Responder[ψ] (n=41)	Non-responder (n=43)	p-Value
Sex	Men n (%)	35 (79.6)	20 (76.9)	0.80[1)]	30 (73.2)	35 (81.4)	0.37[1)]
	Women n (%)	9 (20.5)	6 (23.1)		11 (26.8)	8 (18.6)	
Age (year), Mean±S.D.[♦]		45.8±10.4	42.9±11.5	0.27[2)]	46.7±11.0	46.2±11.7	0.85[2)]
Height (cm), Mean±S.D.[♦]		168.0±6.9	169.2±8.6	0.49[2)]	166.3±9.9	167.5±8.6	0.55[2)]
Body weight (kg), Mean±S.D.[♦]		73.7±14.5	80.1±12.4	0.07[2)]	73.5±14.4	81.2±16.1	0.02*[2)]
Smoking	Non-smoker n (%)	12 (27.3)	11 (42.3)	0.41[1)]	18 (43.9)	18 (41.9)	1.00[4)]
	Ex-smoker n (%)	8 (18.2)	3 (11.5)		4 (9.8)	5 (11.6)	
	Smoker n (%)	24 (54.6)	12 (46.2)		19 (46.3)	20 (46.5)	
Alcohol consumption	Drinker n (%)	32 (72.7)	17 (65.4)	0.37[4)]	26 (63.4)	28 (65.1)	1.00[4)]
	Ex-drinker n (%)	2 (4.6)	0 (0.0)		2 (4.9)	2 (4.7)	
	Non-drinker n (%)	10 (22.7)	9 (34.6)		13 (31.7)	13 (30.2)	
Duration of alcohol consumption (year)	n	32	17	0.20[3)]	26	28	0.71[3)]
	Mean±S.D.[♦]	22.7±11.7	17.8±9.9		21.4±11.2	20.9±9.4	
ALT (IU/L)		104.8±46.6	90.6±26.5	0.16[2)]	112.0±66.1	85.5±23.8	0.02*[2)]
AST (IU/L)		79.9±83.2	51.8±17.7	0.04*[2)]	88.3±79.7	66.0±67.9	0.17[2)]
γ-GTP (IU/L)		171.2±231.0	91.9±86.3	0.04*[2)]	239.8±370.1	142.8±288.3	0.18[2)]
TB (mg/dL)		1.8±4.8	0.8±0.3	0.20[2)]	1.1±1.3	0.9±0.5	0.33[2)]
ALP (IU/L)		187.3±136.2	121.8±77.9	0.01*[2)]	212.9±148.4	155.0±147.7	0.08[2)]
Patients with non-escalation dose, n (%)		33 (86.8)	5 (13.2)		27 (65.9)	14 (34.1)	0.03[2)#]
Patients with escalation dose, n (%)		11 (34.4)	21 (65.6)		14 (32.6)	29 (67.4)	0.87[2)&]

HPE; human placental extract, LE–FAD; the mixture of liver extract and flavin adenine dinucleotide, ALT; alanine transaminase, AST; aspartate transaminase, γ-GTP; gamma-glutamyl transpeptidase, TB; total bilirubin, ALP; alkaline phosphatase, S.D.; standard deviation. [ψ]A responder was defined as who had 20% decrease in ALT level after 6 weeks of HPE injection or LE–FAD injection as compared to their baseline. [#]The responders with non-escalation dose who received HPE *vs.* LE–FAD was compared. [&]The responders with escalation dose who received HPE *vs.* LE–FAD was compared. 1) Chi-square test. 2) Independent two sample t-test. 3) Wilcoxon rank sum test. 4) Fisher's exact test. *Statistically significant difference.

Table 4. Comparison of Side Effects[$] between HPE Injection Group and LE–FAD Injection Group in Intent-To-Treat

	HPE injection group (n=95)	LE–FAD injection group (n=99)	p-Value
	n (%) [cases]	n (%) [cases]	
Side effect unrelated with the trial medications	19(20.0) [21]	22(22.2) [42]	0.70[1)]
CI	(13.5,28.0)	(1.6,30.2)	
Side effect related with trial medications	1 (1.1) [1]	5 (5.1) [8]	0.21[2)]
CI	(0.1,4.9)	(2.0,10.3)	
Severity[#] of side effect	n (%)	n (%)	0.32[2)]
Grade 1	17 (81.0)	39 (92.9)	
Grade 2	3 (14.3)	2 (4.8)	
Grade 3	1 (4.8)	1 (2.4)	
Grade 4	0 (0.0)	0 (0.0)	

HPE; human placental extract, LE–FAD; the mixture of liver extract and flavin adenine dinucleotide, CI; 90% confidence interval. 1) Chi-square test. 2) Fisher's exact test. [$]Side effects were recorded according to the WHO Adverse Reaction Terminology (WHO-ART). [#]Investigators assessed severity of side effect.

agents, anti-oxidant, and hepatoprotectants have been investigated as medical agents for the control of compound metabolic and hepatotoxic sequences of NASH.[14)] Insulin-sensitizing agents have been considered as a promising agent for medical control of NASH. However, other studies of NASH treatment are still required for a definitive conclusion of standard therapy.[18)] It also needs to overcome the side effects such as body fat redistribution and cardiovascular risk.[19)]

HPE has anti-inflammatory and antioxidant activity.[20,21)] A preclinical study of concanavalin A-induced liver injury model demonstrated that HPE protected hepatocytes during chronic inflammation *via* the suppression of intercellular adhesion molecule-1 (ICAM-1) and myeloperoxidase.[22)] In addition, HPE increased superoxide dismutase (SOD) and decreased oxidative malondialdehyde (MDA) and nitrite oxide (NO), which suggests that HPE has protective effect on liver cells injured from lipid peroxidation.[22)]

In our comparative non inferiority study, both HPE and LE–FAD decreased the levels of AST, ALT, TB, ALP, and γ-GTP throughout this trial. Although it was the randomized study, the baseline levels of some liver function parameters were higher in the control group than in the test group, which may suggest the occurrence of selection bias. Nevertheless, these differences were insignificant between two groups, and the patients with higher baseline levels of liver function parameters responded preferably better as comparing responders *vs.* non-responders in both groups.

Even overall dosing throughout study in both groups was

similar, the number of patients with non-escalating dose in the test group responded better than the control group. Eleven out of 32 patients who received an escalated dosage of HPE improved ALT level remarkably. However, to clarify the correlation between dose and efficacy, further efficacy study needs to be performed. Other secondary efficacy outcome was also similar in both groups. Consequently, results of an open-labeled and multicenter clinical trial lead to confirmation that the clinical efficacy of HPE to ASH and NASH is not inferior to the efficacy of LE–FAD. Safety profile reported in both groups was similar and tolerable.

In summary, although pharmacologic mechanism is not clear, data suggest that HPE Laennec® might be used for the management of ASH and NASH like Adelavin-9®. However, further study is required for HPE itself in order to identify the active substance(s) that is (are) engaged in the treatment of ASH and NASH.

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