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The Carbocyclic Analog of 2′-Deoxyguanosine Induces a Prolonged Inhibition of Duck Hepatitis B Virus DNA Synthesis in Primary Hepatocyte Cultures and in the Liver

ISABELLE FOUREL,[1] JEFFREY SAPUTELLI,[1] PRISCILLA SCHAFFER,[2] AND WILLIAM S. MASON[1]*

Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111,[1] and ViraChem, Inc., and Dana Farber Cancer Institute, Boston, Massachusetts 02115[2]

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The carbocyclic analog of 2′-deoxyguanosine (2′-CDG) is a strong inhibitor of hepatitis B virus (HBV) DNA synthesis in HepG2 cells (P. M. Price, R. Banerjee, and G. Acs, Proc. Natl. Acad. USA 86:8543–8544, 1989). We now report that 2′-CDG inhibited duck hepatitis B virus (DHBV) DNA synthesis in primary cultures of duck hepatocytes and in experimentally infected ducks. Like foscarnet (phosphonoformic acid [PFA]) and 2′-,3′-dideoxycytidine (ddC), 2′-CDG blocked viral DNA replication in primary hepatocyte cultures when present during an infection but failed to inhibit the DNA repair reaction that occurs during the initiation of infection to convert virion relaxed circular DNA to covalently closed circular DNA, the template for viral mRNA transcription. Moreover, as for PFA and ddC, viral RNA synthesis was detected when infection was initiated in the presence 2′-CDG. In another respect, however, 2′-CDG exhibited antiviral activity unlike that of ddC or PFA: a single 1-day treatment of hepatocytes with 2′-CDG blocked initiation of viral DNA synthesis for at least 8 days, irrespective of whether DHBV infection was carried out at the time of drug treatment or several days later. Furthermore, orally administered 2′-CDG was long-acting against DHBV in experimentally infected ducklings. Virus replication was delayed by up to 4 days in ducklings infected after administration of 2′-CDG. These observations of long-lasting efficacy in vitro and in vivo even after oral administration suggest that this inhibitor or a nucleoside with similar pharmacological properties may be ideal for reducing virus replication in patients with chronic HBV infection.

Hepadnaviruses may cause either transient or chronic liver infections (5). Chronic hepatitis B virus (HBV) infection is associated with the eventual development of hepatocellular carcinoma in many patients as well as with morbidity and mortality due to ongoing liver damage (20). Since these viruses cause chronic productive infections of hepatocytes which do not appear to be directly cytopathic, liver damage and cancer are thought to be related to immune-mediated attack on the infected hepatocytes. Why a host response fails to clear an infection in some individuals and completely eliminates the virus in others is unknown. However, the fact that a transient infection is associated with cessation of liver damage, and a much lower risk of liver cancer than a chronic infection, has raised the hope that antiviral therapy to either eliminate an infection or manage an infection by eliminating virus from the majority of hepatocytes would have the same benefits.

The family of hepadnaviruses includes HBV (1), woodchuck hepatitis virus (31), ground squirrel hepatitis virus (12), duck hepatitis B virus (DHBV) (14), and heron hepatitis B virus (27). The animal viruses have been invaluable for characterization of the mechanism of hepadnavirus replication, which was found to involve reverse transcription via a scheme quite different from that used by the retroviruses. After entrance of the hepadnavirus into a target cell, the 3-kb relaxed circular (RC) DNA genome is converted to a covalently closed circular (CCC) DNA which is the template for synthesis of viral RNAs. One of these RNAs, the viral pregenome, is packaged into immature viral nucleocapsids and reverse transcribed by the virus-encoded reverse transcriptase (28). Following viral DNA synthesis, the nucleocapsids are enveloped and exported from the cell. It has been found in studies with DHBV that the number of CCC DNA molecules increases during the first few days of infection, from the single molecule produced from the genome of the incoming virus to 10 to 50 copies per cell (29, 30, 32, 37). This amplification of CCC DNA copy number occurs via the same reverse transcription pathway involved in production of progeny virus, except that nucleocapsids with complete DNA are transported to the nucleus rather than exported in virus. It is generally believed that amplification of CCC DNA is required for virus replication and the maintenance of chronic infection of individual hepatocytes. Thus, inhibitors of virus reverse transcriptase might effect a cure of infection by their ability to not only block virus replication but also to prevent CCC DNA synthesis from keeping pace with cell division.

Short-term treatments with inhibitors of the HBV viral DNA polymerase suppress HBV replication in cultures of hepatoma cell lines transfected with HBV DNA (8, 15, 16) as well as in patients infected with this virus (6). The same phenomenon has been described for animal hepadnavirus infections in vitro, using primary hepatocyte cultures, and in vivo (2–4, 7, 9, 25, 26, 34). Nevertheless, the inhibition of viral replication is transient, and a rebound of viral replication occurs following withdrawal of treatment. This rebound would be a consequence of the persistence of CCC DNA in hepatocytes during short-term therapy. It has not yet been reported whether long-term in vivo treatment with inhibitors of DNA synthesis can actually clear virus from the hepatocyte population.

Price et al. (21) have shown that carbocyclic 2′-deoxyguanosine (2′-CDG) inhibits HBV DNA synthesis at concentrations as low as 5 ng/ml (19 nM) in transfected HepG2 cells. We now report that 2′-CDG was an equally effective

* Corresponding author. Mailing address: Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111. Phone: (215) 728-2402. Fax: (215) 728-3616.

inhibitor of DHBV replication, both in primary duck hepatocyte cultures and in vivo. Moreover, the antiviral activity was novel in nature. In primary hepatocyte cultures, even a single drug treatment given several days prior to infection produced a prolonged block in the initiation of viral DNA synthesis. The antiviral activity of 2′-CDG also seemed to have a long lifetime in vivo.

MATERIALS AND METHODS

Compounds. 2′-CDG {(+/−)-2-amino-1,9-dihydro-9-[(1α, 3β, 4α)-3-hydroxy-4-(hydroxymethyl) cyclopentyl]-6H-purine-6-one}, a deoxyguanosine analog that is efficiently substituted for dGTP for incorporation into DNA chains when phosphorylated (17, 18, 22), was provided by ViraChem, Inc. Foscarnet (phosphonoformic acid [PFA]) and 2′,3′-dideoxycytidine (ddC) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Suramin was obtained from Mobay Chemical Corp. (New York, N.Y.).

Experimental animals, virus, and treatment protocols. Pekin ducks, negative for DHBV infection, were used for the in vivo studies. Virus used for infection was obtained from pooled sera of 2-week-old ducklings congenitally infected with DHBV. At 8 and again at 9 days of age, ducklings were administered orally 100 ng of 2′-CDG per g of body weight. Drug was given in 0.5 ml of distilled water. The control ducklings received the equivalent volume of distilled water. After the second treatment, the ducklings were inoculated intravenously with 200 μl of DHBV-containing sera (6×10^9 virions per ml). Serum samples were taken before and after virus inoculation and analyzed for the presence of DHBV DNA.

Infection and drug treatment of cultured hepatocytes. Primary hepatocyte cultures were prepared from 2-week-old Pekin ducks negative for DHBV infection. The procedures of liver perfusion, hepatocyte isolation, and culture conditions were described previously (33, 37). Hepatocytes were seeded at confluence onto 60-mm-diameter petri dishes, and the serum-free growth medium was changed daily. Infection with DHBV present in 25 μl of serum from congenitally infected ducks (6×10^9 virions per ml) and addition of drugs to the culture medium were carried out as described in Results. DHBV-containing medium was removed after a 16-h incubation period for virus uptake. At the end of an interval of drug treatment, cultures were rinsed two times with phosphate-buffered saline (PBS) to remove the drug remaining in the extracellular medium.

Analysis of viral nucleic acids. Serum DHBV DNA was detected by a DNA spot hybridization assay. Five microliters of serum was spotted directly on nitrocellulose filters. After denaturation and neutralization, filters were hybridized with a full-length DHBV genomic DNA probe labeled with ^{32}P.

Hepatocytes were rinsed with PBS and stored at −80°C for DNA or RNA isolation. Isolation of total DNA and CCC DNA was performed as described by Wu et al. (37). Briefly, for the isolation of total DNA, cell monolayers were incubated at 37°C for 2 h in lysis buffer (50 mM Tris [pH 7.5], 10 mM EDTA, 150 mM NaCl, 0.1% sodium dodecyl sulfate [SDS]) containing 2 mg of pronase per ml. The lysate was extracted two times with phenol-chloroform. Nucleic acids were precipitated in ethanol overnight and dissolved in 10 mM Tris (pH 7.6)–1 mM EDTA (TE). One-fourth of the preparation from each petri dish was then subjected to electrophoresis in 1.5% agarose slab gels containing 40 mM Tris-HCl (pH 7.2), 20 mM sodium acetate, and 1 mM EDTA and transferred to nitrocellulose essentially as described by Wahl et al. (35). DHBV DNA was detected by hybridization with a full-length DHBV genomic DNA probe labeled with ^{32}P. For the isolation of CCC DHBV DNA, hepatocytes were disrupted with lysis buffer without pronase. KCl was added to a final concentration of 500 mM to precipitate detergent complexes. Insoluble materials were removed by centrifugation. The supernatant, depleted of viral DNAs possessing the covalently bound terminal protein, was then extracted with phenol. Nucleic acids were precipitated in ethanol at room temperature and dissolved in TE. One half of the preparation from each petri dish was electrophoresed through a 1.5% agarose gel and transferred to nitrocellulose. The detection of viral DNA was carried out as described above. Polyadenylated RNA was isolated by using Micro-FastTrack mRNA isolation kit (Invitrogen Corp.). Briefly, hepatocytes were lysed in SDS buffer containing RNase/protein degrader, incubated at 45°C, and applied to oligo(dT)-cellulose. Polyadenylated RNA was eluted from the oligo(dT)-cellulose, precipitated in ethanol, and kept at −80°C until use. The purified RNA was electrophoresed on a 1% agarose gel containing formaldehyde and transferred overnight to nitrocellulose (11). The RNA was hybridized with a ^{32}P-labeled DHBV riboprobe specific for the detection of the plus-strand viral transcripts.

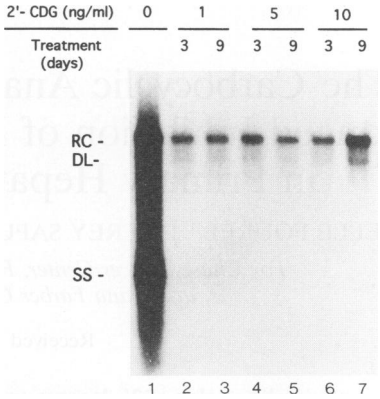

FIG. 1. 2′-CDG inhibited DHBV DNA synthesis in primary hepatocyte cultures. 2′-CDG, at the indicated concentrations, was added 1 day before addition of DHBV-containing sera and was present for either 9 days (lanes 2, 4, and 6) or 3 days (lanes 3, 5, and 7). All cells were harvested at 8 days postinfection, and total DNA was extracted and subjected to Southern blot analysis. The migration positions of RC and single-stranded (SS) 3-kb viral DNAs are indicated. DL, 3-kbp, double-stranded, linear DHBV DNA.

RESULTS

2′-CDG inhibited viral DNA synthesis in primary duck hepatocyte cultures. A preliminary experiment was carried out to determine the antiviral activity of 2′-CDG. The drug, at final concentrations ranging from 1 to 10 ng/ml, was added to hepatocyte culture medium beginning 1 day before DHBV infection and remained until the cells were harvested, at 8 days postinfection. The cells were then examined for the presence of replicative forms of viral DNA. 2′-CDG, at each concentration, blocked viral DNA synthesis, as shown by the absence of intermediates in viral DNA replication (Fig. 1). Surprisingly, viral DNA synthesis did not occur even if 2′-CDG was present in the medium only for 3 days (from 1 day preinfection until 2 days postinfection) followed by 6 days in drug-free medium (Fig. 1). In addition, viral DNA replication intermediates were not detected, at 8 days postinfection, even when 2′-CDG was present in the medium only between 1 and 4 days postinfection

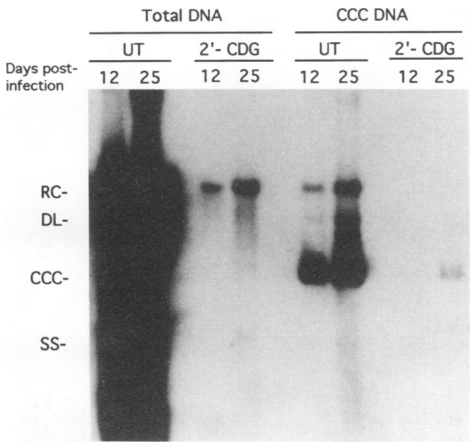

FIG. 2. The inhibitory effect of 2′-CDG on DHBV replication in hepatocyte cultures is partially reversible. 2′-CDG (10 ng/ml) was added 1 day before infection and removed at 2 days postinfection. Cells were harvested 12 and 25 days postinfection. Total DNA and non-protein-bound nucleic acid (CCC DNA) were extracted for Southern blot analysis as described in Materials and Methods. The migration positions of RC, CCC, and single-stranded (SS) DHBV DNAs are indicated. UT, untreated cells. DL, 3-kbp, double-stranded, linear DHBV DNA.

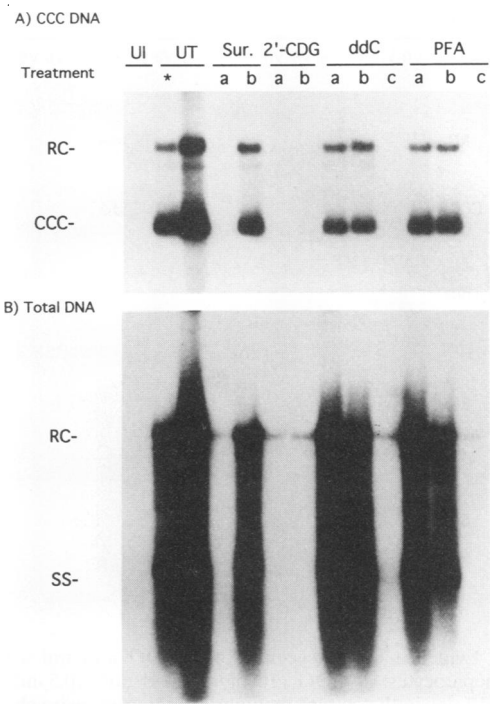

FIG. 3. The antiviral activity of 2′-CDG in hepatocyte cultures differs from those of ddC, PFA, and suramin. Hepatocytes were treated with ddC (0.5 mM), suramin (0.1 mg/ml), PFA (1 mM), and 2′-CDG (10 ng/ml). Drug was added 1 day before infection and maintained until 2 days postinfection (2′-CDG, ddC, and PFA) or until the cells were harvested (suramin [Sur.]). Drug was added 1 day after infection (lanes a) and remained in culture medium until 4 days postinfection (lanes b) or remained throughout (lanes c). Suramin was present in all cultures beginning at 1 day postinfection. After discontinuation of treatment with 2′-CDG, ddC, or PFA, cells were rinsed two times with PBS and maintained in medium lacking these compounds for either 6 or 4 more days, respectively. Cells were harvested 8 days postinfection. The sample in the lane marked with an asterisk was harvested 4 days postinfection. Non-protein-bound nucleic acid (A) and total DNA (B) were extracted for Southern blot analysis as described for Fig. 1. The migration positions of RC, CCC, and single-stranded (SS) DNAs are indicated. UI, uninfected cells; UT, untreated cells. CCC DNA was detectable in the 2′-CDG-treated cultures with longer film exposures.

(data not shown). This result indicated that the effect of 2′-CDG upon DHBV replication was not dependent on the presence of the drug during the initiation of infection, i.e., during CCC DNA formation from incoming viral genomes. In other words, the effect of 2′-CDG in DHBV DNA synthesis was long-lasting even after formation of CCC DNA from virion would presumably have occurred.

To determine whether the 2′-CDG effect could be reversed by a long-term chase, we kept hepatocytes for several weeks in culture. 2′-CDG was present in the hepatocyte culture medium for 3 days beginning 1 day before DHBV infection. The cells were then harvested at 12 and 25 days postinfection. As evidenced by the presence of CCC DNA and replicative forms of viral DNA (Fig. 2) at 25 days postinfection, a rebound of viral replication to ca. 6% of the control value took place. Interestingly, the rebound occurred between 10 and 20 days after the removal of the drug. Primary hepatocyte cultures may become relatively nonpermissive to infection after a week or more in culture (22a), which may have contributed to the slow and inefficient rebound of replication when the drug was withdrawn.

We next examined whether some other inhibitors of DHBV infection, including ddC (0.5 mM) and PFA (1 mM), would show behavior similar to that of 2′-CDG when compared in a single experiment. PFA and ddC, like 2′-CDG, apparently exert their activity as inhibitors of viral DNA synthesis. Drugs were added 1 day before or 1 day after DHBV addition. 2′-CDG, ddC, and PFA were left in for 3 days. Suramin (0.1 mg/ml) was added to all cultures beginning at 1 day postinfection to block secondary rounds of infection (19, 37). One culture also received suramin beginning at 1 day before infection. Cells were harvested 7 days postinfection and assayed for the accumulation of replicative forms of viral DNA and CCC DNA (Fig. 3). As expected, when suramin was present at the time of DHBV addition, no viral replication could be detected, but when suramin was added 1 day after DHBV addition, viral infection and replication took place. The reduced level of DNA synthesis in the culture treated with suramin from 1 day postinfection probably reflects an ability of suramin to dissociate virus that has attached to but not yet penetrated the cell surface after 1 day, as well as its ability to block secondary rounds of infection.

In contrast to the results with suramin, the inhibitory effects of ddC and PFA on DHBV replication were rapidly reversible in this culture even when these drugs were present during virus uptake (Fig. 3). Neither of these inhibitors of viral DNA synthesis was able to block initiation of infection. Again, 2′-CDG inhibition was not rapidly reversible irrespective of whether it was added before or after DHBV.

We next considered whether the prolonged inhibition of viral DNA synthesis by 2′-CDG might be due to toxicity to the hepatocyte cultures (data not shown). Visually, the treated hepatocytes appeared healthy in all experiments, and the monolayers remained intact. To further test for toxic effects, cultures were treated for 8 days with 2′-CDG, at which time the cells were maintained for 4 h in the presence of [^{35}S]methionine. Labeled cellular proteins were then resolved by

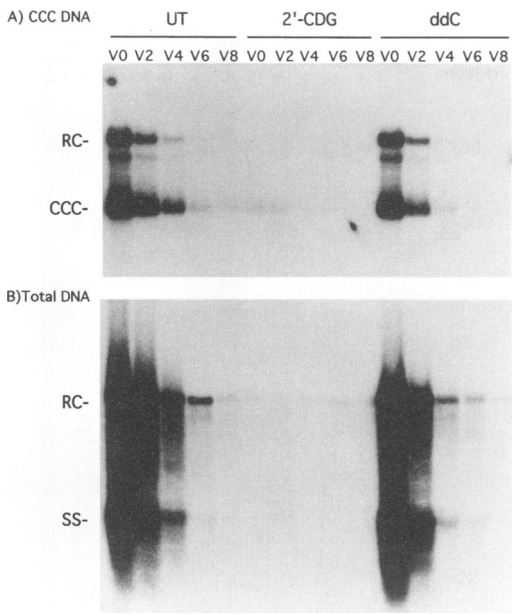

FIG. 4. Evidence for persistence of 2′-CDG antiviral activity in cultured hepatocytes. 2′-CDG (10 ng/ml) and ddC (0.5 mM) were maintained 1 day in the culture medium. Hepatocytes were challenged with DHBV at the time of (V0) or 2 (V2), 4 (V4), 6 (V6), or 8 (V8) days after drug release. Cells were harvested 8 days postinfection. Extracted non-protein-bound DNA (A) and total DNA (B) were analyzed by agarose gel electrophoresis and hybridized with a DHBV-specific probe. The positions of RC, CCC, and single-stranded (SS) DNAs are indicated. UT, untreated cells.

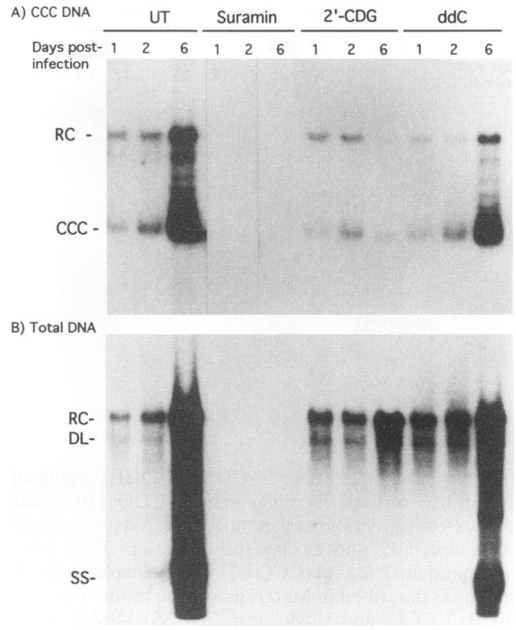

FIG. 5. 2′-CDG does not inhibit CCC DNA formation from input virus. Hepatocytes were treated with ddC (0.5 mM), suramin (0.1 mg/ml), and 2′-CDG (10 ng/ml) 1 day before DHBV addition for 4 days. Cells harvested 1, 2, and 6 days postinfection were extracted for non-protein-bound DNA (A) and total DNA (B). DNA was analyzed by Southern blot hybridization. The positions of RC, CCC, and single-stranded (SS) DNAs are indicated. UT, untreated cells; DL, 3-kbp, double-stranded, linear DHBV DNA.

SDS-polyacrylamide gel electrophoresis (PAGE) and detected by autoradiography. No qualitative or quantitative differences were observed between the drug-treated and untreated controls. To assess the capacity of treated cells to synthesize viral protein, primary hepatocytes were prepared from congenitally infected ducklings, treated for 8 days with 2′-CDG (10 ng/ml) or ddC (0.5 mM), and again labeled by maintenance in culture medium containing [^{35}S]methionine. Compared with the untreated control, labeling of the viral core gene product was reduced about twofold in the 2′-CDG- and ddC-treated cultures. This reduced labeling was correlated with a similar reduction in the amounts of CCC DNA and viral mRNA within the treated cultures. In summary, our results did not support the idea that the long-lasting effects of 2′-CDG were due to toxicity to the hepatocyte cultures.

Experiments described above showed that 2′-CDG, if present during or after initiation of infection, was effective in preventing DHBV DNA synthesis. Experiments were therefore carried out to determine whether de novo infection was inhibited in cultures that had been pretreated with 2′-CDG. 2′-CDG and ddC were maintained for 1 day in culture media, and hepatocytes were challenged with DHBV either at the time of drug release or 2, 4, 6, or 8 days later. In every case, cells were harvested 8 days postinfection and examined for the presence of replicative forms of viral DNA. It has been reported that hepatocyte cultures become less permissive to infection with DHBV at ca. 7 days after plating (22a, 33). In agreement with this report, we observed a decrease of viral replication in relation to the time of infection, and no viral replication could be detected in hepatocytes to which DHBV was added at 10 days after plating (V8 in Fig. 4). 2′-CDG prevented viral DNA synthesis even when present for only a 1-day period that ended 4 to 6 days before infection (Fig. 4). By contrast, ddC only slightly reduced replication following subsequent infection with DHBV. Taken together, our results indicated that the antiviral activity of 2′-CDG was distinct in character from that of the other three inhibitors that we examined.

2′-CDG did not inhibit initiation of infection. Experiments were carried out to determine whether 2′-CDG blocked initiation of infection, as defined by the conversion of virion DNA to transcriptionally active CCC DNA. Such an antiviral activity could be important for maximum in vivo efficacy. PFA does not have this activity (13). We therefore assayed for the appearance of viral CCC DNA and of viral mRNAs when infection was carried out in the presence of either 2′-CDG (10 ng/ml [38 nM]), ddC (0.5 mM), or suramin (0.1 mg/ml). The drugs were added to cultures 1 day before DHBV addition and were maintained for 4 days in the culture medium. Suramin (0.1 mg/ml) was also added to all cultures beginning at 1 day postinfection to block secondary rounds of infection. Cells were harvested 1, 2, and 6 days postinfection and were examined for the presence of viral nucleic acids. As shown in Fig. 5, with the exception of the cells receiving suramin before infection, CCC DNA was detected 1 and 2 days postinfection in the control and treated cultures. Amplification of CCC DNA was observed at 6 days postinfection not only in the untreated cultures but also in the ddC-treated cells, as expected from the reversibility of inhibition of DNA synthesis by ddC. We also examined whether the CCC DNA formed in the presence of 2′-CDG was transcriptionally active. In this same experiment, hepatocytes were harvested at 3 days postinfection

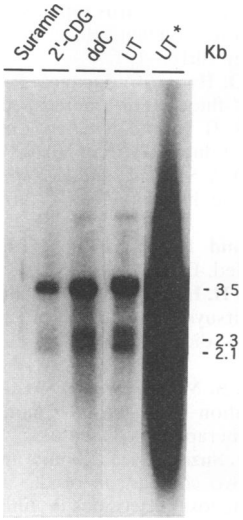

FIG. 6. Viral transcription occurs in the presence of 2′-CDG. In the same experiment as described for Fig. 4, cells were harvested 3 days postinfection. The sample in lane UT* was harvested 8 days postinfection. Polyadenylated RNAs were extracted and analyzed by Northern (RNA) blot hybridization using a DHBV riboprobe. The positions of pregenomic RNA (3.5 kb) and subgenomic RNAs (2.3 and 2.1 kb) are indicated. UT, untreated cells.

and analyzed for viral mRNAs. Figure 6 shows that the major viral transcripts of 3.2, 2.3, and 2.1 kb were produced in the presence of 2′-CDG, as well as in cells receiving ddC, but not in suramin-treated cells. This result was consistent with the idea that the antiviral activity of 2′-CDG was exerted entirely at a stage of infection following CCC DNA formation.

2′-CDG had a long-lasting effect in vivo. We next examined whether 2′-CDG exhibited the same effect in vivo. At 1 day and again at 8 h before infection, five ducklings received oral doses of 2′-CDG and five other ducklings received distilled water as described in Materials and Methods. The ducklings were then bled daily for 10 days. As shown in the Fig. 7, viremia was delayed by several days in 2′-CDG-treated ducklings compared with the control ducklings. Two 2′-CDG-treated ducklings (ducklings 3 and 4) developed a low-level viremia, which was confirmed by the detection of core DHBV antigen in the liver by immunofluorescence, carried out on liver collected at 10 days postinfection (data not shown). These results suggested that, as observed previously in cell culture, 2′-CDG displayed a long-acting effect on DHBV replication.

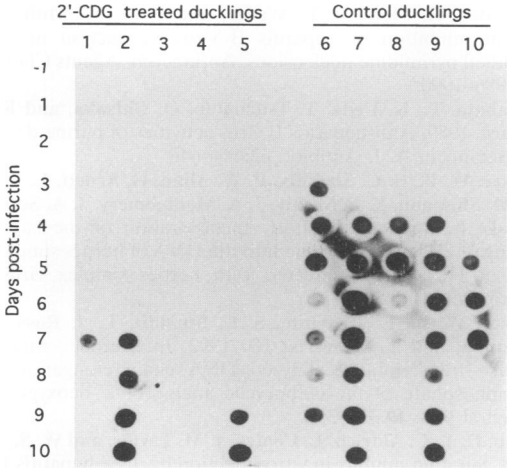

FIG. 7. 2′-CDG treatment delayed the appearance of a viremia in ducklings inoculated with DHBV. One day and again 8 h before infection, five ducks received 2′-CDG (lanes 1 to 5) and five ducks received distilled water (lanes 6 to 10). Serum samples obtained daily (−1 to +10 days) were assessed by dot hybridization.

DISCUSSION

At the present time, there is no uniformly effective treatment for HBV-induced chronic liver disease. Both antiviral and immunomodulatory agents, alone or in combination, have been evaluated extensively in recent years. Some studies have indicated that approximately 30 to 40% of appropriately selected individuals will respond to antiviral therapy (6). Clearly, more effective agents are needed for the treatment of HBV-induced hepatitis. Recent advances in the development of antiviral agents have identified agents such as 2′-CDG, which is active against herpes simplex virus types 1 and 2 (24), human cytomegalovirus (23), and HBV (21) as candidate drugs for the treatment of human infections.

In this study, we have examined the antiviral activity of 2′-CDG on DHBV replication both in duck hepatocyte cultures and in experimentally DHBV-infected ducklings. Our results showed that 2′-CDG strongly inhibited DHBV replication in both cases. In primary hepatocyte cultures, 2′-CDG blocked DHBV replication but, like PFA and ddC, was unable to inhibit the first major step in a round of infection, i.e., the DNA repair that occurs during the initiation of infection to convert virion RC DNA to transcriptionally active CCC DNA. However, 2′-CDG displayed an unusual property: a single drug treatment even 4 days before infection blocked DHBV DNA replication for several days. By contrast, the antiviral effects of PFA and ddC quickly dissipated when these compounds were removed from the culture medium. Furthermore, in vivo, administration of 2′-CDG before infection delayed the appearance of a detectable viremia. No visual evidence of toxicity, at the concentrations used, was detected in hepatocyte cultures. To our knowledge, 2′-CDG is the first antihepadnaviral nucleoside described with a potent and long-lasting activity in cell culture and, apparently, in vivo.

Previous studies, both in cell culture and in vivo, showed that short-term treatment (1 to 2 weeks) of animal hepadnavirus infection with inhibitors of reverse transcriptase suppressed hepadnavirus replication. However, the antiviral effect was only partial, and after withdrawal of these agents, levels of hepadnavirus replication rebounded to pretreatment levels (4, 9, 7, 25, 26). Most of these compounds are nucleoside analogs and, like 2′-CDG, act at a late event of infection, on viral DNA synthesis. Our expectation, in studying 2′-CDG, was that this compound would act as other known nucleoside analogs with potent antiviral activity. This seems not to be the case.

The actual mechanism for the antiviral activity of 2′-CDG is still uncertain. Unlike ddC, 2′-CDG does not appear to be an effective chain terminator. Recently, studies of the endogenous DNA polymerase reaction of purified HBV indicated that 2′-CDG triphosphate is efficiently incorporated in place of dGTP (22). Furthermore, as shown with herpes simplex virus DNA polymerase (17), HBV DNA polymerase can incorporate 2′-CDG into internal sites of viral DNA strands, potentially but not necessarily leading to a nonfunctional genome. Interestingly, a recent study on the effects of 2′-CDG on human immunodeficiency virus replication showed that 2′-

CDG is a good substrate for the human immunodeficiency virus reverse transcriptase. However, the growing DNA chain is poorly extended beyond the site of incorporation of 2′-CDG (18).

A factor which may contribute to the antiviral activity of 2′-CDG against DHBV is that the drug may inhibit the priming of reverse transcription. Since a G residue is the first nucleoside of minus-strand DNA (10, 36), 2′-CDG possibly can block this reaction and further DNA chain extension. Our results are consistent with this possibility, since DHBV replication, in the presence of 2′-CDG, was blocked at the level of CCC DNA and pregenomic RNA and no minus-strand DNA could be detected.

Apart from the question of how 2′-CDG might block viral DNA synthesis, there remains the apparently distinct problem of explaining the long-lasting effect of the drug. Probably the most reasonal explanation for the long-lasting activity is that once phosphorylated, this compound has a very long half-life in cells. Our in vitro and in vivo results are consistent with this possibility.

Among the three factors discussed above that are probably important for the antiviral activity of 2′-CDG, only the third is likely to be unique to this compound. However, pharmacokinetic studies need to be done to test this hypothesis. Other factors or even a combination of factors may also contribute to the potent antiviral activity of 2′-CDG against hepadnavirus replication. One such possibility is that 2′-CDG blocks a normal cellular function that is also required for viral DNA synthesis. If this is the case, however, it is apparently not a function essential for liver function, as ducks have been subjected to over a year of treatment with virus inhibitory doses of 2′-CDG without any obvious health effects (12a).

This present study has interesting clinical implications since it showed that 2′-CDG was one of the most potent of anti-DHBV and anti-HBV agents, effective at lower concentrations than other nucleoside analogs and displaying properties distinct from those of other inhibitors. Both properties of 2′-CDG (oral efficacy and long-acting effect) lead us to suggest that this compound or others with the same properties could be ideal for management of HBV infections. Even if viral infection cannot be completely eliminated from the liver, such compounds could be useful to control the extent of viral infection and hence the level of liver disease resulting from the host immune response to viral antigens.

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