Combination of Simvastatin and Entecavir for Chronic Hepatitis B: A Phase 1 Trial

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Abstract

Background: Hepatitis B Virus (HBV) is the most common chronic infectious disease worldwide, accounting for millions of deaths per year. Twenty-three years have passed since the introduction of the last class of anti-HBV drugs, nucleoside analogs. The goal of new drug development is the loss of hepatitis B surface antigen (HBsAg). Nucleoside analogs or placebos given for up to 5 years do not differ in eliminating HBsAg. Synergistic lowering of HBV DNA with a second drug added to a nucleoside analog has not been demonstrated. Entecavir is used for long-term therapy to suppress viral replication. Simvastatin has potent anti-HBV properties in vitro.

Aim: This phase 1 study examined the short-term combination of entecavir and simvastatin in patients chronically infected with HBV. Efficacy was measured by change in HBV DNA levels and safety by liver tests and creatine kinase.

Materials and Methods: Six control patients took 0.5 mg/day entecavir monotherapy for 14 days. Eighteen patients took combination therapy consisting of 0.5 mg/day entecavir and different doses of simvastatin (5, 10, 20, 40 mg daily) for periods of 14 to 60 days.

Results: A significant separation of decline in serum HBV DNA between entecavir monotherapy and entecavir + simvastatin was achieved in only 14 days for the 5, 20, and 40 mg simvastatin combination groups. The 60-day group had a greater change in HBV DNA than controls at all time points. Unexpectedly, 2 patients achieved immunological cure (loss of surface antigen, undetectable HBV DNA) that persisted upon one-year follow-up. One patient received 14 days of 0.5 mg entecavir plus 5 mg simvastatin; the second took 28 days of 0.5 mg entecavir plus 20 mg simvastatin. ALT declined by a mean of 30 IU/ml in all patients taking combination therapy.

Conclusion: The combination of simvastatin and entecavir significantly improved the rate of decline of HBV DNA compared to entecavir monotherapy. Moreover, 2/18 (11%) loss HBsAg with only four weeks of combined therapy. There were no safety issues. These data warrant further examination in phase 2 trial.

Keywords: Chronic Hepatitis B; Entecavir; Simvastatin; Hepatitis B treatment; Nucleoside Analogs

Introduction

Hepatitis B virus (HBV) persistently infects an estimated 350 million people, making it the most common chronic infectious disease worldwide. HBV is the predominant cause of hepatocellular cancer globally. Together with complications from cirrhosis, HBV causes more than a million deaths per year [1].

A new class of antivirals for treatment of hepatitis B (HBV) has not been introduced since the first nucleoside analog, lamivudine, was marketed in 1995. Nucleoside and Nucleotide Analogues (NA), which include lamivudine, entecavir, tenofovir, and others, suppress HBV DNA by breaking nascent viral protein chains through incorporation of pseudo-nucleic acids.

Current therapy for HBV can be simplified by identifying the most responsive group. The immunocompetent patient who has "e" antigen positive (HBeAg+), wild-type virus, an HBV DNA level ≥ 105 IU/ml, and an ALT that is above the upper limits of normal (ULN) is most likely to respond. Other patient categories respond less well [2].

NA given for years can suppress HBV DNA; and less frequently, HBeAg seroconversion can occur. Stopping a NA before two years of use often leads to relapse of viral replication; however, even when HBV replication becomes undetectable, serum hepatitis B surface antigen (HBsAg) persists. Still, viremia can relapse. Putting two NAs together does not improve outcome. The five-year administration of entecavir, or any other NA, does not increase the proportion of patients who clear HBsAg (5%) over placebo. Forty-eight weeks of peginterferon monotherapy can lead to long-term clearance of HBsAg that is marginally better than placebo at 11%. Used as monotherapy, peginterferon, entecavir or tenofovir are considered first-line agents [2].

Given that covalently closed circular DNA (cccDNA) of HBV persists in the nucleus of hepatocytes, the term “cure” has been historically avoided. It is not yet clear what proportion of patients who achieve seroclearance of HBsAg will have persistent cccDNA. However, seroclearance of HBsAg is considered "immunological cure." Clearance of serum HBsAg is now the goal of new drug development in HBV [3].

While this report focuses on hepatitis B, our group was the first to give 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors, known as statins, intentionally to patients with hepatitis C under FDA license [4]. Most statins as monotherapy suppress HCV RNA replication modestly, but there is a rank order of efficacy [5]. We, and others, have demonstrated in randomized, controlled trials, a substantial improvement of viral cure for HCV when fluuvastatin was combined with peginterferon and ribavirin [6].

Dr Korba evaluated statins for their anti-HBV effects. His laboratory was the world's designated by the National Institute of Allergy and Infectious Diseases Antiviral Acquisition program to discover novel compounds that might possess antiviral activity against HBV. The cell line used, HepG2.2.15, has been used to discover all the currently FDA-approved NA for HBV. Only one statin, simvastatin, showed anti-HBV effect; however, the in vitro effect was very potent [7]. Simvastatin worked just as well against drug resistance strains to HBV as with wild

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type HBV [8]. Significant in vitro synergy of entecavir + simvastatin was demonstrated [7].

The goals of this small phase 1 study were to investigate the safety and efficacy of combining different doses of simvastatin with entecavir for 14-60 days in chronic hepatitis B patients without evidence of advanced liver disease.

Materials and Methods

Patients

Adult subjects between 18 and 70 years of age were referred if they were known to be chronic carriers of HBV infection as evidenced by HBsAg tested six months apart. All subjects gave written informed consent. A history and physical examination was followed by testing for HBsAg, HBeAg, HBV DNA, HBV genotype, HBV drug resistance panel, alpha-fetoprotein, HCV RNA, HIV RNA, a 28-test chemistry panel, CBC, TSH, creatine kinase, urinalysis, and a liver ultrasound. The first patient was enrolled on January 3, 2012. The last patient was withdrawn from the study protocol on July 30, 2013.

No patient had received statins or anti-HBV treatment within 30 days. None had ever received peginterferon. Patients were not co-infected with hepatitis C or human immunodeficiency virus. No subject had evidence for decompensated cirrhosis. Subjects were HBeAg positive and HBsAg positive with a minimum HBV DNA level ≥ 10^6 IU/ml. All patients had only wild-type HBV detected.

Study Design

In the development of the trial and in the current report, CONSORT (Consolidated Standards of Reporting Trials) recommendations were adhered to.

Six patients were controls with 0.5 mg entecavir monotherapy for 14 days.

Fourteen patients were given simvastatin and entecavir for 14 days: four patients were given 5 mg of simvastatin + 0.5 mg entecavir; four patients were given 10 mg of simvastatin + 0.5 mg entecavir; four were given 20 mg of simvastatin + 0.5 mg entecavir; and two were given 40 mg simvastatin + 0.5 mg entecavir.

Four patients took 60 days of 20 mg simvastatin + 0.5 entecavir.

For the entecavir controls and 14-day drug combination studies, HBV DNA, ALT, and other safety laboratory tests were drawn at baseline, days 3, 7, 14. The study medications were given for 14 days and then repeat laboratories were drawn on day 21.

In the subsequent 60-day study, HBV DNA, ALT, and other safety laboratory tests were drawn at baseline, days 30 and 60. Study medications were given for 60 days.

HBV DNA was measured with the Roche COBAS TaqMan® Analyzer at the VA Hospital in Oklahoma City, Oklahoma. Real-time amplification and detection of RNA was performed with fully automated sample transfer from the COBAS AmpliPrep Instrument.

Statistical Evaluation

The outcome of interest was the drop in log10 HBV DNA at each time-point relative to the baseline log10 HBV DNA. Group statistics are given as mean ± Standard Deviation (SD). The performance of entecavir against HBV has been well delineated in the medical literature in terms of the log10 HBV drop over time from the baseline [9]. These historical curves, along with known standard errors, were used for comparison (historical control). Comparison with historical control at individual time-points used Students t-tests. For the 60-day study comparison to historical control over the entire time used generalized estimating equations to account for the repeated measures over time. Data analyses were conducted using IBM SPSS Statistics (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Results corresponding to p-values lower than 5% are significant. Chris Aston, Ph.D., University of Oklahoma, provided the statistical interpretation.

Results

The 24 patients who entered the protocols were male veterans, between the ages of 30 and 60. The distribution of HBV genotypes among subjects was A - 11, B - 3, C - 5, D - 3, E - 2.

Controls given Entecavir

The six patients given the control entecavir dose of 0.5 mg for 14 days did not differ significantly from the decline in HBV DNA reported in the historical literature (Figure 1). Whether we added the primary non-responder or not, our control group data did not differ from historical data reported for entecavir monotherapy.

Combination Therapy using Entecavir and Simvastatin

In the 14-day protocol, the use of the 5, 20, or 40 mg dose of simvastatin plus 0.5 mg entecavir reduced the HBV DNA levels significantly compared to the current control group (Figure 2). (entecavir versus 5 mg simvastatin + entecavir, p= 0.0090; entecavir versus 20 mg simvastatin + entecavir, p= 0.016; and entecavir versus 40 mg simvastatin + 0.5 mg entecavir, p= 0.033). The 10 mg dose of simvastatin added to 0.5 mg entecavir did not achieve statistical significance at 14 days when compared to the contemporary entecavir control group (p= 0.091).

Four patients were given 60 days of 20 mg simvastatin and 0.5 mg entecavir (Figure 3). Considering all time points at once with generalized estimating equations, the log10 HBV drop over time from the baseline in treated subjects was generally greater than controls at all time points.

Three patients were remarkable. In the 60-day study arm, a 40-year-old Caucasian male, HBV genotype A, had a normal pre-treatment
ALT. After 28 days of 20 mg simvastatin and 0.5 mg entecavir, his ALT rose to 2,084 IU/ml (Figure 4). The test drugs were stopped. On day 48, (27 days after stopping drugs) his ALT returned to 63 IU/ml and his entry HBV DNA of 94,000,000 IU/ml had declined to 73 IU/ml. On day 97 (14 weeks after drugs were stopped) his HBsAg and HBV DNA were non-detectable, and his ALT was normal. The HBsAg and HBV DNA were undetectable one year after treatment.

Patient two was a 45-year-old Caucasian male, HBV genotype A, who had never received treatment for HBV. During a previous study, he had been given two 14-day courses of simvastatin monotherapy. One with 5 mg simvastatin and one with 20 mg simvastatin (Figure 5). With 5 mg of simvastatin alone his HBV DNA did not change. The ALT declined from 235 to 195 IU/ml. After a period of 35 days, he was given 20 mg simvastatin alone. His HBV DNA declined from 15,000,000 to 1.5 million IU/ml. His ALT went from 319 to 251 IU/ml.

Figure 2: All doses of simvastatin added to 0.5 mg entecavir with control groups. The simvastatin doses of 5 mg, 20 mg, 40 mg when added to 0.5 mg were statistically significant (p = 0.0090, p = 0.016, p = 0.033, respectively) compared to the contemporary entecavir controls given 0.5 mg/day. The 10 mg simvastatin plus 0.5 mg entecavir did not reach significance (p = 0.091).

Figure 3: Sixty-day study of combination simvastatin and entecavir. Controls on 0.5/mg/day entecavir monotherapy (green line and circles). Cases took 20 mg/day simvastatin + 0.5 mg entecavir. Considering all time points at once with generalized estimating equations, the log10 HBV DNA drop over time from the baseline in treated subjects was generally greater than controls at all time points.

Figure 4: Patient one took 20 mg/day simvastatin + 0.5 mg/day entecavir. Medications were stopped at day 28 because of the elevated ALT. His HBsAg and HBV DNA remained undetectable one year later.

Figure 5: Patient two was given two courses of simvastatin monotherapy as noted. After six months, he was given 5 mg/day simvastatin + 0.5 mg/day entecavir for 14 days. His HBsAg became undetectable in three months and remained negative one year later.

Figure 6: Patient three. The blue charts the course of a control course of 0.5 mg/day entecavir. After a 30-day without study medications, he restarted 5 mg/day simvastatin + 0.5 mg entecavir.
No further testing was performed until six months later; it was then noted that his HBV DNA had declined to 432 IU/ml. His HBeAg and HBsAg remained positive. He was given 5 mg simvastatin + 0.5 mg entecavir for 14 days. His HBV DNA became undetectable in 14 days. His ALT declined from 44 to 39 IU/ml. One year later his HBeAg and HBsAg were undetectable.

The third patient, a 35-year-old Vietnamese-American male, HBV genotype B, was remarkable for a different reason. He had a normal ALT and was given 0.5 mg entecavir as a control patient for two weeks. He exhibited no reduction of HBV DNA during the two weeks. A 30-day wash-out period was done. He was then given 5 mg of simvastatin + 0.5 mg entecavir. In 14 days, his HBV DNA fell from 109,000 to 89 IU/ml. His ALT remained < 20 IU/ml during both sessions of medication (Figure 6).

Discussion

The purposes of a phase 1 trial are to evaluate potential treatment response, explore dosing, and examine safety. Phase one trials are not randomized or controlled. The data from a phase 1 trial should either encourage or discourage the pursuit of a larger phase 2 trial.

The small number of patients tested was typical for a phase 1 trial. The pre-study expectation was to collect safety data for combination therapy with simvastatin and entecavir. Pre-study safety concerns also limited enrollees. At the time of study design, many thought giving statins to patients with active liver disease was unwise [10].

The limitations of this study were an all-male Veteran population. The smaller proportion of HBV genotypes B and C reflected in a Midwestern United States population does not reflect the proportion of HBV genotypes present globally. There was a pre-study concern that our North American patients, with different hepatitis B genotypes, might respond differently. However, our active control patients treated with entecavir monotherapy did not differ from historical controls with a larger proportion of Asian genotypes.

While the viral kinetics of HBV disappearance with NA can be complex, it is believed that the rapid decrease in HBV DNA in the first four weeks seen in responders reflects clearance of free virions in the plasma; thereafter, the slower rate of decline of HBV DNA reflects the clearance of infected hepatocytes from cytotoxic T lymphocytes [9,11].

Safety

The idea of statin hepatotoxicity was maintained for many years [10]. The author has been an advocate of the safety of statins for patients with liver disease [10]. The advocacy was based on the marked improvements in ALT reported in our patients with HCV or HBV when statins were intentionally administered. The controversy has largely disappeared. Statins are now being explored for their possible therapeutic value in several liver diseases (Janicko et al., 2016; Tschochatzis and Bosch, 2017).

There were no safety issues during this study. In the 18 subjects given combination therapy, ALT values dropped by a mean of 30 IU/ml. No muscle symptoms or significant creatine kinase laboratory changes took place in any patient.

Efficacy measured by HBV DNA

There is no record in the medical literature of two nucleoside analogues, when combined with each other, leading to a significantly greater viral kinetic decline than that occurring from either agent alone. In this case, the second drug added, simvastatin, has not been known to have an in vivo anti-HBV effect. A significant separation of decline in serum HBV DNA between entecavir monotherapy and entecavir + simvastatin was achieved in only 14 days. The 5, 20, and 40 mg doses of simvastatin showed statistical difference when added to entecavir. The 10 mg combination group did not.

The pre-study expectation was that safety would be demonstrated. It was thought that 14 days would be too little time to show efficacy. However, frequent HBV DNA testing was included for total assessment. It came as a bonus that the HBV DNA response curves separated in only 14 days.

The data for the different doses were too limited to determine the best dose of simvastatin to be combined with entecavir. The 20-mg dose of simvastatin seemed to perform best. The last group of patients were then given 20 mg simvastatin + 0.5 mg entecavir for 60 days.

Why the 10 mg combination group did not perform as well is not readily explained. However, this group came close to the pre-study definition of p< 0.05 group with a result of p< 0.09.

Effects on loss of HBsAg: The natural history of untreated clearance of HBsAg involves years of a stepwise clearance of HBeAg and HBsAg [12]. The clearance of these antigens is usually preceded by a marked elevation in serum ALT of more than 10X ULN. The first case is remarkable in that he had a normal ALT to begin with and received only four weeks of combination therapy (Figure 4).

The causal link between therapy and outcome in the second patient is not as compelling (Figure 5). Since the patient began the first course of simvastatin monotherapy with an ALT of 235 IU/ml, one could argue that a serendipitous observation of a natural history event took place. Little information is available in relation to either the natural history or response to NA in HBV carriers with extremely low HBV DNA levels. One prospective, controlled study used lamivudine for one year in 18 anti-HBe+, HBsAg+ subjects who started with an HBV DNA mean of 214 IU/ml. All the treated subjects and half of the 37 control subjects began with an ALT > ULN. None of the treated or control subjects achieved undetectable HBV DNA levels or loss of HBsAg [13]. The latter data suggest that a patient with low-level HBV DNA does not quickly lose HBsAg when given a NA.

Effect upon primary non-response of entecavir: Entecavir has the lowest primary non-response rate of any NA at 16%. In contrast to other NA, entecavir has a significant reduction of HBV DNA even if the starting ALT is within expected limits [14]. The third patient represents conversion of a primary non-responder to a NA to a responder by adding a second non-antiviral drug, a phenomenon not yet reported.

Possible Mechanisms of Action

Two in vitro mechanisms for the anti-HBV effect of simvastatin have been demonstrated. Production of the cholesterol precursor mevalonate by HMG CoA reductase represents a potential bottleneck for cholesterol production. Inhibition of HMG CoA reductase is a well understood pharmacological effect of statins. Others have shown that if this latter reaction is responsible for the effect of a statin, it can be reversed with addition of mevalonate back to the culture [5].

We showed in a 2010 report that the addition of mevalonate to our test system abolished the anti-HBV effect of simvastatin. Moreover, mevalonate monotherapy did not display an anti-HBV effect, nor did it reduce the anti-HBV effectiveness of lamivudine [7]. However, since we and others have been unable to make other statins work against HBV the reduction of mevalonate is unlikely to be the sole source of anti-HBV effect for simvastatin.

A second mechanism is that nuclear Mini-Chromosome Maintenance protein seven (MCM7) correlates with HBV infection [15]. There are ten MCM proteins functioning in gene replication. Of the ten conserved factors, MCM2-7 is connected to form a complex.
The complex serves to restrict DNA replication to a single round per cell cycle. The MCM complex is a host factor that participates in the genome replication of viruses in host cells, such as the influenza virus [16]. In this context, Li, et al demonstrated that simvastatin reduced the expression of MCM7 protein at the translational level in HepG2.2.15 cells. The reduction of MCM7 attenuated the expression of HBV DNA [17].

Conclusion

The field of anti-HBV therapy has failed to produce drugs in the past two decades with a novel mechanism of action. Moreover, there does not appear to be any innovative anti-HBV drug in the pipeline that might win FDA-approval within the next five years. Thus, any signal that might provide a new clue deserves further exploration. The distinct advantages here are that simvastatin and entecavir as individual drugs have an excellent and well-known safety record. They are also inexpensive. Given that the bulk of HBV carriers worldwide are in countries that struggle with health care cost, an inexpensive approach is important.

In summary, we conducted a phase one trial for the combination of simvastatin and entecavir. Simvastatin at doses of 5 mg, 20 mg and 40 mg potentiated the effect of entecavir when given even for a brief period. Simvastatin not only improved the rate of decline of HBV DNA compared to entecavir monotherapy, but 2/18 (11%) patients underwent immunological changes involving HBsAg loss with only four weeks of combined therapy or less. There were no safety issues. These data warrant further examination in phase 2 trial.

References