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ORIGINAL ARTICLE

High plasma levels of the soluble receptor for advanced glycation endproducts in patients with symptomatic carotid atherosclerosis

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ABSTRACT

Background Advanced glycation endproducts (AGEs), particularly carboxymethyl(lysine)-adducts (CML), exert part of their cellular effects by binding to a receptor, named receptor for AGEs (RAGE). The soluble form of this receptor (sRAGE) has been shown to have an athero-protective role. We hypothesized the existence of a relationship between the AGE–RAGE axis and the occurrence of symptoms related to carotid atherosclerosis in nondiabetic conditions.

Materials and methods We evaluated plasma levels of CML and sRAGE (by ELISA), and tissue levels (tAGEs and tRAGE, semiquantitatively, by immunohistochemistry) in endarterectomy carotid plaque tissue in 29 nondiabetic patients. At the time of surgery, 10 patients were asymptomatic and 19 were symptomatic.

Results Plasma levels of sRAGE were higher in symptomatic patients than in asymptomatic patients [median (interquartile range): 676 (394–858) pg mL^{-1} vs. 347 (284–479) pg mL^{-1}, $P = 0{\cdot}009$]. In symptomatic patients, plasma levels of sRAGE correlated positively with CML ($r = 0{\cdot}60$, $P < 0{\cdot}01$), C-reactive protein (CRP) ($r = 0{\cdot}618$, $P < 0{\cdot}01$) and fibrinogen ($r = 0{\cdot}522$, $P<0{\cdot}005$), while in asymptomatic patients, no correlation was observed. Although tissue and plasma levels of AGEs and RAGE did not correlate between each other, tAGEs and tRAGE were also positively correlated only in symptomatic patients ($\chi^2 = 8{\cdot}93$, $P = 0{\cdot}003$).

Conclusions Plasma levels of sRAGE are higher in symptomatic than asymptomatic carotid atherosclerosis. Higher levels of sRAGE in symptomatic patients may be markers of a higher degree of vascular inflammation in such patients.

Keywords Advanced glycation endproducts, atherosclerosis, inflammation, RAGE.

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Introduction

Cardiovascular events are related to the composition and stability of atherosclerotic plaques. At present, no inflammatory or immune mediators have been shown to consistently predict the risk of stroke in patients with carotid artery disease [1,2]. Ligands for the receptor (RAGE) for advanced glycation endproducts (AGEs) may play a role as inflammatory triggers in atherosclerosis. The most commonly encountered AGEs *in vivo* are carboxymethyl(lysine)-adducts (CML) [3–5]. CML can be generated not only via glycation and lipoxidation of proteins, but also via the reaction of proteins with myeloperoxidase products in inflammatory conditions [6,7]. The inflammatory response driven by RAGE within the diabetic vessel wall may contribute to accelerated progression of atherosclerosis also by amplifying and sustaining other glucose-independent pro-atherogenic mechanisms [8]. Indeed, the inhibition of RAGE activation by a soluble, truncated form of the receptor (sRAGE) or by anti-RAGE antibodies in genetically manipulated mice stabilizes atherosclerosis in both euglycaemic and diabetic mice deficient in apoE, without affecting glucose levels or the lipid profile, suggesting that the biology of RAGE and its ligands extends beyond diabetes [8].

In addition to the cell-bound full-length RAGE, several truncated variant forms of this receptor have been described [9].

These include a soluble form lacking the intracellular domain, but still able to bind its ligands. This is secreted extracellularly and can be detected in plasma [10]. Soluble RAGE may thus function as a decoy for circulating AGEs, indirectly inhibiting AGE-mediated RAGE activation [11]. High plasma levels of sRAGE have been reported in association with a lower incidence of coronary artery disease (CAD) in nondiabetic men [12], and low plasma levels of sRAGE have been reported in association with CAD, carotid and femoral atherosclerosis, as well as some components of the metabolic syndrome [13,14]. Altogether, these results have suggested an athero-protective role for sRAGE; however, it is also conceivable that high levels of sRAGE are part of a counter-regulatory mechanism elicited by vascular inflammation and aimed at its suppression.

We hypothesized the existence of a relationship between AGE–RAGE levels, in plasma and in the plaque tissue, and the occurrence of symptoms related to carotid atherosclerosis in nondiabetic conditions, marking the occurrence of more vulnerable plaques than that in asymptomatic carotid atherosclerosis. In this study, we report on measurement of sRAGE and CML levels in plasma and in tissue retrieved at carotid endarterectomy in a population of patients with carotid atherosclerosis.

Materials and methods

Patients

Plasma and carotid plaque samples were collected from 29 nondiabetic patients undergoing endarterectomy for the removal of a carotid plaque, according to the North American Symptomatic Carotid Endarterectomy (NASCET) and the Asymptomatic Carotid Atherosclerosis Study (ACAS) criteria. Diabetes was excluded by normal fasting blood glucose and glycosylated haemoglobin levels.

Patients were classified according to plaque symptomaticity. We specifically compared a group of 10 patients who were asymptomatic (AS) for carotid artery disease at the time of surgery, with a group of 19 symptomatic patients (S), the latter comprising 11 patients having suffered recent (< 3 months) transient ischaemic attacks (TIA) and eight patients with a recent (< 3 months) stroke. Patients and specimens were collected at the EMO Center Cuore Columbus (Milan, Italy) and at the IRCCS Humanitas Health Center (Rozzano, Italy) between September 2005 and February 2006. Blood samples were collected immediately before surgery. The study was approved by local Scientific and Ethical Committees.

The degree of carotid stenosis before endarterectomy was evaluated first by high-resolution vascular colour Doppler ultrasound and later confirmed by carotid angiography. Endarterectomy was carried out for carotid artery stenoses (% diameter) ≥ 70%.

Laboratory methods

Blood sample handling. For CML and sRAGE determinations, blood samples were collected in tubes containing Na_2EDTA, centrifuged at 4 °C and immediately divided into aliquots. Plasma samples were stored at −80 °C until the analysis.

Determination of plasma CML levels. Plasma CML levels were measured in triplicate with an in-house developed competitive enzyme-linked immunosorbent assay (ELISA) using the mouse $F(ab')_2$ anti-AGE monoclonal antibody 6D12 (ICN Pharmaceuticals, Costa Mesa, CA, USA), which recognizes specifically CML-protein adducts, as previously described elsewhere [15]. Intra-assay and interassay coefficients of variation were 3·2% and 8·7% respectively. The lower limit of detection of CML was 3·12 $\mu g\ mL^{-1}$.

Determination of plasma sRAGE levels. Plasma sRAGE levels were determined using a commercial ELISA kit (DuoSet ELISA development kit; R&D systems, Minneapolis, MN, USA) containing the basic components required for the development of double-sandwich ELISAs, as described previously elsewhere [14]. Intra-assay and interassay coefficients of variation values were 5·9% and 8·2% respectively. The lower limit of detection of sRAGE was 21·5 $pg\ mL^{-1}$. We evaluated the specificity of this ELISA, by performing some assays in the presence of exogenously added high levels of AGEs or CML; no interference of these ligands was observed in sRAGE detection.

Assessment of plaque morphology. Carotid plaques were immediately fixed in 10% buffered formalin, and then embedded in paraffin blocks. Plaque morphology was evaluated by light microscopy on 5-μm haematoxylin–eosin (HE)-stained sections. Plaque composition was classified according to the updated eight-stage Stary classification [16]. Histological sections were analysed semiquantitatively according to the following scoring system: for atheromatous core (0, 1, 2, 3: absence, small atheroma, large atheroma and extensive atheroma respectively); for inflammatory infiltrates (0, 1, 2, 3: absence, minimal infiltrates, infiltrates present in aggregates or widespread infiltrates respectively); for fibrosis (0, 1, 2, 3: absence, discrete presence, predominant and complete respectively); for calcium nodules (0, 1, 2, 3: absence, isolated areas, multiple areas with large amounts of calcium or widespread infiltration respectively) and for neoangiogenesis (0, 1, 2, 3: absence, isolated neovessels, minimal aggregates or abundant neovessels respectively).

Detection of AGEs and RAGE in carotid plaques. Immunohistochemistry for AGEs and RAGE was performed on

formalin-fixed, paraffin-embedded carotid plaques. Five micrometres thick fixed sections were cut and mounted on positively charged slides.

For the immunohistochemical detection of AGEs, we omitted any thermal treatment of tissue sections to avoid false positive results. Slides were de-paraffinized in a Bioclear solvent buffer (Bioclear; Bioptica, Milan, Italy) and rehydrated in solutions of ethanol at decreasing concentration. Antigen retrieval with trypsin for 11 min at 37 °C was followed by endogenous peroxidase blocking with 3% H_2O_2 TRIS-buffered saline solution (TBS) for 10 min. Nonspecific binding was avoided with a universal blocking serum (Chemicon International, Temecula, CA, USA) for 5 min at room temperature. Incubations were carried out at room temperature for 90 min with a chicken polyclonal anti-AGE serum (60 μg mL^{-1}, kindly supplied by Dr A.M. Schmidt, Columbia University, New York, NY, USA) in a wet chamber, followed by washing with TBS plus Tween 20 (TBST). Incubations with the secondary biotinylated anti-chicken antibody (Chemicon) diluted 1 : 200 were carried out for 30 min at room temperature, followed by washing with TBST. Subsequently, slides were incubated with streptavidin-conjugated peroxidase (Lab Vision Corp., Fremont, CA, USA) for 25 min, followed by washing with TBST. The binding reaction was detected using 3,3′-diaminobenzidine (DAB) (Dako, Glostrup, Denmark), developing a brown colour. Slides were then counterstained with haematoxylin.

For the immunohistochemical detection of tissue RAGE, slide de-paraffinization and antigen retrieval were performed by a single treatment in wax-capture, antigen-retrieval solution (W-CAP)-citrate buffer, pH 6·0 (Bioptica), at 95–98 °C for 30 min, followed by cooling at room temperature for 30 min. The sections were washed with distilled water, and endogenous peroxidases were blocked in 3% H_2O_2 TBS solution for 10 min. The detection system of the Labelled-Streptavidin-Biotin Kit (LSAB; Dako) used for the purpose did not require antigen blocking. Incubations were at this point carried out for 90 min at room temperature with rabbit polyclonal anti-RAGE serum (200 μg mL^{-1}, kindly supplied by Dr A.M. Schmidt) in a wet chamber, followed by washing in TBST. The sections were then incubated with LSAB for 25 min at room temperature, washed in TBST and incubated with streptavidin-conjugated peroxidase for 20–25 min, then washed in TBST and incubated with DAB. The slides were then counterstained with haematoxylin.

Scoring for AGEs and RAGE deposition in carotid plaque. The immunohistochemical depositions of AGEs and RAGE were evaluated by two independent readers, according to a semiquantitative scoring system. The scoring was as follows: (0) no staining, (1) minimal/occasional staining, (2) moderate staining and (3) diffuse staining.

Additional immunohistochemical analyses. Immunohistochemical analyses were also carried out on fixed plaques to detect macrophages (Mac 387, dilution 1 : 1000, Dako), T lymphocytes (UCHL1, dilution 1 : 300, Dako) and B lymphocytes (CD20, dilution 1 : 150, Dako), and smooth muscle cells (smooth muscle cell α-actin, dilution 1 : 300, Dako). Detection was performed with the ABC Elite system (Vector Laboratories, Burlingame, CA, USA), with DAB peroxidase substrate.

Statistical analysis

Normally distributed data are given as mean ± SD. Skewed data are given as median (interquartile range).

Categorical variables were compared by chi-square analysis, and continuous variables with normal or non-normal distributions were compared by the Student's *t*-test or the Mann–Whitney *U*-test respectively.

Relationships between quantitative parameters were tested by Pearson correlation analysis. Variables showing a positively skewed distribution were natural logarithmic-transformed (ln) before the correlation analysis. Statistical analysis of semiquantitative immunohistochemical values was conducted after grouping for both AGE and RAGE plaque deposition: group 1 comprised of cases with low-grade staining (no staining/occasional staining) and group 2 of cases with high-grade staining (moderate/diffuse staining). Correlations between categorical variables were analysed by chi-square analysis. Data were analysed with version 10·2 of the SYSTAT software for Windows (Cranes Software International, Bangalore, India). Probability (P) values of $< 0{\cdot}05$ were considered significant.

Results

Clinical and biochemical parameters

Clinical parameters were evaluated for the whole group of patients ($n = 29$) and for the two subgroups of asymptomatic ($n = 10$) and symptomatic ($n = 19$) patients (Table 1).

The two groups did not differ significantly with regard to age, gender and main clinical features. We also found no differences in lipid parameters as well as in plasma levels of C-reactive protein between the two study groups. In contrast, fibrinogen levels were higher in symptomatic patients ($P = 0{\cdot}032$), with nine symptomatic and two asymptomatic patients showing fibrinogen levels ≥ 400 mg dL^{-1}. The levels of sRAGE were significantly higher in symptomatic patients compared with that in asymptomatic patients ($P = 0{\cdot}009$) (Table 2). In particular, the median values of sRAGE for asymptomatic and symptomatic patients were 347 and 676 pg mL^{-1} respectively (Table 2). No significant differences in CML values were evident between groups.

Levels of sRAGE and CML were positively and strongly intercorrelated in symptomatic patients ($P < 0{\cdot}01$, $r = 0{\cdot}60$)

Table 1 Main clinical and biochemical characteristics of study patients†

	All patients (*n* = 29)	Asymptomatic (*n* = 10)	Symptomatic (*n* = 19)	*P*-value*
Age (years)	73 ± 7·8	70.1 ± 5·6	74 ± 8·3	0·135
Male (*n*, %)	21 (72)	8 (80)	13 (68)	0·608
F amily history (*n*, %)	8 (27)	2 (20)	6 (31)	0·722
Smoking (*n*, %)	4 (14)	2 (20)	2 (10)	0·890
Hypertension (*n*, %)	23 (79)	7 (70)	16 (84)	0·228
Triglycerides (mg dL^{-1})	149 (125·5–170·5)	158 (133–172)	146 (112–169)	0·580
Total cholesterol (mg dL^{-1})	211·5 ± 44	193·7 ± 41	219·8 ± 44	0·173
Carotid plaque stenosis (mean ± SD)	79 ± 8	79 ± 6	79 ± 9	0·82

*Symptomatic vs. asymptomatic.
†Diabetes was an exclusion criterion for the enrollment.

Table 2 Plasma levels of inflammatory markers

	All patients (*n* = 29)	Asymptomatic (*n* = 10)	Symptomatic (*n* = 19)	*P*-value*
C-reactive protein [mg L^{-1}, median (interquartile range)]	4 (1–19·3)	3·1 (1·2–6·4)	6 (8–21)	0·502
Fibrinogen [mg dL^{-1}, mean ± SD]	346·9 ± 106·6	283·5 ± 95·2	378·7 ± 99·8	0·036
sRAGE [pg mL^{-1}, median (interquartile range)]	472 (347·5–731)	347 (284–479)	676 (394–858)	0·009
CML [μg mL^{-1}, median (interquartile range)]	14·6 (7·9–37·9)	23 (7–60)	14·6 (8·4–27·6)	0·614

sRAGE, soluble receptor for advanced glycation endproducts; CML, N-e-(carboxymethyl)lysine; SD, standard deviation.
*Symptomatic vs. asymptomatic patients.

(Fig. 1a), while this was not the case in asymptomatic patients. Further, sRAGE values were positively correlated with CRP values ($P < 0·01$, $r = 0·618$) and fibrinogen ($P < 0·05$, $r = 0·522$) in symptomatic patients (Fig. 1b–c), but not in asymptomatic patients.

Plaque histological features

All plaques retrieved at endarterectomy, both in symptomatic and in asymptomatic patients, were Stary types V and VI.

Lipid core, fibrosis and calcium were present in variable percentages in all carotid plaques evaluated by light microscopy. Calcium was usually found within the lipid core. Calcium was present with a diffuse or nodular organization. Diffuse and focal inflammatory infiltrates were also present, usually in association with intraplaque blood vessels of small diameters.

The evaluation of plaque morphology and composition between asymptomatic and symptomatic patients showed that fibro-atheromatous plaques were more common in asymptomatic patients than in symptomatic patients (60% vs. 26% respectively, approaching statistical significance). In both groups, the majority of plaques showed a high degree of vascularity and marked plaque inflammation (Table 3). Inflammatory cells mostly consisted of macrophages and T lymphocytes (Fig. 2). Such features were, however, similarly distributed between groups (Table 3). Plaque morphology scoring was also not significantly different between the two groups.

Deposition of AGEs and RAGE within plaques

A positive staining for AGEs and RAGE was found in the plaques of both asymptomatic and symptomatic patients. Staining for tAGEs was associated with both cellular elements and the extracellular matrix in the plaque (Fig. 2). Cellular positive staining was associated with the cytoplasm of macrophages/foam cells or smooth muscle cells. In areas of fibrosis and inflammatory infiltrates, positive staining for RAGE was associated with foam cells and lymphocytes (Fig. 2). No differences in tAGEs and tRAGE staining were found between plaques from asymptomatic and symptomatic patients (Table 4). Tissue depositions of AGEs and RAGE were positively correlated with each other in symptomatic patients ($\chi^2 = 8·93$, $P = 0·003$), but not in asymptomatic patients.

Discussion

RAGE may be involved in endothelial dysfunction and intimal hypertrophy, and may have a role in plaque formation

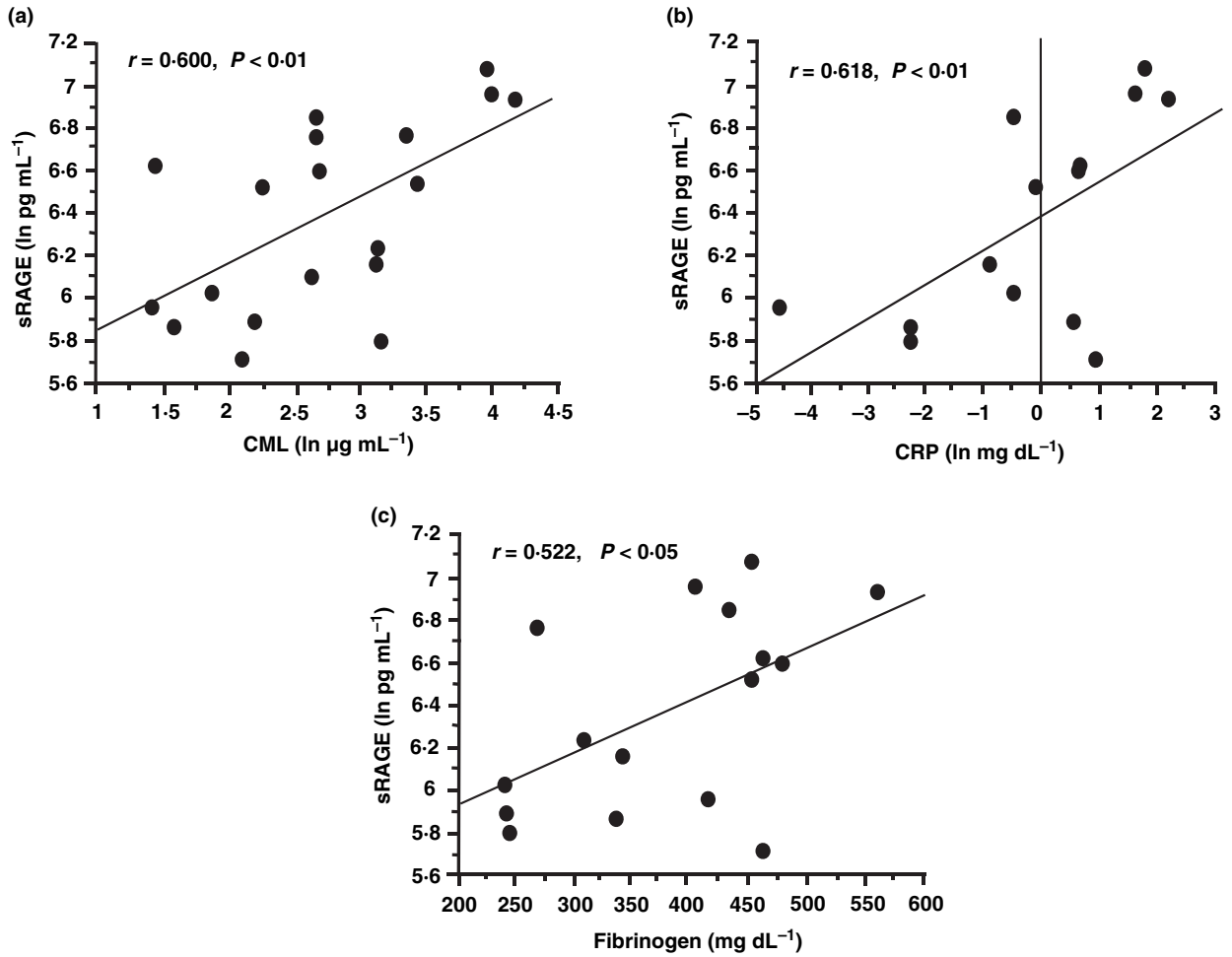

Figure 1 Linear regression analysis of the relationship between plasma sRAGE with CML (a), CRP (b) and fibrinogen (c) in symptomatic patients. Data are correlation coefficients and *P* values. sRAGE, soluble receptor for advanced glycation endproducts; CML, N-ε-(carboxymethyl)lysine; CRP, C-reactive protein.

Table 3 Plaque morphological evaluation

	Asymptomatic patients, *n* (%)	Symptomatic patients, *n* (%)	*P*-value*
Atheromatous core	1 (10)	6 (32)	0·197
Fibro-atheroma	6 (60)	5 (26)	0·076
Calcific atheroma	3 (30)	8 (42)	0·523
Vascularity	8 (80)	14 (74)	0·706
Plaque inflammation	8 (80)	17 (89)	0·482

Definitions: Atheromatous core: lipid component > 80% of the entire lesion area; Fibro-atheroma: similar lipid and fibrous components; Calcific atheroma: lipid component with the presence of calcium nodules; Vascularity: presence of intimal neovessels; Plaque inflammation: presence of lymphocyte/macrophage infiltration.
*Symptomatic vs. asymptomatic.

and complications following the engagement with its ligands [5]. Previous reports have also characterized that cellular RAGE expression increases in clinical settings characterized by enhanced cell activation and prolonged exposure to RAGE ligands [17,18]. Soluble (s)RAGE, which is secreted extracellularly and can be detected in plasma [10], may compete with the engagement of RAGE by its pro-inflammatory ligands, acting as a decoy molecule. Treatment with sRAGE indeed has been found to dose-dependently inhibit the development of atherosclerosis in animal models [19,20].

Levels of sRAGE have been the object of intense clinical research in diabetic [14,21,22], as well as nondiabetic subjects [12,23,24]. All such researches, based on case-control studies, have overall shown low levels of sRAGE in several settings of

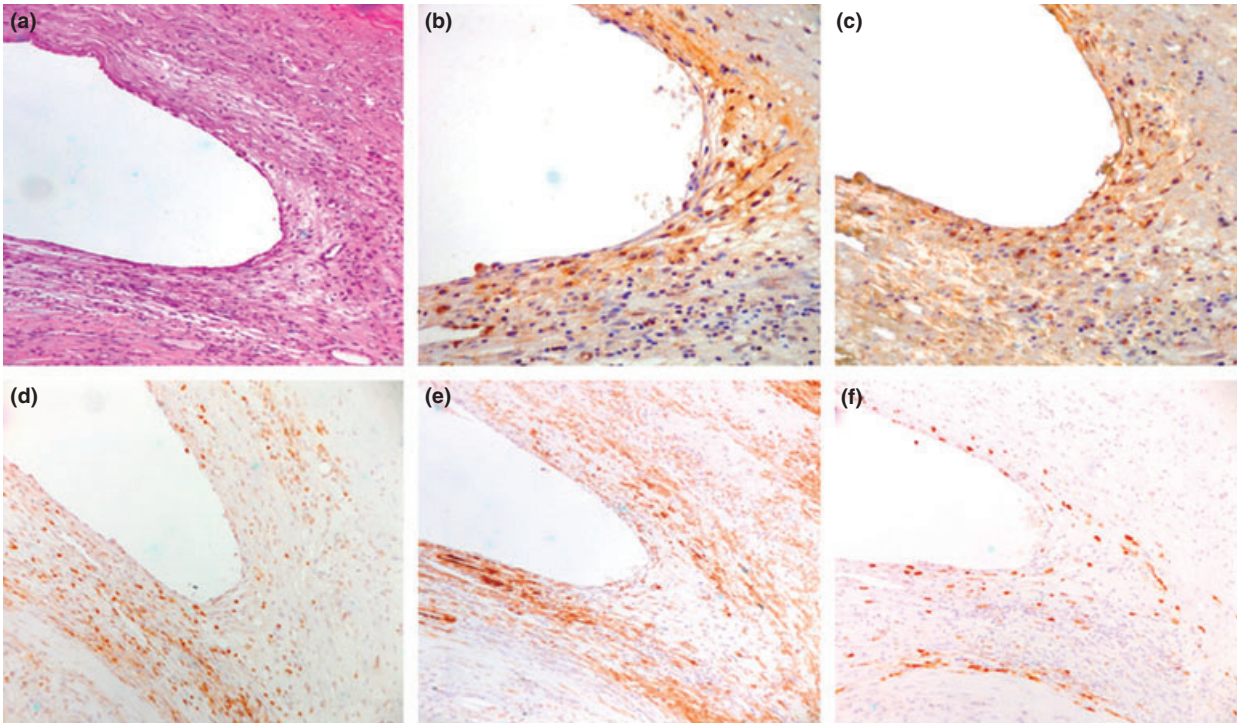

Figure 2 Co-localization of AGEs, RAGE and inflammatory infiltrates in atherosclerotic plaques. Panels are representative samples (10X) from the shoulder region of a plaque from a symptomatic patient. (a) Haematoxylin and eosin staining. (b) Immunohistochemistry for AGEs, showing the association with matrix and cellular components of the plaque. (c) Immunohistochemistry for RAGE, showing the expression on cellular components of the plaque. (d) T lymphocytes (UCHL1). (e) Smooth muscle cells (α-actin). (f) Macrophages (Mac 387). Note that the expression of RAGE co-localizes with AGE staining and inflammation.

Table 4 Plaque morphology scoring (median values)*

	Asymptomatic patients (n = 10)	Symptomatic patients (n = 19)	P-value†
Atheromatous core	2	2	0·866
Calcium nodules	0	1	0·170
Fibrosis	2	1·5	0·115
Neoangiogenesis	0·5	1	0·866
Inflammatory infiltrates	1·25	1	0·270

*See text for definitions.
†Symptomatic vs. asymptomatic.

disease compared with control conditions, therefore suggesting an athero-protective role for this molecule.

This is the first report of sRAGE plasma concentrations in patients with a recent acute complication of a carotid atherosclerotic plaque, compared with asymptomatic patients. In this study, we demonstrate that circulating sRAGE levels are significantly higher in symptomatic patients than in asymptomatic ones. Therefore, in this study, we highlight higher – rather than lower – levels of sRAGE, discriminating symptomatic patients from asymptomatic ones, *i.e.* in patients associated with an *a posteriori* defined vulnerable plaque compared with those associated with a 'stable' plaque. Reasons for the discrepancy between previous studies, suggesting an inverse relation between sRAGE and CVD, and the current findings of a direct relationship are unclear. However, they may be attributable to different study designs and patient groupings. Indeed, in our study, we compared two groups of subjects with carotid atherosclerosis, while previous studies compared case subjects with controls. In addition, as the method of sRAGE assay is not standardized, comparisons between studies are difficult.

We hypothesize that a more active inflammatory status of our patients may be reflected by sRAGE levels [23]. In support of a different status of systemic inflammation in our two groups of patients, fibrinogen levels were higher in symptomatic patients. Additionally, in our study, sRAGE levels were positively associated with CRP and fibrinogen in symptomatic

patients, suggesting that sRAGE, originating as a splice variant of RAGE or a split-off variant of the cell surface RAGE, might be a marker of inflammation, in this case related to the clinical status of the patient better than CRP.

We can say little about the functional significance of sRAGE in our patients. The assay we used quantifies concentrations of total sRAGE in plasma. This assay cannot differentiate between endogenous secretory RAGE (esRAGE) and the soluble RAGE, which results from the cleavage of the cell-surface receptor by proteases [25]. Such a further assessment has to be the object of future research prompted by our current findings.

AGE accumulation in human atherosclerotic plaques has been documented extensively. Sakata *et al.* [26] highlighted that CML, a prominent AGE, localizes both in the cytoplasm of foam cells and in the extracellular space, while non-CML AGEs localize mainly in the extracellular components of the plaque. For the detection of tissue AGE expression, the antibody we used was not specific for CML, which is a rather specific ligand of RAGE. Our results of the tissue distribution of AGEs, using a nonspecific antibody, are therefore in agreement with both the cellular and extracellular localizations of tissue AGEs.

Our data also suggest that AGE levels may represent one of the determinants of sRAGE levels. In fact, AGEs and RAGE co-localize at sites of vascular damage, and AGE accumulation may induce enhanced expression/release of sRAGE by upregulating RAGE expression [17,18]. Accordingly, although total levels of tRAGE and tAGEs did not differ between symptomatic and asymptomatic patients, we still found a strong correlation between tAGE and tRAGE in symptomatic patients.

The lack of correlation between tAGE and tRAGE in asymptomatic patients might depend on the small sample size of this population; otherwise, it might suggest that the amount of tissue AGEs and RAGE within each plaque is not as critical as their association/co-localization, which might conversely be more implicated in plaque instability. In other words, the AGE–RAGE interaction at the tissue level might be involved in transforming structurally vulnerable plaques into functionally unstable ones. Hence, it is likely that compensatory anti-inflammatory phenomena in response to tissue injury might affect total sRAGE plasma levels.

As sRAGE was shown to bind pro-inflammatory AGEs in a saturable and dose-dependent manner [27], as well as to neutralize AGE effects on endothelial cells in culture [10], our data suggest that higher levels of sRAGE may constitute a counter-regulatory mechanism by which cells reduce AGE-dependent cellular activation, thus exerting protection against the effects of AGE–RAGE interaction. Similar mechanisms appear to be in place when exceedingly high cytokine signalling is limited through the generation of soluble cytokine receptors that bind target cytokines and thus antagonize their biological activity [28]. For instance, it has been described that cytokine-induced TNF receptor shedding can suppress potentially deleterious TNF-mediated inflammatory responses [29,30].

Pichiule *et al.* [31] have recently provided insight into the potential role of RAGE in the pathophysiology of stroke using mice genetically deficient for RAGE. In that study, the authors showed that RAGE mRNA and protein levels were stimulated in the mouse brain after experimental stroke and systemic hypoxia. Activation of RAGE-dependent post-ischaemic pathway(s) appears to have a neuroprotective role, as mice genetically deficient in RAGE featured increased cerebral infarct size after injury [31]. Similar activations of anti-inflammatory mechanisms might have been in place in our symptomatic patients. Accordingly, other districts might show similar phenomena in our symptomatic patients and have consequently contributed to the increased sRAGE plasma levels.

Similarly as in other reports [1,2], plaque histological features, such as calcification, fibrosis, the extent of the lipid core, vascularity and the number of inflammatory cells, failed to discriminate lesions from symptomatic patients from those retrieved from asymptomatic patients in our study. This suggests that the possible identification and quantification of these features by imaging techniques *in vivo* in the future might be unable to distinguish lesions at high risk for ischaemic events from those that are likely to remain clinically silent. Rather, a better knowledge of the molecular features of the plaque and a thorough investigation of more specific circulating markers might be useful tools to predict plaque vulnerability.

In conclusion, our data suggest that higher levels of sRAGE occur in symptomatic patients with carotid atherosclerosis, possibly as a result of a counter-regulatory mechanism by which vascular cells reduce RAGE ligand-dependent activation. Our results are overall consistent with the concept that inflammation is a critical factor in carotid plaque symptomaticity. Further progress in understanding the role of the AGE–RAGE axis in carotid artery disease is warranted to identify patients at risk, and possibly to devise novel preventive strategies.

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