Eur Arch Psychiatry Clin Neurosci (2002) 252 : 63-67

ORIGINAL PAPER

György Paragh · Petra Balla · Evelin Katona · Ildikó Seres · Anikó Égerházi · István Degrell

Serum paraoxonase activity changes in patients with Alzheimer's disease and vascular dementia

Received: 30 November 2001 / Accepted: 24 January 2002

Abstract The prevalence of Alzheimer's disease (AD) and vascular dementia (VAD) increases with aging of the population. The role of lipoproteins in the pathogenesis of AD is unclear: $apoE_2$ offers protection and $apoE_3$ is neutral, while $apoE_4$ promotes the development of the disease.

Recently, several studies have confirmed the role of oxidative stress in the pathogenesis of AD and VAD. HDL-associated paraoxonase is one of the antioxidative enzymes that may reduce LDL oxidation. In our study, we investigated the lipid parameters of the sera and the serum paraoxonase activity in patients with AD and VAD.

Lipid parameters were determined by an autoanalyzer in 30 AD patients, 40 VAD patients and 40 healthy, age-matched control (C) subjects. Paraoxonase activity was measured spectrophotometrically using paraoxon as the substrate. The phenotypic distribution of paraoxonase was determined by the dual substrate method, using paraoxon and phenylacetate as substrates.

In our results, we found that most of the patients with AD had the $apoE_4$ isoform, consistent with other studies. In the VAD and AD patients we found significantly higher total-cholesterol compared to the control group (C: 4.71 ± 0.89, VAD: 6.3 ± 0.8, AD: 6.52 ± 0.7 mmol/l; $p < 0.01$) and LDL-cholesterol levels (C: 2.6 ± 0.6, VAD: 3.96 ± 0.8, AD: 3.84 ± 0.6 mmol/l; $p < 0.001$). The HDL-associated antioxidant, paraoxonase activity did not differ significantly in the patient groups, but compared to the healthy control subjects, paraoxonase activity was significantly lower in both of the patient groups (C: 188 ± 55 U/l; AD: 131 ± 37, VAD: 151 ± 50 l; $p < 0.05$).

Our results suggest that the defect in HDL-associated antioxidant capacity plays a role in the pathogenesis of Alzheimer's disease and vascular dementia.

Key words Paraoxonase · Alzheimer's disease · vascular dementia · HDL

György Paragh, MD, DSc (✉) · E. Katona · I. Seres · A. Égerházi
University of Debrecen
1st Department of Internal Medicine
Nagyerdei krt. 98
4012 Debrecen, Hungary

P. Balla · I. Degrell
Department of Psychiatry
Nagyerdei krt. 98
4012 Debrecen, Hungary

Introduction

The prevalence of dementia is increasing in developed countries. New therapeutic possibilities and the development of preventive measures have led to an increase in the life span of the population, thereby increasing the prevalence of vascular cerebral disease, and dementia with organic etiology [36].

One of the characteristic neuropathological features of AD is the presence of amyloid-containing senile plaques. The senile plaques comprise aggregates of β-amyloid, which is derived from the amyloid precursor protein [7].

Increasing evidence suggests that cholesterol plays a role in the pathophysiology of Alzheimer's disease, and elevated serum total-cholesterol level has been shown to be a risk factor for AD [17, 37].

The connection between apoE isoforms and Alzheimer's disease (AD) was first described in 1993 [8, 33]. The presence of $apoE_2$ isoform inhibits the development of AD, while the presence of $apoE_4$ raises the risk of Alzheimer's disease 3- to 8-fold [4, 8, 20, 29]. Earlier studies suggest that $apoE_2$ and $apoE_3$ form a stable bond with tau protein, and this binding inhibits the phosphorylation of tau protein, thereby stabilizing microtubules and cytoskeleton in the neuron. On the other hand, the connection between $apoE_4$ and tau protein is unstable, and can not protect against phosphorylation and neuron degeneration [27]. The aggregation of tau isoforms into intraneuronal filaments is an important pathological event in the pathogenesis of AD. Until recently, it was thought that an abnormal phosphorylation of tau proteins was responsible for the aggregation in AD. Buée et

al. suggest that in addition to phosphorylation, other mechanisms may be involved in the formation of pathological tau filaments [5]. In the pathogenesis of AD, neuron degeneration is caused by a combination of beta amyloid production, oxygen deficiency, and lipid peroxidation [18]. $ApoE_3$ and $apoE_2$ have an effective support repair mechanism [4, 20, 29]. Oxidative stress is an important noxious agent; Behl et al. established that amyloid produces free radicals, which damage the neurons [3]. Dyrks et al. demonstrated that oxidative stress is responsible for transforming soluble amyloid into the insoluble fibril form [11]. Troncoso et al. found a strong association between oxidative stress and the polymerization of tau protein [35]. These findings suggest that beside beta amyloid, oxidative stress also plays a role in the development of the disease. In this respect, there are some similarities between atherosclerosis and AD, because oxidative stress and lipid metabolism also play important roles in atherosclerosis. Ross et al. found that on the surface of monocytes in the circulation, integrins appear, which bind to vascular adhesion molecules on the endothelial surface [30].

The adherent monocytes penetrate the subendothelial space, where they become macrophages. Simultaneously, one of the circulating lipoproteins, mainly the small, dense LDL cholesterol enters into the subendothelial space, where it is oxidized by free radicals, which are produced during macrophage activation [30]. Macrophages present in the subendothelial space take up the oxidatively modified LDL through their scavenger receptor, although this uptake is not able to mediate the negative feed back mechanism, which should be able to stop the cholesterol accumulation in the cells, resulting in macrophages becoming foam cells that initiate atherosclerosis [14, 25, 32].

VAD is caused by vessel occlusion related to arteriosclerosis leading to progressive white matter degeneration [28]. This similar pathomechanism raises the possibility of the protective HDL-cholesterol playing an important role in the development of AD and vascular dementia, since HDL inhibits oxidative modification and atherosclerosis. This protective effect is partly the result of HDL's reverse-cholesterol transport function, and partly due to HDL associated paraoxonase (PON) [24]. Paraoxonase can inhibit low-density lipoprotein (LDL) oxidation and prevents accumulation of lipid peroxides in LDL [22]. This enzyme is a glycoprotein of 43 000 Da molecular weight, its gene is located on chromosome 7 [12], and the enzyme's activity and stability require the presence of Ca^{++} [13]. Paraoxonase is synthesized in the liver, and is comprised of three phenotypes: BB with high activity, AA with low activity and AB type with intermediate activity [10, 16, 36].

The aim of our present study was to determine the lipid parameters and paraoxonase activity in 30 AD and 40 VAD patients, compared to that of healthy controls. We would like to study the connection between lipid abnormalities and HDL-associated oxidative changes in AD and VAD.

Methods

Thirty patients with AD (20 females, 10 males, mean age 64.3 ± 11.7 years), 14 of whom had coronary artery disease (CAD); and 40 patients with VAD (27 females, 13 males, mean age 76.1 ± 12.4 years), 34 of whom had CAD; 40 voluntary, healthy, control subjects from the same age group (26 females and 14 males, mean age 72.3 ± 9.6 years) were included in the study.

The patients were selected from the in- and outpatient units of the Psychiatric Department, Medical and Health Science Center University of Debrecen.

Patients were diagnosed according to the ICD–10 (and DSM-IV) diagnostic criteria system [2], and NINCDS-ADRDA criteria [26], MMSE score (< 24), California Verbal Learning Test (CVLT) score (< 60 %). Dementia caused by other possible diseases was excluded. All participants had laboratory tests: serum ion, enzyme levels, thyroid-, liver-, and kidney function, lipid levels, urine examination, blood count, erythrocyte sedimentation rate, vitamin B_{12} level, folic acid level, hemostasis, VDRL, apoE genotype, chest X-ray, EEG, CT/MR examination. Cerebral SPECT and carotid Doppler examination were also performed when it was clinically indicated. The differential diagnosis between AD and VAD was based on the Hachinski Ischemic Scale, laboratory tests, anamnestic data, imaging investigations and attending illnesses. Healthy controls were included in the study after general physical examination, and routine laboratory tests, in cases of negative neurological and psychiatric anamnesis, and MMSE score between 27 and 30 points. The use of antilipidemic or antioxidant drugs was an absolute exclusion criterion for all the three groups.

Blood sampling

Blood sampling was taken after an overnight fast. From these samples, hemoglobin, hematocrit, leukocyte count, liver enzymes, urea, creatinine, CK, fibrinogen, C-reactive protein, bilirubin, uric acid, serum glucose, total serum cholesterol, HDL-cholesterol, triglyceride, apoA1, apoB100, lipoprotein(a) and activity of serum paraoxonase were measured.

Lipid measurements

Serum cholesterol and triglycerides were assayed with a Boehringer Mannheim GmbH Diagnostic enzyme kit, while the HDL-cholesterol was measured by the phospho-tungstic-magnesium precipitation method. The LDL-cholesterol fraction was calculated indirectly using the Friedewald equation (Tg < 4.5 mmol/l). Apolipoprotein examination was performed with immuno-nephelometric assay (Orion Diagnostic kit).

Paraoxonase (PON) activity

Paraoxonase activity was determined, using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma Chemical Co) as the substrate, by measuring the increase in the absorbance at 412 nm due to formation of 4-nitrophenol. Activity was measured by adding 50 µl serum to 1 ml Tris/ HCl buffer (100 mmol/l, pH = 8.0) containing 2 mmol/l $CaCl_2$ and 5.5 mmol/l paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm, 25 °C, by the use of a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzymatic activity was calculated from the molar extinction coefficient 17 100 $M^{-1}\,cm^{-1}$. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above assay conditions [1]. Salt-stimulated PON activity was measured in the presence of 1M NaCl.

Arylesterase assay

Arylesterase activity was also measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris/HCl (pH 8.0). The

reaction was started by the addition of serum, and the increase in absorbance was read at 270 nm. Blanks were included to correct the spontaneous hydrolysis of phenylacetate. Enzyme activity was calculated using the molar extinction coefficient 1310 $M^{-1}cm^{-1}$. Arylesterase activity was expressed in units per liter. One unit is defined as 1 μmol phenylacetate hydrolyzed per minute.

ApoE polymorphism

Deteminations were carried out from peripheral blood lymphocytes. The polyacrylamide gel electrophoresis was performed on fragments of genomic DNA, obtained by salt extraction, using the PCR technique [15].

Statistical methods

The SAS TM for Windows TM 6.11 computer program was used to perform the statistical analysis. Data were presented by descriptive analysis (case number, mean, standard deviation). The comparisons between groups were performed by *t*-test and ANOVA. The $p < 0.05$ probability was accepted as the significance level.

Results

The serum cholesterol levels were significantly higher both in AD and VAD patients compared to the controls (C: 4.71 ± 0.89; VAD: 6.3 ± 0.8; AD: 6.52 ± 0.7 mmol/l; $p < 0.001$). Similar differences were found in serum LDL-cholesterol levels (C: 2.6 ± 0.6; VAD: 3.96 ± 0.8; AD: 3.84 ± 0.6 mmol/l; $p < 0.001$). The serum triglyceride level was higher in both patient groups compared to the controls, but not significantly (C: 1.06 ± 0.52; VAD: 1.47 ± 0.8; AD: 1.68 ± 0.1 mmol/l). The protective HDL's level was significantly higher in AD patients compared to the controls and to the VAD patients (C: 1.47 ± 0.1 mmol/l; VAD: 1.43 ± 0.31; AD: 1.95 ± 0.1; $p < 0.001$) (Fig. 1). The decrease in HDL-associated paraoxonase activity in both patient groups was significantly lower compared to the control group (C: 188 ± 55 U/l; VAD: 151 ± 47; AD: 131 ± 40; $p < 0.05$). The NaCl stimulated paraoxonase activity decreased significantly in both patient groups compared to the control group (C: 422 ± 120; VAD: 343 ± 89; AD: 272 ± 100 U/l; $p < 0.05$). No difference was observed in the arylesterase activity of the three groups (C: 130 ± 35 U/l, VAD: 128 ± 40; AD: 123 ± 34) (Fig. 2). To determine the connection between paraoxonase activity and HDL level, we corrected the enzyme activity for HDL concentration. The PON/HDL ratio in both patient groups decreased significantly compared to the healthy control group (C: 194 ± 79; VAD: 98.4 ± 40; AD: 88.4 ± 34; $p < 0.001$) (Fig. 3). In the AD patient's group apoE3/4 isoform was found in 44 % of the samples, the frequency of apoE3/3 was 33 %, and the apoE4/4 isoform's frequency was 19 %, apoE2/3 isoform was found in 4 % of samples, while in VAD patients, the distribution of apoE genotype was 3/3:56 %, 3/4:38 %, 2/3:6 %. (Table 1)

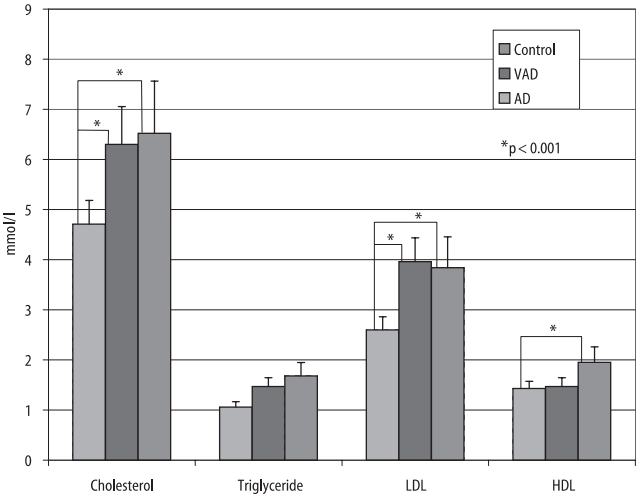

Fig. 1 Lipid parameters of the studied groups.

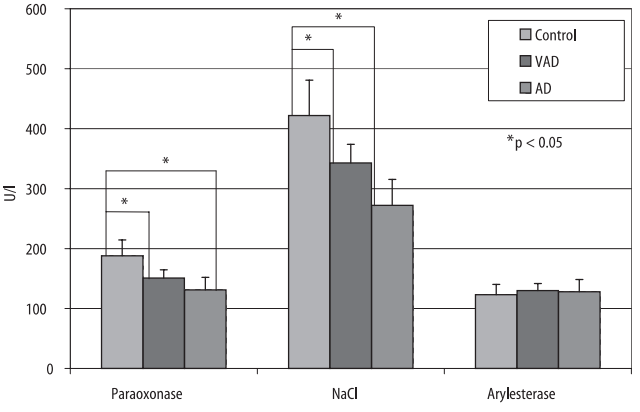

Fig. 2 Serum paraoxonase, salt-stimulated paraoxonase and arylesterase activity.

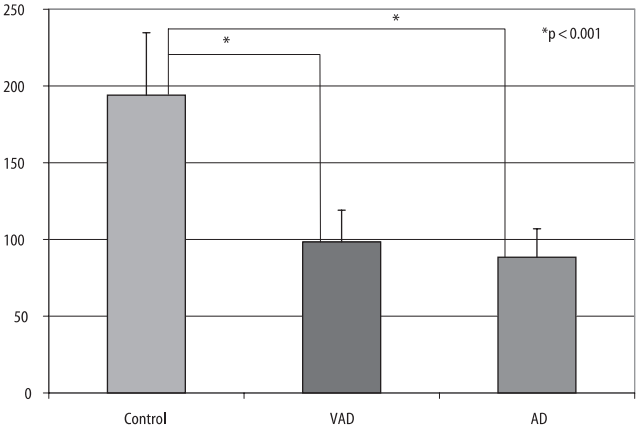

Fig. 3 Paraoxonase activity corrected for the HDL-C concentration (PON/HDL ratio).

Table 1 Percentile distribution of ApoE isoforms

	AD	VAD	C
ApoE 3/4	0.44	0.38	0.23
ApoE 3/3	0.33	0.56	0.65
ApoE 2/3	0.04	0.06	0.08
ApoE 4/4	0.19		0.01
ApoE 2/2			0.02
E2 (%)	1.77	3.57	6.4
E3 (%)	58.72	76.78	80.4
E4 (%)	39.78	19.74	12.9

Discussion

There are many similar events in the pathomechanisms of AD and atherosclerosis. The altered lipid metabolism and the importance of oxidative stress are noticeable in both processes. Because of the increasing number of Alzheimer and vascular dementia cases, more investigations are required to determine the exact details of the pathomechanism. We found that occurrence of apoE4 isoform in the AD patients was 39.8 % and in the VAD patients 19.7 %, consistent with other studies [4, 31].

The HDL particle has an antiatherogenic function, due partly to its antioxidant effect [6, 19]. Mackness et al. found that HDL inhibits lipid peroxide accumulation in LDL particles in vitro [21]. The activity of HDL associated paraoxonase has been reported to be significantly reduced in patients with diabetes mellitus, hypercholesterinemia, after myocardial infarction, chronic uremia, and after kidney transplantation [24].

In our present study, we found that in patients with AD and VAD, the serum triglyceride level was not significantly increased compared with healthy controls. In contrast, the serum cholesterol levels in AD and VAD patients were significantly higher compared with the control subjects (Fig. 1). We have found significant differences between the activities of HDL associated paraoxonase enzyme in the patients' groups and normal subjects. The NaCl stimulated paraoxonase activity was significantly decreased in the two patient groups.

These results suggest that paraoxonase in these patients is able to do less work in the presence of NaCl. The studies by Corrigan et al. raised the possibility that the altered components of HDL particle might cause decreased paraoxonase activity [9]. Since paraoxonase is a HDL-associated enzyme and in the patients with AD we found significantly higher serum HDL level, the question has been raised about how paraoxonase activity correlates with one unit HDL in the patients' group compared with control subjects. We found a significantly decreased PON/HDL ratio in both patients' groups compared to control subjects. Thome et al. found significantly decreased superoxide dismutase in an AD patient group [34]. This suggests that altered antioxidant capacity of the serum plays a role in the pathomechanism of Alzheimer's disease.

These results suggest that beside altered lipid metabolism and increased oxidative stress, a defect in the antioxidant system capacity and altered paraoxonase activity play important roles in the above described pathomechanism.

Acknowledgments This study was supported by a grant from ETT (398/2000), Hungary.

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