EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

DNA Structure in Peripheral Blood Lymphocytes from Patients with Chronic Viral Liver Damages

V. I. Reshetnyak, T. I. Sharafanova*, L. Yu. Il'chenko*, and G. G. Poroshenko**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 4, pp. 459-461, April, 2002
Original article submitted December 13, 2001

We studied DNA damages (single-strand breaks and alkali-labile sites) in peripheral blood lymphocytes from patients with chronic viral hepatitis and cirrhosis of mixed etiology. The structure of DNA was estimated fluorometrically by changes in the intensity of ethidium bromide fluorescence. Monoinfection with hepatitis B and C viruses was not accompanied by considerable changes in DNA structure in peripheral blood lymphocytes from patients with chronic diseases. The incidence of DNA damages in lymphocytes increased in patients with hepatitis G virus and TTV monoinfection. This is probably related to replication of these viruses in nucleated blood cells. Our results suggest that hepatitis C virus potentiates damaging effect of hepatitis G virus on DNA in lymphocytes.

Key Words: *chronic viral hepatitis; liver cirrhosis; DNA; lymphocytes*

Recent studies indicate that chronic viral hepatitides are systemic infections. Replication of hepatitis B (HBV) and C viruses (HCV) in mononuclear cells of the blood, bone marrow, spleen, and other organs was demonstrated. This contributes to polymorphism of clinical manifestations of these infections and explains inefficiency of antiviral therapy with interferon-α preparations [1-3]. The role of hepatitis G virus (HGV) in the development of autoimmune disorders is discussed [3]. The phenomenon of escape of B and TT viruses from the immune control is related to impaired functional activity in infected lymphocytes and monocytes [2]. Replication of viruses in nucleated blood cells led to disturbances in the genetic apparatus of lymphocytes and triggers immunopathogenetic reactions.

Here we studied structural changes in DNA of peripheral blood lymphocytes (PBL) from patients with chronic liver diseases.

MATERIALS AND METHODS

We examined 39 patients (26 men and 13 women) with chronic liver diseases. The age of patients was 16-77 years (average age 40.8±17.7 years). These patients had chronic viral hepatitis (n= 37) caused by HBV (n=5), HCV (n=11), HBV+HCV (n=1), HGV (n=8), TTV (n=4), HBV+HGV (n=2), HCV+HGV (n= 3), and HBV+HCV+HGV (n=3) and liver cirrhosis of mixed etiology (alcohol and HGV). The control group included 10 healthy donors (5 men and 5 women, age 18-54 years, mean 29.6±16.7 years). Functional state of the liver was evaluated by alanine and aspartate transaminase activities and parameters of cholestasis (alkaline phosphatase and γ-glutamyltranspeptidase activities and contents of cholesterol and bilirubin). Markers of hepatitides B (HBsAg, HBeAg, HBeAb,

State Research Center for Antibiotics; *Central Research Institute of Gastroenterology; **Institute of General Reanimatology, Moscow. ***Address for correspondence:*** entero@aha.ru, v_reshetnyak@yahoo.com. Reshetnyak V. I.

0007-4888/02/1334-0399$27.00

HBcAb IgG, HBcAb IgM, and HBV DNA), C (HCVAb IgG, HCVAb IgM, and HCV RNA), G (HGV RNA), and TT (TTV DNA) were detected by enzyme immunoassay and polymerase chain reaction. The content of IgM, IgG, and IgA was measured by immunodiffusion. Liver biopsy was performed in most patients with chronic hepatitides.

The structure of DNA in PBL was estimated by the method of direct fluorometry [4] with modifications [5], which allows detection of single-strand breaks and alkali-labile sites in DNA of human nucleated cells [5]. This method is based on different fluorescence of complexes containing ethidium bromide and normal or damaged double-strand DNA (dDNA).

Peripheral blood (20 ml) taken from healthy donors and patients with chronic liver diseases was layered on a Ficoll-Verografin density gradient (4 ml) and centrifuged at 400*g* for 30 min. Interphase lymphocytes were suspended in 2 ml 10 mM phosphate buffer and counted in a Goryaev chamber. The suspension was adjusted to a concentration of 2×10^6 cells/ml and the cells were lysed with sodium dodecyl sulfate. The lysate was treated with ethidium bromide, which selectively binds dDNA with the formation of fluorescent complexes. The mixture was divided into 3 samples: sample 1 was used to estimate the content of native DNA in lymphocytes, sample 2 contained DNA which was completely destructed by alkaline and mechanical disintegration, and sample 3 was used to study single-strand breaks and alkali-labile sites in alkali-treated DNA. Damages to cell DNA were calculated as the ratio of dDNA by the formula: $(P\text{-}B)/(T\text{-}B)\times100\%$, where P is fluorescence of complexes containing ethidium bromide and native dDNA in PBL, T is fluorescence of complexes containing ethidium bromide and DNA in alkali-treated PBL, and B is baseline fluorescence of ethidium bromide. Changes in the intensity of fluorescence (%) reflected the severity of DNA damages.

Fluorescence of complexes containing ethidium bromide and DNA was measured on a Jasco FP 550 spectrofluorometer at excitation and emission wavelengths of 520 and 590 nm, respectively (aperture width 8 nm). The results were analyzed by Statistica 5.5A software.

RESULTS

Viral replication was found in all patients with chronic viral hepatitides and liver cirrhosis of mixed etiology.

The content of single-strand breaks and alkali-labile sites in DNA from these patients did not differ from the control. The incidence of DNA damages in PBL tended to increase in patients with chronic liver diseases.

The average contents of dDNA in PBL from healthy donors and patients infected with HBV, HCV, HGV, TTV, HCV+HGV, HBV+HCV+HGV, and HBV+HGV were 83.5, 89.3, 86.2, 78.3, 69.6, 65.3 ($p<0.05$ compared to the control), 89.1, and 76.8%, respectively.

The count of single-strand breaks and alkali-labile sites considerably increased in patients with HCV+HGV infection (65.3±12.9%, $p<0.05$ compared to the control). In other patients of this group the content of dDNA did not differ from the control.

Evidently, HBV and HCV do not damage PBL DNA in patients with chronic diseases. The number of DNA damages in PBL tended to increase in patients with HGV and TTV monoinfections, which is probably related to replication of these viruses in nucleated blood cells [3]. HCV potentiated the damaging effect of HGV on DNA in patients with chronic hepatitides of mixed etiology (HCV+HGV). We studied DNA damages in patients with chronic hepatitides of different severity. No correlation was found between structural changes in DNA and severity of hyperenzymemia.

Our results show that HBV and HCV monoinfections do not affect the structure of PBL DNA in patients with chronic diseases. HGV and TTV monoinfections increase the number of single-strand breaks and alkali-labile sites in DNA of PBL, which indirectly indicates that these viruses are replicated in nucleated cells. HCV probably potentiates the damaging effect of HGV on DNA in lymphocytes.

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