A polymorphism upstream from the human paraoxonase (PON1) gene and its association with PON1 expression

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Abstract

Human serum paraoxonase (PON1) is an esterase that has been shown to decrease the susceptibility of lipoproteins to lipid peroxidation. We found a polymorphism of cytosine/thymidine (−108C/T, the number is from the ATG codon) in the upstream region of the PON1 gene. The luciferase activity was lower in the −108T allele than in the −108C allele. The serum PON1 concentrations in 132 normal subjects were as follows: −108CC > −108CT and > −108TT genotypes. The polymorphism upstream from the PON1 gene is associated with transcription of the PON1 gene and the serum PON1 concentration. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Paraoxonase; Lipid peroxidation; Genetic polymorphism; Coronary heart disease

1. Introduction

Human serum paraoxonase (PON1) is associated with high-density lipoprotein (HDL) [1] and has been shown to decrease the susceptibility of low-density lipoprotein (LDL) to lipid peroxidation in vitro,[2,3] which may prevent the development of atherosclerosis [4]. Recently, PON1-knockout mice were developed and were found to be more susceptible to atherosclerosis than their wild-type littermates [5]. This protein in humans has two polymorphic sites: Leu→Met at position 55 of the amino acid sequence (L→M), and Gln→Arg at position 192 (Q/R) [6]. Q/R polymorphism is associated with PON activity which is estimated using paraoxon as a substrate, and the PON activity in subjects with the QQ genotype is lower than that in subjects with the RR genotype [7]. On the other hand, L/M polymorphism is related to the serum PON1 concentrations [8]. Little information is available concerning other factors which influence the PON1 concentration. We detected polymorphisms in the upstream of the PON1 gene. We examined the relationship between the polymorphisms and the promoter activity of PON1 or the serum PON1 concentrations.

2. Materials and methods

Samples from 161 normal healthy subjects used in a previous study of PON1 [9], were employed in this study. The subjects visited a medical center in Kochi and were confirmed as being of normal status by physical and laboratory examinations including normal resting electrocardiography. No subject had diabetes mellitus, as confirmed by an oral glucose tolerance test, or a history of cardiovascular disease. As the serum from 29 subjects was completely used in previous study, only 132 of 161 samples (63 males and 69 females) were available to undergo complete detection of polymorphisms upstream from the PON1 gene and protein concentration examination. Their mean age was 57 ± 8 (S.D.) years. The data of the 55L/M and 192Q/R polymorphisms were used from the previous study [9].
The 5′ end of exon 1 of the PON1 gene was not mapped. Therefore, we applied the nucleotide number from the ATG start codon to a nucleotide position in the upstream region. PON1 in the human sera was assessed by ELISA (WAK-CHEMIE MEDICAL GMBH, Bad Soden, Germany). Briefly, ELISA plates (Corning, Corning, NY), were coated with antigen at room temperature and left overnight. The plates were blocked with 1% BSA for 1 h at 37°C. The mixtures of test sera and anti-paraoxonase antibody were incubated in a pretreated ELISA plate for 3 h at 37°C. The bound anti-paraoxonase antibody was detected with alkaline phosphatase conjugated antibody (Sigma, St Louis, MO).

We detected the 5′ upstream region of the PON1 gene using a method of ‘walking upstream’ (Genome-Walker Kits, Clontech, Palo Alto, CA). We used an antisense primer whose sequence was obtained from Genbank (U55877). Variations upstream from the promoter region of the PON1 gene were detected by a cycle sequencing method. The DNA fragment was amplified by the PCR method using a sense primer (5′-GGGTCCCACAGCAA-CAATATC-3′) and an antisense primer (5′-GGTCCCACAGCAA-CAATATC-3′), which were chosen from exon 1 and exon 6. The PCR product (475 bp) was detected in 2% agarose gel and the sequence of the product was confirmed by the cycle sequencing method.

Transient transfection into HepG2 cells was performed by a cationic lipid method using Tfx™-20 (Promega, Madison, WI). The cells were incubated in a 35 mm dish, and 13.2 μl of Tfx™-20 and 2.0 μg DNA were added. After incubation for 1 h, the cells were incubated for an additional 48 h in medium with serum. The luciferase assay was performed using a commercial kit (Luciferase Assay System, Promega, Madison, WI) according to the procedure. The luciferase activities were normalized using protein concentrations (luciferase light units/μg protein) and expressed as relative values compared with the activity of –108C DNA. The protein concentration was measured using the Protein Assay Kit (Bio-Rad Lab, Richmond, CA).

All data were presented as the mean ± S.D. The distribution of two PON1 genotypes of –108C/T and 192Q/R were estimated by contingency table analysis. Comparison of variables among groups was performed using one-way ANOVA and each comparison was estimated by Fisher’s protected least significant difference. Comparison of two groups was estimated by the unpaired t-test.

3. Results

Three polymorphisms were detected in the upstream fragment (–269/+65) as follows: –108C/T, –126G/C, and –160G/A. The –126G/C and –160G/A polymorphisms were almost linked. All –160GG and –160AA homozygotes showed –126GG and –126CC homozygotes, respectively. Only 1.8% of –160GC heterozygotes showed –126GG homozygotes. The frequencies of the –126C allele and –160A allele were low (0.09 and 0.10, respectively).

The frequencies of –108CC, –108CT, and –108TT genotypes were 25.8, 44.7, and 29.5%, respectively. Allele frequencies of C and T were 0.48 and 0.52, respectively. Table 1 shows the distributions of –108C/T and 192Q/R. There was a significant relationship between the –108C/T and 192Q/R polymorphisms. The frequency of the 192RR genotype in the –108TT genotype was higher than that in the –108CC genotype.

The HepG2 cell line that we used expressed PON mRNA (Fig. 1). As shown in Fig. 2, the luciferase activity of the –108T allele was significantly lower than that of the –108C allele. We examined the assay
four times, and the mean activity of the \(-108\)T allele was 0.67–0.77-fold lower than that of the \(-108\)C allele.

The mean concentration of serum PON1 was as follows: \(-108\)CC \(>\) \(-108\)CT \(>\) \(-108\)TT genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CC (%)</th>
<th>CT (%)</th>
<th>TT (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-108)CC</td>
<td>7 (20.6)</td>
<td>6 (10.2)</td>
<td>1 (2.6)</td>
<td>14</td>
</tr>
<tr>
<td>(-108)CT</td>
<td>23 (67.6)</td>
<td>39 (67.1)</td>
<td>16 (41.0)</td>
<td>78</td>
</tr>
<tr>
<td>(-108)TT</td>
<td>4 (11.8)</td>
<td>14 (22.7)</td>
<td>22 (56.4)</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>34 (100.0)</td>
<td>59 (100.0)</td>
<td>39 (100.0)</td>
<td>132</td>
</tr>
</tbody>
</table>

\(^a\) \(\chi^2; P < 0.001\).

Fig. 1. Expression of PON mRNA in HepG2 cell line and human liver tissue. Lane (a), maker (\(\phi\)X174-HaeIII digest); lane (b), PCR product from the cDNA of HepG2 cell; lane (c), PCR product from the cDNA of human liver tissue.

Fig. 2. The relative luciferase activities of the upstream region in the \(-108\)C allele and the \(-108\)T allele of the PON1 gene. \(*P < 0.001\), unpaired \(t\)-test. Control shows the activity by pGL3-Basic without introducing DNA.

4. Discussion

PON1 exhibits several enzymatic activities associated with artificial substrates [10], but its physiological function is unknown except for a protective effect against lipid oxidation of LDL or HDL [2,3,11]. The enzyme activity for an artificial substrate may not always indicate the physiological effect of PON1. The 192Q/R polymorphism may be involved in CHD, and the RR is related to an increased risk of CHD [12,13]. However, PON activity in the RR genotype is higher than that in the QQ genotype. The serum PON1 concentration is related to 55L/M polymorphism although the 192 polymorphism had little impact on the concentration [8]. The relationships among enzyme activities, mass concentrations, and clinical diseases have not been established.

The \(-108\)C/T variation is a common polymorphism at least in Japanese populations, although the polymor-
phism has been reported in the Genebank (accession No. AF051133) from Switzerland. PON1 is distributed in many tissues including the liver [10], and the HepG2 cell line expresses the PON1 mRNA [14]. Therefore, we studied the reporter gene assay of PON1 gene using HepG2 cells, and the results showed that the −108C/T polymorphism was associated with the transcription of PON1. Moreover, the relationship between the genotypes and serum concentrations of PON1 suggested that the polymorphism was also associated with the serum concentration. Although the distributions of 54L/M and −108C/T polymorphisms were linkage disequilibrium, the concentration of PON was related to the −108C/T polymorphism not only in all subjects, but also in the group of 54LL genotype. Therefore, the relationship between the serum concentration and the −108C/T polymorphism might be independent of the 54L/M polymorphism.

The sequence of the −108C allele (ggggcggggc) showed a consensus sequence of the Sp1 binding site [15]. The region of polymorphism may be involved in the transcription through Sp1. However, the promoter activity of the −108T allele by the luciferase assay decreased by 0.7-fold compared with that of the −108C allele. Therefore, the region only partly regulates PON1 transcription. Our reporter gene assay was estimated without any stimulation. The promoter activity may be influenced by many factors in vivo, and changes in various conditions. In addition, the inserted DNA of the PON1 upstream region was within 600 bp. There may be other stronger regulatory sites further upstream. The relationships among other sites in the PON1 upstream region and the −108C/T polymorphism should be examined.

Recently, Mackness et al. showed that the PON1 QQ-HDL was the most efficient at protecting LDL against oxidative modification and PON1 RR-HDL was the least efficient [16]. The 192Q/R polymorphism may affect the ability of HDL to protect LDL from oxidation. However, Cao et al. could not confirm lower activity of the R allele towards lipid peroxides [17]. Our result showed that the −108C/T polymorphism was linked to the 192Q/R polymorphism. The association of 192Q/R polymorphism with CHD may be partly reflected by the −108C/T polymorphism. Furthermore, the relationship between polymorphisms of the PON1 gene and CHD must be clarified.

References