Interferon Alone or Combined with Ribavirin for Acute Prolonged Infection with Hepatitis C Virus in Chimpanzees

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Key Words

Chimpanzee · Hepatitis C virus · Interferon · Ribavirin

Abstract

Infection with hepatitis C virus (HCV) persisted for longer than 29 weeks in 2 chimpanzees after they had been inoculated with it experimentally. One of them (C-210) received short-term subcutaneous interferon- α (IFN- α) 6 million units (MU) daily for 7 days at week 29. He cleared HCV RNA from the serum and remained negative for it during 25 weeks after the withdrawal of IFN. The other (C-224) did not respond to 2 courses of a short-term IFN monotherapy at weeks 20 and 23. Twelve weeks thereafter, he received IFN- α 3 MU daily for 2 weeks and then 3 times a week for 14 weeks combined with oral ribavirin 600 mg daily during 16 weeks. HCV RNA disappeared from the serum and stayed negative until the last follow-up 24 weeks after the completion of combination therapy.

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Due to a very narrow species-specificity of hepatitis C virus (HCV), chimpanzees remain the only animal that can be infected with it. Once they served as the sole means

of identifying the infection with HCV that had been referred to as non-A, non-B hepatitis virus until its discovery in 1989 [1]. HCV infection can persist in chimps at rates ranging from 30 to 60%, depending on the age and gender as well as viral strains in inocula they have received [2, 3]; the persistence rate is comparable to that of 55–85% in humans [4, 5]. The long-term outcome of chimpanzees infected with HCV is not known, nor have there been any attempts to treat them with either interferon (IFN) alone or IFN in combination with ribavirin.

Two chimps with acute prolonged HCV infection received antiviral treatment. They were chimps No. 210 (male, 14 years old and weighing 62.8 kg) and No. 224 (male, 14 years old and weighing 59.1 kg). Both of them were kept in individual cages and received humane care, in accordance with all relevant requirements for the use of primates in an approved facility. Chimp No. 210 participated in the experimental transmission study for determining the minimum infectious dose of HCV [6]. He received 1 ml of fresh-frozen plasma from a donor in the window period of HCV infection with mixed genotypes (1b plus 2a) containing 7.0×10^6 copies/ml of HCV RNA. Chimp No. 224 was inoculated with 1 ml of fresh-frozen plasma from another donor in the window period of HCV infection with genotype 1b containing 8.4×10^6

C-210

IFN-α 6 MU daily for 1 week

HCV RNA

Anti-HCV (2^N)

HCV 7.0 × 10⁶ copies

ALT and AST (U/l)

Weeks after the inoculation

Weeks after the inoculation	ALT (U/l)	AST (U/l)	HCV RNA (copies)	Anti-HCV (2 ^N)
0	28	18	2.0	+
1	28	18	2.0	+
2	30	18	2.0	+
3	30	18	2.0	+
4	30	18	2.0	+
5	22	15	2.0	+
6	22	15	2.0	+
7	35	18	2.0	+
8	40	18	2.0	+
9	38	18	2.0	+
10	28	18	2.0	+
11	68	65	7.0 × 10 ⁶	2333
12	38	18	2.0	2333
13	30	18	2.0	2333
14	32	22	2.0	2333
15	35	22	2.0	2333
16	32	22	2.0	2333
17	25	18	2.0	2333
18	25	18	2.0	2333
19	25	18	2.0	2333
20	32	22	2.0	2333
21	32	22	2.0	2333
22	30	22	2.0	2333
23	30	22	2.0	2333
24	35	22	2.0	2333
25	25	25	2.0	2333
30	25	25	2.0	56
35	28	28	2.0	6
40	30	30	2.0	-
45	30	30	2.0	-
47	30	32	2.0	5
50	28	28	2.0	-
53	20	10	2.0	4
55	20	10	2.0	3

C-224

IFN- α 6 MU daily for 2 weeks

IFN- α 3 MU (daily, 2 weeks; tiw, 14 weeks)

Ribavirin 600 mg daily for 16 weeks

HCV RNA

Anti-HCV

HCV 8.4×10^6 copies

(●—●) ALT and (○—○) AST (U/l)

Weeks after the inoculation

the highest twofold dilution of serum (2^N) that induced hemagglutination. They received IFN- α (Sumiferon®, Sumitomo Pharmaceutical Co. Ltd., Tokyo, Japan) alone or in combination with ribavirin.

The 2 chimps were bled under anesthesia with ketamine hydrochloride weekly for the initial 21–24 weeks, and then at intervals until the completion of this study. HCV RNA was determined qualitatively by Amplinat MPX (Roche Diagnostics K.K., Tokyo, Japan). Antibody to HCV (anti-HCV) was determined semiquantitatively using a commercially available hemagglutination assay system (the second-generation HCV PHA, Abbott Japan, Co. Ltd., Tokyo, Japan), and the results were expressed by

whereupon the IFN monotherapy was discontinued. He stayed negative for HCV RNA until the last observation 25 weeks after the withdrawal of IFN monotherapy.

Figure 2 depicts the clinical course of chimp No. 224 who was inoculated with 8.4×10^6 copies of HCV of genotype 1b. HCV RNA was detected in his serum at week 1. HCV RNA stayed positive through 20 weeks, and he was considered to have developed persistent infection. IFN- α 6 MU was given daily for 7 days since the 21st week. Because HCV RNA was positive at the next examination, IFN monotherapy was given again during the 23rd week.

However, HCV RNA did not disappear from the serum after 2 courses of IFN monotherapy. At 36 weeks when HCV RNA was confirmed to be present in the serum, he received a combination therapy with IFN- α 3 MU, daily for 2 weeks and then 3 times a week for 14 weeks, along with oral ribavirin 600 mg daily in 2 divided doses. HCV RNA decreased 1 week after the institution of combination therapy, and became undetectable the next week; the loss of HCV RNA continued throughout the following 15 weeks on treatment. He was confirmed negative for serum HCV RNA at tests performed 4, 12 and 24 weeks, respectively, after the completion of combined IFN and ribavirin. Transaminase levels increased moderately 6 weeks after the initiation of combination therapy, but thereafter they returned to normal through the observation till 24 weeks after the completion of therapy. Chimp No. 224 did not respond to HCV infection by raising anti-HCV, and remained seronegative throughout 76 weeks since he received inoculation.

The biggest problem with HCV infection in human beings is its strong propensity to persist in up to 85% of individuals who contract it, although chances of persistence depend on sex, age and route of transmission [4, 5]. We have reported that HCV replicates very rapidly in chimpanzees inoculated with it at a doubling time of 6.3–8.6 h [7]; it is much shorter than that of HBV estimated at 1.9–3.4 days [8]. Such a fast replication velocity of HCV might contribute toward a high persistence rate after the primary infection; cellular immune responses to clear HCV may not be able to catch up with exponentially increasing population and rapidly evolving HCV quasispecies.

The sustained virological response to pegylated-IFN combined with ribavirin in patients with chronic hepatitis C remains insufficient; it is achieved in merely one half of the patients infected with HCV genotype 1 in a high viral load [9]. This stands in sharp contrast to the excellent efficacy of IFN on patients with acute prolonged hep-

atitis C [10]. Hence, we started treating 2 chimpanzees in whom acute infection with HCV had prolonged after they were experimentally transmitted with HCV [6, 7]. The preacute serum from one of them (chimp 210) served for illustrating the early dynamics of HCV infection, and provided blood centers with the standards of HCV RNA, containing defined copy numbers per milliliter, for calibrating nucleic acid amplification test (NAT).

Chimp 210 cleared HCV infection after he had received IFN- α 6 MU daily for 1 week (fig. 1). Chimp 224 failed to clear HCV after 2 courses of the IFN monotherapy. Thereafter, he responded to IFN 3 MU daily for 2 weeks followed by 3 times a week for 14 weeks in combination with oral ribavirin 600 mg daily. The virological response with loss of HCV RNA from the serum was achieved during treatment, and sustained 24 weeks after the completion of combination therapy (fig. 2). They both had kept HCV for 29 and 36 weeks before treatment, respectively, exceeding 6 months for the clinical definition of persistent infection. There remains a possibility, however, that chimp 210 may have been clearing HCV naturally without therapeutic intervention, in view of his remarkable response to a short-term IFN monotherapy. Chimp 210 was infected with HCV of genotype 1b and 2a, and chimp 224 with HCV of genotypes 1b. HCV of genotype 2a might have disappeared earlier than HCV of genotype 1b in chimp 210, in view of different sensitivity to IFN of these 2 HCV genotypes in clinical trials [11, 12].

We have shown that acute prolonged HCV infection can be cured in chimps if they receive IFN alone or combined with ribavirin soon enough after they have been infected, as in the treatment of acute hepatitis C in patients [10]. Hopefully, the efficacy of IFN with or without ribavirin would be extended in additional chimps with acute prolonged HCV infection after they have completed transmission studies. Furthermore, such treatments would need to be considered in many chimps who have acquired persistent HCV infection after experimental transmission during the long past.

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