PARAOXONASE 1 ACTIVITY IN NORTHERN CHINESE DIABETIC PATIENTS WITH CHRONIC RENAL FAILURE

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Abstract

The objective of this study is to investigate the relationship between paraoxonase 1 (PON1) activity and dyslipidaemia in Northern Chinese diabetic patients with chronic renal failure (DM-CRF). For this purpose, 45 diabetic patients with CRF, 63 non-diabetic patients with CRF, 90 type 2 diabetic patients without CRF were investigated, as well as 70 subjects without diabetes and CRF. The serum PON1 activity and serum lipids, high-density lipoprotein cholesterol (HDLc) were determined.

The results showed that, as compared to control subjects, serum ArE1/PON1 activities were significantly decreased in patients with diabetes, CRF and DM-CRF. In a further investigation of the relationship among ArE/PON1 and lipid parameters in all groups, not only serum ArE/PON1 activity, but also ratios of serum ArE/TC and ArE/HDL3c were found to be significantly decreased in the three groups, and the degree of decrement was DM-CRF>CRF>diabetes. In DM-CRF group, multiple regression analysis showed that ArE/PON1 was closely related to HDL2C, Apo A1 and HDLC. ArE/TC was also related to HDL2C, Apo A1, HDLC/TC and HDLC.

In conclusion, serum ArE/PON1 can be one of the signals reflecting the disorder of lipid metabolism of CRF, especially in DM-CRF patients.

Key words: Paraoxonase I, polymorphism, lipoprotein, chronic renal failure, renal transplant.

INTRODUCTION

Arylesterase/Paraoxonase-1 (ArE/PON1) is a protein of 354 amino acids with a molecular mass of 43 kDa. In serum, it is almost exclusively located on high-density lipoprotein (HDL) (1). Purified ArE/PON1 is significantly more efficient than Apo A1 or lecithin cholesterol acytransferase (LCAT) in preventing oxidation of low-density lipoprotein (LDL) (2, 3), although addition of the latter components

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slightly enhances the effect of ArE/PON1. The capacity of ArE/PON1 to accept and detoxify lipid peroxides provides general protection for all membranes. Furthermore, the resemblance of LDL to a cell membrane explains its capacity to protect LDL from oxidative modification (4, 5).

Diabetic patients with chronic renal failure (CRF) suffer from a primary and secondary form of complex dyslipidemia consisting of both quantitative and qualitative abnormalities in serum lipoproteins resulting from alterations in lipoprotein metabolism and composition. The main features of dyslipidemia in CRF are increment in triglyceride level and decrement in HDL cholesterol level as GFR declines (6, 7). Poor glycemic control in subjects with diabetes can be associated with dyslipidemia (8, 9). Dyslipidemia appears to be an increase in serum triglyceride level (due to elevated very low density lipoprotein [VLDL]-remnants and intermediate-density lipoprotein [IDL]) and decreased high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) cholesterol, especially small and dense LDL subclass (sdLDL) or oxLDL increase (7, 10). The apolipoprotein B (apoB)-containing part of the lipoprotein may undergo modifications (enzymatic- and advanced glycation end-product [AGE] - peptide modification, oxidation, or glycosylation). These alterations can contribute to impaired LDL receptor-mediated clearance from plasma and promote prolonged circulation.

Decreased ArE/PON1 activity has been found in patients with end-stage renal disease (ESRD) in several studies. This decrease was unrelated to differences in ArE/PON1 phenotype distribution or its serum concentration (11-13). ArE/PON1 status in Chinese diabetic patients with CRF has never been evaluated. In this study, we investigate serum PON1 activities in diabetic patients with CRF (DM-CRF) and relate them to levels of lipid parameters.

SUBJECTS AND METHODS

Fortyfive diabetic patients (20 males; age: 35~65 years; history of diabetes >10 years; fasting blood glucose levels 12.56 ± 2.50mmol/L) with CRF were recruited (creatinine clearance 13.38 ± 1.79 ml/min, urine protein +++~++++). None of these patients was hemodialyzed, or received any immunosuppressive treatment or lipid lowering drugs either. Most of them were treated with erythropoietin and supplemental iron orally. Exclusion criteria were the presence of liver disease or other infectious disease.

Sixty three non-diabetic patients (35 males, aged 25-65 years, fasting blood glucose levels 5.03 ± 0.52 mmol/L) with CRF (creatinine clearance 14.58 ± 2.16 ml/min; urine protein +++~++++) were selected.

Ninety type 2 diabetic patients (55 males; aged 48-66 years; fasting blood glucose levels were 12.68 ± 2.45 mmol/L, urinary levels of micro-albumin <15 μg/min.) and seventy healthy volunteers as control group (30 males; aged 25-44 years) were selected in this study.

The patients were recruited among outpatients and inpatients admitted to both 1st affiliated hospital and Zhengzhou 2nd hospital. Subjects receiving drugs known to affect lipid level as well as subjects with kidney or liver disease were excluded from the study. All subjects were of Han Ethnicity, resided in Henan Province, and gave their informed consent to participate in the study.

Sample collection. All subjects were admitted to the hospital at 08:00 AM after 16 hrs of fasting. Blood samples were obtained before 10:00 the next morning. All serum and plasma samples were stored at -80°C until processed.

Laboratory measurements. Serum ArE/PON1 activities were measured using the modified method described previously (18). Plasma glucose was measured by the hexokinase method. Serum total cholesterol (TC), serum and urine creatinine were measured with enzymatic methods. HDLc and HDL3c were determined as described (19). Apoliprotein A1 (Apo AI) and Apo B100 were measured with immunoturbidimetric assay. All tests were analyzed by a Hitachi 7105 Autoanalyzer except for HDLc and HDL3C. Serum level of total oxLDL particles was directly measured by a sandwich ELISA assay. All reagents were purchased from Sigma Chemical Company (USA), Beijing Zhongsheng Biological engineer Company and Shanghai Rongsheng Bological Reagent Company.

Statistical analysis. Data are presented as mean±S.D. Differences between continuous variables were evaluated by one-way analysis of variance (ANOVA). Multiple regression anaysis was used to evaluate the relationships among ArE/PON1 and other variables in patients with CRF. A $P<0.05$ was considered significant.

RESULTS

A comparison among lipid parameters and ArE/PON1 activities in patients and control subjects is shown in Table 1. Serum activities of ArE/PON1 in all patients' groups were lower than in control group ($P<0.01$). Furthermore, ArE/PON1 activity in diabetic patients with CRF (DM-CRF) was much lower than in non-diabetic CRF patients. The levels of HDL2c and Apo A1 were also significantly lower. There were relatively higher levels of Apo B100 and oxLDL in patients as compared to control group.

Table 1. Lipid parameters and ArE/PON1 activities in investigated patients

	ArE/PON1 (uml $^{-1}$)	TC (mmol L^{-1})	HDLc (mmol L^{-1})	HDL2c (mmol L^{-1})	oxLDL (mg L^{-1})	ApoA1 (g L^{-1})	ApoB100 (g L^{-1})
DM	0.221±0.033#	4.75±0.70#	1.25±0.21*	0.38±0.16*	0.403±0.109#	1.28±0.18#	0.99±0.32*
DM-CRF	0.161±0.036#	5.55±0.68#	0.98±0.22#	0.26±0.17*	0.481±0.088#	0.96±0.19#	1.34±0.30#
CRF	0.183±0.042#	5.27±0.67#	1.07±0.25#	0.32±0.16*	0.442±0.110#	1.11±0.17#	1.21±0.30#
Control	0.261±0.018	4.33±0.64	1.34±0.20	0.53±0.15	0.336±0.102	1.44±0.15	0.87±0.12

Compared with control group, *$P<0.05$; #$P<0.01$

Table 2. The ratio of ArE/PON1 and lipid parameters in investigated patients

	ArE/TC	ArE/ HDL3c	HDLc/TC	HDL2c/TC	HDL2c/HDL3c	ApoA1/Apo B100
DM	0.055±0.005#	0.270±0.060#	0.290±0.09#	0.120±0.040*	0.500±0.230*	1.29±0.26#
DM-CRF	0.036±0.002#	0.200±0.048#	0.210±0.069#	0.08±0.050*	0.336±0.195#	0.81±0.24#
CRF	0.041±0.003#	0.218±0.049#	0.239±0.070#	0.100±0.049*	0.410±0.210*	0.99±0.25#
Control	0.065±0.003	0.315±0.100	0.34±0.06	0.132±0.030	0.60±0.18	1.55±0.17

Compare with control *P<0.05, #P<0.01

To further explore the role of ArE/PON1 activity in hyperlipaemia of DM-CRF, we examined the ratio of ArE/PON1, cholesterol (ArE/TC) and HDL3c (ArE/HDL3c). As shown in Table 2, the ratios of ArE/TC to ArE/HDL3c in all patients groups were significantly lower than in controls: DM-CRF <CRF <diabetes <controls. Furthermore, comparing the ratios of lipid parameters, the tendencies of HDLc/TC, HDL2c/TC, and Apo A1/ Apo B100 were similar to ArE/TC.

The above data suggested that the activity of ArE/PON1 played a role in the development of hyperlipaemia in diabetic patients with CRF. Therefore, we explored the relationships among ArE/PON1 activity and other lipid parameters in diabetic patients with CRF by multiple regression analysis. A close relationship was found among ArE/PON1, ArE/TC, HDLC, Apo A1, HDL2C/TC, Apo A1/Apo B100 and HDLc/TC.

The regression equations were ArE/PON1 = 0.0079 + 0.0675ApoA1 + 0.03814 ApoA1/ApoB100 + 0.237 HDL2C + 0.0453 HDLC, ArE/TC = -0.0064 + 0.0293 ApoA1 + 0.0108 ApoA1/ApoB100 + 0.0536 HDL2c + 0.0122HDLC + 0.0187 HDLc/TC.

DISCUSSION

The risk of cardiovascular disease is highly increased in patients with chronic renal failure. Diabetes aggravates this situation (14, 15). Increased susceptibility of LDL to oxidation has been reported in chronic renal failure (7, 10). In the view of HDL ability to protect LDL against oxidation, the aim of our study was to investigate the activities of serum ArE/PON1 in diabetic patients with chronic renal failure and to assess whether changes in its activity might contribute to the accelerated hyperlipaemia.

As previously described, serum PON1 activity levels are decreased in patients with uremia. Conversely, the PON1 genetic polymorphisms, 55 Leu/Met (L/M) and 192 Gln/Arg (Q/R) were not different in CRF patients undergoing hemodialysis

(12). At present, this is difficult to explain, but it is well known that factors influencing lipid peroxides and lipid metabolism may contribute to cardiovascular death. Furthermore, ArE/PON1 might play an important role in regulation of lipid peroxides and lipid metabolism. In our study, ArE/PON1 activities in patients with CRF, diabetic or not, were lower than in controls. At the same time, ArE/PON1 activities were lower in diabetic patients, with CRF or not. The ArE/PON1 activities in this study were as follows: DM-CRF <CRF <diabetes mellitus <control.

ArE/PON1 is a constituent of HDL that protects LDL from oxidative stress. ArE/PON1 destroys oxidized phospholipids that are biologically active and thereby prevents the proatherogenic effects induced by oxidized LDL (16). As a result of many factors such as age, smoking and dysfunction of liver, in humans it has been difficult to clearly link changes in ArE/PON1 activity to dyslipidaemia. In mice, however, studies showed that decrease in ArE/PON1 level, in response to hyperlipidic diets segregates with aortic lesion development (2). In support of this, we observed whether the decrease of ArE/PON1 activity is relative to other lipid parameters. Table 1 shows that the concentrations of HDLc, HDL2c and Apo A1 were also decreased while the concentrations of TC and Apo B100 increased significantly.

The activities of ArE/PON1 were associated with diabetes, which appears to induce low ArE/PON1 activities. An impaired ArE/PON1 activity was also observed in patients with CRF (Table 1). Therefore, we explored ArE/PON1 activities and hyperlipidaemia in CRF of different causes. As shown in Table 2, regardless of the cause of CRF, the ratios of ArE/PON1 to total cholesterol (ArE/TC), and HDL2c (ArE/HDL2c) were significantly lower than in control subjects, and the activities of ArE/PON1 in diabetic patients with CRF were lower than in all of groups. It is conceivable that the extent of this decrease depends on the presence of primary and secondary dyslipaemia and other environmental factors.

These observations raise some interesting considerations. ArE/PON1 activity was reported to be reduced by oxidative incubation with Cu^{2+}-induced peroxidation of LDL. Oxidized LDL appears to inactivate ArE/PON1 through interactions between the enzyme-free sulfhydryl group and oxidized lipids that are formed during LDL oxidation (17). ArE/PON1 activity may be partially inactivated in the presence of oxidative stress, which occurs in diabetic patients with CAD (data not shown). Therefore, the low ArE/PON1 activity in CRF patients is probably a consequence of increased oxidative stress. In this study, we found that the oxLDL increased, which was associated with the decreased activities of ArE/PON1, the higher concentration of oxLDL and the lower activities of ArE/PON1 in diabetic patients with CRF (Table 1).

Our previous research showed that the decrease in ArE/PON1 activities is associated with the decreased concentration of HDL2c (data not shown). We considered ArE/PON1 was a major factor in improving anti-dyslipaemia capacity. We investigated the correlations among ArE/PON1 activity and other clinical data in diabetic patients with CRF using multiple regression analysis. ArE/PON1 activity was closely related to HDL2C, Apo A1 and HDLC. Also, ArE/TC ratio correlated to HDL2C, Apo A1, HDLC/TC and HDLC. These data could indicate

that the decreasing ArE/PON1 activity is related to the dyslipaemia in patients with CRF, especially in diabetic patients. Therefore, serum ArE/PON1 can be considered as a indicator of the disordered metabolism of lipids in chronic renal failure, especially in diabetic patients.

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