Sustained Viral Response in a Hepatitis C Virus-Infected Chimpanzee via a Combination of Direct-Acting Antiviral Agents

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Efforts to develop novel, interferon-sparing therapies for treatment of chronic hepatitis C (HCV) infection are contingent on the ability of combination therapies consisting of direct antiviral inhibitors to achieve a sustained virologic response. This work demonstrates a proof of concept that coadministration of the nucleoside analogue MK-0608 with the protease inhibitor MK-7009, both of which produced robust viral load declines as monotherapy, to an HCV-infected chimpanzee can achieve a cure of infection.

There is a need to develop direct antiviral therapy for chronic hepatitis C virus (HCV) infection to improve both the limited response rate (40 to 60%) and the poor tolerability of presently approved therapies consisting of combinations of pegylated interferon α and ribavirin (2, 7, 8). MK-0608 (2'-C-methyl-7-deaza-adenosine) (10) was previously administered orally to HCV-infected chimpanzees at 1 mg/kg once daily, and profound decreases in circulating viral load were observed (1). To determine the durability of the antiviral efficacy of MK-0608 at a higher dose, chimpanzees X7 and X8, chronically infected with HCV (predose viral loads of −6,500 and −3,000 IU/ml, respectively), were dosed with 2 mg/kg MK-0608 for 37 days (1). Study protocols described in this work were reviewed and approved by the Institutional Animal Care and Use Committee at both Merck Research Laboratories and at the New Iberia Research Center (University of Louisiana at Lafayette) where the experiments were conducted to ensure compliance with all federal regulations. As shown in Fig. 1A, the circulating viral titer in both chimpanzees rapidly decreased to levels below the lower limit of quantitation (LOQ) of an HCV quantitative PCR (qPCR) viral load assay (20 IU/ml; Analyte Specific Reagent; Roche, Nutley, NJ), remained there throughout the duration of dosing and for at least 6 and 20 days after the last dose in chimpanzees X7 and X8, respectively, and then rebounded. Select samples were analyzed by transcriptional-mediated amplification (Bayer Diagnostics, Berkeley, CA); both chimpanzees were viral positive prior to viral rebound. The concentration of MK-0608 in plasma samples collected 4.5 h postdosing averaged 450 ± 140 nM (1). Isolation of viral RNA from the plasma of X7 from study days 4 and 49 and X8 from day 65 and genotyping via population sequencing (limit of detection approximately 20%) did not detect the presence of the S282T variant known to be resistant to MK-0608 (9). Thus, MK-0608 dosed at 2 mg/kg for 37 days produced a profound, durable antiviral response but did not result in a cure or sustained virological response (SVR) of viral negativity 6 months post-treatment.

The antiviral efficacy of the NS3 protease inhibitor MK-7009 (5) was assessed in chimpanzees X9 and X10 which were administered the compound orally as a suspension in chocolate milk at a dose level of 5 mg per kg twice daily for 7 consecutive days. As shown in Fig. 1B, circulating viral loads rapidly declined after initiation of dosing and fell below the LOQ by day 2 (chimpanzee X9) and day 5 (chimpanzee X10), corresponding to a >5-log10 decrease. Viral loads rapidly rebounded after dosing ended. Analysis of the viral genomic sequence from the day 2 sample from chimpanzee X10 with use of reverse transcription-PCR (RT-PCR) rescue and population sequencing revealed that an R155K variant of the NS3 gene was present as the major circulating viral species (5). In vitro analysis of the susceptibility of an R155K mutant in assays of protease activity revealed a 200-fold loss of inhibitory potency for MK-7009 (6), thus confirming that the virus was resistant to the compound. Despite the appearance of the resistant viral variant, viral titers continued to decline. Population sequencing of virus early in the rebound phase after dosing ended revealed that virus from both chimpanzees was predominantly the R155K variant. Over the course of the next several months the viral population reverted to the initial baseline population.

To determine whether combination dosing of MK-0608 and MK-7009 could result in SVR, three chimpanzees, X6, X11, and X12, all chronically infected with HCV, were codosed via oral administration of MK-0608 at a dose level of 2 mg/kg once daily and MK-7009 also by oral administration at a dose level of 5 mg/kg twice daily for 37 days. MK-0608 dosing then ended, and MK-7009 dosing continued for a total of 84 days. Chimpanzee X6 had previously been enrolled in a study with MK-0608 at 1 mg/kg (1); the other two chimpanzees had not previously been treated with either drug.
As shown in Fig. 2, the starting plasma viral titers of the three chimpanzees varied from 3,000 to 340,000 IU/ml (chronic HCV-infected patients have viral loads typically between $10^5$ and $10^7$ IU/ml). After administration of the combination of compounds was initiated, plasma viral titers in all three chimpanzees rapidly decreased to levels below the LOQ and remained there in all three chimpanzees throughout the period of coadministration of the compounds. After administration of MK-0608 had ended but before the end of dosing of MK-7009, the viral load in chimpanzee X11 became quantifiable by day 65 of the study. Analysis of the viral genomic sequence from the day 72 sample from chimpanzee X11 via RT-PCR rescue and population sequencing revealed the R155K variant of the NS3 gene was present as the major circulating viral species. Viral load in chimpanzee X12 remained below the LOQ throughout the dosing duration but then rebounded 21 days after the end of dosing. RT-PCR rescue of viral NS3 sequences from a day 105 sample and sequencing of the viral cDNA revealed that the major circulating species of chimpanzee X12 contained the R155K variation. Viral load in chimpanzee X6 remained below the LOQ of the assay throughout the duration of dosing and for at least 6 months after the end of dosing, thus demonstrating SVR. The dashed vertical lines represent the end of dosing of MK-0608 at day 37 and MK-7009 at day 84.

FIG. 1. Plasma viral loads in two HCV-infected chimpanzees. Plasma viral loads were assessed by the HCV qPCR assay (Roche, Nutley, NJ) with a lower limit of quantitation (LLOQ) of 20 (1.3 log_{10}) IU/ml dosed. The vertical dashed line represents the time of administration of the last dose, and the horizontal dashed line represents the LLOQ. Values that were below the LOQ are graphed as 1.3 log_{10} IU/ml. Viral detection by transcription-mediated amplification (TMA; Bayer Diagnostics, Berkeley, CA) where data are available is indicated. Shown are graphs representing dosages of 2 mg/kg MK-0608 orally once daily for 37 days for chimpanzees X7 (○) or X8 (□) starting at day 0 and ending at day 36 (A); or dosages of 5 mg/kg MK-7009 orally twice daily for 7 days for chimpanzees X9 (○) or X10 (□) dosed starting at day 0 and ending at day 6.5 (B). Plasma samples taken on day 5 were retested using the HCV TagMan assay, and HCV was detected in the repeated assays with values of 99 (1.99 log_{10}) and 328 (2.51 log_{10}) IU/ml in chimpanzees X9 and X10, respectively. The values depicted in the graph represent the results when the entire set of samples was evaluated in one run.

FIG. 2. Plasma viral loads before, during, and after coadministration of MK-0608 and MK-7009 to HCV-infected chimpanzees. MK-0608 was dosed orally at 2 mg/kg once daily for 37 consecutive days, and MK-7009 was dosed orally at 5 mg/kg twice per day for a total of 84 consecutive days to three chimpanzees infected with HCV genotype 1a as determined by a line probe assay (Versant HCV genotype assay [LiPa]; Bayer Diagnostics/Innogenetics) and confirmed by RT-PCR rescue of HCV genetic material and DNA sequencing. Plasma samples were collected periodically during the study, and viral titer was determined using the HCV TagMan assay (Roche) with a limit of quantitation of 20 IU/ml (1.3 log_{10} IU/ml). Circulating viral load rebounded in chimpanzee CBO X11 (○) during the monotherapy phase with MK-7009, at 21 days after the last dose of MK-7009 in chimpanzees X12 (□), but remained below the limit of quantitation in chimpanzee X6 (Δ) for at least 6 months after the end of dosing, thus demonstrating SVR. The dashed vertical lines represent the end of dosing of MK-0608 at day 37 and MK-7009 at day 84.
SVR, representing to our knowledge the first case of SVR due to administration of a combination of direct antiviral agents in the absence of interferon α. Relapse rates after SVR with interferon therapy are typically less than 10% (3), and analysis of a plasma sample drawn from this chimpanzee more than 3 years after study completion demonstrated that the viral load was still below the LOQ (data not shown). Improvements to the SVR rate will likely come from lengthening therapy, increasing the dose level of one or both compounds, utilizing combinations of other compounds, or some combination thereof. In this regard, results from the clinical testing of a combination of protease and nucleoside inhibitors (the INFORM Trial) are promising, with an average viral load reduction of 5.1 log among treatment-naïve patients who received 13 days of combination treatment (4). The ability of a combination of two direct antivirals to achieve SVR in an animal model of chronic HCV infection stands as a proof of concept of direct antiviral therapy without the need for interferon α to treat this important viral disease.

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REFERENCES