

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

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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 recrudesced to pretreatment 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 \approx 5 mg/kg. © 2000 Elsevier Science B.V. All rights reserved.

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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 Bicegale, 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 α ,2 β ,3 α)-2-amino-9-[2,3-bis(hydroxymethyl)cyclobutyl]-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 (+ 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°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 hybridization (Korba and Gerin, 1992), or for endogenous viral DNA polymerase (EPA) activity which measures serum content of replication-competent virions (Hantz et al., 1984; Miller et al., 1984; Nassal, 1996).

Briefly, for the hybridization assay, alkali-extracted DNA from test sera, affixed to nylon filters, were incubated with a whole genomic, randomly radiolabeled (^{32}P), linear WHV DNA probe. Hybridized serum DNA was measured for radioactivity by image analysis. WHV DNA content calculation was based on comparison to a standard curve of cloned WHV DNA. The lower WHV DNA assay detection limit was ≈ 200 pg viral DNA per millilitre of serum. For WHV-carriers of this study, pretreatment WHV DNA ranged from 4.0×10^4 to 6.0×10^5 pg per millilitre of serum. For each serum sample of each respective animal, the WHV DNA quantity was divided by the animal's pretreatment serum viral DNA content, to obtain data as the percent of pretreatment WHV DNA. Results for each time point were graphically presented for each animal treatment group, as the arithmetic mean (± 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 suspensions, obtained from test sera by centrifugal concentration, were mixed in a buffered solution with dGTP, dCTP, TTP and [α - ^{33}P]-dATP, then incubated to allow enzymatic formation of radiolabeled DNA. Product DNA was collected by trichloroacetic acid precipitation and measured for incorporated radioactivity by liquid scintillation counting. EPA data for each animal was calculated as a percentage of incorporated radioactivity in test samples relative to the respective animal's pretreatment serum. This assay was only used to evaluate samples of one treatment study (see Figs. 1 and 2). EPA results were graphically presented for each treatment group, as the arithmetic mean (± 1 SEM) of the percent incorporated radioactivity of pretreatment sera.

Lobucavir was previously reported to be effective 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 lobucavir 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 recrudesced (Fig. 1). In the subsequent 4-week period, 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 (≤ 200 pg DNA per millilitre), $\leq 0.1\%$ of pretreatment 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 significant change in mean serum WHV DNA was

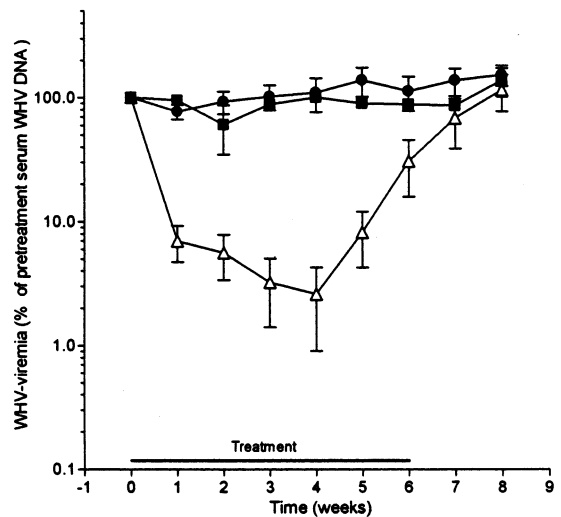


Fig. 1. WHV-viremia in WHV-carriers treated per os daily with lobucavir for 6 weeks. The drug-treatment time interval of this experiment is indicated by the horizontal line, parallel to the x-axis. Viremia was measured by WHV DNA hybridization assay. Data at each time point for each treatment group are presented on the ordinate as the arithmetic mean (± 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 (Δ) 5 mg/kg per day ($n = 5$ animals), (\blacksquare) 0.5 mg/kg per day ($n = 6$ animals) and (\bullet) placebo treated controls ($n = 6$ animals).

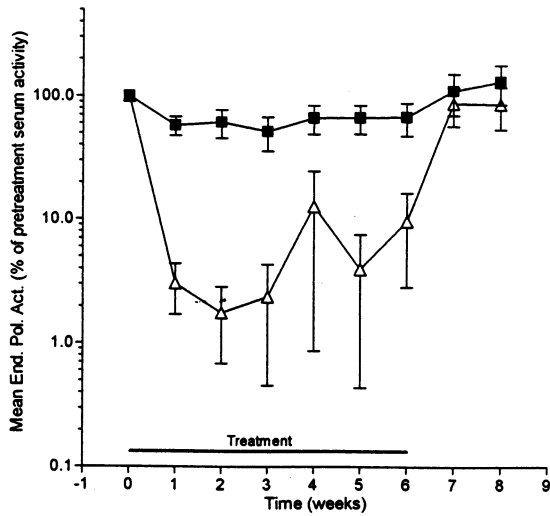


Fig. 2. Viremia, as measured by EPA, in WHV-carriers treated per os daily with lobucavir for 6 weeks. Data for each treatment group are presented as the arithmetic mean (± 1 SEM) of the percent of pretreatment serum viral EPA. Symbols, depicting the treatment groups, are lobucavir at (Δ) 5 mg/kg per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

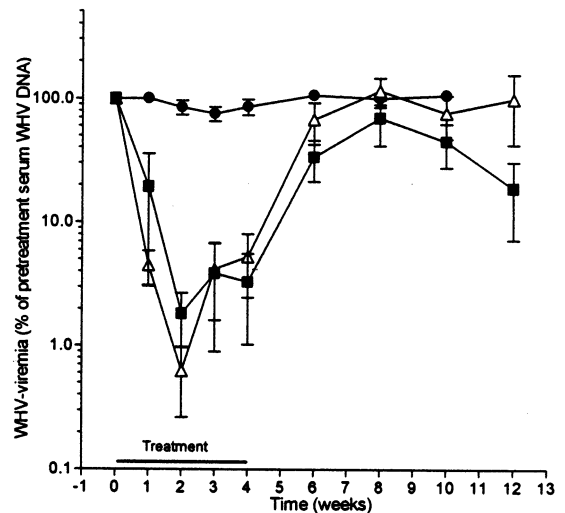


Fig. 3. WHV-viremia in WHV-carriers treated per os daily for 4 weeks with 10 or 20 mg/kg per day lobucavir. Data are presented as detailed in Fig. 1, as the percent of pretreatment serum WHV DNA content. Symbols, depicting the treatment groups, are lobucavir at (Δ) 20 mg/kg per day ($n = 6$ animals), (\blacksquare) 10 mg/kg per day ($n = 6$ animals), and (\bullet) placebo treated controls ($n = 5$ animals).

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 (Fourel 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 $\leq 0.01\%$ 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 emtricitabine [(–)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 (Fourel 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.

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References

- Braitman, A., Swerdel, M.R., Olsen, S.J., Toumari, A.V., Lynch, J.S., Blue, B., Michalik, T.M., Field, A.K., Bonner,

- D.P., Clark, J.M., 1991. Evaluation of SQ34514: Pharmacokinetics and efficiency in experimental herpes virus infections in mice. *Antimicrob. Agents Chemother.* 35, 1464–1468.
- Colacino, J.M., Staschke, K.A., 1998. The identification and development of antiviral agents for the treatment of chronic hepatitis B virus infection. *Progr. Drug Res.* 50, 259–322.
- Cullen, J.M., Smith, S.L., Davis, M.G., Dunn, S.E., Botteron, C., Cecchi, A., Linsey, D., Linzey, L., Frick, M.T., Paff, D., Goulding, A., Biron, K., 1997. In vivo anti-viral activity and pharmacokinetics of (–)-*cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathio-lan-5-yl] cytosine in woodchuck hepatitis virus-infected woodchucks. *Antimicrob. Agents Chemother.* 41, 2076–2082.
- Enriquez, P.M., Jung, C., Josephson, L., Tennant, B.C., 1995. Conjugation of adenine arabinoside 5'-monophosphate to arabinogalactan: synthesis, characterization, and antiviral activity. *Bioconj. Chem.* 6, 195–202.
- Field, A.K., Toumari, A.V., McKeever-Rubin, B., Terry, B.J., Mazina, K.E., Haffey, M.L., Hagen, M.E., Clark, J.M., Braitman, A., Slusarchyk, W.A., Young, M.G., Zahler, R., 1990. (±)-{1 α ,2 β ,3 α }-9-amino-[2,3-bis(hydroxymethyl)]-guanine [(±)-BHCG or SQ33,054]: a potent and selective inhibitor of herpes viruses. *Antiviral Res.* 13, 41–52.
- Fiume, L., Di Stefano, G., Busi, C., Mattioli, A., Rapicetta, M., Giuseppetti, R., Ciccaglione, A.R., Argenti, C., 1995. Inhibition of woodchuck hepatitis virus replication by adenine arabinoside monophosphate coupled to lactosaminated poly-L-lysine and administered by intramuscular route. *Hepatology* 22, 1072–1077.
- Fourel, I., Hantz, O., Watanabe, K.A., Jacquet, C., Chomel, B., Fox, J.J., Treppe, C., 1990. Inhibitory effects of 2'-fluorinated arabinosyl-pyrimidine nucleosides on woodchuck hepatitis virus replication in chronically infected woodchucks. *Antimicrob. Agents Chemother.* 34, 473–475.
- Genovesi, E.V., Lamb, L., Medina, I., Taylor, D., Seifer, M., Innaimo, S., Colonno, R.J., Standring, D.N., Clark, J.M., 1998. Efficacy of the carbocyclic 2'-deoxyguanosine nucleoside BMS-200475 in the woodchuck model of hepatitis B virus infection. *Antimicrob. Agents Chemother.* 42, 3209–3217.
- Gust, I.D., Burrell, C.J., Coulepis, A.G., Robinson, W.S., Zuckerman, A.J., 1986. Taxonomic classification of human hepatitis B virus. *Intervirology* 25, 14–29.
- Hantz, O., Ooka, T., Vitvitski, L., Pichoud, C., Treppe, C., 1984. Comparison of properties of woodchuck hepatitis virus and human hepatitis B virus endogenous DNA polymerases. *Antimicrob. Agents Chemother.* 25, 242–246.
- Hoofnagle, J.H., Di Bicegle, A.M., 1997. The treatment of chronic viral hepatitis. *New Engl. J. Med.* 336, 347–356.
- Hostetler, K.Y., Beadle, J.R., Hornbuckle, W.E., Bellezza, C.A., Tochkov, I.A., Cote, P.J., Gerin, J.L., Korba, B.E., Tennant, B.C., 2000. Antiviral activities of oral 1-*O*-hexadecylpropanediol-3-phosphoacyclovir and acyclovir in woodchucks with chronic woodchuck hepatitis virus infection. *Antimicrob. Agents Chemother.* 44, 1964–1969.
- Ikeda, N., Kaneko, S., Shimoda, A., Inagaki, Y., Unoura, M., Okada, M., Yonekawa, Y., Takashashi, K., Kobayashi, K., 1994. Efficiency of oxetanocin-G, a novel nucleoside against the woodchuck hepatitis virus. *J. Antimicrobial Chemother.* 33, 83–89.
- Innaimo, S.F., Seifer, M., Bisacchi, G.S., Standring, D.N., Zahler, R., Colonno, R.J., 1997. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrob. Agents Chemother.* 41, 1444–1448.
- Korba, B.E., Gerin, J.L., 1992. Use of a standardized cell culture assay to assess activities of nucleoside analogs against hepatitis B virus replication. *Antiviral Res.* 19, 55–70.
- Korba, B.E., Schinazi, R.F., Cote, P.J., Tennant, B.C., Gerin, J.L., 2000. Effect of oral administration of emtricitabine on woodchuck hepatitis virus replication in chronically infected woodchucks. *Antimicrob. Agents Chemother.* 44, 1757–1760.
- Locarnini, S.A., Civitico, G.M., Newbold, J.E., 1996. Hepatitis B: new approaches for antiviral chemotherapy. *Antiviral Chem. Chemother.* 7, 53–64.
- Malik, A.H., Lee, W.M., 2000. Chronic hepatitis B virus infection: Treatment strategies for the next millennium. *Ann. Intern. Med.* 132, 723–731.
- Margolis, H.S., Alter, M.J., Halder, S.C., 1991. Hepatitis B: evolving epidemiology and implications for control. *Semin. Liver Dis.* 11, 84–92.
- Mason, W.S., 1996. The problem of antiviral therapy for chronic hepadnavirus infection. *J. Hepatol.* 17 (Suppl. 3), 137–142.
- Mason, W.S., Cullen, J., Moraleda, G., Saputelli, J., Aldrich, C.F., Miller, D.S., Tennant, B.C., Frick, L., Averett, D., Condreay, L.D., Jilbert, A.R., 1998. Larnivudine therapy of WHV-infected woodchucks. *Virology* 245, 18–32.
- Miller, R., Marion, P.L., Robinson, W.S., 1984. Hepatitis B viral DNA-RNA hybrid molecules in particles from infected liver are converted to viral DNA molecules during an endogenous DNA polymerase reaction. *Virology* 139, 64–72.
- Nassal, M., 1996. Hepatitis B virus morphogenesis. *Curr. Top. Microbiol. Immunol.* 214, 297–337.
- Ponzetto, A., Fiume, L., Forzani, B., Song, S.Y., Busi, C., Mattioli, A., Spinelli, C., Marinelli, M., Smedile, A., Chiaberge, E., Bonino, F., Gervasi, G.B., Rapicetta, M., Verme, G., 1991. Adenine arabinoside monophosphate and acyclovir monophosphate coupled to lactosaminated albumin reduce woodchuck hepatitis virus viremia at doses lower than do the unconjugated drugs. *Hepatology* 14, 16–24.
- Roggendorf, M., Tolle, T.K., 1995. The woodchuck: an animal model for hepatitis B virus infection in man. *Intervirology* 38, 100–112.
- Schodel, F., Sprengel, R., Weimer, T., Fernholz, D., Schneider, R., Will, H., 1989. Animal hepatitis B viruses. *Adv. Viral Oncol.* 8, 73–102.
- Seeger, C., Summers, J., Mason, W.S., 1991. Viral DNA synthesis. *Curr. Top. Microbiol. Immunol.* 168, 41–59.

- Seifer, M., Hamatake, R.K., Colonna, R.J., Stranding, D.N., 1998. In vitro inhibition of hepadnavirus polymerases by the triphosphates of BMS-200475 and lobucavir. *Antimicrob. Agents Chemother.* 42, 3200–3208.
- Tencza, M.G., Newbold, J.E., 1997. Heterogeneous response for mammalian hepadnavirus infection to acyclovir: drug arrested intermediates of minus strand viral DNA are enveloped and secreted from infected cells as virion-like particles. *J. Med. Virol.* 51, 6–16.
- Tennant, B.C., 1998. Woodchuck hepadnaviruses. In: Zuckerman, A.J., Thomas, H.C. (Eds.), *Viral Hepatitis*. Churchill Livingstone, Div. Harcourt Brace & Company, London, UK, pp. 129–240.
- Tennant, B.C., Baldwin, B.H., Hornbuckle, W.E., Korba, B.E., Cote, P.J., Gerin, J.L., 1996. Animal models in the preclinical assessment of therapy for viral hepatitis. *Antiviral Ther.* 1 (Suppl. 4), 47–52.
- Tennant, B.C., Baldwin, B.H., Graham, L.A., Ascenzi, M.A., Hornbuckle, W.E., Rowland, P.H., Toshkov, I.A., Yeager, A.E., Erb, H.N., Colacino, J.M., Lopez, C., Engelhardt, J.A., Bowsher, R.R., Richardson, F.C., Lewis, W., Cote, P.J., Korba, B.E., Gerin, J.L., 1998. Antiviral activity and toxicity of fialuridine in the woodchuck model of hepatitis B virus infection. *Hepatology* 28, 179–191.
- Tennant, B.C., Baldwin, B.H., Bellezza, C.A., Hornbuckle, W.E., Erb, H.N., Scott, D.W., Ascenzi, M.A., Toshkov, I.A., LaCreta, F., Manitpitsikul, P., Colonna, R.J., Korba, B.E., Cote, P.J., Gerin, J.L., 2000. Antiviral activity of lobucavir in the woodchuck model of hepatitis B virus infection: 12-week oral dosing study in adult woodchucks with chronic woodchuck hepatitis virus infection. *Antimicrob. Agents Chemother.* (in press).
- Yamanaka, G., Wilson, T., Innaimo, S., Bisacchi, G.S., Egli, P., Rinehart, J.K., Zahler, R., Colonna, R.J., 1999. Metabolic studies on BMS-200475, a new antiviral compound active against hepatitis B virus. *Antimicrob. Agents Chemother.* 43, 190–193.
- Yang, H., Drain, R.L., Franco, C.A., Clark, J.M., 1991. Efficacy of BMS-180194 against experimental cytomegalovirus infections in immunocompromised mice. *Antiviral Res.* 29, 233–241.
- Zoulim, F., 1999. Therapy of chronic hepatitis B: virus infection: inhibition of the viral polymerase and other antiviral strategies. *Antiviral Res.* 44, 1–30.