Acta Diabetol (2009) 46:239–242
DOI 10.1007/s00592-008-0066-3

SHORT COMMUNICATION

Low human paraoxonase predicts cardiovascular events in Japanese patients with type 2 diabetes

Yukio Ikeda · Mari Inoue · Tadashi Suehiro · Kaoru Arii · Yoshitaka Kumon · Kozo Hashimoto

Received: 22 January 2008 / Accepted: 12 September 2008 / Published online: 2 October 2008

Abstract Human serum paraoxonase (PON1) is associated with HDL and inhibits oxidative modification of LDL. PON1 enzymatic activity has been shown to decrease in diabetic patients; however, the effect of PON1 status on long-term outcome has not been reported. In this study, we examined the association between baseline PON1 status and the development of cardiovascular disease (CVD) during 10 years of follow-up in 88 type 2 diabetic patients whose enzymatic activities, concentrations, and genetic polymorphisms of PON1 had been determined. A total of 20 CVD events were recorded during the follow-up period. Using Kaplan–Meier survival curves, we found a significantly increased incidence of CVD in patients with a lower concentration or paraoxonase activity of PON1 than each median value (log-rank 7.460; $P < 0.01$, and log-rank 4.187; $P < 0.05$, respectively). By Cox regression analysis, both concentration and paraoxonase activity were significantly associated with the development of CVD, even after correction for gender, age, and preexisting CVD ($P < 0.05$). Low concentration and enzymatic activity of PON1 may be an independent predictor of cardiovascular events in diabetic patients.

Keywords Serum paraoxonase · PON1 · Cardiovascular disease · Type 2 diabetes

Y. Ikeda (✉) · M. Inoue · T. Suehiro · K. Arii · K. Hashimoto
Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Kohasu, Oko-cho, Nankoku, Kochi 783–8505, Japan
e-mail: ikeday@kochi-u.ac.jp

Y. Kumon
Department of Laboratory Medicine, Kochi Medical School, Kochi University, Nankoku, Japan

Introduction

The oxidative modification of low-density lipoprotein (LDL) plays a central role in the initiation and acceleration of atherosclerosis. Human serum paraoxonase (PON1), an esterase associated with high-density lipoprotein (HDL), reduces the susceptibility of LDL to lipid peroxidation in vitro [1]; therefore, this enzyme is thought to have a central role in the inhibitory effect of HDL on lipid peroxidation.

We [2, 3] and other investigators [4, 5] have shown that PON1 levels are decreased in diabetic patients. Recently, Lakshman et al. [6] showed a clear inverse correlation between PON1 enzymatic activity and the severity of cardiovascular disease (CVD) in a cross-sectional study of type 2 diabetic subjects; however, the effect of PON1 status on the long-term outcome of diabetic patients remains unknown. We therefore examined the association between baseline PON1 status and CVD in an observational study of an outpatient diabetic population.

Patients and methods

We had previously recruited 108 patients with type 2 diabetes (53 males and 55 females) from our outpatient clinic between June and October 1996, and determined PON1 enzymatic activity, concentrations, gene polymorphisms, and other clinical parameters [2, 3]. Among these patients, we were able to follow-up 88 patients (41 males and 47 females) until October 2006. At baseline, mean age of the 88 patients was 57.0 ± 9.0 years (range 33–69), and duration of diabetes was 10.0 ± 8.4 years (range 1–31).

CVD was defined as the presence of coronary heart disease (CHD), a cerebrovascular accident (CVA), peripheral vascular disease (PVD) or congestive heart failure (CHF);

13 patients already had CVD at the baseline examination. A detailed description of the study population and definitions of CHD, CVA, and PVD have already been reported [2].

PON1 enzymatic activities, concentrations, and the PON1 gene polymorphisms, determined in previous studies [2, 3], were used for this study. Briefly, paraoxonase assays were performed on serum samples. Paraoxonase activity was measured using paraoxon as the substrate [2]. PON1 concentrations were quantified by competitive enzyme immunoassay as described in detail [3]. Genomic DNA extracted from blood cells was used to determine PON1 genotypes. The coding region 192Q/R and 55L/M polymorphisms were evaluated by PCR–RFLP [2], and the -108C/T promoter polymorphism was evaluated by a cycle sequencing method [3].

All values were shown as mean ± SD. Kaplan–Meier event-free survival curves were analyzed separately in participants with baseline PON1 status above and below the median value and compared using the log-rank test. Prognostic variables for survival were evaluated using Cox-proportional hazards regression models, and summarized as hazards ratio and 95% confidence interval (CI).

Results

During follow-ups over a period of 10 years, we recorded 20 CVD events, including CHD ($n = 9$), CVA ($n = 8$) and CHF ($n = 3$). Eight patients died from CVD ($n = 4$), cancer ($n = 2$), or infectious diseases ($n = 2$) during the period.

The median values of PON1 concentration and paraoxonase activity at baseline were 17.6 U/ml and 103.8 U/l, respectively. First, we compared event-free survival curves for CVD between the subgroup with PON1 concentrations of 17.6 U/ml or greater ($n = 44$) and the subgroup with concentrations lower than 17.6 U/ml ($n = 44$). As shown in Fig. 1, overall CVD events were significantly frequent in the group with lower PON1 levels by Kaplan–Meier estimation (log-rank 7.460, $P < 0.01$). We also compared survival curves between the subgroup with paraoxonase activity of 103.8 U/l or greater ($n = 44$) and that with the activity lower than 103.8 U/l ($n = 44$). Overall CVD events were significantly frequent in the group with lower paraoxonase activity (log-rank 4.187, $P < 0.05$).

We next used Cox-proportional hazards models to identify significant predictors for the development of CVD. By univariate analyses, PON1 concentration and paraoxonase activity were found to be significant predictors for CVD ($P < 0.01$ and $P < 0.05$, respectively), as was preexisting CVD at entry ($P < 0.05$). A multivariate analysis model, which included PON1 concentration or paraoxonase activity in addition to age, gender and preexisting CVD, showed that both PON1 concentration and paraoxonase activity were independent predictors (Table 1). Age-, gender- and preexisting CVD-adjusted hazard ratio for every 1 U/ml increment in PON1 concentration was 0.905 ($P < 0.05$) and for every 1 U/l increment in paraoxonase activity it was 0.988 ($P < 0.05$). On the other hand, no associations of the PON1 gene polymorphisms with the development of CVD during the follow-up period were detected by Kaplan–Meier methods or Cox-proportional hazards regression models (data not shown).

Discussion

It has been shown that PON1 enzymatic activity is decreased in CHD patients [7, 8]. Recently, decreased PON1 activity was also shown in ischemic stroke patients [9, 10]. However, data obtained from such cross-sectional analyses are limited, because it remains unclear whether the decrease in PON1 precedes the development of the clinical events considered to be the end point of atherosclerosis. In this study, we showed that both PON1

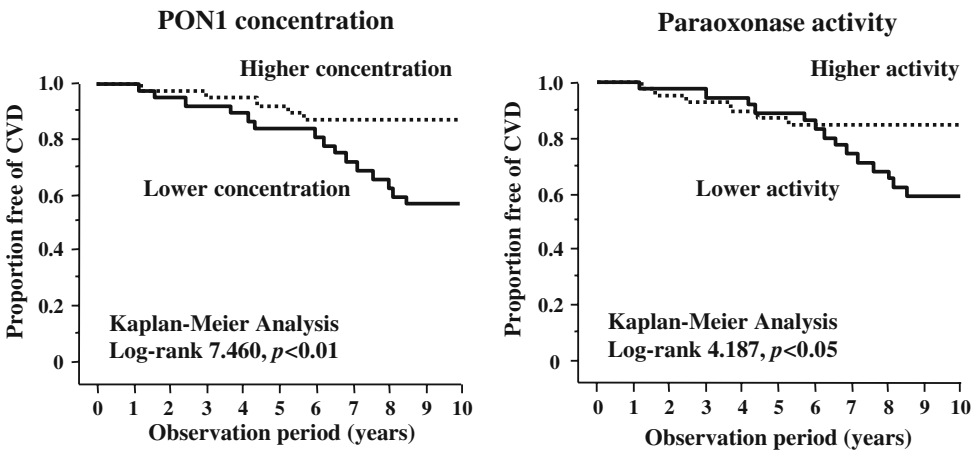

Fig. 1 Kaplan–Meier event-free survival curves showing cardiovascular events ($n = 20$) in 88 diabetic patients grouped into higher concentration (*dotted line*, $n = 44$) and lower concentration (*solid line*, $n = 44$) groups according to the median baseline PON1 concentration (*left*). A similar analysis in those patients grouped into higher activity (*dotted line*, $n = 44$) and lower activity (*solid line*, $n = 44$) groups according to the median baseline paraoxonase activity (*right*)

Table 1 Adjusted hazard ratios for cardiovascular events in an 10-year follow-up of type 2 diabetic patients

Variable	Model 1 Adjusted HR (95% CI)	Model 2 Adjusted HR (95% CI)
Age (per 1 year)	1.036 (0.980–1.095)	1.056* (0.997–1.117)
Male gender (*vs* female)	2.109 (0.813–5.475)	1.905 (0.733–4.951)
Preexisting cardiovascular disease	2.821** (1.019–7.806)	3.107** (1.131–8.539)
PON1 concentration (per 1 U/ml)	0.905** (0.822–0.997)	–
Paraoxonase activity (per 1 U/l)	–	0.988** (0.976–1.000)

Prognostic variables were evaluated using multivariate Cox-proportional hazard regression models. Model 1 includes PON1 concentration in addition to age, gender and preexisting cardiovascular disease, and model 2 includes paraoxonase activity instead of PON1 concentration

HR hazard ratios

* $P < 0.1$; ** $P < 0.05$

concentration and enzymatic activity are independent predictors of the development of CVD in diabetic patients. This is the first study to show an association between PON1 status and long-term clinical outcome in this category of patients. Having a history of CVD is one of the most important predictors of a cardiovascular event. Our data may be criticized for the analysis of a mixed population including patients both with and without preexisting CVD at baseline. Indeed, preexisting CVD has been shown to be a significant risk factor for CVD in univariate Cox-proportional hazards modeling. However, multivariate modeling clearly demonstrated that both low concentration and low activity were significant independent risks for incident CVD, even after adjustment for preexisting CVD as well as age and gender. Very recently, Bhattacharyya et al. [11], in a large-scaled prospective study of patients undergoing diagnostic coronary angiography, reported that low PON1 activity is associated with increased risk of CVD events. These findings strongly support our results, although they did not evaluate PON1 concentrations.

Many investigators have reported associations of PON1 polymorphisms, including 55L/M, 192Q/R and -108C/T, with the presence of CHD in case-control studies. Especially, 192RR genotype has been emphasized as a risk factor for CHD [12] though some studies present negative results [13]. Mackness et al. [14] demonstrated, in vitro, that HDL from PON1 192QQ homozygotes, which show the lowest paraoxonase activity, is effective in protecting LDL, while that from RR homozygotes, which show the highest activity, and is less effective. In concordance with this, a recent study showed that malondialdehide concentration, a marker of oxidative stress, was significantly higher in CHD patients with RR phenotype than in those with QQ phenotype [15]. In the present study, however, none of PON1 gene polymorphisms was associated with the development of CVD during the observation period. Similarly, previous prospective studies failed to identify direct associations of PON1 polymorphisms with CHD events in a healthy male population [16] and a CHD patient population [17]. Furthermore, the most recent prospective study of Bhattacharyya et al. [11] demonstrated for the first time that 192QQ genotype is associated with increased risk of CVD events. This is opposite to previous case-control studies, which suggested 192RR genotype to be a risk factor for CHD [12]. Thus, the effect of PON1 polymorphism on the development of CVD remains unclear, and it may be quite different depending on the study population. Both concentrations and functional activity of PON1 can be influenced not only by genetic polymorphisms but also many environmental factors. Therefore, circulating PON1 status, which probably reflects the consequence of an interaction between genetic and environmental factors, may be a better risk marker for CVD than genetic polymorphisms.

Although the subject number in this study was too small to arrive at any definitive conclusions, our findings are suggestive that PON1 levels are associated with the development of CVD in diabetic patients. More extensive prospective studies involving larger cohorts need to be undertaken to confirm any causative link between PON1 status and the development of CVD in this type of patient.

References

1. Mackness MI, Arrol S, Abbott C, Durrington PN (1993) Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated praoxonase. Atherosclerosis 104:129–135
2. Ikeda Y, Suehiro T, Inoue M, Nakauchi Y, Morita T, Arii K, Ito H, Kumon Y, Hashimoto K (1998) Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. Metabolism 47:598–602
3. Inoue M, Suehiro T, Nakamura T, Ikeda Y, Kumon Y, Hashimoto K (2000) Serum arylesterase/diazoxonase activity and genetic polymorphisms in patients with type 2 diabetes. Metabolism 49:1400–1405

4. Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, Miller JE, Boulton AJM, Durrington PN (1998) Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis 139:341–349
5. Boemi M, Leviev I, Sirolla C, Pieri C, Marra M, James RW (2001) Serum paraoxonase is reduced in type 1 diabetic patients compared to non-diabetic, first degree relatives; influence on the ability of HDL to protect LDL from oxidation. Atherosclerosis 155:229–235
6. Lakshman MR, Gottipati CS, Narasimhan SJ, Munoz J, Marmillot P, Nylen ES (2006) Inverse correlation of serum paraoxonase and homocysteine thiolactonase activities and antioxidant capacity of high-density lipoprotein with the severity of cardiovascular disease in persons with type 2 diabetes mellitus. Metabolism 55:1201–1206
7. McElveen J, Mackness MI, Colley CM, Peard T, Warner S, Walker CH (1986) Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clin Chem 32:671–673
8. Ayub A, Mackness MI, Arrol S, Mackness B, Patel J, Durrington PN (1999) Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol 19:330–335
9. Kim NS, Kang K, Cha MH, Kang B-J, Moon J, Kang BK, Yu B-C, Kim Y-S, Choi SM, Bang O-S (2007) Decreased paraoxonase-1 activity is a risk factor for ischemic stroke in Koreans. Biochem Biophys Res Commun 364:157–162
10. Can Demirdögen B, Türkanoglu A, Bek S, Sanisoglu Y, Demirkaya S, Vural O, Arınc E, Adalı O (2008) Paraoxonase/arylesterase ratio, PON1 192Q/R polymorphism and PON1 status are associated with increased risk of ischemic stroke. Clin Biochem 41:1–9
11. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, Fu X, Shao M, Brennan DM, Ellis SG, Brennan M-L, Allayee H, Lusis AJ, Hazen SL (2008) Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA 299:1265–1276
12. Durrington PN, Mackness B, Mackness MI (2001) Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol 21:473–480
13. Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J (2004) Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. Lancet 363:689–695
14. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN (1998) Effect of the human serum paraoxoanse 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett 423:57–60
15. Kotur-Stevuljevic J, Spasic S, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V, Stefanovic A, Vujovic A, Memon L, Kalimanovska-Ostric D (2008) PON1 status is influenced by oxidative stress and inflammation in coronary heart disease patients. Clin Biochem 41:1067–1073
16. Robertson KS, Hawe E, Miller GJ, Talmud PJ, Humphries SE (2003) Human paraoxonase gene cluster polymorphisms as predictors of coronary heart disease risk in the prospective Northwick Park Heart Study II. Biochim Biophys Acta 1639:203–212
17. Turban S, Fuentes F, Ferlic L, Brugada R, Gotto AM, Ballantyne CM, Marian AJ (2001) A prospective study of paraoxonase gene Q/R192 polymorphism and severity, progression and regression of coronary atherosclerosis, plasma lipid levels, clinical events and response to fluvastatin. Atherosclerosis 154:633–640