Short communication

Antiviral efficacy of lobucavir (BMS-180194), a cyclobutyl-guanosine nucleoside analogue, in the woodchuck (Marmota monax) model of chronic hepatitis B virus (HBV) infection


Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut, CT 06492-7660, USA

Received 13 April 2000; accepted 1 September 2000

Abstract

Lobucavir (BMS-180194), a cyclobutyl-guanosine nucleoside analogue, effectively reduced WHV-viremia in chronically infected carrier woodchucks (Marmota monax) by daily per os treatment. WHV-viremia in the animals was measured by the serum content of hybridizable WHV-genomic DNA. Lobucavir, given at daily doses of 10 and 20 mg/kg body weight, reduced WHV-viremia by a 10- to 200-fold range during therapy. Lobucavir, given at 5 mg/kg, suppressed WHV-viremia by a 10- to 30-fold range, whereas a 0.5 mg/kg dose had no significant effect. WHV-viremia was also measured by hepadnaviral endogenous polymerase activity (EPA) in sera of animals treated for 6 weeks at 5 and 0.5 mg/kg. Changes in EPA in sera of lobucavir treated animals were comparable to changes in WHV DNA levels. Viremia in treated carriers recurred at levels by 2 weeks of therapy cessation. These results indicated that the minimally effective antiviral daily per os dose of lobucavir in WHV-carrier woodchucks was ≈ 5 mg/kg. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Hepatitis B virus (HBV); Woodchuck hepatitis virus (WHV); Lobucavir; Woodchucks (Marmota monax); Carbocyclic guanosine nucleoside analogues; Chronic virus infection

Human hepatitis B virus (HBV), a prototype member of the hepadnaviridae, is a small enveloped, partially double-stranded circular DNA virus (Gust et al., 1986; Schodel et al., 1989), recognized as a major etiologic agent of the current hepatitis pandemic. While primary human
HBV infections are often self-limited and resolved, chronic persistent infections occur in up to 10% of primary infection cases, leading to a virus-carrier state, marked by recurrent episodes of liver inflammation and progression to such debilitating liver diseases as cirrhosis and hepatocellular carcinoma. There are an estimated 350 million HBV-carriers in the world and the clinical need for effective treatments against chronic HBV disease is critical (Margolis et al., 1991; Locarnini et al., 1996; Mason, 1996; Hoofnagle and Di Bicegle, 1997; Colacino and Staschke, 1998). Since the hepadnavirus replicative cycle involves a virally encoded polymerase (POL) that catalyzes a complex multistep process of viral genomic synthesis coordinated with virion maturation in the cytoplasm of infected cells, a major antiviral research effort for the therapeutic management of chronic HBV has been on development of potent inhibitory nucleoside analogues against POL (Seeger et al., 1991; Locarnini et al., 1996; Nassal, 1996; Colacino and Staschke, 1998; Zoulim, 1999).

There has been interest in the chemotherapeutic application of lobucavir (BMS-180194 or SQ-34514), for HBV infections (Malik and Lee, 2000). Lobucavir, a cyclobutyl-guanosine nucleoside, \(1R-(1\alpha,2\beta,3\alpha)}\)-2-amino-9-[2,3-bis(hydroxymethyl)cyclo-butyl]-1,9-dihydro-6H-purin-6-one, has in vitro and in vivo efficacy against herpetic viruses (Field et al., 1990; Braitman et al., 1991; Yang et al., 1991). Lobucavir also reversibly inhibited HBV production by the HBV-expressing HepG 2.2.15 cell line (Innaimo et al., 1997). In molecular studies, lobucavir nucleotide triphosphates acted as a non-obligate chain terminator of POL, inhibiting all three major enzymatic functions in genomic replication: oligodeoxynucleotide primer synthesis, reverse transcriptase, and DNA-dependent DNA (\(+\text{strand}\) synthesis (Seifer et al., 1998). This inhibitory mechanism operated against POL of other hepadnaviruses, including woodchuck hepatitis virus (WHV) and duck hepatitis virus (Seifer et al., 1998).

Experiments were undertaken to assess the in vivo efficacy of lobucavir against chronic HBV infections, using woodchucks (Marmota monax) with chronic WHV infection. WHV-infection of its natural woodchuck host has served as a suitable animal virus model for studies on the pathogenesis and immunobiology of HBV infections and related liver disease conditions. The chronically infected WHV-carrier is considered useful to evaluate the efficacy of agents for the therapy of human HBV infections (Roggendorf and Tolle, 1995; Tennant et al., 1996; Tennant, 1998).

Procedures involving woodchucks were reviewed and approved by the Animal Care and Use Committee of Wallingford, CT, site of the Pharmaceutical Research Institute of Bristol-Myers Squibb Co. WHV-carrier woodchucks were obtained from the breeding colony of Marmotech Inc. (Cortland, NY). Animals were WHV-inoculated within 1 week of birth with virus derived from sera of woodchuck carriers infected with WHV strain WH7. Inoculated woodchucks were determined as chronically infected at 6 months of age by detection of circulating WHV-genomic DNA. WHV-carriers were sero-positive for WHV-surface antigen (viral envelope protein) and for antibody to WHV-core antigen (Roggendorf and Tolle, 1995; Tennant, 1998).

For antiviral efficacy studies, lobucavir was administered per os, once daily to WHV-carriers for selected time periods. The compound was suspended in either pyrogen-free sterile water or Avicel (FMC Corp., Newark, DE) then admixed, according to individual animal body weight, to the desired concentration in a liquid diet formulation (Liquid Woodchuck Control Diet; Dyets Inc., Bethlehem, PA) for oral delivery in a 5-ml volume. Liquid diet was given to animals in the placebo control treatment groups. At selected weekly time intervals, animals were anesthetized by intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and bled from either the femoral vein or artery for serum samples. Until needed for assay, all samples were stored at \(-20^\circ\text{C}\).

Antiviral effectiveness of lobucavir treatment was based on WHV-viremia reduction in serum, obtained at times during and after therapy relative to samples taken before treatment. Viremia was monitored as previously described (Genovesi et al., 1998) for serum WHV-genomic DNA con-
tent by a modified method of dot-blot hybridiza-
tion (Korba and Gerin, 1992), or for endogenous
viral DNA polymerase (EPA) activity which mea-
sures serum content of replication-competent viri-
ons (Hantz et al., 1984; Miller et al., 1984; Nassal,
1996).

Briefly, for the hybridization assay, alkali-ex-
tacted DNA from test sera, affixed to nylon
filters, were incubated with a whole genomic, ran-
domly radiolabeled ($^{32}$P), linear WHV DNA
probe. Hybridized serum DNA was measured for
radioactivity by image analysis. WHV DNA con-
tent calculation was based on comparison to a
standard curve of cloned WHV DNA. The lower
WHV DNA assay detection limit was $\approx 200$ pg
viral DNA per millilitre of serum. For WHV-car-
riers of this study, pretreatment WHV DNA
ranged from $4.0 \times 10^4$ to $6.0 \times 10^5$ pg per millilitre
of serum. For each serum sample of each respec-
tive animal, the WHV DNA quantity was divided
by the animal’s pretreatment serum viral DNA
content, to obtain data as the percent of pretreat-
ment WHV DNA. Results for each time point
were graphically presented for each animal treat-
ment group, as the arithmetic mean ($\pm$ 1 SEM) of
the percent of pretreatment WHV DNA.

The EPA assay was adapted to a 96-well plate
format (Seifer et al., 1998). WHV-virion suspen-
sions, obtained from test sera by centrifugal con-
centration, were mixed in a buffered solution with
dGTP, dCTP, TTP and [a-$^{33}$P]-dATP, then incu-
bated to allow enzymatic formation of radiola-
teled DNA. Product DNA was collected by
trichloroacetic acid precipitation and measured
for incorporated radioactivity by liquid scintilla-
tion counting. EPA data for each animal was
calculated as a percentage of incorporated ra-
dioactivity in test samples relative to the respec-
tive animal’s pretreatment serum. This assay was
only used to evaluate samples of one treatment
study (see Figs. 1 and 2). EPA results were graphi-
cally presented for each treatment group, as the
arithmetic mean ($\pm$ 1 SEM) of the percent incor-
porated radioactivity of pretreatment sera.

Lobucavir was previously reported to be effec-
tive in reducing WHV-viremia in carriers when
given per os at a daily dose of 20 mg/kg for 3
months (Tennant et al., 2000). Based on this
information, a study was conducted to test lobu-
cavir at lower daily doses of 5 and 0.5 mg/kg in a
6-week treatment regimen, with a 6-week post-
therapy observation interval (Fig. 1). At different
times during lobucavir treatment at 5 mg/kg,
mean viremia levels were reduced from 3% to 10%
of pretreatment serum WHV DNA content. By 2
weeks (e.g. week 8) after therapy, viremia re-
crudesced (Fig. 1). In the subsequent 4-week pe-
riod, viremia was at pretreatment serum WHV
DNA levels with no viral flares noted (data not
shown). The magnitude of WHV DNA reduction
among the animals was not uniform and was
independent of pretreatment WHV DNA levels.
Viremia reductions to the assay detection limit
($\leq 200$ pg DNA per millilitre), $\leq 0.1\%$ of pre-
treatment serum load, were noted for two of the
five animals of this group (data not shown). For
carriers ($n = 6$) treated with 0.5 mg/kg, no signifi-
cant change in mean serum WHV DNA was

![Fig. 1. WHV-viremia in WHV-carriers treated per os daily
with lobucavir for 6 weeks. The drug-treatment time interval
of this experiment is indicated by the horizontal line, parallel
to the x-axis. Viremia was measured by WHV DNA hy-
bridization assay. Data at each time point for each treatment
group are presented on the ordinate as the arithmetic mean
($\pm$ 1 SEM) of the percent of serum viral DNA content of the
'week 0' pretreatment blood samples. Symbols, depicting the
treatment groups, are lobucavir at (△) 5 mg/kg per day ($n = 5$
animals), (■) 0.5 mg/kg per day ($n = 6$ animals) and (●) place-
bo treated controls ($n = 6$ animals).]
noted (Fig. 1). Serum WHV DNA content of placebo control WHV-carriers ($n = 6$) was unchanged (Fig. 1).

Serum samples of animals of this study, treated with lobucavir at 5 and 0.5 mg/kg, were also quantitated for viremia by EPA (Fig. 2). Relative viremia changes of lobucavir treated animals, as determined by EPA, paralleled those results obtained by the WHV DNA hybridization assay. For animals given 5 mg/kg lobucavir, there was a mean viral EPA reduction in the 10- to 100-fold range during therapy. By 2 weeks after therapy cessation, EPA returned to pretreatment levels. Mean serum EPA activity was unchanged in animals treated with lobucavir at 0.5 mg/kg. The parallel viremia pattern noted in this trial by the EPA assay (Fig. 2) and by the WHV DNA hybridization assay (Fig. 1) suggested that circulating WHV DNA was largely detected as virion-encapsidated genomic material and was not freely released from livers by hepatotoxic events, which can occur in the course of natural infection or by drug-related toxicity.

A 12-week trial was conducted in which animals were treated per os daily with 5 mg/kg lobucavir. Such extended therapy did not improve the antiviral efficacy of lobucavir, as reductions in circulating WHV DNA were comparable to those of the former study (data not shown).

The magnitude of viremia reduction and the non-uniformity in responses of treated WHV-carriers, by the daily 5 mg/kg lobucavir dose, indicated that this regimen was minimally effective for therapy. Accordingly, to determine if a greater virus suppressive effect could be achieved, a short-term 4 week lobucavir treatment experiment was conducted, at higher daily per os doses of 10 mg/kg ($n = 6$ animals) and 20 mg/kg ($n = 6$ animals). These two lobucavir doses appeared more effective and comparable in reducing viremia (Fig. 3). Animals of both lobucavir treated groups responded with reductions in mean serum WHV DNA, ranging from 10- to 200-fold of pretreatment content during therapy. Serum WHV DNA reductions, to the detection limit of the WHV DNA hybridization assay, were noted for three of six animals treated at 10 mg/kg and in four of six

![Fig. 2. Viremia, as measured by EPA, in WHV-carriers treated per os daily with lobucavir for 6 weeks. Data for each treatment group are presented as the arithmetic mean ($\pm$ 1 SEM) of the percent of pretreatment serum viral EPA. Symbols, depicting the treatment groups, are lobucavir at ($\triangle$) 5 mg/kg per day ($n = 5$ animals) and ($\Box$) 0.5 mg/kg per day ($n = 6$ animals).](image)

![Fig. 3. WHV-viremia in WHV-carriers treated per os daily for 4 weeks with 10 or 20 mg/kg per day lobucavir. Data are presented as detailed in Fig. 1, as the percent of pretreatment serum WHV DNA content. Symbols, depicting the treatment groups, are lobucavir at ($\triangle$) 20 mg/kg per day ($n = 6$ animals), ($\Box$) 10 mg/kg per day ($n = 6$ animals), and ($\bullet$) placebo treated controls ($n = 5$ animals).](image)
animals treated at 20 mg/kg; the reductions were ≤ 0.1–0.3% of pretreatment viral load (data not shown). Following therapy termination, WHV DNA subsequently returned to pretreatment levels for all treated carriers. Serum WHV DNA of placebo controls (n = 5) remained unaffected in this study (Fig. 3).

Results of this study demonstrated that oral administration of lobucavir to WHV-carrier woodchucks significantly suppressed WHV-viremia. These results also extend prior reports on the in vitro efficacy of lobucavir against HBV (Innaimo et al., 1997; Seifer et al., 1998) and support the recent report on the in vivo efficacy of lobucavir against WHV in chronically infected carriers (Tennant et al., 2000). The minimum in vivo effective daily per os dose of lobucavir in the WHV-carrier woodchuck was determined to approximate 5 mg/kg.

Compared to published reports on other nucleoside and nucleotide derived compounds tested in the WHV-carrier woodchuck (Fourer et al., 1990; Ponzetto et al., 1991; Ikeda et al., 1994; Enriquez et al., 1995; Fiume et al., 1995; Cullen et al., 1997; Tencza and Newbold, 1997; Genovesi et al., 1998; Mason et al., 1998; Tennant et al., 1998; Hostetler et al., 2000; Korba et al., 2000), the lobucavir doses used in this study were quite effective in reducing WHV-viremia. Moreover, these doses were tolerated as treated animals did not exhibit adverse clinical signs and remained healthy (data not shown). The recent report on lobucavir efficacy in WHV-carriers indicated that daily per os 20 mg/kg treatment profoundly suppressed WHV-viremia and WHV-replication in infected livers (Tennant et al., 2000). In reference to these results, WHV-viremia, expressed as WHV-genomic equivalents per millilitre of serum, was reduced to a mean range of 0.3–0.03% of pretreatment load by the third treatment week and was further reduced to ≤ 0.01% by the fourth treatment week. The relative magnitude of this WHV-viremia decline was significantly greater than the mean viremia reduction to the range of < 5–1% of pretreatment levels, as achieved in our experiments by lobucavir treatment in the same time interval (Fig. 3). In comparison to the effective antiviral dose range of lobucavir determined in this study, daily oral treatment of WHV-carriers with entecitabine [(-)-FTC] was similarly reductive; however, a 10- to 100-fold greater mean reduction in WHV-viremia was observed by 30 mg/kg treatment doses of emtricitabine (Korba et al., 2000). Entecavir (BMS-200475) and 2′-fluorinated arabinosyl-pyrimidine nucleosides (Fourer et al., 1990; Genovesi et al., 1998; Tennant et al., 1998), were more potent than lobucavir in reducing WHV-viremia with a 5- to 200-fold lower minimum effective dose range. However, whereas entecavir did not cause overt toxicity, the 2′-fluorinated arabinosyl-pyrimidine nucleosides caused anorexia and death in treated WHV-carriers.

The lower efficacy of lobucavir against WHV in infected carrier woodchucks compared to entecavir, another carbocyclic guanosine nucleoside, also reflected quantitative dose differences noted in inhibition of HBV replication in HBV-expressing cells (Innaimo et al., 1997). Moreover, mechanism studies on hepadnaviral POL inhibition did not reveal differences between the nucleotide triphosphates of these two compounds (Seifer et al., 1998), suggesting the lower antiviral effectiveness and potency of lobucavir may be due to its poor quality as a substrate for hepatic cellular phosphorylation (Yamanaka et al., 1999). A better understanding of the potential of lobucavir for HBV antiviral treatment in persistently infected humans may perhaps be obtained from continued in vivo studies on pharmacokinetic and cellular parameters of compound metabolism, and on compound effects on the dynamics of virus-cell persistence and growth in the woodchuck host.

Acknowledgements

We thank Dr B.C. Tennant (Cornell University, Ithaca, NY) for his helpful discussions concerning all aspects of the woodchuck animal model.

References


Field, A.K., Toumari, A.V., McKeever-Rubin, B., Terry, B.J., Mazina, K.E., Haffey, M.L., Hagen, M.E., Clark, J.M., Brautman, A., Slusarchyk, W.A., Young, M.G., Zahler, R., 1990. (\( \pm \))-{1,2,3,4-tetrahydro-2-aminobenzimidazol-3-yl} guanine [\( \pm \)]-BHCG or SQ33,054]: a potent and selective inhibitor of herpes viruses. Antiviral Res. 13, 41–52.


