Research Article

JOURNAL OF HEPATOLOGY

Viral Hepatitis

Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B

Stephan Menne[1,*,†], Daniel B. Tumas[2,†], Katherine H. Liu[3], Linta Thampi[1], Dalal AlDeghaither[1], Betty H. Baldwin[3], Christine A. Bellezza[3], Paul J. Cote[1], Jim Zheng[4], Randall Halcomb[5], Abigail Fosdick[6], Simon P. Fletcher[2], Stephane Daffis[2], Li Li[7], Peng Yue[7], Grushenka H.I. Wolfgang[6], Bud C. Tennant[3]

[1]*Department of Microbiology and Immunology, Georgetown University Medical Center, Washington, DC 20057, United States;* [2]*Department of Biology, Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, United States;* [3]*Department of Clinical Sciences, Gastrointestinal Unit, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, United States;* [4]*Department of Drug Metabolism, Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, United States;* [5]*Department of Chemistry, Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, United States;* [6]*Department of Drug Safety Evaluation, Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, United States;* [7]*Department of Biomarkers, Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, United States*

Background & Aims: New therapies for chronic hepatitis B (CHB) are urgently needed since current treatments rarely lead to cure. We evaluated whether the oral small molecule toll-like receptor (TLR7) agonist GS-9620 could induce durable antiviral efficacy in woodchucks chronically infected with woodchuck hepatitis virus (WHV), a hepadnavirus closely related to human hepatitis B virus (HBV).

Methods: After evaluating the pharmacokinetics, pharmacodynamics and tolerability of oral GS-9620 in uninfected woodchucks, adult woodchucks chronically infected with WHV (n = 7 per group) were dosed with GS-9620 or placebo for 4 or 8 weeks with different treatment schedules.

Results: GS-9620 treatment induced rapid, marked and sustained reduction in serum viral DNA (mean maximal 6.2 $\log_{10}$ reduction), and hepatic WHV DNA replicative intermediates, WHV cccDNA and WHV RNA, as well as loss of detectable serum WHV surface antigen (WHsAg). GS-9620 treatment also induced a sustained antibody response against WHsAg in a subset of animals. Strikingly, treatment reduced the incidence of hepatocellular carcinoma (HCC) from 71% in the placebo group to 8% in GS-9620-treated woodchucks with sustained viral load reduction. GS-9620 treatment was associated with reversible increases in serum liver enzymes and thrombocytopenia, and induced intrahepatic $CD8^+$ T cell, NK cell, B cell and interferon response transcriptional signatures.

Conclusions: The data demonstrate that short duration, finite treatment with the oral TLR7 agonist GS-9620 can induce a sustained antiviral response in the woodchuck model of CHB, and support investigation of this compound as a therapeutic approach to attain a functional cure in CHB patients.

© 2015 European Association for the Study of the Liver. Published by Elsevier B.V. Open access under CC BY-NC-ND license.

Keywords: TLR7; Hepatitis B virus; Hepatocellular carcinoma; Immunomodulation; Seroconversion; Woodchuck animal model.

Received 23 September 2014; received in revised form 26 November 2014; accepted 19 December 2014; available online 2 January 2015

* Corresponding author. Address: Department of Microbiology and Immunology, Georgetown University Medical Center, 3900 Reservoir Road, Washington, DC 20057, United States. Tel.: +1 (202) 687 2949; fax: +1 (202) 687 1800.

E-mail address: sm923@georgetown.edu (S. Menne).

† Authors share first authorship.

Abbreviations: CHB, chronic hepatitis B; TLR7, toll-like receptor 7; WHV, woodchuck hepatitis virus; HBV, hepatitis B virus; WHsAg, WHV surface antigen; HCC, hepatocellular carcinoma; IFN-α, interferon-alpha; HBsAg, hepatitis B surface antigen; HBsAb, anti-HBs antibody; pDC, plasmacytoid dendritic cell; HEK, Human embryonic kidney; WHsAb, anti-WHs antibody; ge/ml, genome equivalents per milliliter; GGT, gamma glutamyl transferase; PK, pharmacokinetic; PD, pharmacodynamic; C_{max}, maximum serum concentration; AUC_{last}, area under the serum concentration versus time curve; RI, replicative intermediate; 2′5′-OAS, 2′,5′-oligoadenylate synthetase; MxA, myxovirus resistance A; FDR, false discovery rate; DEG, differentially expressed gene; IPA, Ingenuity Pathway Analysis; T_{max}, time of maximum serum concentration; ISG, interferon-stimulated gene; QOD, every other day; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SDH, sorbitol dehydrogenase; QW, every week; QOW, every other week; M, module.

Introduction

An estimated 350 million people have chronic hepatitis B virus infection (CHB), and over 500,000 people die each year due to HBV-associated liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC) [1]. Current therapeutics for CHB are limited to nucleos(t)ides and interferon alpha (IFN-α); these reduce viral load, improve long-term outcome, but rarely lead to a cure [2]. Immunologic control of CHB, recognized as a "functional cure," is defined by control of viremia, loss of HBV surface antigenemia (HBsAg), and seroconversion to anti-HBs antibody (HBsAb) and occurs in only 2–3% of patients per year with antivirals [2]. Functional cure with long-term IFN treatment occurs in <10% of patients at doses associated with treatment-limiting adverse effects [2]. There is therefore an urgent need for a curative therapy.

Natural infection with woodchuck hepatitis virus (WHV), a hepadnavirus closely related to the HBV, occurs in the Eastern

Research Article

woodchuck (*Marmota monax*). Chronic WHV infection is a model for studying CHB, hepadnavirus-associated HCC, and is used to evaluate HBV therapeutics [3]. Importantly, comparison of the intrahepatic transcriptional profiles in woodchucks and humans with chronic hepadnaviral infection identified important parallels in the antiviral immune responses and demonstrated molecular similarities in HCC induced by WHV and HBV [4]. As this establishes the translational value of the WHV model, we utilized it to explore therapeutic immunomodulation with the small molecule TLR7 agonist GS-9620.

TLR7 is expressed predominantly in plasmacytoid dendritic cells (pDCs) and B lymphocytes and recognizes viral single-stranded RNA [5]. TLR7 activation induces innate and adaptive immune responses via induction of specific cytokines (including multiple IFN-α subtypes [6]) and chemokines, activation of B cells [7], and cross-priming of cytotoxic lymphocytes [8]. GS-9620 is a selective, oral small molecule TLR7 agonist that has activity in rodents, nonhuman primates and humans [9–12]. A single dosing regimen (three times a week for 8 weeks) of GS-9620 has previously been evaluated in a small number of chimpanzees (n = 3) with CHB [10]. Here we report a placebo-controlled study in which the activity of different regimens and treatment durations of GS-9620 were evaluated in woodchucks chronically infected with WHV. Importantly, this model provided a unique opportunity to evaluate the antiviral activity of GS-9620 in a model of vertical transmission and to determine treatment impact on development of hepadnavirus-associated HCC.

Materials and methods

Investigational drug

GS-9620, (8-(3-(pyrrolidin-1-ylmethyl)benzyl)-4-amino-2-butoxy-7,8-dihydropteridin-6(5H)-one), a small molecule, selective TLR7 agonist, was manufactured by Gilead Sciences, Inc. [9]. Doses were administered in a liquid diet (1 mg/ml; 79002 Liquid Woodchuck Control Diet, Dyets, Inc., Bethlehem, PA) in a dose volume of 10 ml. Placebo-treated woodchucks were administered liquid diet alone.

Woodchuck TLR7 cloning and sequencing

Total RNA was isolated from woodchuck PBMC using RNeasy extraction kit (QIAGEN, Redwood City, CA) and DNA was synthesized using SuperScript® III First-Strand Synthesis System (Life Technologies, Chicago, IL) according to the manufacturer's instructions. Woodchuck TLR7 (wTLR7) sequence was identified by a PCR-based strategy using various primers that were designed based on highly conserved sequences between human TLR7 (NM_016562.3) and mouse TLR7 (NM_133211.3). The 5′- and 3′-ends of wTLR7 were identified using 5′ and 3′ RACE System kits respectively according to the manufacturer's instructions (Life Technologies, Chicago, IL). All amplified PCR fragments were sequenced to obtain the complete wTLR7 sequence, deposited into NCBI (*Accession TBD Prior to Publication*). wTLR7 was synthesized and cloned into pUNO1-hTLR7-HA3x vector (InvivoGen, San Diego, CA) in place of human TLR7 to generate pUNO1-wTLR7-HA3x.

HEK293 TLR7 assay

Human embryonic kidney (HEK) 293 cells (CRL1573-ATCC) were seeded in 96 well plates in DMEM-GlutaMAX-I supplemented with 10% FBS. Eight hours post seeding, the cells were co-transfected with either human TLR7 (pUNO1-hTLR7-HA3x) or wTLR7 (pUNO1-wTLR7-HA3x) along with the NF-κB luciferase reporter pNiFty2-Luc (InvivoGen, San Diego, CA) using TransIT®-293 transfection reagent (Mirus, Pittsburgh, PA) according to the manufacturer's instructions. Eighteen hours post-transfection the cells were stimulated with various concentrations of GS-9620 for 6 h. Cells were lysed using ONE-Glo Luciferase assay system (Promega, Madison, WI) and NF-κB luciferase activity measured using a VICTOR X Light Luminescence plate reader (Perkin-Elmer, Waltham, MA).

Animals

Protocols were approved by the Institutional Animal Care and Use Committee of Cornell University. Neonatal woodchucks were infected at 3 days of age with a WHV7P1 inoculum containing 5×10^6 WID50 of WHV strain WHV7-11 and were then raised to adulthood prior to use in this study. All infected woodchucks were monitored serologically through 1 year post infection. At the start of the study all woodchucks had antibody to WHV core antigen (anti-WHc antibody), were negative for antibody to WHV surface antigen (anti-WHs antibody; WHsAb), had serum WHV DNA levels $\geqslant 10^9$ ge/ml and detectable WHV surface antigen (WHsAg) at pretreatment, and were free of HCC by ultrasound liver examination and serum gamma glutamyl transferase (GGT). Uninfected control woodchucks were negative for anti-WHc antibody, anti-WHs antibody, WHsAg, and WHV DNA.

Single dose pharmacokinetic (PK) and pharmacodynamic (PD) study in uninfected woodchucks

Uninfected healthy, adult male woodchucks (n = 3 per dose group) were orally administered 1, 5, or 10 mg/kg of GS-9620. Evaluations included clinical observations, clinical pathology, pharmacokinetics and pharmacodynamics. Serum levels of GS-9620 were measured over time to calculate maximum serum concentration (C_{max}) and exposure, calculated as area under the serum concentration *vs.* time curve (AUC_{last}).

Efficacy study in woodchucks chronically infected with WHV

The study design is described in Table 1. Briefly, five groups of woodchucks (n = 7 per group, mixed sex) were treated with GS-9620 for approximately 4 weeks (groups 1, 2, and 3) or 8 weeks (groups 4 and 5) and evaluations continued through 35 weeks (time of scheduled euthanasia). Woodchucks that developed HCC were euthanized; HCC was diagnosed by ultrasound and serum GGT and confirmed at necropsy. Woodchucks that died unexpectedly during the study were evaluated by necropsy. Serum GS-9620 levels were evaluated at week 4, 4 h post-dose, the approximate time of peak exposure.

Determination of GS-9620 in serum

Woodchuck serum was mixed with acetonitrile containing an internal standard. After protein precipitation and centrifugation, the supernatant was mixed with water with 0.1% formic acid. An aliquot was injected to a TSQ Ultra Quantum LC/MS/MS system (Thermo Finnigan, San Jose, CA).

Clinical pathology

Clinical pathology was assessed at pretreatment, approximately weekly during treatment (24 h post-dose), at least 1 and 2 weeks after the last dose, and approximately monthly through the end of the 35 week study [13].

WHV serum viremia levels

WHV DNA was quantified by two different methods depending on concentration: dot blot hybridization or real-time PCR assay on a 7500 Real-Time PCR System instrument (Applied Biosystems, Foster City, CA) as described previously [14].

Hepatic levels of WHV nucleic acids

Liver levels of WHV DNA replicative intermediates (RI), WHV cccDNA, and WHV RNA were determined in liver biopsies collected 2 weeks prior to treatment and at selected time-points during and after treatment. Biopsies were obtained under general anesthesia with a 16-gauge needle directed by ultrasound imaging. Biopsy specimens for WHV nucleic acid analyses were placed immediately in liquid nitrogen and stored at −70 °C. WHV RNA was measured quantitatively by Northern blot hybridization as previously described [15,16]. WHV DNA RI

Download English Version:

https://daneshyari.com/en/article/6101905

Download Persian Version:

https://daneshyari.com/article/6101905

Daneshyari.com