Apolipoprotein E polymorphism and low density lipoprotein (LDL) oxidation in patients with dementia

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In patients with dementia, 29 diagnosed as probably suffering from Alzheimer’s disease and 46 subjects with dementia of vascular origin, and in 41 non-demented control subjects LDL oxidation in vitro was compared in carriers of various apolipoprotein E alleles. Restriction isotyping was performed by gene amplification and cleavage with HhaI, LDL oxidation was investigated by determination of conjugated dienes and vitamin E (α-tocopherol) plasma level was measured by HPLC.

In subjects with dementia oxidation of LDL was shown to be higher in carriers of e4 allele as compared with non-carriers of this allele. It was especially observed in the propagation phase, which illustrates oxidation intensity after the exhaustion of the antioxidant reserve in LDL. Vitamin E level did not show differences between carriers of different alleles.

It is concluded that the differences in oxidation susceptibility of LDL between demented subjects possessing particular apolipoprotein E forms can result partially from differing antioxidant properties of apolipoprotein E isoforms and, in a substantial degree, from the size and quality of LDL.

key words: Apolipoprotein E, LDL oxidation, dementia

INTRODUCTION

Polymorphism of apolipoprotein E (ApoE) plays a significant role in the development of some diseases, such as atherosclerosis and dementia. ApoE appears in humans as three types named E2, E3 and E4, differing from each other by a single amino acid substitution and coded by three alleles e2, e3 and e4 at a single gene locus. The isoforms differ to some extent in their biological activity, ApoE4 representing the most disadvantageous form, especially in the case of dementia. This form is present with markedly increased frequency in Alzheimer’s disease patients. In our previous work [14] we found it in as many as 32% of demented individuals, as compared with 11.5% in subjects of the same age without dementia.

Oxidative stress plays an important role in the pathogenesis of dementia [8]. Beta amyloid accumulating in the brain of Alzheimer’s disease patients produces free radicals and exerts a cytotoxic effect on nervous cells [1, 7]. This effect can be combated by antioxidative agents. The main plasma antioxidant is α-tocopherol (vitamin E). Oxidation of low density lipoprotein (LDL) contributes substantially to the development of atherosclerotic changes in dementia of vascular origin.

It was found that apolipoprotein E also has an antioxidant action. The plasma level of ApoE is lower in subjects with ApoE4 as compared with persons with other
polymorphic species [10]. ApoE4 has a lower level of antioxidative action than the other forms [5, 12]. LDL contains very little ApoE. Nevertheless, the lower antioxidative properties of the ApoE4 form could influence the total antioxidative defence of the organism.

In this work an attempt was made to evaluate whether being an APOE4 carrier had an influence on LDL oxidation in patients with dementia.

MATERIAL AND METHODS

Patients: 29 subjects diagnosed as probable Alzheimer’s disease (AD) — 11 males and 18 females, 48–83 years old (mean age 68.4 ± 9.1 y.) and 46 subjects with dementia of vascular origin (VD) — 23 males and 23 females, 46–90 years old (mean age 70.7 ± 7.5 y.). Controls: 41 subjects without dementia — 16 males and 25 females, 47–84 years old (mean age 67.3 ± 7.0 y.).

Dementia was diagnosed on the basis of DSM IV. The differential diagnosis between AD and VD was based on neurological and neuropsychological examinations and neuroimaging investigations according to NINCDS-ADRDA and NINCDS-AIREN criteria.

The patients were divided into two groups: carriers and non-carriers of \( \varepsilon^4 \) allele (coding ApoE4). The carrier group comprised 24 persons with genotype \( \varepsilon^4/4 \) and \( \varepsilon^4/3 \), the non-carrier group 51 persons with genotypes \( \varepsilon^2/3 \) and \( \varepsilon^3/3 \). There were 14 carriers and 15 non-carriers in the AD group, 10 and 36 in the VD group and 7 and 34 in the control group respectively.

The patients gave their informed consent and the study was approved by the Ethics Committee of the Institute of Psychiatry and Neurology in Warsaw, which is in agreement with European laws of ethics.

Apolipoprotein E genotype identification: leukocyte DNA was isolated by phenol extraction. The genotype was identified by the method of Hixson and Vernier [4], consisting in the amplification of the gene section, digestion with the restricting enzyme HhaI and identification of the DNA fragments after polyacrylamide gel electrophoresis.

LDL oxidation: LDL was isolated from blood plasma by ultracentrifugation [3]. The kinetics of LDL oxidation in vitro in the presence of Cu++ was evaluated by measuring the appearance of conjugated dienes at 3-min intervals over 3 hours at 243 nm according to Esterbauer et al. [2]. In the course of LDL oxidation two principal phases can be observed: the lag phase expressed in minutes indicating a delay in oxidation caused by the presence of antioxidants (the main antioxidant is \( \alpha \) tocopherol), and a propagation phase expressed as nmoles of conjugated dienes arising per milligram of LDL protein per minute and illustrating the rate of oxidation after the exhaustion of the antioxidants. The rate of oxidation depends on Cu++ availability and, probably, on LDL characteristics.

\( \alpha \) tocopherol (vitamin E) was determined by HPLC [9].

Cholesterol and triglycerides were determined by enzymatic methods using RANDOX kits and a Synchron Cx7 Beckman spectrophotometer.

Statistical methods: differences between the groups were evaluated by the Mann-Whitney test. \( P < 0.05 \) was considered as statistically significant.

RESULTS

Table 1 presents the results of LDL oxidation measurements in carriers and non-carriers of \( \varepsilon^4 \) allele.

<table>
<thead>
<tr>
<th>Patients</th>
<th>AD</th>
<th>VD</th>
<th>AD + VD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carriers of ( \varepsilon^4 ) allele</td>
<td>Non-carriers of ( \varepsilon^4 ) allele</td>
<td>Carriers of ( \varepsilon^4 ) allele</td>
<td>Non-carriers of ( \varepsilon^4 ) allele</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>15</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>Lag phase [min]</td>
<td>56.7 ± 12.4</td>
<td>67.0 ± 25.7</td>
<td>50.4 ± 12.4</td>
<td>64.4 ± 29.1</td>
</tr>
<tr>
<td>P</td>
<td>0.230</td>
<td>0.027</td>
<td>0.060</td>
<td>0.136</td>
</tr>
<tr>
<td>Propagation rate [nmoles of dienes/mg LDL protein/min]</td>
<td>5.67 ± 0.79</td>
<td>4.17 ± 1.25</td>
<td>5.63 ± 1.61</td>
<td>4.39 ± 1.29</td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td>0.014</td>
<td>0.00004</td>
<td>0.808</td>
</tr>
</tbody>
</table>

AD — Alzheimer’s disease, VD — vascular dementia
In the patients with dementia the lag phase was shorter in the carriers as compared with non-carriers and this difference was statistically significant in the VD group. The propagation phase showed a significant increase in the carriers of e4 allele in all the demented subjects. In the controls no differences between LDL susceptibility towards oxidation were observed between carriers and non-carriers of e4 allele.

Table 2 presents α-tocopherol levels in the same groups. No statistically significant differences between e4 carriers and non-carriers were observed.

**DISCUSSION**

Miyata and Smith [5] have observed in their experiments on cell cultures that the antioxidative effect of apolipoprotein E did not cause the prolongation of the lag phase which depends on the presence of α tocopherol. On the contrary, it affected mostly the propagation phase, which was illustrated by differences in the metal binding between various ApoE types. This could depend on LDL properties. In our study the difference in LDL oxidation between carriers and non-carriers of the APOE4 gene mostly concerned the propagation phase and, to a lesser extent, the lag phase. We did not observe a low α tocopherol level in our e4 carriers, in whom a greater susceptibility of LDL toward oxidation was found. In these subjects some degree of lag phase shortening could depend on other antioxidants.

Nikkila et al. [6] have stated that possessing a particular ApoE isoform had a significant influence on LDL size, which decreased in the order E2/2, E3/3, E3/4 and E4/4. It was also observed that the oxidative susceptibility of LDL increased with their increasing density and decreasing particle diameter [13]. This has also been confirmed by other authors [11].

We therefore consider that the higher oxidation of LDL observed in our group of e4 carriers with dementia could depend only partially on the antioxidant properties of various ApoE isoforms. The difference could also be caused by different LDL properties, such as their size and density. The e4 gene is frequent in dementia and in atherosclerosis and such LDL properties in its carriers could contribute to the disadvantageous role played by e4 in the development of disease. It could then intensify the pathological processes taking place in the brains of demented subjects.

The question remains as to why the APOE4 gene influenced LDL oxidation only in the demented patients and not in the controls. The possibility is that this was caused by some deficiency in the patients’ antioxidative reserve.

**REFERENCES**


