Circulating Levels of Soluble Receptor for Advanced Glycation End Products in Alzheimer Disease and Vascular Dementia

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Background: The receptor for advanced glycation end products (RAGE) is a cell surface receptor that has been implicated in vascular disease and neurodegeneration. Low levels of its secreted isoform, soluble RAGE (sRAGE), have been regarded as a putative risk factor for atherosclerosis. In addition, administration of sRAGE has been shown to reduce development of cerebral β-amyloidosis in an Alzheimer disease mouse model.

Objective: To investigate the role of sRAGE as a biological marker for Alzheimer disease and vascular dementia.

Design: Cross-sectional study of 152 patients with a clinical diagnosis of Alzheimer disease, 91 with vascular dementia and 161 control subjects.

Main Outcome Measure: Plasma levels of sRAGE.

Results: Levels of sRAGE were significantly reduced in the plasma of patients with Alzheimer disease compared with that for those with either vascular dementia (P<.05) or with controls (P<.001).

Conclusions: Patients with Alzheimer disease have reduced levels of sRAGE in plasma compared with patients with vascular dementia and controls. The striking reduction of circulating sRAGE in Alzheimer disease further supports a role for the RAGE axis in this clinical entity and requires further investigation.

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ASCULAR RISK FACTORS, including indicators of atherosclerosis, have been suggested to play a role not only in vascular dementia (VaD) but also in Alzheimer disease (AD).

The receptor for advanced glycation end products (RAGE) is a multiligand, signal transduction receptor of the immunoglobulin superfamily that is expressed by a variety of cell types including endothelial cells, smooth muscle cells, and neurons. Several endogenous ligands have been shown to activate the RAGE transductional pathway, including advanced glycoxidation products, S100/calgranulins, and amphotatin/HMGB1. Binding of ligands to RAGE stimulates expression of RAGE itself and generates chemotaxis and oxidative stress, as well as the production of proinflammatory cytokines with an established role in atherosclerosis. In addition, RAGE has been shown to bind with the high-affinity amyloid-beta (Aβ) peptide as demonstrated by its ability to rapidly transport circulating free Aβ into the central nervous system and brain-derived Aβ into the blood. This finding suggests that RAGE might be implicated not only in vascular disease but also in neurodegeneration.

A secreted isoform of RAGE, termed soluble RAGE (sRAGE), has the ability to prevent the adverse effects of RAGE signaling by acting as a decoy. Accordingly, sRAGE has been used successfully in several animal models to antagonize RAGE-mediated vascular damage. In addition, administration of sRAGE has been shown to reduce development of cerebral β-amyloidosis in an AD mouse model, thereby suggesting that sRAGE may be a target for inhibiting pathogenic consequences of Aβ-vascular interactions.

We have recently reported a reduced concentration of endogenous sRAGE in patients with coronary artery disease. These results indicated that low levels of sRAGE could be regarded as a putative risk factor for atherosclerosis in humans.

Given the potential importance of sRAGE not only in vascular disease but also in neural dysfunction, circulating levels of sRAGE were measured in patients with clinically diagnosed AD and compared with those of patients with VaD and a group of cognitively healthy control subjects.
METHODS

Patients with dementia were recruited consecutively from the Memory Clinic, IRCCS Centro San Giovanni di Dio, Brescia, Italy, and from the Regional Neurogenetic Center, Lamezia Terme, Italy. Neuropsychological testing, a detailed structured interview, and clinical examinations were performed. All patients underwent morphologic and/or functional neuroradiological testing together with the usual battery of screening blood tests to exclude treatable causes of dementia. The severity of the dementia was assessed by the Mini-Mental State Examination (MMSE) and the Hachinski Ischemic Score estimated cerebrovascular risk factors.

Two groups of patients were involved in the study: patients with probable AD (n = 152) and patients with probable VaD (n = 91). The NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association) criteria were fulfilled by patients with AD and the NINDS-AIREN (National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l’Enseignement des Neurosciences) criteria by patients with VaD.

Cognitively healthy controls (n = 161) were recruited consecutively from individuals who attended a health screening in the outpatient clinics of the participating institutions. Assessment of controls included a full medical history and a physical examination. Controls were also tested with the MMSE to exclude unknown cognitive disturbances and all scored above 28 points. None of the patients or controls was diabetic nor did any have renal, liver, or thyroid disease. All patients or legal guardians and control subjects gave informed consent to participate and the approval of the local ethical committee was obtained.

Blood specimens for the measurement of plasma sRAGE concentrations were drawn in EDTA-containing tubes, centrifuged at low speed, and the plasma aliquots stored at −20°C. Levels of sRAGE were measured in duplicate using an enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Minneapolis, Minn) as reported earlier. All tests for each sample underwent morphologic and/or functional neuroradiological testing together with the usual battery of screening blood tests to exclude treatable causes of dementia. The severity of the dementia was assessed by the Mini-Mental State Examination (MMSE) and the Hachinski Ischemic Score estimated cerebrovascular risk factors.

Data were analyzed with the use of statistical software SPSS 11.0 (SPSS Inc, Chicago, Ill.). Concentrations of sRAGE were not normally distributed and nonparametric analysis was used. An overall comparison for differences in plasma sRAGE levels between the 3 groups was made by the Kruskal-Wallis analysis followed by post hoc Dunn tests. Correlations between the study variables were tested by simple correlation analysis (Pearson).

Plasma sRAGE levels were also dichotomized in sRAGE below 776 pg/mL as the cutoff for an abnormal sRAGE concentration since it has been previously shown to be a reliable cutoff for identifying subjects at an increased vascular risk. Multivariate logistic regression analyses were performed to assess the independent contribution to AD and VaD of sRAGE levels below 776 pg/mL after adjustment for age, sex, and the carriage of at least 1 ApoE ε4 allele. Statistical tests were made at the .05 level.

RESULTS

Plasma sRAGE concentrations in the study groups are presented in Table and Figure. Levels of sRAGE were significantly different (P < .001, Kruskal-Wallis test) among the 3 study groups. Concentrations of sRAGE had significantly decreased in patients with AD (median [interquartile range]: 402 [229-879] pg/mL, P < .001) and in patients with VaD (662 [441-952] pg/mL, P < .001) compared with cognitively healthy controls (1240 [911-1800] pg/mL). Furthermore, the AD group also showed significantly lower sRAGE levels than the VaD group (P < .05).

The AD group had a significantly higher prevalence of subjects with sRAGE levels below 776 pg/mL (69.7%) in comparison with controls (43.4%, P < .001), but not with the VaD group (67.9%, P = .18). Results of multivariate logistic regression analyses showed that a plasma sRAGE level below 776 pg/mL was independently associated with both AD (odds ratio, 13.934; 95% confidence interval; 7.830-24.795; P < .001) and VaD (odds

| Table. Demographic Data and Plasma sRAGE Levels of Patients and Healthy Subjects |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | AD (n = 152)    | VaD (n = 91)    | Controls (n = 161) |
| Age (SD), y                    | 73.11 (10.45)   | 80.10 (6.58)    | 72.28 (3.82)     |
| Age at onset (SD), y           | 70.08 (10.24)   | 76.65 (7.06)    | . . .             |
| Male sex, No. (%)              | 46 (30.2)       | 59 (42.8)       | 51 (31.6)        |
| Education (SD), y              | 9.18 (4.12)     | 8.95 (3.64)     | 9.58 (3.33)      |
| MMSE score (SD)                | 13 (8)          | 11 (7)          | 29 (1)           |
| Hachinski Ischemia Score (SD)  | 2.9 (0.9)       | 11.3 (2.3)      | . . .             |
| ApoE ε4 carriers, No. (%)      | 75 (49.3)       | 24 (26.4)       | 46 (28.5)        |

Abbreviations: AD, Alzheimer disease; ApoE ε4, apolipoprotein E ε4 allele; MMSE, Mini-Mental State Examination; sRAGE, soluble receptor for advanced glycation end products; VaD, vascular dementia.

Figure. Scatter diagram for plasma soluble receptor for advanced glycation end products (sRAGE) concentrations in patients with Alzheimer disease (AD) (n = 152), vascular dementia (VaD) (n = 91), and cognitively healthy controls (CTR) (n = 161). Horizontal lines across the scatter diagram represent mean values.
Simple correlation analysis revealed that log-transformed sRAGE concentrations correlated positively with the MMSE scores in AD (r=0.167; P=.04), but was not the case in either healthy controls (r=0.012; P=.87) or patients with VaD (r=0.094; P=.25).

No significant correlations were found between log-normalized levels of sRAGE age, sex, duration of dementia, and Hachinski scores. All patients with AD had Hachinski scores less than 4, making it unlikely that cerebrovascular risk factors contributed significantly to the reduced levels of sRAGE that were seen.

We designed a cross-sectional study of plasma sRAGE concentration in a clinical series of different dementing disorders. Our results provide important evidence that sRAGE levels below 776 pg/mL are significantly associated with VaD and, above all, AD. These associations were found to be independent of age, sex, and the ApoE status of the study participants.

Vascular dementia is characterized by small and large brain infarcts usually associated with vascular changes. Hence, the atherosclerotic process has been discussed as an important component of the vasculopathy observed in VaD.1 Because we have previously reported that a low plasma sRAGE concentration could be a marker for vascular disease,10 its association with VaD is not surprising.

More importantly, we observed a highly significant reduction of sRAGE levels in AD. Indeed, sRAGE concentrations in patients with AD were not only significantly lower than those in the control group, but they were also lower than those in the VaD group. A correlation was also found between log-normalized plasma sRAGE levels and the severity of AD, although this did not prove to be very strong. In view of the role played by cell surface RAGE in mediating Aβ toxicity,19 it could be speculated that subjects with a decreased concentration of the endogenous decoy sRAGE could display an exaggerated sensitivity to the Aβ-induced neuronal perturbation.

Limitations of our study should be considered. First, its cross-sectional design does not allow us to establish whether the reduced level of sRAGE in AD is a cause or rather a consequence of the disease. A second limitation is that we did not measure levels of sRAGE in cerebrospinal fluid. Therefore, it will be important in future investigations to test the correlation between cerebrospinal fluid and plasma levels of sRAGE in demented persons.

Given these caveats, our results support the possibility that sRAGE may aid in the assessment of the laboratory diagnosis of AD. Further longitudinal studies will be necessary to determine whether there is a relationship between plasma sRAGE levels and progression of this clinical entity.

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