The effect of twelve weeks of treatment with ezetimibe on HDV RNA level in patients with chronic hepatitis D

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ABSTRACT

Background/Aims: *Sodium taurocholate co-transporting polypeptide (NTCP) is the receptor for the hepatitis B virus (HBV) and hepatitis D virus (HDV) entry into hepatocytes. Ezetimibe is a cholesterol-lowering drug that possesses the pharmacophore features to inhibit NTCP. This study evaluates the efficacy of ezetimibe in patients with chronic HDV infection in a nonrandomized trial.*

Materials and Methods: *This proof of concept phase 2 trial evaluated the efficacy and safety of ezetimibe 10 mg daily in (interferon treatment-experienced or interferon ineligible) patients with chronic hepatitis D (CHD). Forty-four patients with CHD were recruited, 38 male and 6 female patients, mean age 35.2±8.7 (range 19-64). Fifteen (34%) patients were on concomitant nucleoside therapy, and cirrhosis was present in 14 subjects. The primary therapeutic endpoint was a decline in HDV RNA at one log or more from the baseline at week 12.*

Results: *The mean HDV RNA level was 5.4±1.3 log10 IU/mL. HBeAg was non-reactive in 43 (98%). HBV DNA was undetectable in 28 (64%). One patient stopped treatment at week 4, and one patient did not follow-up. One log or more reduction in the HDV RNA levels was observed in 18/44 (41%) patients. No log reduction occurred in 16 patients, and 8 experienced a log increase. No adverse effects from the concomitant nucleoside analogue use or clinical cirrhosis were observed. The drug exhibited a positive safety profile.*

Conclusion: Treatment of CHD patients with ezetimibe resulted in a one log reduction of viral load in 43% (18/42) of the patients who completed the 12 weeks of therapy.

Keywords: *Hepatitis D, ezetimibe, HDV RNA, sodium taurocholate co-transporting polypeptide*

INTRODUCTION

There are over 240 million individuals chronically infected globally with hepatitis B virus (HBV), including 15-20 million co-infected with hepatitis delta virus (HDV) (1). HDV is a small RNA virus that requires the hepatitis B surface antigen (HBsAg) to enter the hepatocyte. Chronic hepatitis D is a severe form of chronic liver disease. There is a rapid progression to cirrhosis and hepatocellular carcinoma (2). Cirrhosis generally occurs within 5 to 10 years in up to 80% of these cases.

The lipoprotein envelope of HDV is provided by the HBV and consists of the same proteins that are found in the hepatitis B virion. The sodium taurocholate co-transporting polypeptide (NTCP), which is the receptor for the hepatitis B virus, has also been identified as the receptor for HDV (3). The virus gets attached to NTCP via myristoylated preS1 (myr-preS1) peptide domain of its large hepatitis B surface protein.

There is no approved treatment for hepatitis D, and this infection has been given the status of an orphan disease in the European Union and the United States. Treatments available for HDV infection are interferon-alpha, standard, or pegylated. Therapy with pegylated interferon is accompanied by a myriad of adverse effects, and the efficiency is limited to a quarter of the patients (4). The nucleoside inhibitors used to treat hepatitis B are not efficient against HDV. The discovery of human NTCP may lead to a very promising novel therapy for the treatment of chronic HBV and HDV infections (5).

Myrcludex B (Bulevirtide), a novel peptide derived from the preS1 region of the large envelope protein of HBV and HDV, is currently undergoing clinical trials as a promising approach for the intervention (6-8). However, currently, there are already some FDA approved drugs available like irbesartan, ezetimibe, and ritonavir, which can inhibit NTCP (9, 10). Since binding to NTCP is an initiating step in HDV infection, we hypothesized that therapy with ezetimibe could lead to a decline in hepatitis D virus levels. This study evaluated the therapeutic use of ezetimibe in patients with chronic HDV infection.

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MATERIALS AND METHODS

Design of the clinical trial

This proof of concept phase 2 trial used a primary therapeutic endpoint of one log or more reduction in the HDV RNA levels from the baseline at 12 weeks of therapy. The primary safety endpoint was the ability to tolerate ezetimibe at the prescribed dose for 12 weeks of therapy. Discontinuation of the medication was considered a failure to tolerate the medicine. The secondary endpoint was a normalization of the serum alanine aminotransferase (ALT) at the end of therapy. Each patient remained in the study for 12 weeks.

Patient information

Patients who presented as an outpatient with evidence of chronic HDV infection were selected based on the criteria mentioned in Table 1. Forty-four patients were included, 38 male and 6 female, mean age 35.2±8.7 (range 19-64). Clinical cirrhosis was present in 14 patients and was determined via clinical examination, laboratory findings, and imaging studies. Fifteen (34%) patients were already using concomitant nucleoside therapy.

Ethics

The study was approved by the Ethical review committee (ERC) of Ziauddin University (00281116ZAGE). This study was registered at ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT03099278). Written informed consent was taken. The patient's data, including demographics, concomitant illnesses, drug history, and previous treatments, were gathered and recorded on a pre-designed survey form. A standard symptom questionnaire was used to get information on the categorical presence of symptoms as well as their severity, Child-Pugh score, and relevant investigations, which were then recorded.

Table 1. Inclusion and exclusion criteria for the study patients.

Inclusion criteria
Treatment-experienced patients who were non-responders or had relapsed after pegylated interferon therapy
Interferon ineligible patients
Age ≥ 18 years
Presence of anti-HDV antibody and quantifiable HDV RNA in serum
ALT levels higher than the upper limit of normal
Exclusion criteria
Decompensated liver disease (Child B and C) defined by bilirubin >2 mg/dl, albumin < 3.5 mg/dL, INR >1.7, and history of bleeding varices, ascites, or hepatic encephalopathy
ALT levels greater than 10 times ULN (400 U/L)
Pregnancy or the inability to practice adequate contraception
Significant systemic or major illnesses other than liver disease, including, but not limited to congestive heart failure, renal failure (eGFR<50 mL/min), organ transplantation, serious psychiatric disease or depression, and active coronary artery disease
Systemic immunosuppressive therapy
Evidence of another form of liver disease in addition to viral hepatitis
Active substance abuse such as alcohol or injectable drugs
Concurrent HCV or HIV infection
Hepatocellular carcinoma
Diagnosis of any malignancy in the last five years
Concurrent usage of statins
Concurrent use of any other drug known to inhibit NTCP
Inability to understand or sign the informed consent
Any other condition, which in the opinion of the investigators would impede the patient's participation or compliance in the study

HDV: hepatitis D virus; RNA: ribonucleic acid; ALT: alanine aminotransferase; INR: international normalization ratio; ULN: upper limit of normal; eGFR: estimated glomerular filtration rate; HCV: hepatitis C virus; HIV: human immunodeficiency virus; NTCP: sodium taurocholate co-transporting polypeptide.

Baseline investigations

The baseline investigations used to evaluate all of the HDV infected patients included abdominal ultrasound, complete blood counts, international normalization ratio (INR), liver function tests, creatinine kinase, lactate dehydrogenase, blood sugar, creatinine and electrolytes, lipid profile, alpha-fetoprotein, thyroid-stimulating hormone, and a pregnancy test. Virology tests included HBeAg, determination of HBV DNA, and HDV RNA levels. HBV DNA was amplified by polymerase chain reaction using Cobas AmpliPrep/ COBAS TaqMan HBV Test, v2.0 (Roche Diagnostics, CA, USA). HDV RNA was amplified by polymerase chain reaction using the Robogene HDV RNA quantification kit Analytik Jena (Analytik Jena AG, Jena, Germany). Patients were followed up at weeks 4, 8, and 12 in the outpatient clinic. Compliance was monitored via patient diaries and prescription records.

Monitoring side effects

The therapy was stopped if intolerance to ezetimibe was found. If therapy for HBV had been started with a nucleos(t)ide analogue prior to enrolling in this study, the specific analogue was continued. However, the use of statins was prohibited during the study. All patients were monitored by evaluating their quantitative HDV RNA levels at the end of the 12- week treatment. Routine safety measures and liver function tests were performed at four-week intervals. Patients were monitored for side effects. The discontinuation of ezetimibe was based upon the scoring of adverse events. The scoring of toxicity was performed using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 (11).

Statistical analysis

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS) software version 23 (IBM Corp.; Armonk, NY, USA). A descriptive analysis was conducted for the demographic features, and the continuous variables are presented as a mean±standard deviation. These variables were then analyzed by the Mann Whitney-U test. Baseline versus the end-of-treatment comparison was performed by Wilcoxon signed-rank test or the paired t-test where appropriate. Categorical variables were presented as a number with percentage frequency and analyzed by the Fisher exact test. A $p<0.05$ was considered significant.

RESULTS

The mean HDV RNA level (log10 IU/mL) was 5.4±1.3. HBeAg was non-reactive in 43 (98%) patients. HBV DNA was undetectable in 28 (64%); range when detectable 20-3817 IU/mL. The baseline characteristics of the study patients are given in Table 2. One patient stopped the treatment at week 4, and one patient did not attend the follow-up. According to the intention-to-treat analysis, one log or more reduction in the HDV RNA levels was seen in 18/44 (41%) patients at week 12. Fifteen out of 30 patients with a baseline viral load <6 log10 responded compared to 3/14 patients with viral load >6 log10 (p=0.104 by Fisher exact test, Odds ratio 3.67, 95% CI 0.72-20.1). The presence or absence of detectable HBV DNA did not affect the outcome (p=0.492). According to the per-protocol analysis (n=42), ≥1 log reduction in HDV RNA levels was seen in 18/42 (43%), no-log reduction in 16 (38%), and a log increase was found in 8 (19%) patients. In the 18 patients who responded, their mean HDV RNA level (log10 IU/mL) was 5.0±1.3 at baseline and 3.7±1.6 at week 12 (p<0.001). Furthermore, their ALT levels were 88±64 at baseline and 56±29 at week 12 (p=0.006). At the conclusion of treatment, normal ALT was observed in 7/18 responders and 4/24 non-responders (p=0.159). ALT reduction of >50% was observed in 3 responders and 1 non-responders. No adverse effects from the treatment were observed with the concomitant use of nucleoside analogue or the presence of cirrhosis. Changes

Table 2. Baseline characteristics of the study patients.

Age (mean years)	35.2±8.7 (range 19-64)
Gender (male: female)	38:6
Body mass index (kg/m^2)	24.2±5.7
Hemoglobin (g/dL)	13.5±2.0
Total leukocyte count (x 10^9 /L)	5.6±1.7
Platelet count (x 10^9 /L)	142±82
Total bilirubin (mg/dl)	0.94±0.49
ALT (IU/L)	100±76
AST (IU/L)	85±63
GGT (IU/L)	104±75
ALP (IU/L)	135±89
Cirrhosis	29 (66%)
HBeAg non-reactive	43 (98%)
HDV RNA (mean log10 IU/mL)	5.4±1.3
HBV DNA non-detected	28 (64%)
Concomitant nucleoside therapy	15 (34%)

ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; ALP: alkaline phosphatase; HBeAg: hepatitis B e antigen; HDV: hepatitis D virus; RNA: ribonucleic acid; HBV: hepatitis B virus; DNA: deoxyribonucleic acid

in the HDV viral load at 12 weeks of treatment are depicted in Figure 1.

In the group of 18 responders, two of the patients showed more than a two-log decrease in the HDV RNA levels at week 12. One patient, with a baseline HDV RNA level of 5.5 log10, became HDV RNA negative at week 12. The other patient who had baseline HDV RNA level 5.8 log10, the level decreased to 2.6 at week 12. This patient decided to continue taking the drug, and the level further decreased to 2.00 log10 at week 24. According to the study protocol, treatment had to be stopped at twelve weeks; however, three more patients who had exhibited a response of at least one log reduction in the HDV RNA level opted to continue taking the drug for another twelve weeks. One of these patients experienced another log decrease in the viral load at week 24, while the other two patients exhibited an increase in the HDV RNA levels (Figure 2).

This drug exhibited a good safety profile. The adverse events found during this study were generally mild and included bodyaches in 7, fatigue 1, dyspepsia 1, fever 1, and a decrease in total leucocyte count in 1. However, one patient decided to cease treatment due to a derangement in the liver function tests.

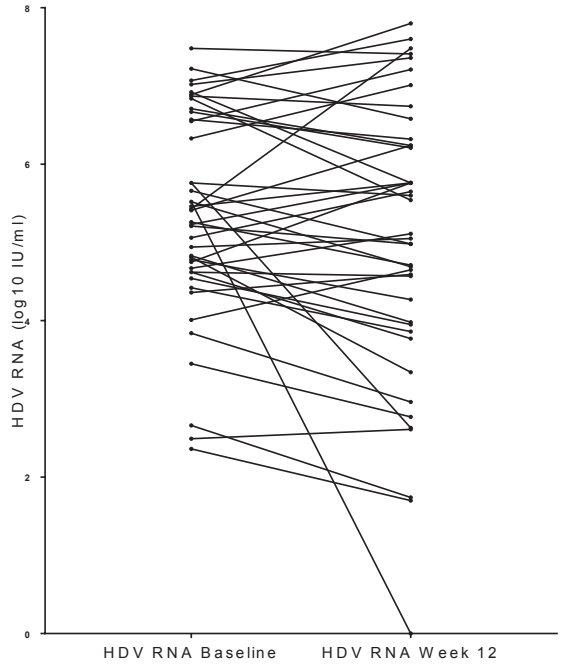

Figure 1. Changes in the HDV RNA levels at 12 weeks of treatment with Ezetimibe.

DISCUSSION

It is expected that the Inhibition of HDV entry will block reinfection and shield naïve hepatocytes that emerge during natural liver turnover (12). There are already available branded drugs that have been shown to inhibit the transport function of NTCP. A recent study investigated the ability of these drugs to impair viral entry using an HDV in vitro infection model based on an NTCP-expressing Huh7 cell line (13). The investigators demonstrated the potential of these FDA approved molecules, including ezetimibe to alter HDV infection *in vitro.*

Ezetimibe impairs dietary and biliary cholesterol absorption at the brush border of the intestine without affecting the absorption of triglycerides or the fat-soluble vitamins. The mechanism of action included Niemann-Pick C1 Like 1 (NPC1L1) protein or an associated protein involved in cholesterol transport (14). This drugs' effect on cholesterol may be due to interference with NPC1L1 in the liver, in addition to this effect in the intestine. Ezetimibe possesses pharmacophore features that inhibit NTCP, the receptor required for HBV and HDV hepatocyte entry, including two hydrogen bonds and one hydrogen bond acceptor (9). The usual dose of Ezetimibe to treat hypercholesterolemia is 10 mg per day, which was the dose used in this study.

In a study conducted by König et al. (10) ezetimibe at a concentration of 100uM significantly inhibited the myrpreS1 peptide binding and the HBV infection of hepatocyte and hepatoma cell lines. Ezetimibe might also have some effect on the already infected cells. Lucifora et al. (15) investigated the effect of ezetimibe on HBV infec-

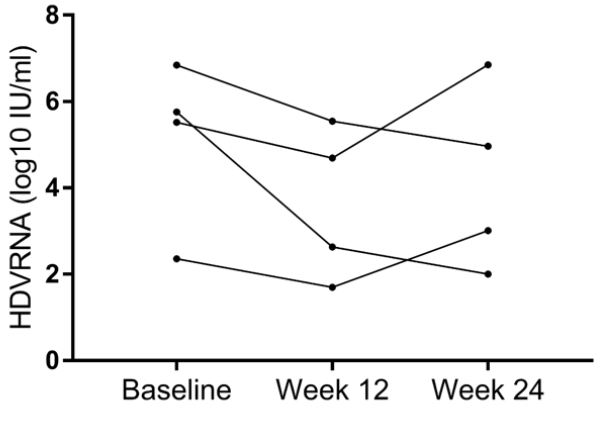

Figure 2. HDV RNA levels at week 24 in patients who had responded at 12 weeks and continued treatment.

tion using differentiated HepaRG cells as a cell-culture infection model. Treatment with ezetimibe inhibited the establishment of the intrahepatic cccDNA and the expression of the viral replication markers when the cells were infected with HBV virions.

In the current study, a reduction of one log or more in the HDV RNA levels was seen in 18 (41%) patients. However, we could not identify the specific factors leading to a favorable response in these 18 responders. Finally, in this study ezetimibe was used as a monotherapy. However, we found that blocking viral entry at just one point did not effectively suppress the viral load. Therefore, combining ezetimibe treatment with another antiviral agent or host immunostimulatory agent might have a more profound effect on achieving a sustained decrease in the viral load.

We chose one log reduction at week 12 as a primary outcome measure, instead of two log reduction at week 24 or a longer duration of therapy as a surrogate marker for initial treatment efficacy as proposed recently by Yurdaydin et al. (16). We chose a shorter time frame because we were not sure of the safety of ezetimibe in patients with liver disease. However, four of our responders who were interferon experienced opted to continue taking the drug. Two of these patients showed a further decrease in their HDV levels (greater than 2 log reduction from the baseline), while the other two patients experienced an increase in the viral load. One of our patients became HDV RNA negative at week 12. This 36-year-old male patient was treatment-experienced, non-cirrhotic, HBV DNA and HBeAg negative patient, with a baseline HDV RNA level 5.5 log10 IU/mL and ALT 46 IU/L which increased to 55 at week 12 suggesting an ongoing immune response.

As this was a pilot study, we assessed the response of oral ezetimibe at 10 mg daily given for twelve weeks. Ezetimibe plasma concentrations at the standard treatment dose of 10 mg daily could be below the value effective for complete HBV inhibition; thus, the use of this drug at the standard dose might not be applicable for treatment of chronic hepatitis D (14). We had not evaluated the initial response to this therapy as we only had the baseline and 12 weeks HDV RNA values and lacked the weekly response values to depict the viral kinetics. Moreover, we could not follow the HDV RNA and ALT levels in all of the study patients after the conclusion of the 12-week trial.

In summary, we have shown that some patients with chronic HDV infection responded to the standard dose of ezetimibe monotherapy. However, we had recruited only difficult to treat patients, including treatment-experienced or interferon ineligible patients for this study. Thus, further studies are warranted using ezetimibe for the treatment of naïve patients, at a higher dose, for a longer duration, or in combination with another antiviral agent.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Ziauddin University, Karachi Pakistan. Reference Code: 00281116ZAGE,

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conflict of interest to declare.

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