Effect of Interferon Therapy on Radionuclide Imaging in Chronic Liver Diseases Due to HCV Infection

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Interferon (alpha-IFN) exerts a modulating effect on the immune system. Kupffer cells of the liver play an important immunological role by their uptake of various agents and particles, including colloids. We sought to discover if alpha-IFN could enhance the colloid uptake function of the Kupffer cells. The effect of alpha-IFN therapy on radioisotope scans of the liver was studied in 20 patients with chronic liver disease due to hepatitic C virus (HCV) infection who received therapy at a dose of 3 million IU for 6 mo, in another 20 patients who received the same therapy for 12 mo and in matched control groups (10 patients with HCV infection for each study group) who did not receive alpha-IFN. **Methods:** A ^{99m}Tc-sulfur colloid scan of the liver was obtained for each group before and after therapy and, for control subjects, at the start and end of the study periods. The liver-to-spleen geometric mean ratio of colloid uptake was assessed. **Results:** In the first study group, the mean rate of improvement in the liver-to-spleen ratio was 48% in 70% of patients, compared to 8% in 20% of controls ($p < 0.05$). In the second study group, mean liver-to-spleen ratio was 88% in 85% of patients, compared to 12% in 40% of controls ($p < 0.001$). **Conclusion:** Alpha-IFN therapy appears to enhance the colloidal uptake function of Kupffer cells, which adds a new dimension to the immunomodulatory effect of interferon.

Key Words: interferon; technetium-99m-sulfur colloid; hepatitis C

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Radioisotope scanning is an established method of diagnosing chronic liver diseases (*1*). Chronic liver disease due to hepatitis C virus (HCV) is associated with generalized decrease in isotope uptake by the liver and increase by the spleen and bone marrow (*2*). Scintigraphically, a decrease in the liver-to-spleen geometric mean ratio of colloid uptake reflects impaired reticuloendothelial extraction of colloid by the diseased liver and enhanced uptake by the spleen. Computerized and semiquantitative methods have demonstrated such liver-to-spleen ratios in patients with fatty liver and chronic hepatitis and in those with cirrhosis (*3*).

Reticuloendothelial cells are distributed uniformly within the liver and spleen, with approximately the same number of cells per gram of tissue in each organ. Since the liver mass in adults is normally 8 to 10 times that of the spleen, the distribution of intravenously injected colloid is roughly 90% to the liver and 10% to the spleen, with a small fraction entering bone marrow (*4*). The type of colloid and the range of its particle size, however, affect relative organ distribution.

The only therapy proven effective in treating chronic HCV is the antiviral agent interferon (alpha-IFN), which showed transient normalization of liver enzymes in preliminary reports (*5,6*). In this study, we sought to evaluate the effect of alpha-IFN therapy on hepatic imaging in patients with chronic liver disease due to HCV infection.

METHODS

Patients

Sixty patients were enrolled in this study. They all had chronic liver disease caused by HCV infection, as proven by liver biopsy and viral markers. All patients were given a complete medical history. Emphasis was placed on history of blood transfusions, surgical procedures and clinical examinations of the liver and spleen. Laboratory investigations included liver function tests [serum bilirubin (total and direct) and serum transaminases (ALT and AST)] and viral markers for HCV antibodies and HCV RNA (*7*). Abdominal ultrasonography to evaluate the condition of the liver and spleen was performed and radioisotope scanning and liver-to-spleen ratio assessments were performed for all patients at the beginning and end of the study periods. Finally, needle liver biopsies were also performed on all patients.

The patients were divided into four groups:

- Group 1 was comprised of 20 patients who were given alpha-IFN therapy, 3 million units subcutaneously 3 times per week for 6 mo.
- Group 2 was comprised of 10 patients who served as controls for the first study group and were not given alpha-IFN therapy. Group 2 patients were matched with Group 1 according to age, sex and clinical and biochemical data. Initially and 6 mo after the therapy, isotopic scanning was performed on both Groups 1 and 2 and liver-to-spleen ratios were assessed.

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- Group 3 was comprised of 20 patients who were given alpha-IFN therapy, 3 million units subcutaneously 3 times per week, for 12 mo.
- Group 4 consisted of 10 patients who served as controls for the third study group and were not given alpha-IFN therapy. Groups 3 and 4 were matched for age, sex, and clinical and biochemical data. Initially and 12 mo after therapy, both groups had radioisotope scanning and liver-to-spleen ratios were determined.

During the therapeutic course, each patient's leukocyte and platelet counts were tested every 2 wk. If the leukocyte count fell below 300/cm^3 or the platelet count fell below 50,000/cm^3, alpha-IFN therapy was suspended until the count rose above these levels.

Scintigraphic Technique

Patients fasted overnight to produce standard physiological conditions because digestion significantly affects intestinal blood flow (*2*).

Imaging was performed with subjects supine beneath a large field of view rectangular gamma camera. The liver, spleen and heart were included in the anterior image. An injection of 4 mCi (150 MBq) ^{99m}Tc-sulfur colloid was administered intravenously as a rapid bolus. An online computer system was used to acquire dynamic anterior data which commenced with the start of bolus injection. Data were recorded in a 64 × 64 matrix as a set of 80 frames for 0.5 sec each.

Anterior and posterior static images of the liver and spleen were acquired 15 min postinjection with a preset time of 60 sec per view. Regions of interest (ROIs) were drawn on a summed image of the initial dynamic study to outline the heart and lung bases. These areas were applied on both anterior and posterior static scans to avoid overlapping of these structures with the activity in the liver and spleen. ROIs were drawn around the outlines of the liver and spleen on the anterior and posterior static images, avoiding overlap with the predrawn cardiac and pulmonary areas. This was particularly useful in patients with marked delay of colloid clearance from the circulation. For the few patients in whom there was overlap between the spleen and left lobe of the liver, the area of possible overlap was excluded when the hepatic and splenic ROIs were drawn. The organ counts were then obtained and the liver-to-spleen geometric mean ratio of liver-to-spleen uptake was calculated by the following formula (*8,9*):

$$L/S = \sqrt{\frac{\text{anterior liver count} \times \text{posterior liver count}}{\text{anterior spleen count} \times \text{posterior spleen count}}}.$$

Data Analysis

Data were statistically analyzed using a personal computer supplied with SAS statistical software. Raw data distribution showed no significant variance for all groups; thus, the Student's t-test was used. The Fisher exact test was used to compare proportions, since the expected value of two cells in Table 2 was less than five.

Calculation of the rate improvement, i.e., percentage of improvement or worsening of the liver-to-spleen ratio, was done for each patient according to the following equation:

$$\frac{\text{L/S ratio after IFN therapy} - \text{L/S ratio before IFN therapy}}{\text{L/S ratio before IFN therapy}} \times 100.$$

A positive result indicated improvement and a negative sign implied worsening of the liver-to-spleen ratio. The rate of improvement or worsening of the liver-to-spleen ratio was similarly determined for the control groups.

TABLE 1
Comparison of Liver-to-Spleen (L/S) Ratios in Groups 1–4 at the End of the Study Periods

L/S ratio	Range	Mean + s.d.	p
Study Group 1 (n = 20)	4.1–0.99	1.94 + 0.74	>0.05
Control Group 2 (n = 10)	1.75–0.25	0.81 + 0.36	
Study Group 3 (n = 20)	5.2–0.70	2.46 + 1.25	<0.05
Control Group 4 (n = 10)	2.57–0.18	1.63 + 0.38	

RESULTS

A beneficial effect of IFN therapy on colloid liver scans was observed. After 6 mo of therapy, there was an insignificant difference in the mean liver-to-spleen ratio between Groups 1 and 2 ($p > 0.05$). After 12 mo of therapy, there was a significant difference in the mean liver-to-spleen ratio between Groups 3 and 4 ($p < 0.05$) (Table 1). Analysis of the rate of improvement in the liver-to-spleen ratio in all four groups, however, demonstrated that 70% of Group 1 patients showed improvement compared to 20% of Group 2 controls, with a statistical significance of $p < 0.05$ (Table 2). The improved liver-to-spleen ratio in Group 1 ranged from 1% to 143% (mean, 48% improvement), while that in Group 2 was from 7% to 9% (mean, 8% improvement). The difference in the mean rates of improvement was also significant ($p < 0.05$) (Table 2). Six patients with cirrhosis in Group 2 (30%), however, showed worse liver-to-spleen ratios after alpha-IFN therapy.

After 12 mo of alpha-IFN therapy, 85% of patients in Group 3 showed improved liver-to-spleen ratios compared to 40% in Group 4 and the difference in these proportions was highly significant ($p < 0.001$). The improved liver-to-spleen ratio in Group 3 ranged from 1% to 393% (mean, 88% improvement), while that in Group 4 was from 5% to 25% (mean, 12% improvement). The difference of the mean values was also highly significant ($p < 0.001$) (Table 2). Three patients with cirrhosis (15%) in Group 3 showed worse liver-to-spleen ratios after alpha-IFN therapy.

Interestingly, in both study groups all patients who showed an improved liver-to-spleen ratio simultaneously had normalized transferase levels.

DISCUSSION

The presence of intrahepatic porto-system shunts and reduction in the number and/or impaired function of Kupffer cells are regarded as the main cause of extrahepatic colloid shift to the spleen (*4*). True depletion of

TABLE 2
Rate of Improvement and Worsening of Liver-to-Spleen Ratios Compared in All Groups

Group no.	Improved L/S ratio			Worse L/S ratio			P† value
	No (%) of patients	Range	X*	No (%)	Range		
1	14 (70)	1%–143%	48%	6 (30)	−5% to −51%	−29%	<0.05
2	2 (20)	7%–9%	8%	8 (80)	−3% to −73%	−23%	
3	17 (85)	1%–393%	88%	3 (15)	−4% to −12%	−8%	<0.001
4	4 (40)	5%–25%	12%	6 (60)	−3% to −67%	−28%	

*Range and arithmatic mean (X) of the rate of improvement and worsening of the liver-to-spleen ratio from the start to the end of study periods.
†Fisher exact test was used when comparing the proportions of different groups. Student's t-test was used for comparison of arithmetic means.

Kupffer cells occurred in patients with chronic liver disease (*10*), and decreased functional capacity of Kupffer cells was observed in patients with alcoholic liver cirrhosis (*11*).

In this study, alpha-IFN administered for 6 mo produced a 48% mean rate of improvement of the liver-to-spleen ratio on colloid scans in 70% of patients compared to 8% improvement in 20% of the controls who did not receive alpha-IFN. The findings were even more striking in patients who received alpha-IFN for 12 mo. Here the mean rate of improved liver-to-spleen ratio was 88% in 85% of patients compared to 12% improvement in 40% of the controls ($p < 0.001$). These results, besides reflecting the potential value of the rate of change in liver-to-spleen ratios in monitoring response to alpha-IFN therapy, indicate that alpha-IFN probably enhances Kupffer cell function.

Our data showed that there was no significant difference in the mean liver-to-spleen ratio between patients who received alpha-IFN and those who did not ($p > 0.05$) 6 mo after therapy. When the rate of liver-to-spleen ratio change was studied in both groups at the start and end of this period, the difference was significant ($p < 0.05$). After 12 mo of therapy, the difference was significant at a much higher statistical level ($p < 0.001$). These results indicate that comparing the liver-to-spleen ratio rate of change between various groups is a more informative tool than a mere comparison of liver-to-spleen ratio values at one time point. These findings agree with previously published results (*9*).

The mechanism whereby alpha-IFN therapy improves the liver-to-spleen ratio of colloid uptake is not clear. The most conceivable mechanism is enhancement of Kupffer cell function at phagocytosis. In this respect, it has been reported that the failure of C3H/HeJ macrophages to phagocytose immunoglobulin G-coated sheep erythrocytes was fully restored by the addition of exogenous IFN to the culture system (*12,13*). Confirmation of such an effect would add a new dimension to the immunomodulatory effect of interferon.

CONCLUSION

The liver-to-spleen ratio determined from colloid liver scans is a useful indicator of the response of patients with HCV infection to alpha-IFN therapy. The latter probably enhances the phagocytic function of Kupffer cells.

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