A Pilot Clinical Trial of Nitazoxanide in the Treatment of Chronic Hepatitis B

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Chronic infection by the hepatitis B virus (HBV) has remained a major public health problem. To achieve an HBV cure, we will likely need to combine antivirals with different viral targets as well as immunotherapy. Here, we report data from a pilot proof-of-concept clinical trial of nitazoxanide in treating chronic hepatitis B. *Conclusion:* Nitazoxanide offers novel mechanisms of antiviral activity, and it would be interesting to evaluate the potential of combining nitazoxanide with oral nucleos(t)ide analogues. (*Hepatology Communications* 2019;3:744-747).

Chronic infection by the hepatitis B virus (HBV) has remained a major public health problem, with approximately 250 million chronic HBV carriers and at least 850,000 deaths per year. There has been major progress in the efficacy of oral nucleos(t)ide analogues (NUCs) suppressing serum HBV DNA and improving clinical outcomes and survival; yet, treating HBV chronic infection is still a major challenge due to the combination of persisting covalently closed circular HBV DNA (cccDNA) and integrated HBV genomes. Hepatitis B surface antigen (HBsAg) loss remains a rare event during NUC therapy; thus long-term, possibly lifetime, administration is deemed necessary with related cost, safety, and compliance issues.[1]

To achieve an HBV cure, we will likely need to combine antivirals with different viral targets as well as immunotherapy. It would also be important to use molecules with different modes of action, including those targeting host cell metabolism, thus avoiding resistance.

In this context, nitazoxanide and thiazolides might offer interesting prospects for HBV therapy. Nitazoxanide is a first-in-class thiazolide originally developed as an antiprotozoal agent licensed in the United States in 2002. It was the first drug effective against the apicomplexan protozoa *Cryptosporidium parvum* and has remained the only drug effective against this parasite. It was later discovered that the drug was also effective against many viruses.[2] Korba et al.[3] first reported the activity of nitazoxanide and its active circulating metabolite tizoxanide in inhibiting HBV DNA as well as the hepatitis B core antigen, hepatitis B e antigen (HBeAg), and HBsAg in cell cultures. These compounds were effective against six drug-resistant HBV mutants, four of which were resistant to lamivudine, with a 50% effective concentration (EC_{50}) ranging from 0.15 to 0.31 μM. These results compared favorably to the EC_{50} for adefovir dipivoxil, which ranges from 1.5 to 11 μM in the same experimental conditions. Recently, Sekiba et al.[4] have shown that nitazoxanide inhibits expression of HBV cccDNA and HBV RNA transcription by targeting the hepatitis B X protein (HBx)–damage-specific DNA-binding protein 1(DDB1) interaction.

Abbreviations: cccDNA, covalently closed circular DNA; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NUC, nucleos(t)ide analogue.

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Here, we report data from a pilot proof-of-concept clinical trial of nitazoxanide in treating chronic hepatitis B. This study was conducted concurrently with other clinical trials in Egypt evaluating the activity of nitazoxanide in treating chronic hepatitis C.[5-7]

Participants and Methods

The study protocol was approved by the ethical committees of the University of Benha Faculty of Medicine and the University of Alexandria Faculty of Medicine. Nine males at least 17 years of age with chronic hepatitis B without cirrhosis were included in the study. The subjects were recruited from the study clinics, and each had a history of chronic hepatitis B. Each subject signed an informed consent in Arabic before screening. At the screening visit, each subject submitted to a complete physical examination; blood and urine samples were collected for hematology, blood chemistry, and urinalysis; a blood sample was collected for HBV DNA, HBsAg, HBeAg, hepatitis C virus, and human immunodeficiency virus; and a needle liver biopsy was obtained to establish a baseline necroinflammatory score and a fibrosis score.

The trial was conducted between March 2005 and April 2007. Each of the 9 subjects was treatment naive and HBsAg positive with HBV DNA quantified (Log IU/ml; COBAS Amplicor; Roche) in serum. Two subjects were HBeAg positive with HBV DNA levels of 3.6 and 6.0 log IU/mL, and 7 were HBeAg negative with HBV DNA ranging from 2.5 to 4.3 log IU/mL (Table 1). On biopsy, inflammatory grading ranged from 2 to 6/18 and fibrosis staging ranged from 1 to 3/6.

Nitazoxanide 500 mg tablets (Romark, Tampa, FL) were administered orally twice daily with food for up to 48 weeks. Subjects visited the clinic every 4 weeks during treatment for physical examination and collection of blood samples for hematology, blood chemistry, HBeAg, HBsAg, quantitative HBV DNA, and antibodies to hepatitis A, C, and D viruses. Adverse events were also recorded.

Results

Three subjects completed 48 weeks of treatment, 5 elected to discontinue after 32 weeks, and 1 elected to discontinue after 12 weeks. Treatment discontinuations were attributed to loss of interest on the part of the patients as new-generation NUCs (entecavir and tenofovir disoproxil fumarate) were becoming available. The drug was well tolerated with occasional mild to moderate side effects primarily related to the gastrointestinal tract, including diarrhea and epigastric pain. These adverse events were transient and resolved during treatment. None of the adverse events required discontinuation of treatment. There were no laboratory abnormalities that were deemed to be related to treatment. None of the patients developed hepatitis A, C, or D infection during the study.

HBV DNA became undetectable (<38 IU/mL) in the serum of 8 of the 9 subjects (89%) after 4 to 20 weeks of treatment with nitazoxanide (Table 1). The 2 subjects who were HBeAg positive became HBeAg negative after 4 and 16 weeks of treatment, respectively. Three of the 9 subjects (33%) became HBsAg negative, 2 of them after 8 weeks and 1 after 48 weeks of treatment. The subject (subject #1) with the later responses (20 weeks to undetectable HBV DNA, 16 weeks to HBeAg negative, and 48 weeks to HBsAg negative) was treated with 500 mg nitazoxanide only once daily. At the end of treatment, all subjects were rolled over to standard NUC therapy. The 2 subjects who lost HBeAg and the 3 who lost HBsAg were not followed up to determine whether

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TABLE 1. QUANTITATIVE HBV DNA, HBeAg, HBsAg, AND ALT DURING TREATMENT

		Week of Treatment						
Subject		0	4	8	12	24	36	48
#1	HBV DNA	3.6	4.3	4.6	3.6	–	–	–
Male, age 43 years	HBeAg	+	+	+	+	–	–	–
Grade 3/18	HBsAg	+	+	+	+	+	+	–
Stage 1/6	ALT	141	33	26	17	22	14	13
#2	HBV DNA	3.6	3.6	3.7	4.0	4.3		
Male, age 29 years	HBeAg	–	–	–	–	–		
Grade 4/18	HBsAg	+	+	+	+	+		
Stage 1/6	ALT	23	31	29	39	33		
#3	HBV DNA	4.0	–	–	–	–		
Male, age 37 years	HBeAg	–	–	–	–	–		
Grade 6/18	HBsAg	+	+	+	+	+		
Stage 3/6	ALT	48	54	49	57	37		
#4	HBV DNA	3.5	–	–	–	–		
Male, age 32 years	HBeAg	–	–	–	–	–		
Grade 4/18	HBsAg	+	+	–	–	–		
Stage 1/6	ALT	23	48	31	42	31		
#5	HBV DNA	4.3	–	–	–	–		
Male, age 42 years	HBeAg	–	–	–	–	–		
Grade 2/18	HBsAg	+	+	–	–	–		
Stage 1/6	ALT	38	34	26	22	10		
#6	HBV DNA	3.8	–	–	–			
Male, age 43 years	HBeAg	–	–	–	–			
Grade 2/18	HBsAg	+	+	+	+			
Stage 1/6	ALT	29	33	26	29			
#7	HBV DNA	2.5	2.6	–	–	–	–	–
Male, age 40 years	HBeAg	–	–	–	–	–	–	–
Grade 2/18	HBsAg	+	+	+	+	+	+	+
Stage 1/6	ALT	20	14	17	18	21	16	16
#8	HBV DNA	4.0	4.1	–	–	–	–	–
Male, age 34 years	HBeAg	–	–	–	–	–	–	–
Grade 2/18	HBsAg	+	+	+	+	+	+	+
Stage 1/6	ALT	23	68	53	48	40	75	53
#9	HBV DNA	6.0	4.7	4.0	–	–		
Male, age 34 years	HBeAg	+	–	–	–	–		
Grade 3/18	HBsAg	+	+	+	+	+		
Stage 1/6	ALT	53	60	32	40	40		

+, positive; –, HBV DNA < 38 IU/mL; grade, inflammation score; stage, fibrosis score.
Abbreviation: ALT, alanine aminotransferase.

they seroconverted to antibody to HBeAg or antibody to HBsAg.

Discussion

We appreciate that this report is based on an uncontrolled study involving a limited number of subjects. Also, lack of follow-up did not allow the determination of whether the response to nitazoxanide was sustained. However, the results, which were obtained a few years ago, are intriguing, in particular with regard to the rapid decrease of serum HBV DNA following treatment; they are consistent with the *in vitro* anti-HBV activity of nitazoxanide and in fact bridge with the recent finding of nitazoxanide

inhibiting HBV transcription from cccDNA by targeting the HBx–DDB1 interaction. Overall, they further support the potential interest of nitazoxanide and second-generation thiazolides in the treatment of chronic hepatitis B. Moreover, our study suggests that nitazoxanide might not only suppress serum HBV DNA but also lead to serum HBsAg loss in a significant number of cases; this is also consistent with previous *in vitro* evaluations. Nitazoxanide offers novel mechanisms of antiviral activity, most of them likely emanating from inhibition of oxidative phosphorylation in the mitochondria. Thus, in light of our preliminary clinical results, it would be interesting to evaluate the potential of combining nitazoxanide with NUCs. Ongoing randomized clinical trials of nitazoxanide as an "add-on treatment" to NUCs in subjects with chronic hepatitis B will provide further insight into the potential for use of nitazoxanide in treating chronic hepatitis B.

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REFERENCES

1) World Health Organization. Hepatitis B fact sheet. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b. Published July 18, 2018. Accessed April 2017.
2) Rossignol JF. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. Antiviral Res 2014;110:94-103.
3) Korba BE, Montero AB, Farrar K, Gaye K, Mukerjee S, Ayers MS, et al. Nitazoxanide, tizoxanide and other thiazolides are potent inhibitors of hepatitis B virus and hepatitis C virus replication. Antiviral Res 2008;77:56-63.
4) Sekiba K, Otsuka M, Ohno M, Yamagami M, Kishikawa T, Suzuki T, et al. Inhibition of HBV transcription from cccDNA with nitazoxanide by targeting the HBX-DDB1 interaction. Cell Mol Gastroenterol Hepatol 2019;7:297-312.
5) Rossignol JF, Kabil SM, El-Gohary Y, Elfert A, Keeffe EB. Clinical trial: randomized, double-blind, placebo-controlled study of nitazoxanide monotherapy for the treatment of patients with chronic hepatitis C genotype 4. Aliment Pharmacol Ther 2008;28:574-580.
6) Rossignol JF, Elfert A, El-Gohary Y, Keefe EB. Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. Gastroenterology 2009;136:856-862.
7) Rossignol JF, Elfert A, Keeffe EB. Treatment of chronic hepatitis C using a 4-week lead-in with nitazoxanide before peginterferon plus nitazoxanide. J Clin Gastroenterol 2010;2010(44):504-509.