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Increased Formation of Oxidative DNA Damage, 8-Hydroxydeoxyguanosine, in Human Livers with Chronic Hepatitis[1]

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ABSTRACT

8-Hydroxydeoxyguanosine (oh^{8}dG) is a promutagenic DNA lesion produced by oxygen radicals. We examined alterations in the oh^{8}dG level in human livers which have chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The oh^{8}dG content in livers with chronic hepatitis was significantly higher than the oh^{8}dG content in normal livers ($P < 0.05$). There was also a significant correlation between the oh^{8}dG content in noncancerous liver tissues with individual serum alanine aminotransferase concentration ($r = 0.515$; $P < 0.001$). Thus, chronic inflammation in the liver produces oxidative DNA damage, which may increase the risk for genomic alterations causing hepatocarcinogenesis.

INTRODUCTION

HCC[3] is one of the most frequent human cancers in the world. Pathoanatomical studies have revealed that hepatocarcinogenesis occurs in multiple stages including chronic liver disease, adenomatous hyperplasia, atypical adenomatous hyperplasia, early HCC, and advanced HCC (1). It is well accepted that cancer is attributed by genetic alterations accumulating in the cells during the stage of chronic liver disease. Thus, it is very important to identify risk factors for genomic instability which is responsible for the occurrence of genetic alterations for carcinogenesis. oh^{8}dG[3] is a promutagenic DNA lesion produced from deoxyguanosine by oxygen radicals (2, 3). Formation of oh^{8}dG in DNA induces targeted G:C-to-T:A transversions unless repaired prior to DNA replication not only *in vitro* (3) but also *in vivo* (4–6). G:C-to-T:A transversions frequently occur in the *p53* gene with the development of hepatocellular carcinoma (7, 8). Aflatoxin B1 is a potential contributor to hepatocarcinogenesis, and dietary exposure to it is correlated with G:C-to-T:A transversions at the third base of codon 249 of the *p53* gene (9, 10). Though Japan is, geographically, a low-exposure area and the mutation site is not accumulated at codon 249 in Japanese patients, G:C-to-T:A transversion is one of the most common mutation types (8). Hepatitis might provoke free radical production and promote DNA replication through continuous cell death and regeneration. Pathologically, a number of active Kupffer cells, which are known to generate oxygen radicals (11), appear in livers with chronic active hepatitis. Serum ALT is a sensitive indicator of liver cell injury (12). Therefore, we evaluated the content of oh^{8}dG in DNA of various types of human liver tissues and furthermore, we estimated correlation between the oh^{8}dG content in noncancerous liver tissues and the individual serum ALT concentration to attest to the risk of chronic inflammation for carcinogenesis through the generation of oh^{8}dG in DNA of the liver.

MATERIALS AND METHODS

Fresh human liver tumors and noncancerous liver tissues were collected in operating rooms from Japanese patients undergoing resections. Serum ALT concentrations were evaluated within 3 days before operations. Normal livers were collected from patients with metastatic liver tumors. To prevent oxidation by air exposure, all solutions and instruments which came in contact with the specimens were filled with argon gas. DNA was isolated from the homogenate of each sample (150–400 mg) using a nucleic acid extractor (Applied Biosystems). In this instrument, the homogenate was digested with proteinase K, and DNA was directly precipitated from the lysate with ethanol. Phenol/chloroform extraction was skipped, since it is known to induce oh^{8}dG formation in DNA by subsequent exposure to the air (13). The extracted DNA was treated with nuclease P1 and alkaline phosphatase as described previously (14). oh^{8}dG in digested DNA was assayed by an electrochemical detector, and dG was assayed by UV monitor, simultaneously, coupled with high-pressure liquid chromatography. The amount of dG was calculated from the absorbance at 290 nm, and the amount of oh^{8}dG was expressed as the number of oh^{8}dG for every 10^5 dG in DNA. Statistical Package for Social Sciences was used for statistical analyses.

RESULTS AND DISCUSSION

The oh^{8}dG contents in chronic hepatitis livers, cirrhosis livers, HCCs, and normal livers were evaluated by the method described above. We found that oh^{8}dG level was increased in livers with chronic hepatitis but not in cirrhosis livers and HCCs. The oh^{8}dG content in livers with chronic hepatitis was significantly higher than the oh^{8}dG content in normal livers ($P = 0.016$) (Fig. 1). In contrast, the differences between cirrhosis livers and normal livers and between HCCs and normal livers were not statistically significant ($P = 0.132$ and $P = 0.174$, respectively).

In addition, we noticed significant correlation between the oh^{8}dG content in livers and serum ALT concentration ($r = 0.51$; $P < 0.001$; Fig. 2). This correlation was persistently observed even when the effect of the transcatheter arterial embolization or the transluminal arterial infusion of anticancer drugs on the oh^{8}dG level was taken into account by analysis of covariance.

Persistent infection of either HBV or HCV is epidemiologically associated with the development of HCC. Thus, we evaluated the difference in the oh^{8}dG level in livers with the evidence of HBV and/or HCV infection (Table 1). There were no significant differences in the oh^{8}dG level between the HBV-positive group and the HCV-positive group. There was no case which was positive for both HBV and HCV in this study. The oh^{8}dG level in one case with no evidence of HBV and HCV infection was 2.94, which was similar to those in the virus-positive groups. Neither the multifocality (15) of tumors nor the histological grade of tumors proved to have any significant difference among the oh^{8}dG levels in the surrounding noncancerous liver tissues. Thus, correlation of the serum ALT unit with the oh^{8}dG

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[3] The abbreviations used are: HCC, hepatocellular carcinoma; oh^{8}dG, 8-hydroxydeoxyguanosine; dG, deoxyguanosine; HBV, hepatitis B virus; HCV, hepatitis C virus; ALT, alanine aminotransferase.

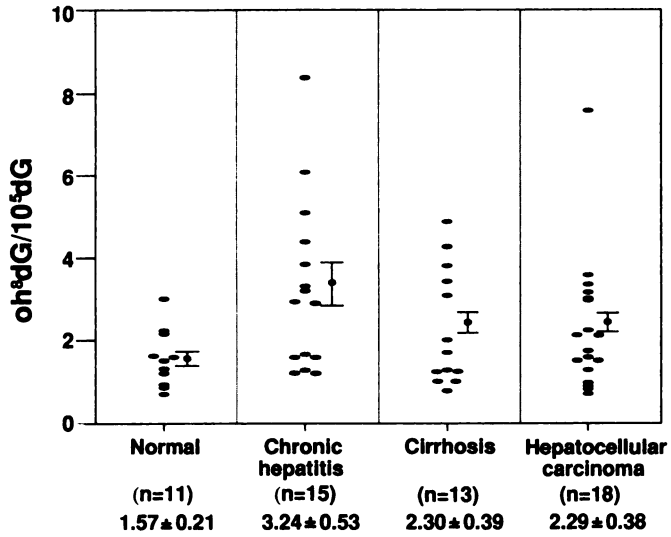

Fig. 1. oh^{8}dG content in human livers under various conditions. Individual data is shown with a *filled oval*, and the mean ± SE in each group is indicated at the bottom.

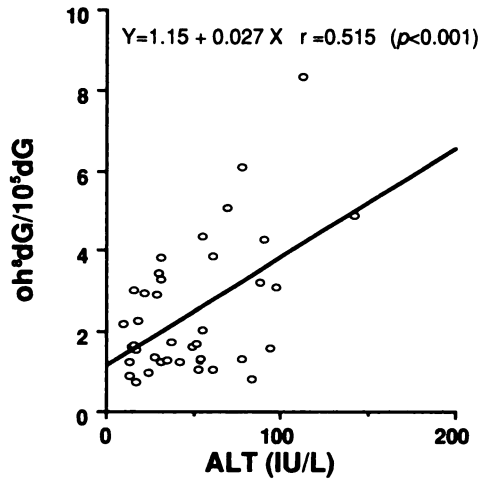

Fig. 2. Correlation of the oh^{8}dG content in livers with the serum ALT concentration. Individual data is shown with an *open oval*.

content in livers suggests that tissue inflammation may directly cause the formation of oh^{8}dG in DNA.

We previously demonstrated that the oh^{8}dG level in the liver DNA was elevated in Long-Evans cinnamon rats, which have abnormal copper metabolism, hereditary hepatitis, and subsequent HCCs (14). It was speculated that oxygen radicals were produced by the copper accumulated in their livers and induced oh^{8}dG formation in DNA. In addition, it has been shown that various carcinogens, ionizing radiation, and psychological stress increase the oh^{8}dG content in the DNA of murine livers (16, 17). Hence, we now obtained the evidence showing that tissue inflammation in the liver generates oxidative DNA damage, leading to genomic instability, and that chronic hepatitis is a potent mutagenic stage for liver cells. Clinically, high correlation of serum ALT concentration with the oh^{8}dG content may imply that the treatment of chronic liver disease to repress serum ALT concentrations might reduce genomic instability by decreasing the oh^{8}dG content in the liver.

Table 1 *oh^{8}dG content in DNA of the liver with persistent viral infection*

Type of tissue	oh^{8}dG/10^5 dG
HBV positive[a] (n = 5)	3.22 ± 0.94[b]
HCV positive[c] (n = 22)	2.71 ± 0.39[b]

[a] HBs antigen positive.
[b] Mean ± SE. There was not a significant difference between these groups.
[c] Second generation anti-HCV antibody positive.

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