ANTIVIRAL EFFICACY AND INDUCTION OF HOST IMMUNE RESPONSES WITH SB 9200, AN ORAL PRODRUG OF THE DINUCLEOTIDE SB 9000, IN THE WOODCHUCK MODEL OF CHRONIC HEPATITIS B VIRUS (HBV) INFECTION

Kyle Korolowicz1, Stefanie Czerwinski1, Radhakrishnan Iyer2, Jeffrey Skee2, Junming Yang3, Robin Tucker4, and Stephen Menne4
1Microbiology and Immunology, Georgetown University Medical Center, Washington, DC, 2Spring Bank Pharmaceuticals, Milford, MA, 3Department of Comparative Medicine, Georgetown University Medical Center, Washington, DC, United States

BACKGROUND

Activation of the viral sensor proteins, RIG-I and NOD2, by viral nucleic acids results in the production of type I and III IFNs and subsequent induction of ISGs and antiviral immune cells (1). SB 9200, an oral produg of the dinucleotide SB 9000, activates RIG-I and NOD2 by binding at the nucleotide binding domain of both proteins (2, 3). This interaction also inhibits the synthesis of viral nucleic acids by steric blockage of the viral polymerase from accessing nucleic acid template for replication. SB 9200 is being developed for the treatment of chronic hepatitis B and C and has completed Phase I evaluation in humans for therapy of HCV infection (4).

The host immune stimulating and direct antiviral activities of SB 9200 were evaluated for single agent efficacy in the woodchuck model of chronic HBV infection (5).

MATERIALS & METHODS

Two groups of woodchuck with chronic woodchuck hepatitis virus (WHV) infection (n=9/group) were treated daily, orally with SB 9200 for 12 weeks at doses of 15 or 30 mg/kg. Endpoints included PK, PD, tolerability, and antiviral efficacy. Safety parameters included hematology, clinical chemistry, body weights, body temperatures and daily observations. Plasma values of SB 9000, the active moiety of the produg SB 9200, were quantified by liquid chromatography using an AB Sciex API 5000 triple quadrupole LC/MS/MS system operating in positive ESI (TurboIonSpray®) mode. Serum DNA load was determined by slot blot hybridization and samples below the limit of detection were further evaluated by PCR. Serum WHsAg and anti-WHs antibodies levels were assayed by ELISA. Hepatic levels of WHV RNA, cccDNA, and DNA replicative intermediates (RI) were measured quantitatively by Northern or Southern blot hybridization respectively. Paraffin sections of formalin-fixed liver biopsy samples were stained with H&E and immunostained with an antibody against WHsAg as previously described.

Induction of an antiviral innate immune response to SB 9200 treatment was determined by measuring RNA levels of IFN-α, IFN-β, CXCL10, OAS1 and ISG15 in whole blood and liver. Total RNA was isolated, reverse transcribed to cDNA and evaluated by PCR using woodchuck-specific primers. 18S RNA expression was used to normalize target gene expression.

RESULTS

Treatment with SB 9200 for 12 weeks was well tolerated and resulted in up to 4.2 and 2.2 log10 reductions in individual serum WHV DNA or WHsAg loads, respectively. Treatment also produced lower hepatic levels of WHV cccDNA (up to 32%), WHV DNA (up to 41%) and WHV RNA (up to 50%), resulted in lower hepatic expression of WHV-α and reduced liver inflammation. Following cessation of treatment, recurrence of WHV replication was observed but woodchucks administered the higher SB 9200 dose had delays in viral recrrecurrence. The antiviral effects were associated with the dose-dependent and sometimes long-lasting induction of IFN-α, IFN-β and ISGs in blood and liver.

SB 9200 treatment of chronic WHV carrier woodchucks with the high dose inhibits WHV replication significantly more than the low dose period.

SUMMARY

Treatment with SB 9200 for 12 weeks was well tolerated and resulted in up to 4.2 and 2.2 log10 reductions in individual serum WHV DNA or WHsAg loads, respectively. Treatment also produced lower hepatic levels of WHV cccDNA (up to 32%), WHV DNA (up to 41%) and WHV RNA (up to 50%), resulted in lower hepatic expression of WHV-α and reduced liver inflammation. Following cessation of treatment, recurrence of WHV replication was observed but woodchucks administered the higher SB 9200 dose had delays in viral recrrecurrence. The antiviral effects were associated with the dose-dependent and sometimes long-lasting induction of IFN-α, IFN-β and ISGs in blood and liver.

CONCLUSIONS

Prolonged oral administration of SB 9200 to woodchucks with chronic WHV infection reduced viral markers (DNA, RNA and antigens) in periphery and liver that were associated with (or were a result of) the induction of host antiviral innate immune responses. These results suggest that in addition to the direct antiviral activity, SB 9200 induces antiviral immunity during chronic active hepapnadrinal infection that has the potential for a functional cure in the treatment of chronic hepatitis B, most likely in combination with marketed anti-HBV drugs.

REFERENCES


FINANCIAL SUPPORT

This study was supported by IDIQ contract HHSN27220100011I, task order HHSN2722003002 (D06) to Georgetown University Medical Center and by R01 grant A0894486 to Spring Bank Pharmaceuticals, Inc. from NIAID/DMID.

Contact Information

Stephen Menne, smenne2@georgetown.edu
Radhakrishnan Iyer, ruiyer@springbankpharma.com