Normalization of Elevated Hepatic 8-Hydroxy-2'-Deoxyguanosine Levels in Chronic Hepatitis C Patients by Phlebotomy and Low Iron Diet

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ABSTRACT

Accumulation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in DNA, which may result from the continuous reactive oxygen species (ROS) generation associated with chronic inflammation, has been reported in various human preneoplastic lesions and in cancers. However, no direct causative relationship between the 8-OHdG formation and carcinogenesis has been thus far demonstrated in humans. Directly proving the causality requires showing that depletion of 8-OHdG levels in tissue by interfering with ROS generation results in a reduction in cancer. Chronic hepatitis C virus (HCV) infection is associated with a high risk of hepatocellular carcinoma (HCC). Several studies on patients with chronic HCV have shown that hepatic iron overload is attributable to liver injury and that iron depletion improved serum aminotransferase levels. Excess iron is known to generate ROS within cells, which causes mutagenic lesions, such as 8-OHdG. In this study, therefore, we have evaluated whether therapeutic iron reduction (phlebotomy and low iron diet) with a long-term follow-up (6 years) would decrease the hepatic 8-OHdG levels and the risk of HCC development in patients with chronic HCV. Patients (34) enrolled were those who had undergone standard IFN therapy but had no sustained response. Quantitative immunohistochemistry using the KS-400 image analyzing system and electrochemical detection was used for 8-OHdG detection. With this treatment, elevated hepatic 8-OHdG levels in patients with chronic hepatitis C (8.3 ± 4.6/10^6 dG) significantly decreased to almost normal levels (2.2 ± 0.9/10^6 dG; P < 0.001) with concomitant improvement of hepatitis severity, including fibrosis, whereas HCV titers were unaffected. None of these patients developed HCC. Thus, long-term iron reduction therapy in patients with chronic hepatitis C may potentially lower the risk of progression to HCC.

INTRODUCTION

Chronic inflammation is tightly associated with the continuous generation of ROS, which causes various types of tissue damage (1, 2). Damaging events at the molecular level underlying these various types include lipid peroxidation, degradation of proteins or sugars, strand breakage of DNA, and formation of 8-OHdG (3–5). Through types include lipid peroxidation, degradation of proteins or sugars, strand breakage of DNA, and formation of 8-OHdG. In this study, therefore, we have evaluated whether therapeutic iron reduction (phlebotomy and low iron diet) with a long-term follow-up (6 years) would decrease the hepatic 8-OHdG levels and the risk of HCC development in patients with chronic HCV. Patients (34) enrolled were those who had undergone standard IFN therapy but had no sustained response. Quantitative immunohistochemistry using the KS-400 image analyzing system and electrochemical detection was used for 8-OHdG detection. With this treatment, elevated hepatic 8-OHdG levels in patients with chronic hepatitis C (8.3 ± 4.6/10^6 dG) significantly decreased to almost normal levels (2.2 ± 0.9/10^6 dG; P < 0.001) with concomitant improvement of hepatitis severity, including fibrosis, whereas HCV titers were unaffected. None of these patients developed HCC. Thus, long-term iron reduction therapy in patients with chronic hepatitis C may potentially lower the risk of progression to HCC.

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2 To whom requests for reprints should be addressed, at Fourth Department of Internal Medicine, Sapporo Medical University School of Medicine, South-1, West-16, Chuo-ku, Sapporo 060-8543, Japan. Phone: 81-11-611-2111; Fax: 81-11-612-7987; E-mail: niitsu@sapmed.ac.jp.

3 The abbreviations used are: ROS, reactive oxygen species; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; ALT, alanine transaminase; HIC, hepatic iron concentration; HAI, hepatitis activity index; dG, deoxyguanosine; TNF, tumor necrosis factor.

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designed to consist of phlebotomy and a strict low-iron diet under the direction of a dietitian.

MATERIALS AND METHODS

HCV Patients and Therapy Protocol. This study was approved by the Human Ethics Committee of the Sapporo Medical University School of Medicine. Patients (45) with chronic hepatitis C, who were routinely visiting the Liver Unit of our department between January 1993 and December 1994, provided written informed consent for iron reduction therapy after having met the following criteria: (a) adult (≥20 years of age) with detectable anti-HCV antibodies and HCV-RNA; (b) those who had been treated previously with a standard 6-month IFN therapy (six MU IFN-α given daily for 2 weeks followed by three times weekly for 24 weeks) but had no sustained response; (c) those who showed persistent elevated serum ALT levels (≥80 IU/liter for >6 months) at least 1 year after IFN treatment; (d) those with histopathological evidence of chronic hepatitis without cirrhosis on liver biopsy; (e) those testing negative for antinuclear antibody, smooth muscle antibody, antimitochondrial antibody, or hepatitis B surface antigen; and (f) those with no habitual drug or alcohol use, anemia (hemoglobin <11 grams/dl), or iron deficiency (baseline serum ferritin <12 μg/liter or transferrin saturation <15%). Instead, 80 IU/liter for

However, of these 44 HCV patients [5 men and 3 women; mean (±SD) age, 51.4 ± 9.1 years] subsequently declined to receive the treatments. The remaining 37 patients [20 men and 17 women; mean (±SD) age, 53.4 ± 12.1 years] were enrolled in the iron reduction study, but 3 of them prematurely withdrew because of a change of residence to a distant location (2 patients) or noncompliance (1 patient).

Therapeutic iron reduction for patients with chronic hepatitis C was carried out based on phlebotomy combined with regulation of dietary iron intake, as it is known that iron absorption increases reciprocally in an iron-deficient state (29). At the initial phase of iron depletion, all patients underwent weekly phlebotomy of 200 ml until a state of mild iron deficiency was achieved (defined by either <10 μg/liter serum ferritin and/or 11 grams/dl blood hemoglobin concentration). The mild iron deficiency state was maintained by additional phlebotomies during the study period; patients were followed up at regular intervals of ≥4 weeks for the duration, and a phlebotomy (200 ml) was performed if the hemoglobin level exceeded 11 grams/dl. In addition, all subjects were advised to reduce their intake of iron-rich foods during the intervention and instructed both p.o. and in writing by a registered dietitian (S. K.). To aid with compliance, each subject was given a comprehensive list of iron-rich foods, which they were to avoid, and instructions on how to complete dietary records, which required a listing of all food and drink consumed over a 3-day period once every 3 months throughout the intervention. The subjects were not required to alter their total caloric intake but were expected to replace their iron-rich foods with appropriate substitutes. All patients allocated to the low iron diets were instructed to replace their iron-rich foods with appropriate substitutes. All patients allocated to the low iron diets were instructed to reduce their consumption of beans, shellfish, green vegetables, meat, and seaweed and replace them with refined carbohydrates. Dietary energy (1900–2000 kcal/day), nutritional balance, and iron intake (5–7 mg/day) during study periods were assessed from the dietary records by using the nutrition analyzing software Win Kenkoukun-III (Hokenjyouhou Co, Chiba, Japan). We performed liver biopsies at the end of the initial iron reduction phase (5–16 weeks after commencing treatment) and at the end of treatment (after 6 years). For the surveillance of HCC, all subjects underwent ultrasound examination and/or computed tomography every 3–4 months during the study period.

Laboratory Tests. Complete blood cell counts, iron studies (serum iron, ferritin, and transferrin saturation), and biochemical examinations, including serum ALT, were determined using automated procedures in the clinical laboratories of Sapporo Medical University Hospital at every visit. Serum HCV-RNA level was determined by reverse transcriptase-PCR using a commercial kit (Amplicor HCV, Roche Diagnostics, Branchburg, NJ), and genotyping was performed on PCR products using a second-generation reverse hybridization, line-probe assay (Inno-LiPA HCV-II, Innogenetics, Zwijndrecht, Belgium).

Tissue Samples. Ultrasound-guided percutaneous needle liver biopsy was performed using Monopry needles (16-gauge, 22-mm length; C. R. Bard, Inc., Covington, GA). Each fresh biopsy specimen was divided into three portions: two small and one large (5, 5, and 12 mm, respectively). The large portion (12-mm length) was formalin fixed, paraffin embedded, and submitted to histological evaluation and immunohistochemical analysis. The two small portions (5-mm length) of each specimen were used for determination of HIC and electrochemical detection of 8-OHdG. Similarly, from patients with HCC (n = 21) and metastatic liver cancer (n = 8) who had undergone surgical resection, HIC and histological normal liver specimens, which had been formalin fixed and paraffin embedded, were also submitted for immunohistochemical analysis of 8-OHdG.

Histopathological Grading. Formalin-fixed and paraffin-embedded liver tissue sections were subjected to histological evaluation by light microscopy, including staining by H&E. Each section was reviewed by two independent pathologists (T. I. and H. I.), who were unaware of the clinical outcome or sequence of the liver tissues, under blind fashion. Hepatic inflammation and fibrosis were scored based on Knodell’s HAI (30).

HIC. HIC was determined by using atom-absorption spectrophotometry as described previously (24). The results are expressed as μg/g dry weight of liver tissue (normal range, 250–1100 μg/g).

Immunohistochemical Determination of Hepatic 8-OHdG. Immunohistochemical analysis for formalin-fixed, paraffin-embedded tissue samples was performed using an avidin-biotin-peroxidase complex technique after microwave antigen retrieval as described previously (31). Sections (4 μm) were successively treated with blocking solution, 1 μg/ml anti-8-OHdG monoclonal antibody (Nikken Foods Co., Fukuroi, Japan) or normal mouse IgG (DAKO, Glostrup, Denmark), biotinylated secondary antibody, and a peroxidase-avidin complex (ABC-kit; Vector Laboratories, Burlingame, CA). The intensity of 8-OHdG immunostaining in the sections was assessed by using an AxiolCam photomicroscope and the KS-400 image analyzing system (Carl Zeiss Vision GmbH, Hallbermoss, Germany). A microscopic image of each liver section was imported into the KS-400 image analyzing program and represented by the ratio to that of each sequential section immunostained by normal mouse IgG.

Electrochemical Measurements of Hepatic 8-OHdG Levels. The 8-OHdG levels in the liver DNA were determined by the method of Shimoda et al. (25). To prevent oxidation by air exposure, all solutions and instruments that came in contact with the tissue specimen were filled with argon gas. High molecular DNA was purified from each 5 mm of fresh liver biopsy specimen using a DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Each extracted DNA sample was treated with nuclease P1, alkaline phosphatase and filtrated through an Ultra Free Centrifugal Filter (Millipore Co., Bedford, MA), and total DNA was subjected to electrochemical detection of 8-OHdG via an Ultra Free Centrifugal Filter (Millipore Co., Bedford, MA), and the amount of 8-OHdG was calculated from the absorbance at 290 nm, and the 8-OHdG levels were represented as the number of 8-OHdG per 105 dG in DNA (normal range, 0.8–3.1/105 dG).

Statistical Analysis. Quantitative values are expressed as means ±SD or median (range). Each data set was first evaluated for normality of distribution by the Kolmogorov-Smirnov test to decide whether a nonparametric rank-based analysis or a parametric analysis should be used. Two groups were compared by either the Wilcoxon signed-rank test or the Student’s t test. Multiple groups were compared by the rank-based, Kruskal-Wallis ANOVA test followed by Dunn’s test for differences among groups or standard ANOVA followed by Dunn’s test for multiple comparisons against a control group. The correlations between variables were examined by simple or multiple linear regression analysis or Spearman’s coefficient of rank correlation test. P values of <0.05 were considered to indicate statistical significance.

RESULTS

Frequency of Phlebotomy and Amount of Daily Iron Intake in Therapy-completed Patients. All 34 patients who completed this therapy showed no serious complications except for slight thinning of the fingernails. The median number of phlebotomies required and the volume of blood removed to achieve the initial iron-deficient state were 11 times (range, 5–16 times) and 2.2 liters (range, 1–3.2 liters), respectively.
HIC; the mean maintain the low hepatic iron state as determined by serum ferritin additional phlebotomies (median 0.6 times/month; range, 0.08–1.159 g/dl during the maintenance phase (Table 1 and Fig. 2). The globin levels were significantly reduced, keeping between 11 and 10 g/liter by the end of the treatment. However, serum HCV-RNA levels remained unchanged during the course of this study (Table 2). When the relationship between HIC and hepatic 8-OHdG levels was analyzed in these patients, a significant correlation (R² = 0.567, P < 0.001) was observed. A positive correlation was also evident in between serum ALT levels and hepatic 8-OHdG levels (R² = 0.518, respectively. During the maintenance phase, all patients required additional phlebotomies (median 0.6 times/month; range, 0.08–1.159 g/dl during the maintenance phase (Table 1 and Fig. 2). The globin levels were significantly reduced, keeping between 11 and 10 g/liter by the end of the treatment. However, serum HCV-RNA levels remained unchanged during the course of this study (Table 2). When the relationship between HIC and hepatic 8-OHdG levels was analyzed in these patients, a significant correlation (R² = 0.567, P < 0.001) was observed. A positive correlation was also evident in between serum ALT levels and hepatic 8-OHdG levels (R² = 0.518, respectively.

### Table 1 Changes of iron-related parameters in patients with chronic hepatitis C before and after iron reduction therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (n = 34)</th>
<th>End point (n = 31)</th>
<th>Normal range in adults</th>
<th>p&lt;sub&gt;0&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (grams/dl)</td>
<td>13.9 ± 1.1</td>
<td>10.7 ± 0.1</td>
<td>11.1–16.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum iron (μg/dl)</td>
<td>160.3 ± 25.7</td>
<td>28.0 ± 9.4</td>
<td>60–140</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum ferritin (μg/liter)</td>
<td>258.9 ± 266.4</td>
<td>7.1 ± 4.7</td>
<td>18–250</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HIC (μg/gram liver)</td>
<td>1,159 ± 576</td>
<td>527 ± 279</td>
<td>250–1,000</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Values represent mean ±SD or median (range).
* Comparisons are between variables at baseline and variables at end point.
* Reference normal range was listed.
* HIC was determined in 22 liver samples in which paired tissues were available.
* HIC in the normal liver was determined in eight liver samples.

### Biochemical Parameters during Iron Reduction Therapy

Before treatment, serum ferritin, serum iron, and HIC were high in patients with chronic hepatitis C (Table 1). As expected, mean hemoglobin levels were significantly reduced, keeping between 11 and 10 grams/dl during the maintenance phase (Table 1 and Fig. 2). The mean ±SD values of serum iron and serum ferritin were also significantly decreased after the 6-year treatment (P < 0.001). At baseline, all patients had elevated serum ALT concentrations (150.8 ± 72.9 IU/liter; range, 62–353 IU/liter). The mean ±SD serum ALT levels significantly decreased (P < 0.001) after the initial iron reduction phase. The levels further decreased, and by the end of the 6-year treatment, they were able to be maintained (P < 0.001; Table 2 and Fig. 2), being normalized (5–40 IU/liter) in 22 of the 34 patients (65%). In all patients, serum ALT levels had decreased to <60 IU/liter by the end of the treatment. When the relationship between HIC and hepatic 8-OHdG levels was analyzed in these patients, a significant correlation (R² = 0.567, P < 0.001) was observed. A positive correlation was also evident in between serum ALT levels and hepatic 8-OHdG levels (R² = 0.518, respectively.
Posttreatment biopsies were available, total HAI score (P = 0.005). In those who declined the therapy, serum ALT, serum iron, serum ferritin, and HIC were all elevated with the mean ± SD values of 134.2 ± 93.1 IU/liter, 154.2 ± 48.3 µg/dl, 241.8 ± 155.3 µg/liter, and 1230 ± 622 µg/gram liver, respectively, after a 6-year observation period.

Histopathological Change. In 28 patients in whom paired pre and posttreatment biopsies were available, total HAI score (P = 0.01), including necrosis, inflammation (P = 0.03), and fibrosis subscale scores (P = 0.04), showed appreciably significant improvements at the end of the treatment (Table 2). In 8 patients who declined the therapy, the HAI score (median 11.5; range, 6–15) after the 6-year observation period was clearly higher than that of postiron reduction therapy (Table 2). None of the 34 patients who received iron reduction therapy developed cirrhosis or HCC during the 6-year study, whereas of the 8 patients who declined the therapy, 2 patients developed cirrhosis, and 1 patient developed HCC.

DISCUSSION

In the present study, two independent methods, electrochemical detection and semiquantitative immunohistochemical analysis, were used for measuring 8-OHdG levels in liver specimens. With the electrochemical method, quantitative results could be obtained, but because the specimens were homogenized before applying to the high-performance liquid chromatography column, the values may not necessarily have represented those of hepatocytes. Therefore, we additionally performed immunohistochemical analysis to stain 8-OHdG specifically in the nuclei of hepatocytes and quantify the staining intensity by applying the KS-400 computer program to assure the objectivity of determination. As a result, values obtained by both methods showed a reasonable correlation (Fig. 1), indicating the equal validity of both methods. In terms of practicality, however, the availability of formalin-fixed samples made immunostaining less laborious than the former method, which requires the use of fresh-frozen biopsy samples; therefore, mainly immunostaining was used.

Another practical concern in the present study was in developing an effective protocol to maintain low hepatic iron levels over an extended period of time. The protocol we adopted for iron depletion therapy was phlebotomy combined with a low iron diet. This protocol was well tolerated during the study course, with only 1 patient (3%) showing noncompliance, resulting in early withdrawal from this study. In addition to therapeutic phlebotomies, we advised the patients to reduce their daily iron intake to a maximum of 7 mg each, because it is known that iron absorption is significantly increased in an iron-deficient state (29). The daily iron intake levels were kept at 5.6 mg/day (range, 5–6.8 mg/day) during the intervention period. As a result, mean serum ferritin levels were kept low, and we were able to greatly reduce the mean number of phlebotomies in the maintenance phase.

Selection of patients was also an important issue to be addressed. As the priority treatment strategy for chronic hepatitis C at present is the administration of IFN or IFN/ribavirin (33, 34), we enrolled patients who had no appreciable response to 6 months of IFN therapy completed at least 6 months before being admitted to our therapy program. When we assessed the hepatic nonheme iron levels in these patients before beginning the therapy, we noticed that the mean value was significantly higher than that found in normal liver. The mechanism(s) underlying the elevation of hepatic iron in chronic hepatitis C patients is presently unclear. There is a possibility that the increment of hepatic iron in chronic hepatitis C is attributable to increased hepatocytolysis, release of ferritin, and subsequent uptake by reticuloendothelial cells or by hepatocytes (35). An alternative possibility is that inflammatory-related cytokines, including TNF-α induced by hepatic inflammation, which has been demonstrated to increase in the sera of patients with chronic hepatitis C (36), would stimulate iron uptake via up-regulation of transferrin receptor expression in hepatocytes as described previously (37). However, the possibility that there is a high incidence of genotype associated with genetic hemochromatosis, specifically HFE gene mutation (C282Y and H63D; Ref. 38), among patients with chronic hepatitis C (39) is not likely, at least in our study group, because none of the 28 subjects tested had these mutations (data not shown). Further investigation is needed for elucidation of the mechanism.

We then confirmed that the levels of 8-OHdG both in hepatitis specimens and HCC specimens were higher than those in normal liver, showing close correlation with the levels of nonheme iron in each corresponding specimen. Nevertheless, the close correlation between levels of 8-OHdG and hepatic nonheme iron does not necessarily assure the effectiveness of iron reduction therapy on depletion.

### Table 2 Changes of serum ALT, HCV-RNA levels, and HAI scores in patients with chronic hepatitis C patients before and after the iron depletion therapy

<table>
<thead>
<tr>
<th>Serum ALT (IU/liter)</th>
<th>HCV-RNA (kcopies/ml)</th>
<th>HAI scores</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>150.8 ± 72.9</td>
<td>575.3 ± 421.8</td>
</tr>
<tr>
<td>End point</td>
<td>34.8 ± 11.3</td>
<td>583.4 ± 410.7</td>
</tr>
<tr>
<td>%</td>
<td>&lt;0.001</td>
<td>0.87</td>
</tr>
<tr>
<td>Control group (n = 8)</td>
<td>Baseline</td>
<td>142.2 ± 98.7</td>
</tr>
<tr>
<td>End point</td>
<td>134.2 ± 93.1</td>
<td>605.3 ± 572.8</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Iron reduction therapy group (n = 34)</th>
<th>Control group (n = 8)</th>
</tr>
</thead>
<tbody>
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<td>End point</td>
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*Values represent mean ± SD or median (range). Comparisons are between variables at baseline and variables of end point at 6 years.
of 8-OHdG; i.e., 8-OHdG, once incorporated into DNA, may not be readily removed and substituted with guanine residue. However, our study clearly demonstrated that iron reduction therapy was indeed effective in bringing about substantial decrease of 8-OHdG in the initial phlebotomy phase (5–16 weeks), reducing it to almost a normal range by the end of 6 years of therapy with concomitant suppression of hepatic iron. Furthermore, it was also shown that not only the mean value, but also that for each and every individual patient examined, showed a significant decrease. The improvement of hepatic 8-OHdG levels over a relatively short period during the initial phlebotomy phase indicates that DNA is being repaired; i.e., this particular base is being removed, in the absence of iron, namely ROS. In this regard, the recent discovery of enzymes which specifically repair 8-OHdG, such as 8-oxoguanine DNA glycosylase (40), supports our observation. As current medical ethics regulations in Japan do not generally allow long-term, therapy-free observation of chronic hepatitis C patients, control patients who did not undergo iron depletion therapy were not randomly examined in our study. However, from the 8 patients who had declined therapy, liver biopsy specimens obtained after 6 years of observation period were available, and the analysis of these specimens were considered to provide information equivalent to that of control patients. The 8-OHdG levels in these particular specimens were almost the same as those found in liver just before the iron depletion therapy, indicating that in liver not having undergone iron depletion therapy, hepatic 8-OHdG levels had essentially remained unchanged. In other words, depletion of 8-OHdG during the iron reduction therapy is a therapy-related phenomena and not an incidental phenomena occurring as part of the natural course of chronic hepatitis.

As has been reported previously, a decrease of serum ALT by iron reduction, which suggests both the cessation of ROS-induced damage of hepatocytes and their recovery from the damage, was also observed in our study. In chronic hepatitis C, hepatocellular damage may be initiated by various immunological reactions occurring on the cell surface, including the interaction of Fas on hepatocytes with the Fas ligand on cytotoxic T cells (41, 42) and TNF receptors on hepatocytes with TNF-α released from macrophages, etc., and subsequently substantiated by apoptotic signaling, which generates the most toxic type of ROS (•OH) in the presence of ferrous iron (the Fenton reaction; Ref. 43). Iron depletion of hepatocytes may prevent the generation of toxic ROS, and it may interfere with apoptosis signaling.

In the present study, not only ALT but also histological improvements by iron depletion were observed, though the latter occurrence retarded the former. Periporal/bridging necrosis, and intralobular degradation/necrosis may be primarily ascribed to hepatocellular death caused by ROS. Furthermore, the induction of fibrosis by ROS through the stimulation of fibroblasts is a well-known phenomena (6, 44). Thus, the effect of iron depletion on histological features may be rationalized by the concomitant inhibition of ROS generation. Neither decrease of serum ALT levels nor histological improvement was observed in patients who declined the therapy, suggesting the notion that ALT elevation and histological derangement in chronic hepatitis C are ascribed to the effects of iron. The fact that HCV titers are unaffected by the iron reduction treatment suggests that in chronic HCV infection, this treatment interferes pathogenic process rather than with viral infection. Because ROS is commonly involved in ALT elevation, fibrosis, and 8-OHdG formation, which is a highly mutagenic factor, these empirical risk factors for HCC may be now theoretically rationalized. In other words, 8-OHdG is deemed to be a genuine risk factor. Therefore, reduction of 8-OHdG levels by iron depletion therapy was shown in this study to be one of the reasonable approaches for prevention of HCC. In fact, none of 34 patients who received the therapy developed HCC over the course of the 6 years, but 1 of the 8 patients who declined the therapy did. Although this term may not be sufficiently long to draw definitive conclusions about the lowering risk of HCC, as there have been several reports showing 2.7–3.8% occurrence of HCC a year (16–23% in 6 years) among patients with chronic hepatitis C (20), 0% among our treated patients is noteworthy.

In conclusion, our results demonstrate that a close link exists between oxidative DNA damage, hepatocarcinogenesis, and hepatic iron overload in patients with chronic HCV infection. Iron reduction therapy is a safe and potentially promising means of treating patients with chronic hepatitis C and is associated with great improvements in histological responses and repair of oxidative DNA damage. Alternatively, other methods, such as administration of iron-chelating agents, might be applicable to reducing iron stores in individuals. Although the ultimate therapeutic goal is the eradication of all detectable HCV from the blood, even when more effective, less toxic approaches are developed, long-term iron reduction may be worthwhile as an adjunctive therapy.

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