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SHORT COMMUNICATION

Plasma protein glycation in Alzheimer's disease

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Recent studies have suggested that formation of advanced glycation end-products (AGEs) in some brain proteins could be associated with Alzheimer's disease.These AGEs can be produced by various sugars (hexose, pentose, glyceraldehyde and oxidative products of vitamin C). In this study, we quantified plasma protein glycation specifically derived from glucose in patients with Alzheimer's disease with different grades of cognitive disorders.

Two groups of Alzheimer patients were studied: a group with moderate Alzheimer's disease (n=6, 9<MMS<20) and a severe Alzheimer group (n=13, MMS<9) who were compared with an age-matched control group (n=10, MMS>23) and a group of subjects with diabetes (n=31). Protein glycation was evaluated in plasma with a highly specific HPLC-UV technique, using furosine, wich is the acid hydrolysis product of ε-deoxy-fructosyl-lysine

Plasma furosine was almost two times higher in subjects with Alzheimer's disease ($p < .005$) than in controls, but still 50% lower than in diabetic patients ($P < .02$). Fasting plasma glucose levels were significantly correlated to the furosine concentration.To explain these results, an eventual impairment in glucose peripheral use or an increase in protein glycation rate associated with Alzheimer's disease should be explored.

Keywords: protein glycation, furosine, plasma glucose, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is characterized by the presence of modified proteins in the brain, such as β-amyloid protein in the senile plaque and τ protein in the neurofibrillary tangle. Recently, several studies have stressed the presence of advanced Maillard products in Alzheimer brains [1, 2] and the possible role of protein glycation as a cause of neurodegenerescence has been discussed [3]. Protein glycation is a posttranslational modification of the protein due to the covalent attachment between a sugar residue and the free ε-NH_2 group of proteins and often results in protein aggregation. In an *in vitro* assay, preaggregated β-amyloid protein induced acceleration of soluble β-amyloid protein aggregation [1]. Moreover, two Maillard end-products (pentodisine and pyrraline) have been detected immunocytochemically in neurofibrillary tangles and in senile plaques [2], but could not be found in primary AGE-structures using antibodies to glucose-albumin mixtures [4].

The purpose of this study was to verify whether plasma proteins are more glycated in Alzheimer brains than in controls, providing some evidence of an impairment in glucose metabolism in this disease. Protein glycation was measured with a highly specific HPLC assay using furosine, wich is the acidic hydrolysis product of peptidic ε-fructosyl-lysine.

Methods

Subjects with diabetes

The subjects used included 15 men (mean age 45 ± 16) and 16 women (mean age 43 ± 18), hospitalized for their insulin-dependent diabetes in the Hôtel Dieu Hospital (Paris). Diabetes was determined by checking fasting plasma glucose levels (> 7.7 mM) and glycosylated Hb.

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Elderly subjects with Alzheimer's disease

Nineteen subjects with AD, living at home or in institution and aged more than 72 years, were included in the study. The subjects who attended for cognitive checkup in a day-hospital were assigned to two groups according to the MMS test [5] (Table 1): patients with moderate Alzheimer's disease (n= 6, group 1, 9 <MMS<20) and patients with severe Alzheimer's disease (n= 13, group 2, MMS<9). The diagnosis of Alzheimer's disease used DSM III-R criteria [6] and the NINCDS-ADRDA criteria [7]. Ten control subjects (MMS> 23), who were aged more than 57 years, were selected among persons attending for a checkup in a day-hospital, subjects with diabetes were excluded from the AD and control groups. The characteristics of the different groups are given in Table 1. The three nondiabetic groups had a similar mean age and sex ratio (6 women for 4 men).

Furosine quantification

Blood samples were collected in heparin lithium, using venipuncture, and the plasma was immediately separated by centrifugation; one volume metaphosphoric acid was added to one volume plasma before freezing the sample at − 20°C for 15 days maximum.

After centrifugation, the protein pellet (around 20 mg proteins) was hydrolyzed in HCl 7.8 N, at 110°C for 18 h, allowing conversion of peptidic ε-fructosyl-lysine into furosine. After evaporation, the dry hydrolysate was resuspended in water (1 ml) and filtered with Millipore prefilters. Furosine was quantified by its absorbance at 280 nm after separation on a C18 Spherisorb column and elution with water containing 5.6 mM phosphoric acid, according to Schleicher and Wieland [8]. Standard furosine was obtained from Neosystem (France).

The total amino-acid concentration of the hydrolysate was evaluated by the fluorescamine assay [9]. After dilution of the sample using a ratio of 10:1 in water, 200 µl was allowed to react with fluorescamine (100 µL of 15mg/100mL acetone) in 2.2 mL borate buffer (0.1 M). The fluorescence was measured at excitation 390 nm and emission 475 nm (Spex fluorimeter, Jobin-Yvon). Quantification of the protein hydrolysate was done using known concentrations of bovine serum albumin hydrolyzed under the same conditions.

Fasting plasma glucose was quantified colorimetrically by a combination of hexokinase and glucose-6-phosphate dehydrogenase (Technicon Dax system).

Statistical analysis

Mean values were compared for each group, using the non-parametric Wilcoxon test. Correlations between variables were also calculated.

Results

Table 1 indicates that mean plasma furosine and standard deviation are significantly higher in patients with Alzheimer's disease than in age-matched controls. Plasma furosine was also significantly higher in moderate Alzheimer than in severe Alzheimer ($P < 0.05$, Wilcoxon test) despite a similar mean fasting glucose level. Fasting plasma glucose tended to be higher in the patients, but the difference was not significant. Furosine (F) was, however, significantly correlated to fasting plasma glucose (G_0) ($F = 0.157\ G_0 + 0.53$) $r = 0.48$, $n = 15$, $P < .05$). The insulin-dependent diabetic subjects had almost twice the plasma furosine concentration of patients with Alzheimer's disease but the fasting plasma glucose was also significantly higher than in that of the controls and patients with Alzheimer's disease (Table 1). The fasting plasma glucose and furosine concentration were also significantly correlated in the diabetics, but the slope was higher than in the AD and control groups ($F = 0.177\ G_0 + 1.23$; $r = 0.60$, $n = 31$, $P < .02$) (Figur e 1).

Discussion

Protein glycation has been found in some regions of the brain in patients with Alzheimer's disease [1, 2] and could participate in aggregation and deposition of the β-amyloid protein. Furthermore, *in vitro* glycation of soluble β-amyloid peptide can induce protein aggregation [1]. However,

Table 1. Fasting plasma glucose and proteinglycation in patients with Alzheimer's disease

	Diabetic subjects (n = 31)	*Non diabetic Controls (n = 10)*	*Moderate Alzheimer (n = 6)*	*Severe Alzheimer (n = 13)*
Mean age ± std	44 ± 17	76.7 ± 11.3	82.3 ± 4.8	78.2 ± 5.2
Mean MMS ± std	–	27.7 ± 2.1	15.2 ± 3.5	2.6 ± 2.5
Fasting plasma glucose (mM)	8.42 ± 2.30[a]	5.06 ± 0.44	5.78 ± 1.50	5.89 ± 1.72
Furosine (µmol/g of protein)	2.65 ± 0.65[a]	0.91 ± 0.14	1.89 ± 0.40[b]	1.43 ± 0.49[b]

[a] $P < .02$ Wilcoxon test between Alzheimer groups and diabetic subjects.
[b] $P < .005$ Wilcoxon test between Alzheimer groups and controls.

very little is known about the relationship between AD and glucose tolerance [10]. Furthermore, the higher prevalence of AD amongst NIDD patients is very controversial (3). Some studies show a higher relative risk of dementia, including AD in diabetics [11, 12], while others find no relationship between diabetes and dementia [13, 14]. Some confounding factors such as patient selection or effect of hypoglycemic agents [15] could explain these results.

This study find a significantly higher protein glycation in the plasma of patients with Alzheimer's disease compared with age-matched controls, despite the absence of diagnosed diabetes. Protein glycation was measured as furosine, which is the acid hydrolysate of ε-deoxyfructosyllysine, the first stable product formed between lysine and glucose. The plasma furosine concentration was 1.5 to 2 times higher in patients with Alzheimer's disease than in control subjects, but remained much lower than that found in diabetic subjects (1.5 to 5 μmol/g proteins in diabetic subjects depending on the hyperglycemia level; and 0.8 to 2.3 in patients with Alzheimer's disease). The higher furosine levels in Alzheimer patients are partially explained by nonsignificantly higher fasting plasma glucose, as shown by a significant correlation between both variables (Figure 1). The lower activity of glucose transporters (GLUT 1 and GLUT 3) reported in patients with Alzheimer's disease by Simpson et al. [16] could explain the development of marginal hyperglycaemia.

Our results are contrary to those found by Thome et al. [17] who found no detectable changes in plasma glucose or glycation levels. However, the analytical methods used for determining the Amadori product differed in the two studies. Thome et al. [17] used an indirect colorimetric method, the fructosamine assay, whereas we specifically quantified the acidic product of ε-fructosyl-lysine. The fluorescence of plasma advanced glycation end products (AGEs) was also unchanged in the Thome [17] study, and our analysis (data not shown) confirmed this observation. But the rapid turn over of plasma proteins is unlikely to allow some AGEs accumulation in the plasma, even if higher Amadori products are found. We also were unable to find higher fluorescence (excitation 350 nm, emission 430 nm) in the plasma of diabetic subjects than in normoglycemic subjects (data not shown).

Minor defects in glucose tolerance should be investigated in patients with Alzheimer's disease, which might explain both the higher glycation in plasma proteins and the accumulation of AGEs in some brain regions [3]. However, higher glucose could only partially account for advanced glycated end-products in brain, as vitamin C can also give rise to AGEs on uncontrolled oxidation. Pentosidine and pyrraline, two advanced glycated end-products, were identified by immunoassay technique [2]. The former can be formed by both ascorbate oxidation products and glucose [18], but the latter seems more specific for reducing sugars [19]. Furthermore, there is some evidence of lower plasma vitamin C in patients with Alzheimer's disease [20, 21], suggesting higher radical production in AD. A possible link between brain glucose and vitamin C is based on the evidence of a key role for GLUT 1. The latter transports glucose and dehydroascorbic acid, the precursor of vitamin C in the brain [22], and expresses lower activity in Alzheimer's disease [15].

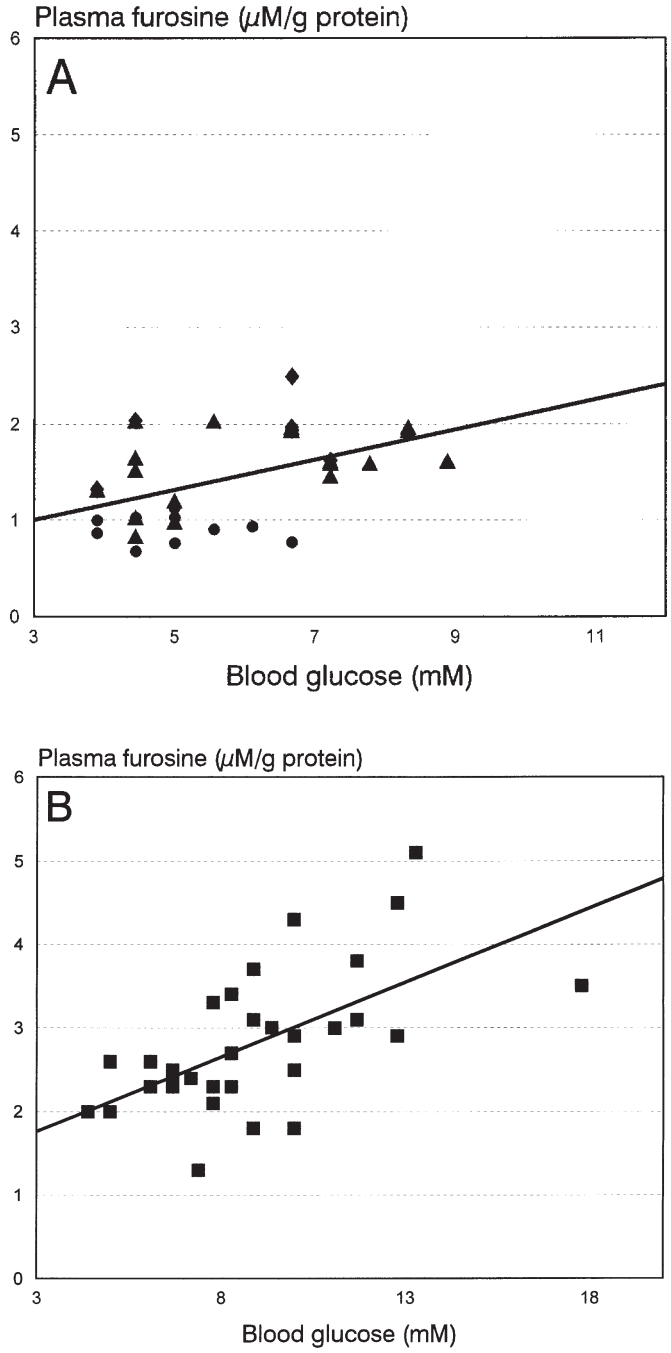

Figure 1. Regression line between fasting plasma glucose and furosine in normoglycemic Alzheimer and control subjects (A) and in Diabetic control subjects (B).

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