Serum arylesterase and paraoxonase activity in patients with chronic hepatitis

Suleyman Sirri Kilic, Suleyman Aydin, Nermin Kilic, Fazilet Erman, Suna Aydin, İlhami Celik

AIM: To investigate the relationship between serum paraoxonase (PON1), AST, ALT, GGT, and arylesterase (AE) activity alterations and the degree of liver damage in patients with chronic hepatitis.

METHODS: We studied 34 chronic hepatitis patients and 32 control subjects, aged between 35 and 65 years, in the Department of Infection and Clinical Microbiology at the Firat University School of Medicine. Blood samples were collected from subjects between 8:00 and 10:00 a.m. following a 12-h fast. Baseline and salt-stimulated PON1 activities were measured by the hydrolysis of paraoxon. Phenyl acetate was used as the substrate and formed phenol was measured spectrophotometrically at 270 nm after the addition of a 10-fold diluted serum sample in AE activity measurements.

RESULTS: The results of this investigation revealed that the levels of AE activity decreased from 29±4.9 to 98±44, baseline PON1 activity from 452±112 to 164±67 (64%), salt-stimulated PON1 activity from 746±294 to 294±220 (61%), HDL from 58.4±5.1 to 86±34 (35%), while a slight increase in the level of LDL from 163±54.1 to 177.3±56.0; 9%) and significant increases in the levels of AST (from 29±9.3 to 98±44), ALP (from 57.2±13.1 to 91±38.1), ALT (from 27.9±3.3 to 89±19.1), GGT (from 24.3±2.10 to 94±48.2), total bilirubin (from 0.74±0.02 to 1.36±0.06; 84%) and direct bilirubin (from 0.18±0.01 to 0.42±0.04; 133%) were detected. However, the levels of albumin, total protein, cholesterol, and uric acid were almost the same in chronic hepatitis and the control subjects.

CONCLUSION: Low PON1 and AE activity may contribute to the increased liver dysfunction in chronic hepatitis patients by reducing the ability of HDL to retard LDL oxidation and might be clinically useful for monitoring the disease of chronic hepatitis.

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Key words: Paraoxonase; Arylesterase; Chronic hepatitis; Lipoproteins

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INTRODUCTION

The paraoxonase (PON1), a 44-ku glycoprotein, is synthesized mainly in the liver, which hydrolyzes organophosphates like pesticides, neurotoxins, and arylesters [4-6]. PON1 activity exhibits a substrate-dependent activity polymorphism in human beings [8-11]. This polymorphism is related to two polymorphic sites at amino acid positions. One at position 192, which is a glutamine→arginine substitution, hydrolyzes paraoxon with a high enzyme activity, and the other at position 55, which is a leucine→methionine substitution causes a low enzyme activity [8]. The activity of PON1 shows great inter-ethnic variability [8,11]. The gene frequency for PON1 192 allele is 0.31 in Turkish population [8,10,11] and in Caucasian population [8,12], 0.41 in Hispanic populations, 0.66 in a Japanese population [8]. Three different phenotypes are reported based on the responses of the two isoenzymes to salt concentrations [8,12]. The ratio of salt-stimulated PON1 activity to arylesterase (AE) activity is used for the definition of phenotypes [8,10,12,13]. Environmental factors that change PON1 activity include tobacco consumption, which has been reported to depress PON1 activity and concentration [14]. Also, several studies have shown that PON1 level is tightly linked with HDL in the serum and contributes to the protection conferred by HDL against LDL oxidation. Human paraoxonase (PON1), an HDL-associated enzyme carried on apo A-I, is believed to protect lipoproteins against oxidative modification [9,10]. As mentioned above, the role of PON1 and AE activity may be particularly meaningful as an index of liver func-
tion status because preliminary studies have revealed a remarkable decrease of serum AE activity in patients with liver cirrhosis[17,18], and AE and PON1 activities have been demonstrated to be a function of a single enzyme[19].

To the best of our knowledge, although AE and PON1 activities have been demonstrated to be a function of a single enzyme[19], both PON1 (only studied by Ferre et al[18]), and AE activity (only studied partly[17,18]) in chronic HBV have not been studied together, as yet. The paucity of data on serum PON1 and AE does not allow for a conclusion about its role in chronic HBV. Therefore, we carried out this study aiming to compare PON1 and AE activities and traditional standard biochemical test of liver function parameters (such as, ALT, AST, and so on), and whether there were changes due to chronic hepatitis.

MATERIALS AND METHODS

We studied 34 chronic hepatitis patients and 32 control subjects, aged between 35 and 65 years, in the Department of Infection and Clinical Microbiology at the Firat University School of Medicine. Control subjects were healthy and evaluated for any symptoms of clinical or analytical evidence of diabetes, renal disease, cardiovascular disease, neoplasia, or hepatic damage and were matched for age and body mass index. Cirrhosis was graded according to Child-Pugh criteria[20]. Blood samples were collected from subjects between 8:00 and 10:00 a.m. following a 12-h fast. The extracted blood samples were then centrifuged at 4 000 r/min for 10 min and stored at -70 ℃ until the assay was performed. This study was performed with the approval of the ethics committee and all subjects volunteered for the study with informed consent.

Assay of paraoxonase activity

PON1 assays were done without additional NaCl (baseline activity) and with 1 mol/L NaCl included in the assay buffer (salt-stimulated activity), following the formation of p-nitrophenol by its absorbance at 405 nm for 5 min. Assay buffer contained 0.125 mol/L Tris-HCl (pH 8.5), 1.25 mmol/L CaCl₂, and 1 mol/L NaCl (pH 8.5)[4,12]. For each set of assays, 6 mmol/L freshly prepared paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma Chemical Co.) substrate solution of 120 µmol/L paraoxon in acetone diluted with 0.125 mmol/L Tris-HCl was used. Paraoxon stock solution was handled very cautiously with protective measures. The assay tube contained 750 µL of Tris buffer, 50 µL of serum (1:2 diluted with water) and 200 µL of 6 mmol/L paraoxon. The reaction was initiated at 37 ℃ by the addition of the substrate solution, and using a Techcomp 8500 II Spectrophotometer, absorbance was continuously monitored at 405 nm and 25 ℃. The PON1 unit was defined as the enzyme quantity that disintegrates 1 µmol paraoxon substrate in one minute[4,12]. The percent stimulation of PON1 was calculated as follows[8]: ([PON1 activity with 1 mol/L NaCl]-(basal activity))/basal PON1 activity]×100.

Assay of arylesterase activity

AE activity was measured with phenylacetate as a substrate as previously described[17,18,22]. AE activity was affected with salt. The assay tube contained 750 µL of 0.1 mol/L Tris-HCl (pH 8.5), 1 mmol/L CaCl₂, 125 µL of 12 mmol/L phenylacetate and 125 µL of serum (1:10 diluted with water). The absorbance was continuously monitored at 270 nm and 37 ℃. The units were expressed as millimoles of phenylacetate hydrolyzed per minute.

Biochemical parameters of liver function

Serum AST, ALT, ALP, GGT, albumin, total protein, indirect bilirubin, total bilirubin and other biochemical parameters were analyzed with an auto analyzer (Olympus 600).

Statistical analysis

Statistical analysis was performed using Student’s t test for group comparisons and data for biochemical analyses were expressed as mean±SD. Pearson’s correlation coefficients were used to test the correlation between each of the two biochemical variables. Multiple linear regression analysis was used between PON1 and AE activity and possible determinants, such as AST, ALT, ALP, and GGT. A P value less than 0.05 was considered statistically significant.

RESULTS

Laboratory results of the standard liver functions tests are documented in Table 1, and provide evidence of the spectrum of disease. As expected, the levels of AST, ALT, ALP, and GGT in the chronic hepatitis patients were significantly increased as compared to the controls. Increased values of transaminase were also matched by a corresponding rise in serum bilirubin. The serum PON1 and AE were obviously correlated with each HDL parameter and triglyceride (TG) in chronic hepatitis patients compared to the controls.

In the present study, we also observed markedly elevated LDL (9%) and decreased HDL (20%) in chronic hepatitis patients as compared to the controls (Table 1). The activities of AE were significantly reduced (by 29%) in the chronic hepatitis group (94±36 U/mL) compared with the control group (132±52 U/mL, P<0.001, Table 1). Furthermore, PON1 baseline activity was significantly decreased (by 64%) in the chronic hepatitis group (164±98 U/mL, P<0.001, Table 1). Also, salt-stimulated serum PON1 activity was decreased (by 61%) in chronic hepatitis group (294±220 U/mL) as compared with the control group (746±394 U/mL, P<0.001, Table 1).

Comparison of serum PON1/AE activity and traditional standard biochemical test of liver function

We observed negative correlations between ALT and AE activities (r = -0.112, P<0.11), as well as between the percent stimulation of PON1 and ALT (r = -0.412, P<0.05) in the chronic hepatitis patients, but not in the controls (r = 0.142). In addition, we found obvious negative
correlations between GGT and AE activities ($r = -0.901$, $P<0.01$), as well as between the percent stimulation of PON1 and ALT ($r = -0.412$, $P<0.05$), but not such correlation in the controls ($r = -0.122$, not significant). We also previously reported similar correlations between AE and PON levels in severe pre-eclamptic women.$^{[23]}$

ALP activity was also correlated with PON1 ($r = -0.41$, $P<0.05$) and AE ($r = -0.44$, $P<0.03$) activities in the chronic hepatitis group, but not in the controls ($r = 0.102$). This moderate correlation might be a result of ALP demonstration in different tissues such as bone and small bowel when compared with ALT, which is demonstrated only in the liver.

**DISCUSSION**

Chronic HBV infection is one of the most important diseases leading to a high morbidity and mortality due to the development of liver failure, liver cirrhosis (LC), and liver cancer.$^{[24,25]}$ There are over 300 million people suffering from HBV infection worldwide.$^{[26]}$ The destruction of liver cells can be extensive, and death follows from liver failure in about 1% of cases. Many of those recover progress to shed the virus for years, while some develop a progressive degenerative liver disease called chronic active hepatitis.$^{[24,25,27]}$ Dramatic alterations in chronic hepatitis remain within laboratory values until gross disease becomes evident. To diagnose such a slow progressive liver disease (chronic hepatitis) before advancing hepatocellular necrosis and fibrosis, besides traditional biochemical tests needs alternative parameters to evaluate liver damage.$^{[28]}$

PON1 and AE activities have been demonstrated in different tissues, such as liver (including microsomes), kidney,$^{[28,29]}$ brain,$^{[30,31]}$ and lung,$^{[32]}$ and it has been studied extensively in relation to cardiovascular diseases$^{[2,3,33,34]}$ whereas there are scarce data available on the hepatic enzyme. Some of these enzymes are released into the circulation and some portions are stored in the liver. Serum PON1, which is carried in circulation bound to HDL particles, protects LDL from peroxidation.$^{[2,3,10,14,16,32]}$ The putative function of PON1 in the liver is to provide hepatic protection against oxidative stress.$^{[33]}$

In the present study, the relation between serum PON1 and AE activity levels and chronic hepatitis was examined. We observed that PON1 and AE activities were obviously lower in the chronic hepatitis patients compared with the control subjects, and were significantly correlated with each HDL parameter, and consistent with reports that this enzyme is bound to a large apo A-I containing HDL subspecies and also confirmed previously reported results.$^{[10,15,34-36]}$ The decrease in PON1/AE activity in the serum was also correlated with serum AST, ALT, GGT, albumin, and bilirubin concentrations. It has been reported that the diagnostic accuracy of PON1 is equivalent to that of ALT in patients with chronic hepatitis and far superior to that of the other tests in patients with cirrhosis.$^{[39]}$ Our results are in agreement with some recent studies in cirrhotic patients$^{[20]}$, rats$^{[31]}$, and chronic hepatitis patients$^{[17,18]}$.

Serum PON1 and AE are mainly the result of liver activity.$^{[17]}$ We have some possible explanations for decreased PON1 and AE activities in chronic hepatitis. One is that even though hepatic PON1 and AE levels may be normal, serum PON1 and AE activity would be lowered as a result of changes in synthesis or secretion of the HDL secondary. This assumption is supported by other researchers.$^{[20,35,36]}$ Alterations in HDL structure and levels related to decreased serum PON1 activities in mice with LCAT deficiency are the consequence of LCAT gene-targeted disruptions. PON1 and AE have also been reported to play a role in the lipid transfer and assembly of VLDL particles in liver microsomes$^{[39]}$. The other is that liver cells damaged via chronic hepatitis do not express the proteome PON1 and AE. Both have been shown to be functions of single enzymes. Supporting this putative function is the report of inhibition of microsomal PON1 activity in rats with chronically administered CCl$_4$.$^{[32]}$ So, elevated LDL and decreased HDL might have a causal role in the pathogenesis of chronic hepatitis. It is known that PON1 is bound to HDL and acts as an antioxidant that protects LDL from oxidative modifications and can reduce oxidized lipids in oxidized lipoproteins.$^{[29]}$

In conclusion, a significant decrement in PON1 and AE is probably the consequence of liver dysfunction. For this reason, serum PON1 and AE activities could be a beneficial tool for chronic hepatitis patients. However, further research is needed to elucidate the mechanism leading to decreased serum PON1 and AE activities in liver diseases and potential pathophysiologic implications.

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