Alerts, Notices, and Case Reports

Development of Hepatocellular Carcinoma After Clearance of Hepatitis C Virus With Interferon Therapy

MYRON J. TONG, PHD, MD
LILLIAN P. LAI, BS
KAORU MURAKAMI-MORI, PHD, MD
Pasadena, California

CHRONIC HEPATITIS C VIRUS (HCV) infection is the most common cause of cirrhosis and hepatocellular carcinoma in the US.[1] Recent studies have demonstrated the presence of HCV RNA in both cancerous and noncancerous liver tissue.[2,3] Whether HCV is directly oncogenic, however, remains to be elucidated.

Interferon-α is an established antiviral therapy for chronic HCV. Eradication of HCV from blood, liver, and peripheral blood mononuclear cells, as measured by sensitive molecular biological techniques, has been reported in long-term responders to interferon treatment.[4,5] It is generally assumed that eradication of HCV by antiviral therapy halts the progression of disease and therefore also prevents the clinical complications associated with this chronic viral infection. A recent case at our institution, however, has caused us to reevaluate this assumption.

Report of a Case

In May 1988, a 50-year-old man came to our clinic for evaluation of his chronic liver disease. He had a history of intravenous drug use that included an episode of acute hepatitis with jaundice approximately 30 years previously. A liver biopsy specimen obtained 8 months before the clinic visit showed marked distortion of the cyto-architecture resulting from periportal fibrosis; a moderate number of proliferating bile ductules; dense lymphocyte infiltration with erosion of the limiting plates; and thin fibrous septae surrounding small nodules of normal-appearing hepatocytes. The histologic diagnosis was chronic active hepatitis with postnecrotic cirrhosis. The patient's chief complaint was fatigue, and physical examination revealed hepatosplenomegaly. Laboratory tests performed at the time of his clinic visit showed that his alanine aminotransferase count was 295 U/l, his aspartate aminotransferase 424 U/l, albumin 3.6 mg/dl, alkaline phosphatase 77 U/l, and bilirubin 0.5 mg/dl, with a prothrombin time of 14.3 seconds (control 12.5 seconds). The patient's HBsAg, HBeAg, and anti-HBe were negative, his anti-HBc and anti-HBs positive, and his serum negative for hepatitis B virus (HBV) DNA.[6] He tested negative for hepatitis A virus (HAV) and nuclear antibody. In addition, his serum ceruloplasmin level, alpha-L-antitrypsin, ferritin, serum iron, and iron saturation were all normal. His initial serum sample proved to be positive for anti-HCV by multiple-antigen enzyme-linked immunosorbent assay (Chiron, Emeryville, CA), his HCV RNA tested positive on reverse transcriptase–polymerase chain reaction (RT-PCR) analysis,[6] and his HCV genotype was 1b.

Beginning in May 1991, interferon α-2b was administered at 3 million units three times a week for a period of 2 years, after which a sustained and complete normalization of serum alanine aminotransferase was achieved. Serum HCV RNA remained consistently negative. Liver ultrasonography findings and serum α-fetoprotein levels were normal from 1988 until April 1995. In October 1995, approximately 2 years after cessation of interferon α-2b therapy, the patient's α-fetoprotein level became elevated for the first time, to 56 ng/ml; in addition, liver ultrasonography and computerized tomography (CT) scanning both showed a 3.5-cm lesion in the lateral segment of the left lobe of the liver. The patient underwent a left-lateral segmentectomy and recovered from the surgery. Microscopic examination of the noncancerous liver tissue showed the presence of numerous nodules of hepatocytes surrounded by fibrous septae. There was minimal portal lymphocyte infiltration, and a broad band of connective tissue separated the cirrhotic nodules from a trabecular type of hepatocellular carcinoma.

Serum samples from before and after interferon treatment and approximately 50 mg of fresh cancerous and noncancerous liver tissue obtained immediately after surgical removal were processed for measurement of HCV RNA by RT-PCR, as previously described.[6,7] Identical tissue samples aseptically cut into small pieces were frozen at –70°C and similarly processed at three other time intervals to check the reproducibility of the procedure. Serum extraction and RT-PCR phases were performed simultaneously with a set of both negative and positive serum controls. The negative control serum was obtained from a healthy person whose anti-HCV antibody test was negative, and the positive control serum from a patient with chronic hepatitis C who repeatedly tested positive for HCV RNA by RT-PCR. The negative liver tissue control sample was obtained from an anti-HCV antibody–negative patient, and the positive control sample from an anti-HCV–positive and serum HCV RNA–positive patient with chronic hepatitis C. Both control tissue samples were frozen at –70°C before use. As seen in Figure 1, the control serum and liver tissue from the anti-HCV–negative patient tested negative for HCV RNA by PCR (lanes 2 and 6), where-

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From the Liver Center and Institute of Molecular Medicine, Huntington Memorial Hospital, Pasadena, California.

Reprint requests to Myron J. Tong, PhD, MD, Liver Center, Huntington Memorial Hospital, 744 Fairmount Ave, Suite 1, Pasadena, CA 91105.

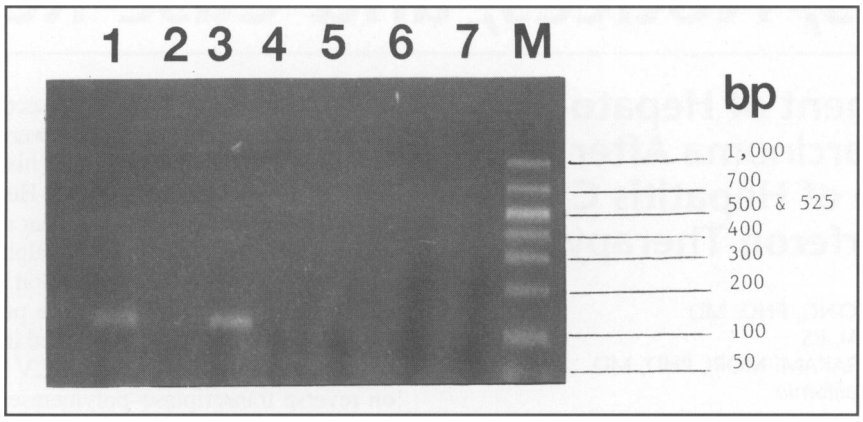

Figure 1.—Detection of HCV RNA by RT-PCR in serum and liver tissue samples obtained from patient. Lane 1 = positive serum control; lane 2 = negative serum control; lane 3 = serum before interferon treatment; lane 4 = serum after interferon treatment; lane 5 = positive liver tissue control; lane 6 = negative liver tissue control; lane 7 = noncancerous liver tissue; lane 8 = cancerous liver tissue; M = marker; bp = base pairs.

as the anti-HCV–positive control serum and liver tissue from the patient with chronic hepatitis C tested positive for HCV RNA (lanes 1 and 5). Furthermore, the patient's pre–interferon treatment serum sample was positive for HCV RNA but became persistently negative after cessation of therapy (lanes 3 and 4). Repeated RT-PCR tests for HCV RNA of cancerous and noncancerous liver tissue obtained in this patient during surgery were consistently negative (lanes 7 and 8).

In addition, approximately 50 mg of frozen cancerous and noncancerous liver tissue was tested for the presence of hepatitis B virus DNA. Each tissue sample was homogenized in DNAzol reagent (Molecular Research Center, Cincinnati, OH), and the hepatitis B virus DNA was amplified by a nested PCR procedure, as previously described.[6] For the positive tissue control, we used a frozen sample of liver tissue obtained from an HBsAg- and HBeAg-positive patient. The negative liver tissue control was obtained from a person who tested negative for HBsAg, hepatitis B core antibody, and hepatitis B surface antibody. The positive control serum was obtained from a patient whose HBsAg and HBeAg tests were positive, and the negative serum control from a person who tested negative for HBsAg and hepatitis B core and hepatitis B surface antibodies. As seen in Figure 2, the control serum and the control liver tissue were negative for HBV DNA (lanes 4 and 2), whereas the HBsAg-positive control serum and liver tissue were both positive for HBV DNA (lanes 3 and 1). The patient's cancerous and noncancerous liver tissue as well as his serum were negative for HBV DNA (lanes 5, 6, and 7).

Discussion

Chronic HCV infection is the most common cause of cirrhosis and hepatocellular carcinoma in many coun-

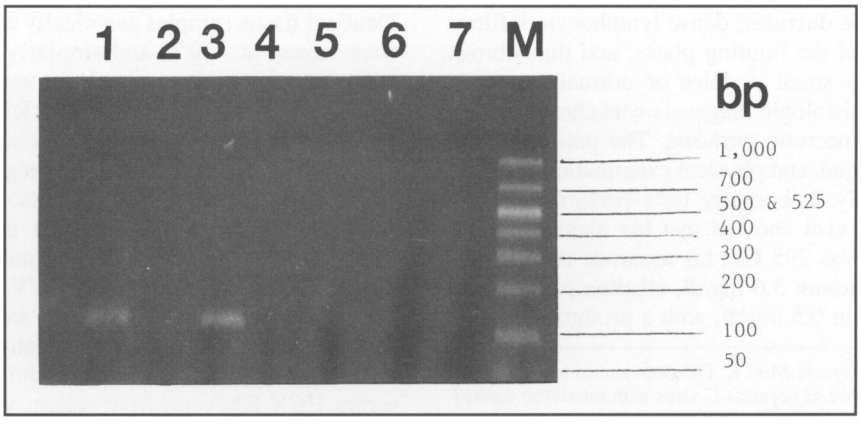

Figure 2.—Detection of HBV DNA by RT-PCR in serum and liver tissue samples. Lane 1 = positive liver tissue control; lane 2 = negative liver tissue control; lane 3 = positive serum control; lane 4 = negative serum control; lane 5 = cancerous liver tissue; lane 6 = noncancerous liver tissue; lane 7 = serum; M = marker; bp = base pairs.

tries.[1] In our recent report on hepatocellular carcinoma in patients positive for anti-HCV, we observed that 92% of cases occurred in association with cirrhosis.[8] The mechanisms of hepatocarcinogenesis in patients with chronic hepatitis C are unknown, and unlike HBV, HCV is not integrated into the genome of the human host. In addition, HCV has not been shown to activate specific oncogenes or inactivate tumor suppressor genes.[9] Chronic HCV infection is an independent risk factor for hepatocellular carcinoma in that it induces cirrhosis. It may also cause hepatocellular carcinoma in patients with already established cirrhosis.[10,11] Thus, it may have been the stimulus to liver regeneration that predisposed our patient to the eventual appearance of his tumor. This may also be the case in primary liver cancers that arise in patients with alcoholic liver disease.

In our patient, elimination of HCV after interferon therapy did not prevent the eventual development of hepatocellular carcinoma. This finding is similar to that of a recent report on a 60-year-old man with chronic HCV and cirrhosis who was successfully treated with lymphoblastoid interferon-α; in this patient, as well, a small hepatocellular carcinoma developed 1 year after cessation of treatment.[12] Our patient's medical history included a bout of acute hepatitis 30 years earlier, although it is not known whether the episode represented acute hepatitis B or C. Hepatitis B serologies in this HBsAg-negative patient were positive for both anti-HBs and anti-HBcs, indicating that he was immune to HBV. Furthermore, a "latent" hepatitis B infection was not likely, since his serum was negative for HBsAg, HBeAg, and anti-HBe, as well as for HBV DNA. In addition, the nontumor cirrhotic tissue and the hepatocellular carcinoma tissue obtained in our patient during hepatic resection in 1995 were both negative for HBV DNA. These findings are in accordance with a previous report in which it was found that HBV DNA was negative in both the serum and liver of HBsAg-negative patients with chronic hepatitis who were positive for both anti-HCV and hepatitis B antibodies.[13]

Two recent studies have indicated an association between treatment of chronic HCV infection with interferon and a decreased incidence of hepatocellular carcinoma in those patients who responded to treatment. In one randomized trial in Japan, 4% of HCV patients with cirrhosis who were treated with interferon developed hepatocellular carcinoma during a mean follow-up period of 4.4 years, compared with 38% of untreated controls.[14] In the two patients treated with interferon who developed hepatocellular carcinoma, therapy was considered ineffective because of persistently elevated alanine aminotransferase levels and positive HCV RNA tests. In another study, 3% of HCV patients with cirrhosis who were treated with interferon therapy developed hepatocellular carcinoma during a mean follow-up period of 4.1 years, compared with 10% of untreated patients.[15] In the latter study, hepatocellular carcinoma developed only in patients who did not respond to interferon treatment. These findings imply that clearance of HCV after interferon therapy significantly reduces the probability that hepatocellular carcinoma will occur. The present case, however, indicates the contrary: in our patient, hepatocellular carcinoma occurred even though HCV RNA was successfully cleared from both serum and liver after treatment with interferon. Thus, in some patients with chronic HCV and cirrhosis, elimination of HCV from serum and liver may not prevent the eventual development of hepatocellular carcinoma. Therefore, continual screening for this malignancy by α-fetoprotein testing and liver ultrasonography is necessary in all patients with HCV and cirrhosis.

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