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Antiviral Effect of Orally Administered (−)-β-d-2-Aminopurine Dioxolane in Woodchucks with Chronic Woodchuck Hepatitis Virus Infection

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(−)-β-d-2-Aminopurine dioxolane (APD) is a nucleoside prodrug that is efficiently converted to 9-(β-d-1,3-dioxolan-4-yl)guanine (DXG). DXG has antiviral activity in vitro against hepatitis B virus (HBV) but limited aqueous solubility, making it difficult to administer orally to HBV-infected individuals. APD is more water soluble than DXG and represents a promising prodrug for the delivery of DXG. A placebo-controlled, dose-ranging efficacy and pharmacokinetic study was conducted with woodchucks that were chronically infected with woodchuck hepatitis virus (WHV). APD was efficiently converted to DXG after oral and intravenous administrations of APD, with serum concentrations of DXG being higher following oral administration than following intravenous administration, suggestive of a considerable first-pass intestinal and/or hepatic metabolism. APD administered orally at 1, 3, 10, and 30 mg/kg of body weight per day for 4 weeks produced a dose-dependent antiviral response. Doses of 3 and 10 mg/kg/day reduced serum WHV viremia by 0.4 and 0.7 log10 copies/ml, respectively. The 30 mg/kg/day dose resulted in a more pronounced, statistically significant decline in serum WHV viremia of 1.9 log10 copies/ml and was associated with a 1.5-fold reduction in hepatic WHV DNA. Individual woodchucks within the highest APD dose group that had declines in serum WHV surface antigen levels, WHV viremia, and hepatic WHV DNA also had reductions in hepatic WHV RNA. There was a prompt recrudescence of WHV viremia following drug withdrawal. Therefore, oral administration of APD for 4 weeks was safe in the woodchuck model of chronic HBV infection, and the effect on serum WHV viremia was dose dependent.

Chronic infection with the hepatitis B virus (HBV) is a major public health problem and is responsible for 1.2 million deaths per year worldwide (45). It is estimated that more than 2 billion people worldwide have serological evidence of previous or current HBV infection and that there are over 350 million chronic carriers of HBV (45). Carriers of HBV are at high risk of developing chronic hepatitis, hepatic cirrhosis, and hepatocellular carcinoma (HCC). Although safe and effective prophylactic vaccines against HBV are available, improvements in drug and/or immunotherapeutic strategies for the treatment of chronic HBV infection are still needed. Therapy with (pegylated) alpha interferon and nucleoside analogues, alone or in combination, can be effective against HBV. However, side effects of interferon and the emergence of nucleoside-resistant mutants often limit treatment outcomes (26, 36). Therefore, there is a continued need for the development of new antiviral compounds for the treatment of chronic HBV infection.

(−)-β-d-2-Aminopurine dioxolane [APD; (−)-(2R,4R)-1-(2-hydroxymethyl-1,3-dioxolan-4-yl)-2-aminopurine] is a purine nucleoside prodrug that is efficiently converted in vivo to the nucleoside 9-(β-d-1,3-dioxolan-4-yl)guanine (DXG) following intravenous and/or oral administration in mice and rhesus monkeys (2, 25) (Fig. 1). Biotransformation of APD to DXG is mediated by the enzyme xanthine oxidase, which is found at high levels in the liver and intestine (25, 37). The closely related prodrug (−)-β-d-2,6-diaminopurine dioxolane (DAPD; amidoxovir) is also converted to DXG following intravenous and/or oral administration to woodchucks, rhesus monkeys, and humans (4, 14, 18, 34, 35). However, the biotransformation of DAPD to DXG occurs through deamination by the ubiquitous enzyme adenosine deaminase (2, 10, 35, 38). DXG and its derivatives exhibit potent antiviral activities in vitro and/or in vivo against wild-type HBV, duck HBV (DHBV), and drug-resistant mutants of both hepadnaviruses (38–41, 46). DXG is relatively insoluble in water, making it difficult to formulate and administer orally to HBV-infected individuals. APD is more water soluble than DXG or DAPD, and studies of mice and rhesus monkeys have demonstrated that APD has increased oral bioavailability (2, 25). Since DXG has antiviral efficacy against HBV (and human immunodeficiency virus), there is increasing interest in examining prodrugs to improve the oral bioavailability of this compound.

Woodchuck hepatitis virus (WHV) and its natural host, the Eastern woodchuck (Marmota monax), is a well-characterized mammalian model available for research on HBV. The woodchuck model has been useful in studies of the pathogenesis of acute, self-limited, and chronic HBV infection and in the pre-
clinical evaluation of the efficacy and safety of drug candidates for the treatment of chronic HBV infection (22, 28, 31) and for the prevention of HCC (43). Results of studies of drug efficacy in woodchucks have been predictive of responses subsequently observed in individuals who are chronically infected with HBV (22).

This study was designed to determine the relationship between the dose of APD and the antiviral response in a placebo-controlled study of woodchucks. APD was effective in suppressing viral replication in woodchucks with wild-type, chronic WHV infection in a dose-dependent manner. Therefore, future studies are planned for the APD treatment of laboratory-derived WHV strains with mutations that confer lamivudine resistance via the rtA566T substitution in the WHV polymerase B domain (17) and for APD treatment in combination with other HBV drug candidates.

MATERIALS AND METHODS

Woodchucks. The woodchucks used for this study were born to WHV-negative females and reared in environmentally controlled laboratory animal facilities at Cornell University. Woodchucks treated with APD were inoculated at 3 days of age with 5 million woodchuck infectious doses of a standardized WHV inoculum (cWHV7P2) (6). Woodchucks selected for use became chronic WHV carriers based on the persistent presence of WHV surface antigen (WHsAg) and WHV DNA in their sera, and this was confirmed prior to the initiation of drug treatment. All woodchucks were free of HCC at the beginning of the study based on hepatic ultrasound examination and normal serum activity of γ-glutamyltransferease (GGT). All experimental procedures involving woodchucks were performed under protocols approved by the Cornell University Institutional Animal Care and Use Committee.

Drug. APD was synthesized as a single batch for this study by C. K. Chu. APD was administered orally to woodchucks, daily for 28 days, with a dose syringe (42). APD was weighed and dissolved in isotonic saline and immediately prior to administration was suspended in a semisynthetic liquid diet formulated for woodchucks (Dyets Inc., Bethlehem, PA) to ensure complete consumption. The liquid diet alone was administered daily as a placebo control for woodchucks.

Pharmacokinetic and oral-bioavailability study. Four WHV-negative, adult woodchucks were used for the pharmacokinetic study. Initially, three woodchucks received APD at 33.3 mg/kg of body weight by intravenous bolus injection. The fourth woodchuck received saline and served as a control. Following a 4-week washout period, the same three woodchucks received 33.3 mg/kg APD orally. The fourth control woodchuck received a saline vehicle orally. Blood samples were collected from each woodchuck prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdosing. A final blood sample was obtained 7 days following treatment. Serum concentrations of APD and DXG were measured using a high-performance liquid chromatography method with UV detection. The limit of quantification was 0.2 μM for APD and DXG (3). Noncompartmental pharmacokinetic analysis was performed on the serum concentration-versus-time data of APD and DXG using the WinNonLin version 5.2 curve fitting program (Pharsight Corp., Mountain View, CA). Single-dose areas under the serum concentration-time curve from 0 h to infinity (AUC0–) were calculated for APD and DXG using the trapezoidal rule from the time of administration to the last available data point, with extrapolation to infinity using the relationship Clast/λz, where Clast and λz are the last observed data point and the first-order rate constant of the terminal elimination phase, respectively. Since DXG, the active metabolite of APD, was too insoluble to administer intravenously, the relative oral bioavailability (Foral) of DXG was calculated using the formula Foral = AUCoral/AUC0– and AUCoral–, where AUCoral and AUC0– are the values of AUC0– for DXG calculated using data collected after the oral and intravenous doses of APD, respectively. The relative percentages of total AUC due to DXG in serum (%AUC0–DXG) were calculated for both routes of administration using the formula AUCoral–,D GX /AUCoral–, APD × 100, where AUCoral–, DXG and AUCoral–, APD are the AUC0– for DXG and APD, respectively, in sera.

Antiviral study. Twenty-five adult woodchucks, all chronically infected with WHV, were stratified equally by age, gender, body weight, serum viral load, and serum GGT activity into five treatment groups of five animals each that were treated daily with oral doses of either 1, 3, 10, or 30 mg APD per kg per day or with vehicle alone as a placebo. The woodchucks were treated daily for 4 weeks and observed for an additional 12 weeks following the cessation of drug treatment.

Blood samples were obtained for analysis from woodchucks under general anesthesia (ketamine at 50 mg/kg and xylazine at 5 mg/kg intramuscularly) 1 week prior to drug administration on the first day of treatment (“week 0”) and then weekly during the 4-week period of drug treatment. Thereafter, samples were obtained at 1, 2, 4, 8, and 12 weeks following the termination of drug treatment. The woodchucks were weighed each time that they were anesthetized and bled, and drug dosages for individual woodchucks were based on the most recent body weight.

Complete blood counts and serum biochemical measurements were performed prior to treatment, at the end of treatment (week 4), and at 4 and 12 weeks following drug withdrawal. Biochemical measurements included determinations of serum GGT, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), total bilirubin, albumin, blood urea nitrogen, creatinine, Na+, K+, Cl−, bicarbonate, total serum iron, iron binding capacity, and percent iron saturation (42). Serum activities of AST, ALT, and SDH are markers of hepatocellular injury in woodchucks.

Serum WHV DNA concentrations were measured before treatment, weekly during treatment, and during the posttreatment follow-up period. Serum WHV DNA was measured quantitatively by two different methods depending on concentration: (i) by dot blot hybridization using three replicate 10-μl volumes of undiluted serum, with results compared with the results from a standard dilution series of a WHV recombinant DNA plasmid, pWHV8 (assay sensitivity, ≤1.0 × 107 WHV genome equivalents [WHV ge] per ml) (6), and (ii) by real-time PCR assay of three replicate samples of WHV DNA extracted from 200 μl of serum, with results compared with the results of parallel PCR assays of 10-fold dilutions of the pWHV8 plasmid standard and of an extracted volume of a standardized serum inoculum with a known content of WHV DNA, WHV7 (6) (assay sensi-
The antiviral effect induced by APD was further assessed by comparing the geometric mean concentrations of serum WHV DNA at the end of the 4-week treatment period, the geometric mean concentration of serum WHV DNA at the end of weeks 1, 2, 3, and 4 of treatment were reduced significantly compared with levels prior to treatment or in placebo control values using Student’s t test (two-tailed) with the SPSS program 11.5 (SPSS Inc., Chicago, IL). P values of < 0.05 were considered statistically significant.

**RESULTS**

**Pharmacokinetic study.** The average serum concentration-versus-time profile for APD and DXG, following intravenous and oral administration of APD at doses of 33.3 mg/kg, are shown in Fig. 2. The noncompartmental pharmacokinetic parameters derived for both routes of administration are summarized in Table 1.

Following intravenous dosing, the initial maximum concentrations in serum (\(C_{\text{max}}\)) of APD and DXG ranged from 100.0 to 122.2 \(\mu\)M and from 15.7 to 35.3 \(\mu\)M, respectively, while the corresponding AUC\(_{\text{0–24}}\) ranged from 167.6 to 240.7 \(\mu\)M \(\cdot\) h and from 89.3 to 239.2 \(\mu\)M \(\cdot\) h, respectively. Therefore, the \(\%\)AUC\(_{\text{DXG}}\) of the total drug exposure was 42% following intravenous administration. The terminal elimination-phase half-lives (\(t_{1/2x}\)) of APD and DXG ranged from 1.4 to 1.9 h and from 3.1 to 4.8 h, respectively.

Following oral dosing, the serum APD and DXG \(C_{\text{max}}\) ranged from 6.3 to 36.2 \(\mu\)M and from 14.5 to 27.4 \(\mu\)M, respectively, while the corresponding AUC\(_{\text{0–24}}\) ranged from 36.6 to 70.2 \(\mu\)M \(\cdot\) h and from 192.2 to 407.0 \(\mu\)M \(\cdot\) h. Therefore, the \(\%\)AUC\(_{\text{DXG}}\) was 86.2%. The mean relative bioavailability of DXG in woodchucks was 212%. The \(t_{1/2x}\) for APD and DXG ranged from 0.7 to 5.8 h and from 7.2 to 15.8 h, respectively.

**Antiviral study.** Oral administration of APD at 3, 10, or 30 mg/kg/day resulted in a dose-dependent decline in serum viremia in woodchucks chronically infected with WHV (Fig. 3). The lowest dose of APD (1 mg/kg/day) did not reduce the geometric mean concentration of serum WHV DNA by week 4 compared with the pretreatment level. APD at 3 mg/kg/day appeared to produce a slight reduction in the geometric mean concentrations of serum WHV DNA, but the 0.4-log\(_10\) reduction observed after 4 weeks of treatment was not statistically significant compared with levels prior to treatment or in placebo-treated controls.

APD at 10 mg/kg/day induced a greater decline in serum WHV DNA than that observed with APD at 3 mg/kg/day. At the end of the 4-week treatment period, the geometric mean concentration of WHV DNA was reduced compared with the pretreatment level by 0.7 log\(_10\) representing a twofold-greater reduction than that observed with APD at 3 mg/kg/day. The geometric mean concentrations of serum WHV DNA at the end of weeks 1, 2, 3, and 4 of treatment were reduced significantly compared with the pretreatment level (\(P < 0.05\)).

With APD at 30 mg/kg/day, reductions in WHV DNA dur-

TABLE 1. Pharmacokinetics of APD and DXG following intravenous and oral administration of APD to WHV-negative woodchucks

<table>
<thead>
<tr>
<th>Compound</th>
<th>(C_{\text{max}}) ((\mu)M)</th>
<th>AUC(_{\text{0–24}}) ((\mu)M (\cdot) h)</th>
<th>(t_{1/2x}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intravenous</td>
<td>Oral</td>
<td>Intravenous</td>
</tr>
<tr>
<td>APD</td>
<td>111.8 ± 11.1</td>
<td>16.8 ± 16.9</td>
<td>203.1 ± 36.6</td>
</tr>
<tr>
<td>DXG</td>
<td>24.6 ± 9.9</td>
<td>20.3 ± 6.6</td>
<td>146.2 ± 81.2</td>
</tr>
</tbody>
</table>

a Three adult, WHV-negative woodchucks received APD at 33.3 mg/kg by a single intravenous bolus administration, followed by an oral administration after a 4-week washout period. Values are means ± standard deviations.

FIG. 2. Pharmacokinetic profiles of APD (A) and its antiviral metabolite DXG (B) in serum following a single intravenous bolus or oral administration of APD at 33.3 mg/kg to three adult, WHV-negative woodchucks (means are presented, and vertical lines denote standard deviations). iv, intravenous.
The initial 2 weeks of treatment were comparable with those observed with APD at 10 mg/kg/day. Thereafter, a high degree of individual variation in antiviral response was observed. In one woodchuck (M7206), a modest reduction in WHV DNA of 0.5 log10 copies/ml was detected. In two others (F7228 and F7221), more remarkable reductions in serum WHV DNA of 0.9 and 1 log10 copies/ml, respectively, were observed, and in the remaining two (M7236 and M7250), more substantial reductions of 3.2 and 4 log10 copies/ml were detected. In the 30-mg/kg/day dose group, the geometric mean concentration of serum WHV DNA was reduced by 1.9 log10 by the end of therapy. The geometric mean concentrations of serum WHV DNA at the end of weeks 1, 2, 3, and 4 of treatment were reduced significantly compared with the pretreatment level (P < 0.01). The mean WHV viremia in woodchucks treated with APD at 30 mg/kg/day was statistically different from the pretreatment value at weeks 1 to 4 of treatment (P < 0.01). The mean WHV viremia in this dose group was also statistically different from that of the placebo-treated control group at weeks 3 and 4 of treatment (P < 0.01). The virions or WHV DNA-containing virus particles are measured.

No apparent changes were observed in the mean concentrations of serum WHV DNA in the placebo-treated control group during the study period. In one woodchuck (M7213), serum WHV DNA was reduced modestly during the last week of treatment and in the first week following drug withdrawal (Fig. 3). In all APD-treated groups, WHV DNA returned promptly to pretreatment levels within 1 to 2 weeks (Fig. 3).

No significant changes were observed in the serum WHSAg of woodchucks treated with APD (data not shown). Occasionally, reductions in levels of WHSAg in the blood were correlated with declines in the serum WHV DNA levels of individual woodchucks treated with APD. In the two woodchucks treated with APD at 30 mg/kg/day in which reductions in serum viral loads were the greatest (M7236 and M7250), transient declines in serum levels of WHSAg in the blood of 1.4- to 1.7-fold from pretreatment levels were observed, based on measurements of optical density units, and the changes were directly related to the observed changes in WHV viremia (Fig. 4).

No changes in anti-WHV core antigen antibody levels were observed in the sera of APD-treated groups, and after 4 weeks of treatment with APD, no anti-WHs antibodies were detected (data not shown). In placebo-treated controls, no significant changes in the levels of WHSAg in sera or of WHV-specific antibodies were observed.

No changes in the mean hepatic concentrations of WHV DNA replicative intermediates (RI) were observed in the placebo control group or after 4 weeks of treatment in woodchucks that received APD at 1, 3, or 10 mg/kg/day (Fig. 5). With APD at 30 mg/kg/day, the concentration of hepatic WHV DNA RI was reduced significantly compared with that in placebo controls (P < 0.05). No statistically significant effects of APD treatment on either hepatic WHV cccDNA or WHV RNA were observed (data not shown). However, in the two woodchucks receiving APD at 30 mg/kg/day (M7236 and M7250) and in which the greatest reductions in serum WHV DNA, serum WHSAg, and hepatic WHV DNA RI were observed, reductions in hepatic WHV RNA were also detected at the end of drug treatment (Fig. 4).

No clinical signs of toxicity were observed in APD-treated woodchucks at any of the drug doses. Changes in the body weights of groups of drug-treated woodchucks were similar to those of placebo-treated control woodchucks. No drug-related changes were observed in the serum biochemical tests per-

![Fig. 3](http://aac.asm.org/Downloadedfrom)
formed, and the hematological parameters of all APD-treated groups were similar to those of the placebo-treated control group. Two of the woodchucks treated with APD at 30 mg/kg/day (M7236 and M7250) and in which the most-robust antiviral responses were observed (Fig. 4) had serum activities of SDH and ALT at the beginning of treatment that were elevated prior to the initiation of drug treatment compared with levels in all the other woodchucks treated with APD (i.e., the average SDH level was 329 ± 48.1 IU/liter and the average ALT level was 12.5 ± 3.5 IU/liter in these two woodchucks, compared with levels of SDH at 129.3 ± 58.3 IU/liter and of ALT at 8.0 ± 2.5 IU/liter in all the other APD-treated woodchucks). After 4 weeks of treatment, both of these woodchucks had reductions in their serum levels of both enzymes (SDH, 211.5 ± 70.0 IU/liter; ALT, 9.5 ± 1.9 IU/liter) that differed from the rising average levels for SDH and ALT (235.4 ± 129.8 IU/liter and 8.4 ± 4.2 IU/liter) observed in all the other APD-treated woodchucks. Importantly, these two woodchucks with the initially higher SDH and ALT levels (M7236 and M7250) had the greatest reductions in serum WHV viremia (Fig. 3), in levels of WHsAg in the blood (Fig. 4), and in hepatic WHV DNA RI (Fig. 5) after 4 weeks of APD treatment.

**DISCUSSION**

This pharmacokinetic study of WHV-negative woodchucks demonstrated that APD was efficiently converted to the parent nucleoside, DXG, following both intravenous bolus and oral administration of APD at 33.3 mg/kg/day (Fig. 2). The simultaneous detection of APD and DXG in serum following oral dosing suggests that APD was readily oxidized to DXG by xanthine oxidase during or after absorption. A higher mean AUC_{0–}\infty for DXG was observed after oral administration than after intravenous administration to woodchucks (Table 1), resulting in an $F_{relative}$ of 212%. APD yielded relatively high levels of DXG in other species as well. In mice, the average AUC_{0–}\infty for DXG following oral and intravenous dosing were 930 and 818 μM·h, respectively ($F_{relative} = 114\%$) (25). $F_{relative}$ could not be calculated for rhesus monkeys, as only oral doses were administered to that species (2). The AUC_{0–}\infty for DXG was larger than that for APD in all three species by all routes of administration tested. The $%AUC_{DXG}$ of the total drug exposure following oral and intravenous administration of APD in woodchucks and mice were 86% and 86% and 69%, respectively. Similarly, the $%AUC_{DXG}$ in rhesus monkeys following oral APD administration was 75% (2). The larger $%AUC_{DXG}$ following oral administration compared to values after intravenous administration in woodchucks and mice support the idea that the metabolism of APD to DXG occurs mainly in the mucosal/epithelial layer of the gastrointestinal tract and/or in the liver during the first-pass metabolism, when the highest levels of xanthine oxidase are reported (25, 32, 37). Following intestinal absorption, substances are delivered to the liver via the hepatic artery. Therefore, it is possible that the liver, the main site of WHV replication, may be exposed initially to higher concentrations of DXG than tissues downstream of the liver.

The elimination rates of APD and DXG observed following the oral administration of APD to mice and rhesus monkeys were also similar to those observed in woodchucks in this study (2, 25). The apparent $t_{1/2\alpha}$ of derived DXG was longer than that of the prodrug in all species. Following the oral administration of APD to mice and monkeys, the $t_{1/2\alpha}$ of APD were
0.3 and 0.5 h, respectively, and those of DXG were 1.0 and 1.5 h, respectively. A similar three- to fourfold-longer $t_{1/2}$ of DXG compared to that of APD was also observed in woodchucks. The overall results of the present pharmacokinetic study indicate that APD can be used as a prodrug for the oral delivery of DXG to woodchucks.

The antiviral activity of APD was assessed against WHV in chronically infected woodchucks in an oral-dosing, placebo-controlled, dose-finding study. In the woodchuck model, it was demonstrated that APD has antiviral activity that would also be expected in human subjects with chronic HBV infection. APD significantly suppressed serum WHV viremia in vivo compared with that in placebo-treated controls during a 4-week period of daily treatment.

No antiviral activity was observed with the lowest dose of APD (1 mg/kg/day) administered in this study. At doses of 3 and 10 mg/kg/day for 4 weeks, an inhibition of WHV replication was demonstrated. The most robust antiviral effect was observed at the highest dose of APD used (30 mg/kg/day), at which a 1.9-log$_{10}$ reduction in serum WHV DNA compared with the pretreatment level was detected (Fig. 3).

The present results are consistent with antiviral studies of DAPD in woodchucks and of DXG or DAPD against DHBV and HBV using transiently or stably transfected cells and primary duck hepatocytes (19, 38–40, 46). Moreover, the antiviral activities of DAPD and DXG have also been demonstrated in vitro against lamivudine-resistant mutants of HBV and DHBV (39, 40, 46).

The magnitude of reduction in serum WHV viremia after 4 weeks of APD treatment and the time to viral recurrence following APD withdrawal observed in this study were similar to those previously reported for other antiviral drugs after short-term administration to chronic WHV carrier woodchucks. Four weeks of treatment with alpha interferon reduced serum WHV DNA by 0.7 log$_{10}$ copies/ml. Serum WHV viremia decreased further during continued treatment and at the end of 24 weeks of treatment was reduced by 2.2 log$_{10}$ copies/ml. WHV DNA returned to pretreatment levels in 1 to 2 weeks in the majority of woodchucks but was extended by 8 to 12 weeks in one woodchuck (20). Famciclovir reduced serum WHV viremia by 1.4 log$_{10}$ copies/ml during 4 weeks of treatment. Recrudescence of viral replication occurred in as little as 1 week in one woodchuck but was extended up to 8 weeks in the other three woodchucks after drug withdrawal (21). Four-week treatment with 1-O-hexadecylpropanediol-3-phosphoacyclovir resulted in a reduction of serum WHV DNA by 1.4 log$_{10}$ copies/ml, and WHV DNA returned to pretreatment levels within 1 to 2 weeks (16). Treatment for 4 weeks with $\beta$-l-2'-deoxyadenosine resulted in a decline of serum WHV viremia by approximately 1.5 log$_{10}$ copies/ml, and recrudescence of viral replication was observed within 1 week following the end of treatment (1). Tenofovir disoproxil fumarate reduced serum WHV DNA by 1.5 log$_{10}$ copies/ml during 4 weeks of treatment, and viral rebound to pretreatment levels occurred within 1 to 4 weeks after the end of treatment (29). Four weeks of treatment with adefovir dipivoxil reduced WHV viremia by 1.8 log$_{10}$ copies/ml and was decreased by more than 2.5 log$_{10}$ copies/ml at the end of treatment at week 12. WHV DNA returned to pretreatment levels within 6 weeks following adefovir dipivoxil withdrawal (8). The average reduction in
serum WHV DNA after 4 weeks of treatment with different doses of lamivudine was approximately 2.5 log_{10} copies/ml, and the average time to recrudescence of viral replication after drug withdrawal was within 1 to 2 weeks (20–22).

Treatment of chronic WHV carrier woodchucks with other antiviral drugs, however, induced greater antiviral effects on serum WHV viremia after 4 weeks of treatment than was observed with APD in this study. Treatment with emtricitabine for 4 weeks reduced serum WHV DNA by 4.9 log_{10} copies/ml, and recrudescence of viral replication occurred within 1 to 2 weeks (9, 23). Four-week treatment with β-L-2′-deoxycytidine reduced serum WHV viremia by 2 to 6 log_{10} copies/ml, and WHV DNA returned to pretreatment levels within 1 to 2 weeks (1). Treatment with entecavir for 4 weeks resulted in a reduction in serum WHV viremia of approximately 7 to 8 log_{10} copies/ml (5, 12). Serum WHV DNA did not appear to decrease much further during continuation of treatment until week 12, and recrudescence of viral replication after drug withdrawal was observed within 6 to 10 weeks (12). Telbivudine (β-L-thymidine) reduced serum WHV DNA by 7 to 8 log_{10} copies/ml during 4 weeks of treatment, and viral recrudescence was observed within 4 to 8 weeks following the end of treatment (1). Treatment with elevudine [1-(2-fluoro-5-methyl-β-L-arabinofuranosyl)-uracil] for 4 weeks reduced serum WHV viremia by 8.2 log_{10} copies/ml (30, 33). WHV DNA reached pretreatment levels within 8 to 12 weeks in the majority of woodchucks after drug withdrawal, but serum WHV viremia was still suppressed in one woodchuck at the end of the study in week 12 (33).

This is the first in vivo study of the antiviral effect of APD against a hepadnavirus infection. In a study of DAPD, a DXG prodrug, against HBV in the duck animal model, there was a significant, but transient, inhibition of DHBV replication (39). After 10 days of intraperitoneal administration of DAPD at 20 or 40 mg/kg/day to ducklings following experimental infection with DHBV, the dose-dependent reduction in the mean DHBV DNA concentrations in serum was between 0.3 and 0.9 log_{10} copies/ml. However, short-term treatment of ducklings with DAPD did not induce a clearance of viral infection or prevent the progression to chronicity.

Four weeks of APD therapy at oral doses as high as 30 mg/kg/day were well tolerated in WHV-infected woodchucks and produced no physical, biochemical, or hematological evidence of toxicity. Because APD was without toxicity, the treatment of woodchucks long-term with an optimal drug dosage can now be considered to assess the effects on the level of hepatic WHV cccDNA, the template for viral transcription (11). The toxicity results from the woodchuck model are consistent with those studies of the efficacy of DAPD and DXG against wild-type HBV and DHBV in cell cultures, where no significant drug toxicity was observed (38, 40). DAPD has been safely administered to human immunodeficiency virus-infected individuals treated for up to 96 weeks or to DHBV-infected ducks treated for 10 days (13, 27, 39, 44).

In the two woodchucks in the group given APD at 30 mg/kg/day (woodchucks M7236 and M7250) in which serum levels of SDH and ALT were higher prior to the start of treatment than in other woodchucks, both enzyme levels decreased by the end of the 4-week treatment period. These two woodchucks had also the greatest reductions in serum WHV DNA and in two other key viral markers, namely, hepatic WHV RNA and serum WHsAg (Fig. 3 to 5). Because APD and DXG concentrations in serum were not determined, it is not known if these two woodchucks had higher serum drug levels than the other APD-treated woodchucks. However, the association of a pronounced antiviral effect with initially elevated liver enzyme activities followed by normalization is similar to observations reported for HBV-infected humans undergoing antiviral drug therapy (15, 24). The observations suggest that in both humans and woodchucks, individuals with a stronger endogenous immune response against hepadnavirus infection may have a more robust response to antiviral therapy.

The antiviral activity observed in chronic WHV carrier woodchucks after 4 weeks of treatment with APD was comparable to that observed after treatment of woodchucks with lamivudine, tenofovir disoproxil fumarate, and adefovir dipivoxil (8, 20–22, 29). The advantages of APD include its water solubility, favorable pharmacokinetics, and safety based on animal studies (e.g., of monkeys and, as described in this paper, woodchucks). APD and DXG (or DAPD) are guanosine analogs, and currently only one other guanosine nucleoside, entecavir, is approved for the treatment of chronic HBV infection. Neither APD nor DAPD is predicted to be cross-resistant with any of the approved drugs, especially those that select for the rtM204V/I mutation, e.g., lamivudine, emtricitabine, and telbivudine, as was shown previously with cell culture (46). In summary, oral APD was safe and significantly effective in reducing concentrations of serum WHV DNA and hepatic WHV DNA in the woodchuck model of chronic HBV infection. Our results with woodchucks and other attributes of APD as described above support the clinical development of APD alone and in combination with other antiviral drugs for the treatment of chronic HBV infection.

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