Specific RNA Drug Therapy of Hepatitis Viruses

WILLIAM A. CARTER,[a,b] ROBERT J. SUHADOLNIK,[c] WERNER E. G. MÜLLER,[d] BRENT E. KORBA,[e] HOWARD R. HUBBELL,[b] GABRIEL GARCIA,[f] AND DAVID R. STRAYER [b]

Chronic infection with hepatitis viruses is an enormous global health care concern.[1] To date, antiviral therapies have been unsuccessful for the majority of chronic hepatitis-positive patients, particularly those who are infected perinatally or during infancy. A number of *in vitro* and *in vivo* preclinical models have demonstrated that RNA drug therapy can effectively inhibit hepatitis viruses and has a different mechanism of action than interferon, thus lending itself to potentially more effective therapeutic intervention than has been previously available. The therapeutic role of RNA drugs is based upon the ways in which double-stranded RNA (dsRNA) acts on cells (FIG. 1). dsRNA activates the 2',5' oligoadenylate (2-5A) synthetase/RNase L pathway,[2] which is important in the control of hepatitis virus and other viral infections. The activated 2-5A synthetase produces bioactive 2-5A molecules, which activate RNase L. RNase L destroys hepatitis virus RNAs. The high molecular weight dsRNA (Ampligen®; poly I:poly $C_{12}U$), can both induce and directly activate 2-5A synthetase, while Oragen™ compounds are low molecular weight structural analogues of the bioactive 2-5A. In addition, bioactive 2-5A potentially can inhibit the hepatitis B virus reverse transcriptase (RT). Inhibition of human immunodeficiency virus (HIV) RT has been demonstrated using bioactive 2-5A and specific Oragen compounds.[3] The bioactive 2-5A can also inhibit topoisomerase I activity,[4] which has been associated with hepatitis B virus DNA integration into the genomes of infected hepatocytes[5] potentially leading to hepatocellular carcinoma.[6]

[a] HEM Pharmaceuticals Corp. One Penn Center, 1617 JFK Boulevard, Philadelphia, PA 19103.

[b] Department of Neoplastic Diseases, Hahnemann University, Broad and Vine Streets, Philadelphia, PA 19102.

[c] Department of Biochemistry, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140.

[d] Institut für Physiologische Chemie, Johannes Gutenberg-Universität, Abt. Angewandte Molekularbiologie, Duesbergweg 6, D-6500 Mainz am Rhein, Germany.

[e] Department of Molecular Virology and Immunology, Georgetown University Medical Center, 5640 Fishers Lane, Rockville, MD 20852.

[f] Division of Gastroenterology, Room H1122, Stanford University School of Medicine, Stanford, CA 94305.

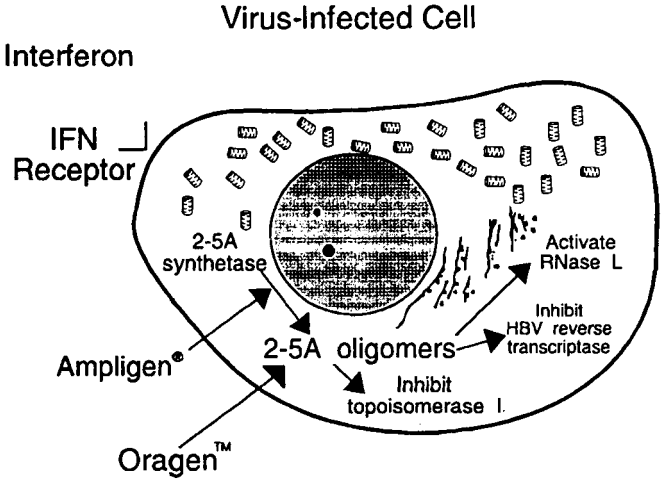

FIGURE 1. Ampligen is a multifunctional biological drug, a high molecular weight double-stranded RNA which activates 2',5' oligoadenylate (2-5A) synthetase, producing bioactive 2-5A. The bioactive 2-5A can activate RNase L, inhibit topoisomerase I activity, and potentially inhibit HBV reverse transcriptase. Oragen compounds are low molecular weight analogues of bioactive 2-5A.

ANTI-HEPATITIS VIRUS ACTIVITY OF AMPLIGEN

Duck hepatitis B virus (DHBV) is a member of the hepadnavirus group and shares many properties, including a unique replicative pathway, with human hepatitis B virus. Ampligen has been used to treat congenitally infected ducks which have become carriers of the DHBV (Locarnini, manuscript submitted). Pekin-Aylesbury crossbred ducklings, with stable and equivalent levels of serum DHBV DNA, were treated with Ampligen (7.5 mg/kg), administered by intraperitoneal injection daily for 28 consecutive days. All treated ducks tolerated the therapeutic regimen. There was no loss of weight or adverse hematologic, liver, or renal function noted. The ducklings treated with Ampligen showed a significant decline in serum DHBV DNA levels during the first two weeks of treatment, which continued for the following two treatment weeks. After the cessation of treatment at 4 weeks, the serum viral DNA levels returned to pretreatment levels. Viral DNA polymerase levels also showed inhibition, similar to the results seen in the serum DHBV DNA assays. Quantitative dot blot analysis of liver samples showed an 83 percent decrease in DHBV genome equivalents in Ampligen treated ducklings compared to untreated controls.

The effect of Ampligen treatment on hepatitis infection has also been studied in both acute and chronic mouse model systems, in which Ampligen extends survival over that seen with untreated mice.[7] These models utilize infection with the coronavirus mouse hepatitis type 3 (MHV-3). Balb/cJ mice received 100 plaque-forming units of mouse hepatitis type 3 (MHV-3) and died from fulminant hepatitis within 5 days post infection. When these mice were pretreated with 10 μg Ampligen by intraperitoneal injection, or given 10 μg Ampligen within 2 hours post infection, they survived 7-10 days. Similarly protected Balb/cJ mice, which received an additional injection 24 hours post infection, survived up to 21 days. In contrast to the Balb/cJ mouse

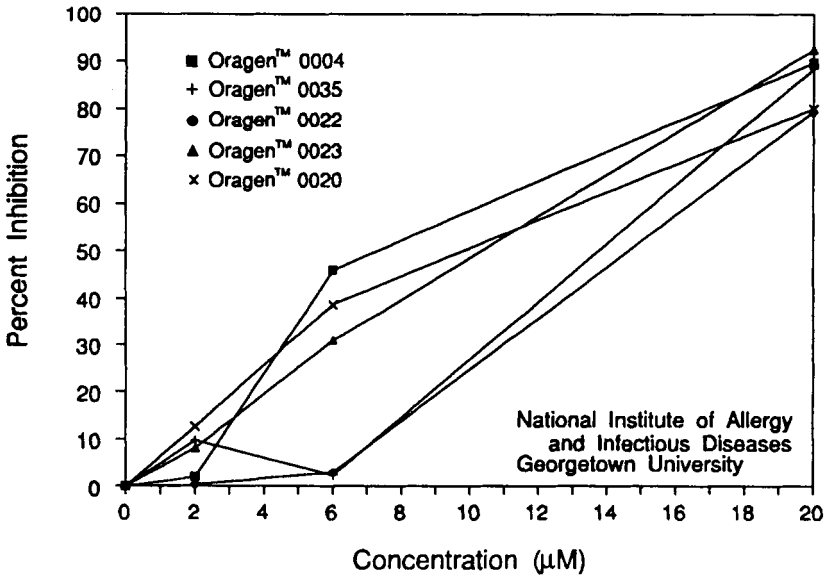

FIGURE 2. Oragen treatment inhibits HBV replication in tissue culture (2.2.15 cells) in a dose-dependent manner.

model system, C3H mice infected with MHV-3 developed chronic hepatitis. C3H mice, pretreated or given 10 μg Ampligen by intraperitoneal injection within 2 hours post infection, cleared the virus quickly and did not develop chronic hepatitis. These results again indicate that Ampligen can be protective against hepatitis virus infection *in vivo*.

ANTI-HEPATITIS ACTIVITY OF ORAGEN COMPOUNDS

Several members of the Oragen family of compounds have been tested for activity against hepatitis B virus. These studies utilized a tissue culture cell line, 2.2.15, derived by transfection of HepG2 human hepatoblastoma cells with a plasmid containing multiple tandem copies of the HBV genome.[8] A standard antiviral assay using this cell line allows analysis of intracellular HBV replication, extracellular production of HBV virions and toxicity of the test drug to the target cells.[9] Intracellular HBV replication was inhibited by five Oragen compounds, in a dose-dependent manner (FIG. 2). The concentration of the Oragen drugs needed to inhibit 90 percent of the viral replication (EC_{90}) was in the range of approximately 19 to 23 μM. Similarly, the production of extracellular virions was inhibited by these compounds in a dose-dependent manner. The EC_{90} for inhibition of extracellular virions ranged from approximately 15 to 22 μM. We have also recently demonstrated that several Oragen compounds inhibit HBV DNA levels when delivered to 2.2.15 cells in liposomes (unpublished results). Cellular toxicity was not seen in the range of therapeutic activity of the Oragen compounds or at concentrations up to nine-fold greater than those that triggered therapeutic activity.

CLINICAL STUDIES OF AMPLIGEN IN HEPATITIS

A phase I/II study of Ampligen therapy in chronic hepatitis B virus infection has recently been initiated at Stanford University and the University of Pennsylvania. The initial patient was treated with 200 mg Ampligen twice per week with a dose escalation to 400 mg twice per week at week 9. At baseline, this patient had significant levels of circulating HBV DNA (138 pg/ml). At eight weeks of therapy, the HBV-DNA remained at baseline levels (140 pg/ml). After the dose escalation, an approximately two-fold decrease (75 pg/ml) was seen at week 17. The SGPT and SGOT, significantly elevated at baseline (SGPT-214 IU/L; SGOT-92 IU/L), decreased approximately two-fold by week 8. However, shortly after the Ampligen dose was increased, the enzyme levels increased. This patient continued on therapy at 400 mg twice per week. Additional patients are being enrolled.

REFERENCES

1. CENTERS FOR DISEASE CONTROL. 1982. MMWR **31:** 317-328.
2. LI, S. W., J. J. MOSKOW & R. J. SUHADOLNIK. 1990. J. Biol. Chem. **265:** 5470-5474.
3. SOBOL, R. W., *et al.* 1990. J. Interferon Res. **10:** S66.
4. CASTORA, F. J., *et al.* 1991. J. Interferon Res. **11:** 143-149.
5. WANG, H. P. & C. E. ROGLER. 1991. J. Virol. **65: 2381-2392.**
6. BEASLEY, R. P. & L.-Y. HWANG. 1991. *In* Viral Hepatitis and Liver Disease. F. B. Hollinger, S. M. Lemon & H. S. Margolis, Eds.: 532-535. Williams and Wilkins. Baltimore, MD.
7. LEE, M. H., *et al.* 1983. Hepatology **3:** 837.
8. SELLS, M. A., A. Z. ZELENT, M. SHVARTSMAN & G. ACS. 1988. J. Virol. **62:** 2836-2844.
9. KORBA, B. E. & G. MILMAN. 1991. Antiviral Res. **15:** 217-228.