Inhibition of Hepatitis C Virus RNA Replication by 2'-Modified Nucleoside Analogs*

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The RNA-dependent RNA polymerase (NS5B) of hepatitis C virus (HCV) is essential for the replication of viral RNA and thus constitutes a valid target for the chemotherapeutic intervention of HCV infection. In this report, we describe the identification of 2'-substituted nucleosides as inhibitors of HCV replication. The 5'-triphosphates of 2'-C-methyladenosine and 2'-O-methylcytidine are found to inhibit NS5B-catalyzed RNA synthesis in vitro, in a manner that is competitive with 3'-substituted nucleosides as inhibitors of HCV replication. The 5'-triphosphates of both nucleosides are detected intracellularly following addition of the nucleosides to the media. However, significantly higher concentrations of 2'-C-methyladenosine triphosphate than 2'-O-methylcytidine triphosphate are detected, consistent with the greater potency of 2'-C-methyladenosine in the replicon assay, despite similar inhibition of NS5B by the triphosphates in the in vitro enzyme assays. Thus, the 2'-modifications of natural substrate nucleosides transform these molecules into potent inhibitors of HCV replication.

Hepatitis C virus (HCV)† infection is the leading cause of sporadic, post-transfusion, non-A non-B hepatitis (1, 2). One hundred seventy million people worldwide are thought to be infected with hepatitis C virus of which an estimated 4 million reside in the United States (3). Approximately 80% of infected individuals progress to chronic infection. Long term chronic HCV infection can lead to liver cirrhosis and to hepatocellular carcinoma (4–6). Currently, the recommended therapy is treatment with a combination of interferon α2b and ribavirin, which results in a sustained viral response in 40% of patients (7, 8). Investigational therapies using a combination of pegylated interferon and ribavirin have lead to an sustained viral response in 54% of patients, but the response rate (42%) of patients harboring HCV genotype 1 is lower (9, 10). Consequently, additional therapies for HCV infection are needed.

Antiviral chemotherapies based on administration of analogs of deoxynucleosides have been widely successful as treatment for HIV, herpes virus, and hepatitis B infection (11, 12). Intracellular phosphorylation of the nucleoside analog to the triphosphate creates the active form of the inhibitor that then serves as a substrate for the viral polymerase. Generally, incorporation of the nucleotide analog at the 3'-end of the replicating viral RNA causes termination of DNA synthesis, owing to the lack of the 3'-hydroxyl required for extension. These successes suggest that an investigation of ribonucleoside analogs as inhibitors of HCV replication would be worthwhile.

The HCV NS5B protein, the RNA-dependent polymerase responsible for the synthesis of the viral RNA genome, is an attractive target for the development of antiviral agents (13). The enzymatic activity of NS5B has been extensively characterized in vitro (13–15, 29). Additionally, cell lines that harbor subgenomic replications capable of supporting HCV replication are available to assess inhibition of replication by compounds within the cellular environment (16, 17). The antiviral effect of interferon α has been documented in these lines (18).

Screening of available nucleosides for inhibitors in the cell-based bicistronic replicon assay have identified two nucleoside analogs, 2'-C-methyladenosine and 2'-O-methylcytidine, that specifically inhibit HCV RNA replication in the absence of cytotoxicity. The biochemical basis for the inhibition by these nucleoside analogs has been investigated. When added to replicons growing in culture, the nucleoside analogs resulted in the intracellular formation of the corresponding triphosphates that were shown to be potent, competitive inhibitors of NS5B-catalyzed reactions in vitro. This study demonstrates the utility of 2'-substituted nucleosides in the inhibition of HCV RNA replication.

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EXPERIMENTAL PROCEDURES

Materials—Nucleotides, α- or γ-32P- and 32P-labeled, were purchased from PerkinElmer Life Sciences. Enzyme nucleoside analogs were purchased from Amersham Biosciences (Piscataway, NJ). 2′-O-Methylcytidine triphosphate was purchased from Trilink (San Diego, CA). 2′-O-Methylcytidine and 2′-O-methyl-5-iodocytidine were purchased from Berry Associates (Dexter, MI). 2′-C-Methyladenosine from the Merck chemical collection was synthesized as previously described (20). The structures of the nucleoside analogs were verified by mass spectrometry and gradient-enhanced homonuclear correlation NMR.

2′-C-Methyladenosine triphosphate was synthesized according to the general procedures previously described (21). The triphosphate was purified by anion exchange chromatography using a 30-× 100-mm Mono Q column (Amersham Biosciences) with a buffer system of 50 mM Tris, pH 8. The elution gradient was 40 mM to 0.8 M NaCl in two column volumes. Appropriate fractions from Mono Q chromatography were collected and desalted by reverse-phase (RP) chromatography using a C18 250-× 21-mm column (Phenomenex) with an elution gradient of 1% to 95% methanol in 5 mM triethylammonium acetate. Mass spectra of the purified triphosphate were determined using in-line RP HPLC mass spectrometry on a Hewlett-Packard (Palo Alto, CA) MSD 1100. The molecular mass was determined using the Hewlett-Packard Chemstation analysis package. LC/MS: 520.0 (calc. for C11H17N5O13P3: 519.9971). The purity of the nucleoside triphosphate was determined using analytical RP and anion exchange HPLC to be 100%.

2′-Bromo-2′-C-methyladenosine was synthesized from 2′-C-methyladenosine by addition of N-bromosuccinimide in dimethylformamide. The mixture was purified on silica gel using methylene chloride (1:9) as eluent. 3′ H NMR (Me2SO- d6): δ 8.06 (s, 1H), 3.78 (m, 2H), 3.89 (m, 1H), 4.45 (dd, 1H), 5.11 (t, 1H), 5.18 (s, 1H), 5.32 (d, 1H), 5.93 (s, 1H), 7.55 (s br, 1H), 8.09 (s, 1H). MS/ES: 360.0299 (calc. for C11H14BrN5O4: 360.0348). The purity of the nucleoside triphosphate was determined in reactions utilizing 5′-end-labeled oligoribonucleotides. HCV (BK strain) NS5B was expressed in Huh-7 cells (25) by transfection with wildtype NS5B RNA and extension of nucleoside analogs catalyzed by HCV NS5B was analyzed using a PhosphorImager (Amersham Biosciences). In a similar manner analogs of cytidine triphosphate were examined for incorporation into reactions with oligoribonucleotide 67N (sequence 5′-GCUAGAGCCCAGUUCCGCCC-3′). Oligoribonucleotides 67N and 68N were synthesized using 2′-acetate ester chemistry (Dharmaco, Lafayette, CO), purified using desalting PAGE gels, and deprotected according to the manufacturer’s instructions.

Human DNA Polymases—DNA polymerase α was supplied by T. Wang (Stanford University). DNA polymerase β was purchased from AB Peptides (St. Louis, MO). DNA polymerase γ was supplied by W. Copeland (NIHES, National Institutes of Health).

HCV NS5B Expression Purification—HCV (BK strain) NS5B was expressed in Escherichia coli strain BL21 (DE3) harboring plasmid pT7(NS5B) and purified as previously described (23). HCV (BK strain) NS5B was expressed in E. coli BL21(DE3) harboring plasmid pT7(NS5B). Plasmid pT7(NS5B) was constructed from plasmid pT7(NS5B-21) by introduction of a stop codon after Leu-537. Protein expression and purification of HCV (BK strain) NS5B was performed by T7 runoff transcription as previously described (22, 24). Protein concentration was determined with the use of quantitative amino acid analysis.

NS5B Enzyme Assay on Template 1500—RNA polymerase activity was determined in reactions catalyzed by NS5B21 and NS5B55 by measuring the incorporation of radiolabeled NTPs into a heteromeric RNA template via a copoly-back mechanism. Template 1500 was generated by T7 runoff transcription as previously described (22), using commercially available kits (Ambion) following the manufacturer’s instructions. The 1500 was purified with RNasey kits (Qigene) and quantified using absorbance at 260 nm. NS5B-catalyzed reaction conditions included 500 nM NS5B, 25 mM NS5B in a 50-μl reaction containing 500 nM T7 RNA, 20 mM Tris, pH 7.5, 50 mM KCl, 2 mM MgCl2, 5 mM DTT, 0.4 unit/μl RNasin (Promega) for 30 min at room temperature. Reactions were initiated by the addition of NTPs. Reactions included 10 μM ATP and/or 2 μM UTP or 1–50 μM 2′-C-methyladenosine triphosphate with or without 2 μM UTP. Reactions were allowed to proceed for 30 or 60 min, and then 5 μl aliquots were removed and quenched with 15 μl of formamide gel load buffer. After denaturing the RNA at 65 °C for 30 min, substrate and one or more products were separated on 20% acrylamide-8% urea gels and analyzed using a PhosphorImager (Amersham Biosciences). In a similar manner, reactions with oligoribonucleotide 76N (sequence 5′-ACGUUGGGCGUUUUCGCCC-3′). Oligoribonucleotides 67N and 68N were synthesized using 2′-acetate ester chemistry (Dharmaco, Lafayette, CO), purified using desalting PAGE gels, and deprotected according to the manufacturer’s instructions.

In Situ Ribonucleic Acid Protection Assay—HBU10A cells (27) were grown and assayed as previously described.2 Replicon cells were passaged at 1:5 and plated at a cell density of 40,000 cells/well in cytostar plates (Amersham Biosciences) in complete Dulbecco’s modified Eagle medium containing 10% FBS and 0.8 mg/ml G418. Compound treatment of the cells was added to the medium containing 25 μg/ml bovine serum albumin (26). In a similar manner, reactions with oligoribonucleotide 76N (sequence 5′-ACGUUGGGCGUUUUCGCCC-3′). Oligoribonucleotides 67N and 68N were synthesized using 2′-acetate ester chemistry (Dharmaco, Lafayette, CO), purified using desalting PAGE gels, and deprotected according to the manufacturer’s instructions.

Modified Nucleosides

Fraction inhibition = 1 − [v / [v]0] = [F(v)]/([v]0 + IC 50) (Eq. 1)

where v is the reaction velocity in the presence of inhibitor, v0 is the reaction velocity in the absence of inhibitor, and IC50 is the half IC50.

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2F. E. Tomassini, E. Boots, K. Getty, S. Shim, Z.-Q. Zhang, and G. Migliaccio (2002), submitted for publication.
at the time of compound addition. Tritiated compound was incubated at 2 μM in the cell culture medium for 3 or 23 h. Cells were then harvested, washed with phosphate-buffered saline, and counted. The cells were then extracted in 70% methanol, 20 mM EDTA, 20 mM EGTA, and centrifuged. The lysate was dried, and radiolabeled nucleotides were analyzed using an ion-pair reverse phase (C-18) HPLC on a Waters Millenium system connected to an in-line β-RAM scintillation detector (IN/US Systems). The HPLC mobile phases consisted of (a) 10 mM potassium phosphate with 2 mM tetrabutylammonium hydroxide and (b) 50% methanol containing 10 mM potassium phosphate with 2 mM tetrabutylammonium hydroxide. Peak identification was made by comparison of retention times to standards.

RESULTS

Inhibition of NS5B Enzyme Activity—NS5B-catalyzed incorporation of nucleotides in reactions with template t500 generates a copy-back or hairpin product (22), as previously described for other RNA templates (28). The rate of product formation catalyzed by NS5BΔ21 or NS5BΔ55 was reduced in the presence of either 2'-C-methyladenosine triphosphate or 2'-O-methylcytidine triphosphate (structures shown in Fig. 1), with IC<sub>50</sub> values as shown in Table I, as determined by monitoring the total incorporation of radiolabeled nucleotide as described under “Experimental Procedures.” The Hill coefficients did not significantly differ from unity. The potency of inhibition by either nucleotide analog was not affected whether the radiolabeled nucleoside triphosphate was GTP or ATP (data not shown), indicating that replacement of the radiolabel by the nucleoside analog was not responsible for the inhibition.

Mode of Inhibition—To determine the mode of inhibition by 2'-C-methyladenosine triphosphate and 2'-O-methylcytidine triphosphate of RNA synthesis catalyzed by NS5BΔ21, reactions were run in which the concentrations of ATP and CTP were varied, respectively, holding the concentrations of the other NTPs constant at concentrations above K<sub>m</sub>. Double-reciprocal plots of the data as shown in Fig. 2 indicated competitive inhibition of activity by 2'-C-methyladenosine triphosphate and 2'-O-methylcytidine triphosphate with varying ATP and CTP, respectively. The K<sub>i</sub> values as determined from a replot of the slopes of the double-reciprocal plot was 0.9 μM for 2'-C-methyladenosine triphosphate and 0.3 μM for 2'-O-methylcytidine triphosphate.

Gel-based Incorporation Assay—To determine whether NS5BΔ55 is capable of incorporating the nucleoside analogs into a growing RNA strand, gel-based analyses of reactions using hairpin RNA templates were carried out. The sequence of the RNA template (68N) was designed such that an intramolecular hairpin could form, allowing the incorporation of AMP followed by UMP. NS5BΔ55 showed a much greater ability to incorporate nucleotides efficiently onto the hairpin RNA substrates than did NS5BΔ21. The relatively low activity of NS5BΔ21 with the hairpin templates is likely a consequence of the low fraction of catalytically competent NS5BΔ21 (~2%, Ref. 22) compared with NS5BΔ55 (~40%, data not shown). The incorporation of AMP leads to the appearance of a single product band (Fig. 3A, lane 3). In reactions that included ATP and UTP, the product resulting from the incorporation of AMP was completely extended by addition of UMP (Fig. 3A, lane 4). NS5BΔ55 was capable of incorporating 2'-C-methyladenosine monophosphate onto the 3'-end of the RNA at the lowest nucleotide concentration tested, 1 μM. However, as shown in Fig. 3A (lanes 9–11), NS5BΔ55 could not add uridine monophosphate onto the 2'-C-methyladenosine-terminated template efficiently, although a trace amount of extended product was evident. In a similar manner, NS5BΔ55 was capable of incorporating 2'-O-methylcytidine monophosphate onto the 3'-end of RNA hairpin 76N as shown in Fig. 3B. However, no detectable extension product was visible when the next correct nucleoside triphosphate, ATP, was added to the reaction.

Inhibition of DNA Polymerases α, β, and γ—To determine the specificity of inhibition the activity of human DNA polymerases α, β, and γ were monitored in vitro in the presence of...

**Table I**

| Enzyme   | 2'-C-Me-ATP IC<sub>50</sub> (μM) | Hill Coeff. | 2'-O-Me-CTP IC<sub>50</sub> (μM) | Hill Coeff. |
|----------|----------------------------------|-------------|----------------------------------|-------------|
| NS5BΔ21  | 1.9 ± 0.4                        | 1.0 ± 0.1   | 3.8 ± 0.3                        | 1.0 ± 0.1   |
| NS5BΔ55  | 1.9 ± 0.3                        | 1.0 ± 0.1   | 2.7 ± 0.2                        | 0.9 ± 0.1   |

**Fig. 2** Mode of inhibition by (A) 2'-C-methyladenosine triphosphate and (B) 2'-O-methylcytidine triphosphate of NS5B-catalyzed synthesis. Reactions included 250 μM NS5BΔ21 and template t500 in reaction buffer as described under “Experimental Procedures.” A, reactions also included 20 μM of GTP, UTP, and CTP and from 1.5 to 30 μM ATP, and no (○), 0.75 (+), 1.5 (×), 3 (●), 4.5 (□), or 6 (○) μM 2'-C-methyladenosine triphosphate. B, reactions included 20 μM of GTP, UTP, and ATP and from 0.1 to 10 μM CTP, and 0 (○), 0.1 (■), 0.2 (+), 0.4 (×), 1 (●), 2 (□), or 4 (○) μM 2'-O-methylcytidine triphosphate. Reactions proceeded for 2 h at RT and were quenched by the addition of EDTA. Product analysis was by DE-81 filter binding. Data were fit to a competitive mechanism, with K<sub>i</sub> values of 0.9 μM and 0.3 μM for 2'-C-methyladenosine triphosphate and 2'-O-methylcytidine triphosphate, respectively.
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**Fig. 3.** PhosphorImager results of the incorporation/extension of nucleotide analogs. A, 2'-C-methyladenosine monophosphate. Lane 1 is a control with no enzyme added. Reactions included 600 nM 5'-23P-end-labeled 68N (sequence shown as a hairpin conformation), 1 μM NS5BΔ55 in reaction buffer as described under “Experimental Procedures,” and either no nucleoside triphosphates (lane 2), 10 μM ATP (lane 3), 10 μM ATP and 2 μM UTP (lane 4), 2 μM UTP (lane 5), 1 μM (lane 6), 10 μM (lane 7), or 50 μM (lane 8) 2'-C-methyladenosine triphosphate. Lanes 9–11 contain the same concentrations of 2'-C-methyladenosine triphosphate as lanes 6–8 with 2 μM UTP. B, 2'-O-methylcytidine monophosphate. Lane 1 is a control with no enzyme added. Reactions included 600 nM 5'-23P-end-labeled 76N (sequence shown as a hairpin conformation), 1 μM NS5BΔ55 in reaction buffer, and either no nucleoside triphosphates (lane 2), 10 μM CTP (lane 3), 10 μM CTP and 2 μM ATP (lane 4), 2 μM ATP (lane 5), 1 μM (lane 6), 10 μM (lane 7), or 50 μM (lane 8) 2'-O-methylcytidine triphosphate. Lanes 9–11 contain the same concentrations of 2'-O-methylcytidine triphosphate as lanes 6–8 with 2 μM ATP. The results indicate that NS5BΔ55 is capable of incorporating either 2'-C-methyladenosine monophosphate or 2'-O-methylcytidine monophosphate onto the 3'-end of the synthetic RNA template but is not capable of efficiently adding the next correct base onto the analog-terminated template.

**Fig. 4.** Inhibition of the cell-based replicon assay. HB110A cells were incubated in the presence of the indicated concentrations of 2'-C-methyladenosine (○) or 2'-O-methylcytidine (□) for 24 h, and the level of HCV RNA was determined by in situ ribonuclease protection assay as described under “Experimental Procedures.” The curves represent the best fit of the data to Equation 1, giving IC50 values of 0.31 μM for inhibition by 2'-C-methyladenosine and 14 μM for inhibition by 2'-O-methylcytidine. The signal-background ratio in the assay was typically 25:1.

2'-C-methyladenosine triphosphate and 2'-O-methylcytidine triphosphate. Less than 20% inhibition of the activity of DNA polymerases α, β, or γ was detected at 50 μM of either nucleoside triphosphate analog.

**Nucleosides 2'-C-Methyladenosine and 2'-O-Methylcytidine Inhibit HCV RNA Replication in Cells—**Nucleosides 2'-C-methyladenosine and 2'-O-methylcytidine were tested for inhibitory activity in a cell-based replicon assay using a stable Huh-7 human hepatoma cell line, which supports the replication of HCV RNA and proteins. The effect of the nucleosides upon RNA replication in a clonal line designated HBI10A (27) was detected by in situ ribonuclease protection assay as previously described. Representative titrations of the compounds in the replicon assay are shown in Fig. 4. Both compounds were active in the assay at 24 h with IC50 values of 0.3 μM for the 2'-C-methyladenosine and 21 μM for the 2'-O-methylcytidine (Table II). The antiviral activity of both compounds was observed in the absence of cytotoxicity in HBI10A cells as measured in the MTS assay when tested up to 100 μM.

**Intracellular Metabolism to the Active Triphosphate—**The intracellular metabolism of the tritiated versions of 2'-C-methyladenosine and 2'-O-methylcytidine was studied in Huh-7 cells in culture for 23 h, after which cells were collected and nucleotides were extracted and chromatographed as described under “Experimental Procedures.” The elution time of a 2'-C-methyladenosine triphosphate standard was the same as that of the last eluting peak on the radiochromatogram. The two smaller peaks eluting at 22 and 10 min probably correspond to the 2'-C-methyladenosine diphosphate and monophosphate, respectively. Lysate corresponding to ~24,000 cells was analyzed per injection. B, [5-3H]-2'-O-methylcytidine (2 μM) was incubated with Huh-7 cells in culture for 23 h, and radioactively labeled nucleotides were extracted and analyzed by ion pairing HPLC as described under “Experimental Procedures.” Lysate corresponding to ~2.4 × 106 cells was analyzed per injection. Peak identification was made by comparing the retention times of unlabeled compounds. Significant amounts of metabolic derivatives of 2'-O-methylcytidine are present. Note the difference in the scales of panels A and B.

**DISCUSSION**

The advent of the cell-based, subgenomic, bicistronic replicon system (16) as a means of assessing the replication of viral
RNA within the cellular environment has permitted the evaluation of analogs of ribonucleosides to complement ongoing efforts aimed at identifying inhibitors of purified NS5B in vitro. Screening of available nucleosides for inhibition of viral replication in the replicon assay identified two inhibitory compounds, 2'-C-methyladenosine and 2'-O-methylcytidine.

The triphosphates of 2'-C-methyladenosine and 2'-O-methylcytidine inhibit the catalytic activity of purified HCV RNA polymerase with IC_{50} values of 1.9 and 3.8 μM, respectively (Table I). Forms of HCV RNA polymerase having two different C-terminal truncations that were investigated have significantly different catalytic efficiencies, with NS5BΔ55 having ~20-fold greater specific activity than NS5BΔ21. However, IC_{50} values varied only slightly between the two enzyme forms for the nucleoside analog triphosphates investigated. As expected the two nucleoside analog triphosphates were competitive inhibitors with varying nucleoside triphosphate, having K_i values that were submicromolar.

Analysis of the incorporation of the nucleoside analogs onto a growing RNA strand was carried out using synthetic RNA templates that are designed to fold into intramolecular hairpins. NS5BΔ55 is capable of incorporating both 2'-C-methyladenosine monophosphate and 2'-O-methylcytidine monophosphate onto the appropriate RNA template, implying that both triphosphates can bind to the enzyme in the substrate NTP binding site and further implying there is some additional room in the vicinity of the 2'-carbon and the 2'-oxygen when bound in the active site that allows HCV NS5B to accommodate either the 2'-C-methyl or 2'-O-methyl substituent. The presence of 2'-substituents likely confers specificity of inhibition of the viral RNA polymerase over inhibition of the human DNA polymerases tested. After incorporation of the nucleotide analog, NS5BΔ55 is not capable of efficiently extending the incorporated analog by addition of the next correct nucleotide, suggesting that with this template system, the nucleoside analogs act as functional chain terminators, despite the presence in both cases of a 3'-OH. The results suggest that after incorporation the 3'-OH is not able to perform nucleophilic attack on the α-phosphorus of the incoming NTP. Further investigation is necessary to understand the molecular details of the apparent chain termination. Chain termination by incorporation of nucleotide analogs that retain a 3'-hydroxyl has previously been observed in the inhibition of DNA polymerase β by arabinofuranosyladenine triphosphate (29) and arabinofuranosylcytidine triphosphate (30).

The nucleosides were converted intracellularly to the corresponding triphosphates, which, in turn, functioned as specific inhibitors of HCV RNA synthesis. The potency of inhibition of HCV replication observed in replicon-containing cells correlated with the levels of triphosphates formed intracellularly. A lesser amount of the intracellular 2'-O-methylcytidine triphosphate was detected than the 2'-C-methyladenosine triphosphate, and the difference is reflected in the reduced potency of 2'-O-methylcytidine in replicon-containing cells, despite the equivalent inhibition of the purified enzyme by the two triphosphates.

The potency of inhibition by 2'-C-methyladenosine in the cell-based replicon assay (0.3 μM) is greater than the potency of the corresponding triphosphate in the enzyme assay (1.9 μM). The greater potency in the cell-based assay likely reflects a combination of the high intracellular concentration of the corresponding triphosphate that is achieved (105 pmol/million cells in the presence of 2 μM extracellular nucleoside) and the fact that the analog acts as a functional chain terminator. Once the nucleotide analog is incorporated into the replicon RNA, the resulting truncated RNA chain, in the absence of a known proofreading activity, is nonfunctional as a template for subsequent rounds of viral RNA synthesis. However, in the enzyme assay, once the nucleotide analog has been incorporated, the truncated RNA is still counted as the product.

Because the in vitro enzyme assay is performed under conditions that differ from physiological, it is conceivable that the enzyme assay may not be truly representative of biological activity. Nonetheless, the use of in vitro enzyme assays is validated by the demonstration of the inhibition of purified HCV NS5B by the triphosphates of nucleoside analogs that also are capable of inhibiting HCV replication in cell culture in the absence of cytotoxicity. A more definitive corroboration of enzyme inhibition with antiviral effect in the replicon assay will be ascertained when resistant mutations are identified that confer resistance both in vitro and in the cell culture assay.

Whether the replicon assay will be a meaningful predictor of antiviral activity in vivo has yet to be determined. Equally uncertain at this point is the question of whether 2'-C-methyladenosine or 2'-O-methylcytidine have pharmacokinetic and safety profiles that are sufficiently attractive to warrant their development as HCV therapeutics. However, the current work establishes the direct inhibition of HCV RNA polymerase activity by 2'-modified nucleotides leading to inhibition of HCV replication in cells.

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