

Anti-Hepatitis B Virus Activity of ORI-9020, a Novel Phosphorothioate Dinucleotide, in a Transgenic Mouse Model

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ORI-9020, a novel dinucleotide, evaluated in transgenic mice expressing hepatitis B virus (HBV), significantly reduced liver HBV DNA ($P \leq 0.001$). Levels of HBeAg and HBsAg in serum and of HBcAg in liver were not affected by treatment. A minimal effective dosage was determined to be between 1.6 and 0.5 mg/kg of body weight/day, which was similar to that observed for adefovir dipivoxil.

The discovery of safe and effective antiviral drugs continues to present considerable challenges for hepatitis B virus (HBV) therapies. The novel dinucleotides ORI-9020, ORI-7246, and ORI-7170 have been developed and identified to be active against HBV in cell culture (2a). The mode of antiviral action of ORI-9020 and ORI-7246 appears to be direct interference of HBV replication at the level of HBV reverse transcriptase and/or DNA polymerase at an early step, either at, or prior to, the production of the first strand of HBV DNA. These experiments show that these compounds have antiviral activities comparable with adefovir dipivoxil (ADV). Furthermore, when used in combination with lamivudine (3TC) possible synergistic antiviral effects were apparent.

Given some of the favorable characteristics of ORI-9020, the anti-HBV activity was evaluated in a previously developed transgenic HBV mouse model (2). Experiments with transgenic mice expressing HBV have demonstrated the model's utility for evaluating potential anti-HBV compounds such as interleukin-12 (1), 3TC (5), alpha interferon (6), ADV (4), and entecavir (3). The results of the evaluation of ORI-9020 in the transgenic mouse model are reported here.

Transgenic HBV mice were originally obtained from Frank Chisari (Scripps Research Institute, La Jolla, Calif.) (2). Animal use and care was in compliance with the Utah State University Institutional Animal Care and Use Committee.

For the first animal experiment, ORI-9020 was prepared fresh daily at a dosage of 100 mg/kg of body weight /day, which was equal to 170 $\mu\text{mol/kg/day}$, and was injected intraperitoneally (i.p.) using cremaphor-ethanol-saline (CES) (10:10:80) or physiological saline as vehicles. ADV (Gilead, Foster City, Calif.), the positive control, was prepared using the CES vehicle. A dosage of 10 mg/kg/day (19.9 $\mu\text{mol/kg/day}$) was used. In

the second experiment to determine the minimal effective concentration, ORI-9020 was prepared in sterile saline in one-half-log dilutions from 50 to 0.05 mg/kg/day. The drug was delivered i.p. in a volume of 0.1 ml.

Liver samples were analyzed for HBV DNA, HBV RNA, and HBcAg, and serum samples were processed for HBV DNA, HBeAg, and HBsAg according to previously published methods (4). HBV DNA and RNA were detected in liver by Southern and Northern blot analysis, respectively. A competitive quantitative PCR was used to detect HBV DNA in the serum. HBeAg and HBsAg were detected using an in-house assay.

For the first experiment, male transgenic mice with HBeAg titers in the upper 85% of the range were block randomized across treatment groups according to HBeAg titers. Mice received i.p. injections once daily for 14 days, and necropsy was performed at least 2 h after the last treatment to obtain liver and serum. For the second experiment female mice received i.p. injections with one-half-log serial dilutions of ORI-9020 in saline. Necropsy was performed as described above. HBV DNA levels in liver were used to identify the minimal effective dosage.

i.p. injection of ORI-9020 at 100 mg/kg/day significantly reduced viral DNA in the liver ($P \leq 0.001$) and showed anti-HBV activity similar to that of ADV (Table 1). ADV typically leaves low-sized viral bands on the Southern blot, whereas ORI-9020 appeared to indiscriminately diminish, but not eliminate, all HBV DNA species, including the bands typically left by ADV (Fig. 1). The reason for the difference between ADV and ORI-9020 is not known, but the difference does suggest that the mechanisms for reducing viral DNA species in the liver may be different between the two compounds.

Serum HBV DNA was not reduced in response to treatment. The reason for this is not known. One possible explanation is that extrahepatic sites of viral production in these transgenic mice may not have been affected by ORI-9020, which in turn may have loaded the serum with virus. Another possibility is that during the course of treatments the titers of virus in

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TABLE 1. Effect of ORI-9020 on HBV DNA in male transgenic mice treated intraperitoneally once daily for 14 days

Compound ^f	Dosage (mg/kg/day)	Mean weight gain ± SD	Mean level in liver ± SD			Mean level in serum ± SD		
			HBV DNA (pg/μg of cellular DNA) (n ^a)	HBV RNA (relative units)	HBeAg (mean no. of stained cells/microscopic field ^e)	HBV DNA (log ₁₀ genome equivalents/ml) (n)	HBeAg (ng/ml)	HBsAg (ng/ml)
ORI-9020/ces	100	1.0 ± 0.6	1.7 ± 1.5 (9) ^d	1.7 ± 0.3	24 ± 15	5.4 ± 0.6 (7)	1,241 ± 279	28 ± 15
ADV ^b /ces	10	0.9 ± 0.7	2.7 ± 1.4 (9) ^d			4.9 ± 0.4 (9)		
CES vehicle ^c		1.5 ± 0.7	18.3 ± 20.8 (10)	1.6 ± 0.4	16 ± 9	5.4 ± 0.2 (10)	1,311 ± 284	46 ± 31
ORI-9020/sal	100	0.4 ± 0.8	0.3 ± 0.4 (8) ^d			5.0 ± 0.3 (8)		
Saline placebo		0.6 ± 1.3	6.9 ± 3.3 (5)			5.9 ± 1.2 (6)		

^a Number of samples. Any difference between number of samples and number alive or total number is because some sample preparation may have been incomplete or undesirable to properly finish the analysis.
^b ADV, adefovir dipivoxil.
^c Cremaphor-ethanol-saline (10:10:30).
^d P ≤ 0.001 compared with vehicle placebo.
^e Microscopic field, ×100.
^f "ces" and "sal" indicate vehicle used.

serum of the placebo groups, as well as the test groups, could be reduced from baseline values (10⁶ to 10⁷ genome equivalents per ml) in these transgenic mice, which can mask the antiviral effects (1).

ORI-9020 also did not affect levels of HBV RNA in liver, levels of HBeAg in serum, or mean titers of HBsAg (Table 1), which was also observed for ADV (4). In transgenic mice expressing the complete infectious virion, unlike in other hepatitis virus animal models, HBV produced in the mice does not reinfect cells within the mouse and cycles of repeated rounds of replication do not occur. Therefore, HBeAg, HBsAg, and HBcAg produced by mRNA from the HBV transgene were not necessarily affected by chemotherapeutic agents that block viral synthesis "downstream," such as blockage of polymerase activities by ORI-9020. Weight gain and total numbers of animals alive per total numbers of animals indicated no signs of toxicity (data not shown).

In the second experiment, the minimal effective dosage of ORI-9020 was determined. The minimal effective dose was identified to be between 1.6 and 0.5 mg/kg/day using liver HBV DNA values (Fig. 2). The anti-HBV activities of different com-

pounds can best be compared using the minimal effective dose rather than by directly comparing the reduction of viral parameters, because the dynamic range of viral parameters detectable in the transgenic mice is small (2- to 3-log reduction) (4). The minimal effective dose of oral ADV was previously determined to be between 1.0 and 0.3 mg/kg/day (4); therefore, the activity of i.p. ORI-9020 was similar to the oral activity of ADV in this animal model.

It is possible that the ORI-9020 does not directly affect the viral polymerase; however, the 5' triphosphate of ORI-9020 was not a competitive inhibitor of endogenous HBV polymerase in a cell-free assay (2a) and was not metabolized by liver microsomes. This supports the possibility that ORI-9020 might inhibit HBV polymerase-directed priming of first-strand synthesis. In this study, ORI-9020 diminished all HBV DNA species, unlike ADV, which preferentially left the small DNA species in the Southern analysis (Fig. 1). A different mode of action for such a compound may provide an opportunity to administer ORI-9020 in combination with chain terminators. Further experiments are necessary to prove mechanism of action of ORI-9020.

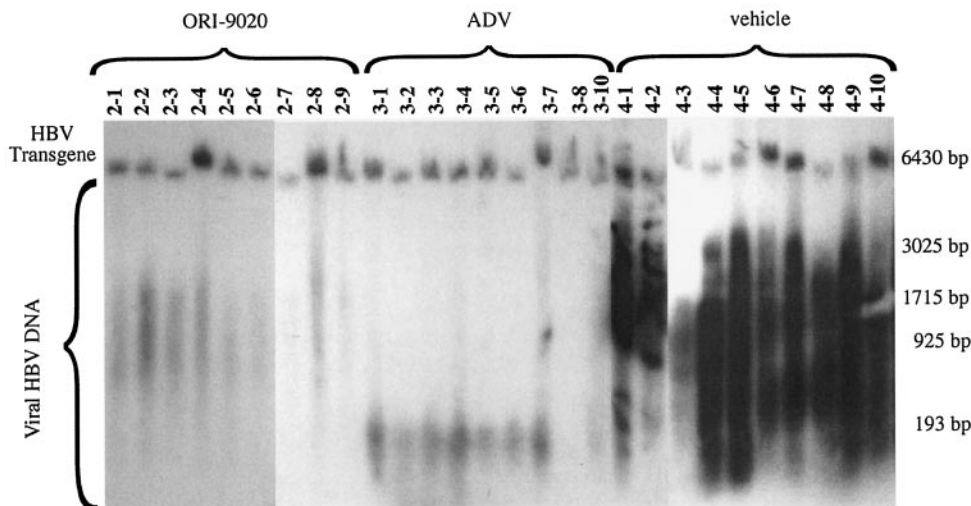


FIG. 1. Southern blot hybridization for HBV DNA in the livers of mice treated i.p. with ORI-9020, ADV, or vehicle only.

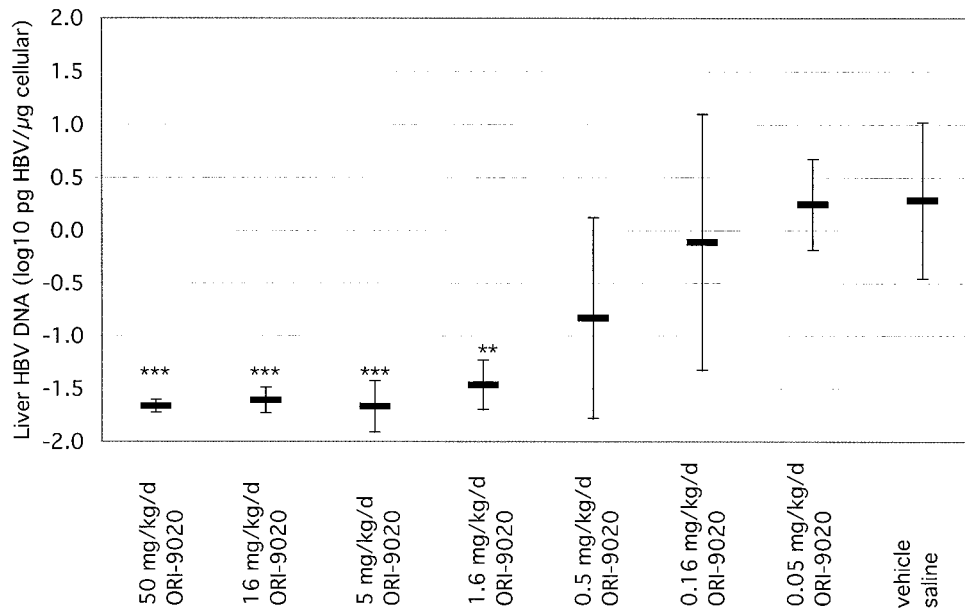


FIG. 2. Determination of minimal effective concentration of ORI-9020 using liver HBV DNA parameter in transgenic mice (geometric mean \pm standard deviation [error bars]). *P* values were based on five animals per group. Symbols: **, $P \leq 0.05$; ***, $P \leq 0.001$.

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