Double-blind Clinical Trial of HD-03/ES versus Placebo in the Management of Chronic Hepatitis B

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ABSTRACT

Background: *In vitro* studies indicated that HD-03/ES has surface antigen suppression and hepatitis B virus (HBV) elimination activities. Acute and subacute toxicity studies indicated that HD-03/ES is devoid of significant toxicity following acute and repeated administration in rats. This study was undertaken to evaluate the safety and efficacy of the formulation HD-03/ES capsules in the management of patients with chronic hepatitis B infection. **Methods:** Double-blind, randomized, placebo-controlled clinical study was carried out in 50 patients with chronic hepatitis B. Loss of hepatitis B surface antigen (HBsAg) and hepatitis B 'e' antigen (HBeAg) as well as alanine transaminase (ALT) normalization were assessed after 16 and 24 weeks of therapy. **Results:** Statistically significant improvements were observed in clinical, biochemical and HBV markers after administration of HD-03/ES capsules. Adverse effects were mild and never warranted withdrawal of the drug. **Conclusion:** A 24-week course of HD-03/ES is safe and effective in the outpatient management of chronic hepatitis B.

Key words: HD-03/ES, chronic hepatitis B, clinical trial, HBsAg, ALT normalization, HBV-DNA

Hepatitis B is the most common serious liver infection in the world. It is caused by the hepatitis B virus (HBV) that attacks liver cells and can lead to liver failure, cirrhosis or cancer of the liver. The WHO estimates that 400 million people worldwide are already chronically infected with hepatitis B. In 2005, about 10-30 million people became infected with HBV and HBV infection leads to over 1 million deaths each year. Approximately two people die each minute from hepatitis B. Unfortunately there are no completely safe and effective treatments available for those who have developed chronic hepatitis B (CHB) infection.[1]

Ideally, an optimal drug to be useful in the treatment of CHB should have the following features: Have potent antiviral effect, inhibit different sites of HBV-DNA replication, excellent safety profile and the ability to induce a sustained response with a limited duration of therapy. Presently, the existing therapies for CHB have limited long-term efficacy. Improvement in treatment options will reduce morbidity and mortality for some individuals who are chronically infected.[2]

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Ayurveda, an indigenous system of medicine in India, has a long tradition of treating liver disorders with plant drugs.[3] On the basis of leads available from folklore usage and recent experimental studies, HD-03/ES (a capsule formulation consisting of 125 mg each of hydroalcoholic extracts of the herbs *Cyperus rotundus* and *Cyperus scariosus*) was developed to elicit hepatoprotective activity.

Surface antigen suppression and HBV elimination activities of herbal extract containing *C. rotundus* and *C. scariosus* were examined using two hepatitis B surface antigen (HBsAg) expressing human hepatocellular carcinoma cell lines, PLC/PRF/5 and HepG2.2.215. Polymerase chain reaction (PCR) for study of amplification of DNA specific to HBV, reverse transcriptase inhibition assay, immunomodulatory effects and hepatoprotective ability against oxidative damage to hepatocytes were some of the other studies performed to evaluate the efficacy of the plant extract. The efficacy of the plant extract to eliminate the DHBV was assessed in experimentally infected Pekin ducks in a duck model study. Our investigations indicated that the extracts could reversibly inhibit cell growth and suppress HBsAg expression in both the human hepatocellular carcinoma cell line models. Acute and subacute toxicity studies conducted in rats indicated that HD-03/ES is devoid of significant toxicity following acute and repeated administration in rats (Data on file).

A preliminary case study report indicated that there was significant reduction of HBsAg along with disappearance of viral DNA in a patient treated with HD-03/ES at a dosage of two capsules twice-daily for a period of six months.[4] At the moment, there is no data to show whether HD-03/ES treatment is adequate for the treatment of HBV infection. We therefore, undertook this clinical study to evaluate the safety and efficacy of HD-03/ES in patients with hepatitis B infection.

Material and Methods

Patients with CHB who attended the Outpatient Department of Medicine, Rajendra Institute of Medical Sciences, Bariatu, Ranchi, India between August 2003 and September 2004 were enrolled in the present study. Eligible patients included males and females aged 18-65 years with positive HBsAg for at least six months. The alanine transaminase (ALT) levels of these patients should be within six times the upper limit of normal at the screening visit. Patients were excluded if they had decompensated liver disease (defined by serum albumin ≤36 g/dl, bilirubin ≥15 g/dl, prothrombin time ≥2 second prolonged or a history of ascites, variceal hemorrhage or hepatic encephalopathy), pancytopenia (defined as hemoglobin <11 g/dl, white cell count <4,000/mm^3 or platelets <10^5/mm^3). Patients with a history of using interferon or antiviral agents were excluded. Women of child-bearing age as well as lactating women were also excluded. Other exclusion criteria included co-infection by hepatitis C virus, a history of hepatocellular carcinoma, serious medical illness, active substance or alcohol abuse and concurrent use of corticosteroids or immunosuppressive agents. The study was approved by the Ethics Committee of the institution and all patients gave witnessed written informed consent before enrolment. The study in general was conducted in accordance with Declaration of Helsinki and GCP Guidelines issued by the Ministry of Health Government of India.

Study Design

This study was a double-blind, randomized trial of HD-03/ES versus placebo for 24 weeks. The study medication was prepared as HD-03/ES, a capsule formulation consisting of 125 mg each of hydroalcoholic extracts of the herbs *C. rotundus* and *C. scariosus* (The Himalaya Drug Company, Makali, Bangalore, Karnataka - 562 123, India) or placebo capsule, and each patient was required to take two capsules (either study drug or placebo) two times daily to make up the tested dosing. The plant materials were identified by Department of Pharmacognosy, The Himalaya Drug Company, Makali, Bangalore and a voucher specimen is being maintained in the department. Randomization and treatment assignment were performed within four weeks after the screening procedures had been satisfactorily completed. Randomization was performed by random numbers generated through computer software Program Rando 1.2 by a third party who was not involved in patient management. The random numbers were placed in concealed envelopes.

Recording and Observation of Symptoms and Signs

The symptoms and signs of patients were recorded in detail using the 'Clinical Observation Table' once a month before and during the treatment.

Etiological Markers of Hepatitis B

Serum samples collected from patients were stored at –20°C until analysis. Serum was assayed for HBsAg, HBeAg (hepatitis B 'e' antigen), and HBV-DNA at baseline, 16 weeks and 24 weeks after therapy using commercially available enzyme-linked immunosorbent assay kits of Roche.

Liver Function

The patients underwent liver function tests every month during the treatment, including contents of serum proteins, total bilirubin and activities of ALT and AST (aspartate aminotransferase).

Safety Analysis

Safety analysis included data for all treated patients during dosing. The primary safety endpoint was discontinuation of study medication because of adverse events. Other safety evaluations included incidence of adverse effects.

Endpoints

The primary endpoint was HBsAg clearance. Secondary endpoints included HBV-DNA levels and ALT normalization to 40 IU/l at the end of treatment.

Table 1. Baseline Characteristics

	Placebo (n = 25)	HD-03/ES (n = 25)
Age (years)		
Mean (SD)	34.3 (8.7)	31.2 (6.8)
Median (range)	18-45	20-45
No. of males	20	22
No. of females	5	3
Body weight (kg)		
Mean (SD)	49 (10)	47 (16)
ALT (IU/ml)		
Mean (SD)	208.9 (28.6)	231.8 (112.1)

Statistical Analysis

The intention-to-treat analysis included all randomized patients who were HBeAg-negative at baseline and received at least one dose of the study medication. Data were expressed as mean ± SD. One-way ANOVA with Bonferroni's multiple comparison test or Dunnett's multiple comparison test was performed wherever appropriate using GraphPad Prism Software, Version 4.00 for Windows (GraphPad Software, San Diego, California, United States). A p value of <0.05 was taken as statistically significant.

Results

A total of 57 patients were screened and 50 patients who met the eligibility criteria were randomized to either HD-03/ES treatment (n = 25) or placebo treatment (n = 25). Of the 50 patients enrolled for the study, five in the placebo group and one in the HD-03/ES group were lost during follow-up during the final visit. Overall, 44 patients completed the study as planned and finished the 24-week treatment phase. Baseline and demographic and disease characteristics are summarized in Table 1. The treatment arms were well-balanced with respect to age, gender ratio, body weight and ALT levels with no statistically significant differences between arms.

Clinical Response

Twenty-four weeks of therapy with HD-03/ES capsules was markedly effective in majority of the patients as it resulted in disappearance or alleviation of chief clinical symptoms such as abdominal pain, and poor appetite. The effect of HD-03/ES therapy on weight loss and jaundice is shown in Table 2. Although there is progressive weight gain in the subjects treated with HD-03/ES capsules, the levels did not reach levels of statistical significance. Jaundice virtually disappeared in all but two of the subjects after 24 weeks of therapy with HD-03/ES (Table 2).

Table 2. Effect of HD-03/ES and Placebo Therapy on Weight and Jaundice

Time (Weeks)	Weight (kg) Mean ± SD		Jaundice			
	HD-03/ES (n = 25)	Placebo (n = 25)	HD-03/ES		Placebo	
			Yes	No	Yes	No
0	49.0 ± 10.0	47.0 ± 16.0	21	4	22	3
4	49.2 ± 8.1	46.1 ± 9.8	16	9	21	4
8	49.3 ± 8.1	46.2 ± 9.6	13	12	21	4
12	49.7 ± 7.9	45.9 ± 8.6	5	20	21	4
16	49.8 ± 9.4	46.1 ± 7.3	4	21	20	5
24	49.9 ± 7.7	46.6 ± 7.8	2	22	15	5

Biochemical Response

Alanine aminotransferase levels were raised in all patients at the time of initiation of therapy. HD-03/ES proved to be more effective than placebo in normalizing ALT levels: An important follow-up marker of biochemical response (Table 3 and 4). ALT normalization occurred in 17 of the 24 patients (70.8%), as compared to just three patients in the placebo group (15%). This occurred more significantly in those who cleared their HBsAg than in those who did not. The effect of HD-03/ES or placebo on other liver function parameters are shown in the Table 3.

Virological Response

The effects of 24 weeks of treatment with placebo or HD-03/ES treatment on virological responses are shown in Table 4. Ten of the 24 patients (41.75%) who were treated with HD-03/ES, had undetectable HBsAg at the end of treatment, as compared to just 5% with placebo. This difference was statistically significant ($p < 0.001$). HBeAg loss occurred more frequently during treatment with HD-03/ES in that five of the nine patients who were positive for HBeAg initially, were negative for the same at the end of therapy (Table 4). HBV-DNA levels became undetectable after 24 weeks of therapy with HD-03/ES therapy in six patients who were positive for the same at the initiation of therapy as compared to just one person losing HBV-DNA in the placebo group ($p < 0.05$).

Table 3. Effect of HD-03/ES Therapy on Liver Function Tests

Liver function test	Time (Weeks)					
	0		16		24	
	HD-03/ES (n = 25)	Placebo (n = 25)	HD-03/ES	Placebo	HD-03/ES	Placebo
Alanine aminotransferase - ALT (U/l)	208.9 ± 28.6	231.8 ± 112.1	66.8 ± 12.3*	161.8 ± 32.4	63.4 ± 13.6*	172.4 ± 63.0
Aspartate aminotransferase - AST (U/l)	128.3 ± 34.1	117.1 ± 28.1	73.6 ± 13.1	87.1 ± 42.3	65.1 ± 11.2*	85.5 ± 26.1
Protein total (g/dl)	7.0 ± 0.2	6.9 ± 0.5	7.2 ± 0.2	6.9 ± 0.6	7.4 ± 0.1*	6.9 ± 0.7
Protein fraction globulin (g/dl)	3.6 ± 0.1	3.5 ± 0.1	3.9 ± 0.1*	3.7 ± 0.1	4.0 ± 0.2*	3.7 ± 0.2
Bilirubin (total) (mg/dl)	4.2 ± 1.3	3.4 ± 1.7	2.5 ± 0.3	2.8 ± 1.9	1.1 ± 0.2*	2.5 ± 1.3
ALT normalization (%)	–	–	56.0	12	70.8	15

*p < 0.05 as compared to 0 weeks.

Table 4. Biochemical and Serological Response of HD-03 Group as Compared to Placebo Group

Variable	HD-03/ES		Placebo	
ALT normalization (%)				
16 weeks	56 (14/25)		12 (3/25)	
24 weeks	70.8 (17/24)		15 (3/20)	
HBsAg loss	**Positive**	**Negative**	**Positive**	**Negative**
0 weeks	25	0	25	0
16 weeks	17	8	24	1
24 weeks	14	10***	19	1
HBeAg loss	**Positive**	**Negative**	**Positive**	**Negative**
0 weeks	9	16	5	20
16 weeks	5	20*	4	21
24 weeks	4	20*	4	16
HBV loss	**Positive**	**Negative**	**Positive**	**Negative**
0 weeks	13	12	14	11
24 weeks	7	17*	13	7

*p < 0.05 as compared to 0 weeks.
***p < 0.01 as compared to 0 weeks.

Table 5. List of Adverse Effects

Adverse effect	Placebo	HD-03/ES
Abdominal discomfort	3	4
Fatigue	2	4
Headache	2	2

Safety

HD-03/ES was well-tolerated in this study, with the incidence of adverse events in the treated group being similar to that for placebo. The majority of adverse events were mild-to-moderate. The adverse events observed during therapy are shown in Table 5. The commonest adverse event was abdominal discomfort. No patient died and none required liver transplantation in this study. No serious biochemical abnormalities were experienced by any other patients in both the study groups. Renal function tests showed normal level of blood urea nitrogen (BUN) and blood creatinine during HD-03/ES treatment.

Discussion

The goal of therapy for patients with HBV infection is to prevent the progression of liver disease to cirrhosis and hepatic cell cancer. In recent years, progress has been made in the treatment of CHB. The issues to be considered include efficacy, safety, resistance and cost.[5] Currently available treatment or re-treatment for hepatitis B achieves sustained biochemical responses in only 15-25% of patients with eventual HBsAg loss and anti-HBs development in a proportion of them.[6] Therefore well-tolerated antiviral agents that provide clinical benefit without producing resistance are needed to manage chronic hepatitis B. The results of the present study showing clinical benefit of HD-03/ES appear promising in the short-term management of hepatitis B.

The virological response (undetectable HBV-DNA) of the currently approved therapies for CHB: Standard or pegylated interferon, lamivudine, adefovir dipivoxil and entecavir ranges between 21 and 44% only[7] and our results of 41.75% HBV-DNA loss is similar to that of the established drugs. This coupled with its excellent safety profile makes HD-03/ES an alternative to conventional therapies whose use is associated with dose limiting side effects.[8-10] The ultimate endpoint of antiviral therapy for CHB infection is loss of HBsAg, which is accompanied by disease remission in terms of ALT normalization.[11] In this study, HBsAg cleared in 41% of patients and ALT normalization was obtained

in 71% of patients. The improvement in treatment options may reduce morbidity and mortality for some individuals who are chronically infected.

Loss of HBeAg either spontaneously or following therapy significantly improves the clinical outcome and survival in chronic HBV patients. Therefore, HBeAg loss has remained as a major endpoint of antiviral therapy in chronic HBV infection.[12] Monotherapy with α-interferon for 16-26 weeks is associated with loss of serum HBeAg in 20-40% of the patients. Our results (55%) are slightly better.

A strong correlation was found between HBV-DNA levels and histology activity index scores in HBeAg-negative patients.[13] As ALT levels are consistent with histological activity index scores, the findings in the present study of ALT normalization, HBsAg loss together with loss of DNA during short-term treatment with HD-03/ES indicate that patients treated with HD-03/ES may lose their infectivity faster and relapse rates would be low.

Although the initial results of this study are promising, it remains to be seen whether virological response will be sustained during chronic dosing and whether relapse rates after cessation of therapy would be low unlike conventional therapies whose relapse rates are high after treatment cessation.[14] Our study has several obvious limitations and among these we should consider the small sample size.

In summary, this trial demonstrated that 24 weeks of HD-03/ES treatment resulted in clinically significant virological and biochemical benefits in patients with CHB infection. Hence to conclude, the potential benefit of HD-03/ES in the management of CHB, HD-03/ES should be studied in long-term comparative trials with standard drugs; extended duration of follow-up are warranted and are under way.

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