Elevated soluble receptor for advanced glycation end product levels in patients with acute coronary syndrome and positive cardiac troponin I

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Objectives **High levels of soluble receptor for advanced glycation end products (sRAGE) have been shown to have an atheroprotective role; however, no data are available on this molecule in acute coronary syndromes (ACS). We evaluated sRAGE levels in patients with non-ST segment elevation ACS (NSTE-ACS) or with chronic stable angina.**

Methods **We studied 265 patients, 190 of whom had NSTE-ACS and 75 had chronic stable angina.**

Results **Plasma sRAGE values were comparable in the two groups ($P=0.19$). However, in the patients with NSTE-ACS, sRAGE levels were significantly higher in patients with cardiac troponin-I (cTnI) of more than or equal to 0.04 μg/l compared with those with cTnI of less than 0.04 μg/l [758 pg/ml (493–1536 pg/ml) vs. 454 pg/ml (167–899 pg/ml); $P=0.0037$]. A significant correlation ($r=0.323$, $P=0.0045$) was found between sRAGE and cTnI levels in patients with NSTE-ACS.**

Conclusion **Plasma sRAGE levels are elevated in patients with NSTE-ACS with positive cTnI, suggesting that they could be related to myocardial cell damage.** ***Coron Artery Dis*** **00:000–000** © **2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Biomarkers play an important role in the diagnosis of acute coronary syndromes (ACS), especially in unstable angina and non-ST-segment elevation (NSTE) myocardial infarction. Among these, cardiac troponin and creatine kinase appear to be the most sensitive and specific markers of myocardial injury [1,2]. However, ideal biomarkers offering early detection, risk stratification, monitoring disease progression, and treatment efficacy remain to be elucidated. As NSTE-ACS are clinically heterogeneous, the assessment of several markers would provide complementary information to that of more established and conventional ones, and enable clinicians to stratify risk more effectively in patients with ACS.

In recent years, the circulating form of receptor for advanced glycation end products (RAGE), called soluble RAGE (sRAGE), able to competitively inhibit ligand binding to cell-surface RAGE, has been reported as an emerging biomarker in cardiovascular, metabolic and inflammatory diseases [3,4].

The interaction of RAGE with its ligands – high-mobility group box 1(HMGB1), advanced glycation end products (mainly *N*-ε-(carboxymethyl)lysine adducts) and S100, among others – leads to cell activation resulting in production of cytokines, growth factors, and adhesion molecules [5,6]. The key finding that RAGE is a multiligand receptor unifies the concept that in diabetes and aging, innate and adaptive inflammatory mechanisms contribute to pathogenesis of tissue injury [7].

The sRAGE, composed of only the extracellular ligand-binding domain, is generated by alternative splicing or proteolytic cleavage of full-length RAGE [8]. It circulates in human plasma and is still able to bind ligands and thus antagonize RAGE signalling [3,4]. This assumption has been supported by animal experiments, in which administration of sRAGE to atherosclerotic mice slowed the progression of atherosclerosis, and by clinical studies in which low levels of sRAGE were associated with coronary artery disease, carotid and femoral atherosclerosis, as well as with metabolic syndrome [9–11], suggesting a vasculoprotective role for this molecule.

Importantly, RAGE ligands are closely involved in the events of ischemia/reperfusion injury through RAGE axis, whereas the administration of sRAGE mitigates the adverse impact of ischemia/reperfusion injury in the heart, revealing a cardioprotective effect [12–14].

It is likely that in an acute inflammatory setting, plasma levels of sRAGE may increase. It has recently been shown that sRAGE levels are elevated in patients with septic shock [3], and in healthy volunteers treated with

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endotoxin they increased after 5 h [15]. Levels of sRAGE are upregulated during intratracheal lipopolysaccharide-induced lung injury [16].

We have recently reported that in patients with carotid atherosclerosis, sRAGE levels were higher in symptomatic than asymptomatic patients [17].

The aim of this study was to investigate the role of sRAGE in patients with NSTE-ACS or chronic stable angina (CSA).

Methods

Patients

Two hundred and sixty-five patients, consecutively admitted to our Coronary Care Unit with coronary artery disease and undergoing diagnostic coronary angiography, were enrolled. A total of 190 patients had NSTE-ACS and 75 had CSA. Exclusion criteria included: ST-segment elevation myocardial infarction (STEMI), previous history of coronary artery bypass grafting, significant heart valve disease, cardiogenic shock, congestive heart failure, renal or liver diseases, infectious, chronic inflammatory or immunologic diseases, malignancies, and serum creatinine level of more than 106.08 μmol/l. None of the participating patients were taking vitamins or antioxidant dietary supplements, nonsteroidal anti-inflammatory drugs, hormone replacement therapy, or anticoagulants. The local ethics committee approved the study protocol, and all patients signed written informed consent. Angiography was performed in all patients according to standard indications [18,19].

Blood samples and laboratory analyses

In patients with CSA, blood samples were collected at the time of angiography but before the injection of contrast material. In patients with NSTE-ACS, blood was drawn on admission, within 24 h after the last ischemic episode and before angiography (mean time was 12.6 ± 5.8 h within 4–24 h).

Blood samples were drawn in tubes without additives, containing heparin or citrate (for routine biochemistry). Creatinine, total cholesterol, high-density lipoprotein cholesterol, triglycerides, glucose and high-sensitivity C-reactive protein (CRP) were determined using routine laboratory procedures. Plasma and serum samples were centrifuged at 4°C, immediately divided in aliquots and stored at −80°C until analysis. All laboratory work was done in blinded manner with respect to the identity of the samples.

Soluble receptor for advanced glycation end products assay

For sRAGE determination, blood samples were collected in tubes containing Na_2EDTA, and plasma sRAGE levels were determined using a double-sandwich enzyme-linked immunosorbent assay (ELISA) kit (DuoSet ELISA development kit; R and D Systems, Minneapolis, Minnesota, USA) as described previously [11]. Intra-assay and interassay values of variation coefficient were 5.9 and 8.2%, respectively. The lower limit of detection of sRAGE was 21.5 pg/ml.

Cardiac troponin I assay

To evaluate cardiac troponin I (cTnI) concentrations, blood samples were collected in tubes with lithium heparin. Plasma samples were analyzed using the Access AccuTnI assay (Beckman Coulter, Villepinte, France). The assay has a detection limit of 0.014 μg/l and a coefficient of variation of 10% at a value of 0.06 μg/l. The 99th percentile of a reference control population is 0.04 μg/l. As the 99th percentile of a reference population has been defined to represent evidence of myocardial necrosis [1,20]. The patients were classified as cTnI positive if the cTnI value was more than or equal to 0.04 μg/l.

Power calculation

Sample size was calculated with the Stata software (version 9.2; Stata Corp. College Station, Texas, USA) by the estimated power for two-sample comparison of means of log-transformed RAGE values. A sample size of 144 patients (72 per arm) would provide 95% power to detect differences of 25–30% in the sRAGE levels between the two groups in a two-sided test at an α level of 0.05. We therefore recruited in excess of this figure to be fully confident in our data.

The subgroup analyses (above vs. below the cutoff of cTnI) had not been prespecified and as the sample size was determined by screening the cutoff of cTnI, the test power associated with the sample size was calculated *a posteriori* for the main variable of the study (sRAGE) and the power value found was 99.9% (with a two-sided type 1 error of 1%).

Statistical analysis

Data with a normal distribution are given as mean ± standard deviation. Variables with a skewed distribution are expressed as median and interquartile range. Group differences were analyzed by the Student *t*-test, and the χ^2-test for normally distributed, and noncontinuous variables, respectively. Variables with a non-normal distribution were logarithmically transformed before each analysis. The correlations were assessed by the Pearson correlation analysis. A two-sided *P* value of less than 0.05 was considered significant. Data were analyzed with the use of statistical software SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA).

Results

The clinical and angiographic details of all patients are summarized in Table 1. The NSTE-ACS and the CSA groups did not differ significantly for sex, age, occurrence

Table 1 Baseline demographic and clinical characteristics of all patients

	NSTE-ACS $n=190$	CSA $n=75$	*P* value
Age (years)	65 ± 10	62 ± 10	0.14
Male (%)	76	86	0.30
Body mass index, kg/m^2	26.8 ± 3.5	25.9 ± 3.6	0.37
Obesity (%)	29	33	0.69
Family history (%)	46	56	0.16
Diabetes mellitus (%)	25	27	0.63
Smoking (%)	56	60	0.39
Hypertension (%)	67	65	0.84
Medications (%)			
Antihypertension treatment	75	85	0.49
Lipid-lowering treatment	56	62	0.42
Hypoglycemic treatment	22	25	0.59
Antithrombotic treatment	80	82	0.83
Coronary angiography			
Left ventricular ejection fraction	54.2 ± 10.2	58.2 ± 7.0	0.23
One-vessel disease (%)	44	55	0.48
Multivessel disease (%)	56	45	0.45

CSA, chronic stable angina; NSTE-ACS, non-ST segment elevation acute coronary syndromes; SD, standard deviation.
Values are means ± SD or frequency (%).

Table 2 Biochemical characteristics of study patients

	NSTE-ACS $n=190$	CSA $n=75$	*P* value
Glucose (mmol/l)	6.55 ± 2.6	6.2 ± 2.05	0.34
Total cholesterol (mmol/l)	4.27 ± 1.34	3.88 ± 1.42	0.06
Triglycerides (mmol/l)	1.49 ± 0.68	1.33 ± 0.69	0.09
HDL-C (mmol/l)	0.98 ± 0.28	1.08 ± 0.23	0.02
LDL-C (mmol/l)	3.26 ± 0.9	3.15 ± 1.06	0.58
Creatinine (μmol/l)	88.85 ± 28.11	82.2 ± 23.9	0.20
CRP (mg/l)	25.9 (8–74)	5.1(1.6–17.9)	0.0001
cTnI (μg/l)	0.34 (0.113–0.648)	0.045 (0.01–0.26)	0.0033
sRAGE (pg/ml)	429 (250–841)	461 (260–678)	0.19

CRP, C-reactive protein; CSA, chronic stable angina; cTn1, cardiac troponin-I; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NSTE-ACS, non-ST segment elevation acute coronary syndromes; SD, standard deviation; sRAGE, soluble receptor for advanced glycation end product.
Values are means ± SD or median (interquartile range).

Table 3 Plasma levels of soluble receptor for advanced glycation end product in patients with non-ST segment elevation acute coronary syndromes

	cTnI negative (<0.04 μg/l) $n=132$	cTnI positive (>0.04 μg/l) $n=58$	*P* value
sRAGE (pg/ml)	454 (167–899)	758 (493–1536)	0.0037
CRP (mg/l)	40 (14.8–100.5)	41 (21.7–203)	0.553

CRP, C-reactive protein; cTn1, cardiac troponin-I; NSTE-ACS, non-ST segment elevation acute coronary syndromes; sRAGE, soluble receptor for advanced glycation end product.
Values are median (interquartile range).

of smoking, obesity, diabetes, family history for coronary artery disease, dyslipidemia, body mass index, coronary angiographic findings, and cardiac medications. Among biochemical parameters, high-density lipoprotein cholesterol values were lower in the NSTE-ACS than in the CSA group ($P = 0.02$). As expected, CRP and cTnI levels were significantly higher in the first group than in the second ($P = 0.0001$ and 0.0033, respectively; Table 2), whereas sRAGE levels did not differ between two groups [429 pg/ml (250–841 pg/ml) vs. 461 pg/ml (260–678 pg/ml), $P = 0.19$; Table 2].

Within the NSTE-ACS group, 58 patients (30.5%) had cTnI of more than 0.04 μg/l (Table 3).

Elevated sRAGE values were found in patients with NSTE-ACS with positive cTnI (cTnI > 0.04 μg/l) than in patients with NSTE-ACS with negative cTnI () [~~cTnI < 0.04 μg/l;~~ 758 ~~pg/ml~~ (493–1536 ~~pg/ml~~) vs. 454 ~~pg/ml~~ (167–899 ~~pg/ml~~); $P = 0.0037$; Table 3].

In contrast, no difference was found in plasma CRP concentrations into the NSTE-ACS group between those patients with raised and those with normal cTnI [41 ~~mg/l~~ (21.7–203 ~~mg/l~~) vs. 40 ~~mg/l~~ (14.8–100.5 ~~mg/l~~); $P = 0.29$; Table 3].

In addition, plasma levels of sRAGE correlated with the levels of cTnI ($r = 0.323$, $P = 0.0045$) in patients with NSTE-ACS but not with all other biochemical parameters or with the number and extent of diseased vessels found on angiography (data not shown).

Discussion

ACS is a complex disease involving macrophage activity, oxidative stress, tissue remodelling, necrosis, and thrombosis. As biomarkers may reflect various pathophysiological processes, the use of multimarker strategy could allow risk stratification and provide prognostic information in patients with ACS. For instance, a multimarker study combining baseline levels of cTnI, CRP, and brain natriuretic peptide among patients with NSTE myocardial infarction identified a 6-fold to 13-fold gradient of mortality risk between those in whom all three markers were elevated and those without elevation of any marker [21]. In contrast to our first hypothesis of the study, we did not find different values of sRAGE between patients with CSA and NSTE-ACS. In agreement with our results, it has been recently reported that sRAGE levels did not differ between ACS and stable angina groups, whereas they were higher in ACS than in control groups [22]. The same study has demonstrated that serum levels of S100 proteins (RAGE ligands) were higher in ACS than in both stable angina and control groups, and the expression of these proteins were related to myocardial injury in patients with ACS and in rat models of myocardial infarction, supporting the role of RAGE and its ligands in myocardial infarction and cardiovascular diseases [22].

The lack of significativity of serum levels of sRAGE between NSTE-ACS and CSA groups could depend by many factors, including the pharmacological treatments, such as statins and antihypertensive drugs [23].

Nevertheless, this is the first study that shows elevated circulating sRAGE levels in patients with NSTE-ACS with an increased cTnI level compared with those with

normal cTnI. Although this is an observational study and thus we cannot draw conclusions about causality, nevertheless these relationships lead us to believe that sRAGE may arise after injury. Acute myocardial ischemia and injury could be prominent stimuli that raise the release of sRAGE. A likely pathway for sRAGE production in patients with NSTE-ACS with positive cTnI is the myocardial injury that induces inflammatory reactions. The cell-surface RAGE is expressed by multiple inflammatory cells, including neutrophils, monocyte macrophages, and lymphocytes. Ligands of RAGE, particularly the endogen danger signal HMGB1 released rapidly during necrosis and actively by inflammatory cells, sustain vascular injury. Accordantly, increased levels of serum HMGB1 have been shown in patients with ACS [24]. Therefore, the increase in HMGB1 and inflammatory cytokines leads to a cascade of inflammation and could stimulate macrophages to secrete sRAGE. In addition, another trigger for the sRAGE increase could be the RAGE-ligand *N*-ϵ-(carboxymethyl)lysine, which recently has been found to be higher in plasma of patients with ACS with positivity to cTnT [25].

Alternatively, an increased proteolytic activity during ACS, by matrix metalloproteinase-9 or disintegrin-like metalloproteinase, ADAM10 (two enzymes involved in RAGE shedding [26]), could generate more sRAGE.

In a recent study in minipigs with ischemia/reperfusion injury, it has been reported that intracoronary sRAGE administration decreases myocardial TGF-β1 expression, myocardial fibrotic lesion formation, and cardiac remodelling, suggesting that inhibition of RAGE-mediated inflammation is crucial for cardioprotection against ischemia/reperfusion injury [27].

In agreement with several reports, we confirm that plasma CRP levels were higher in patients with NSTE-ACS than in those with CSA [28,29]. However, in our study, unlike sRAGE, CRP level was not higher in patients with NSTE-ACS with positive cTnI than in those with negative cTnI and did not correlate with each other, suggesting that CRP and sRAGE may be complementary and reveal different features of the disease. The measurement of sRAGE shows promise and needs to be more thoroughly evaluated for commercial development for implementation into routine clinical and laboratory practice. After all, larger clinical studies are indicated to review the role of sRAGE as a surrogate biomarker of myocardial injury. More widespread use of sRAGE assays for patients with NSTE-ACS will therefore facilitate prognostication and improve tailoring of management. Crossing boundaries from research to clinical application will require replication in multiple settings and experimental evidence supporting a pathophysiologic role and, ideally, interventional trials showing that monitoring single or multiple biomarkers improves outcomes.

Study limitation

The main limitation of this study is that we did not recruit patients with more severe myocardial injury,that is, patients with STEMI. Actually, these data were unexpected because before the beginning of the study, we hypothesized that we might see significant differences in sRAGE levels between CSA and NSTE-ACS groups. Therefore, at the moment we do not know whether plasma sRAGE levels in patients with STEMI are even higher than those in patients with NSTE-ACS. In addition, in a further step, longitudinal studies should be performed to evaluate whether sRAGE measurement might be useful in monitoring the progression or remission of ACS.

A second caveat of this study is the lack of RAGE ligand assessment that could provide a more complete picture of the complex interaction between RAGE tissue, ligands and sRAGE.

Third, the ELISA system for sRAGE determination cannot distinguish among diverse sRAGE splice variants. However, this limitation is only apparent; whether sRAGE or esRAGE is produced, in both cases the ligand-binding domain is intact, enabling soluble receptor isoforms to sequester RAGE ligands and promote their disposal.

Conclusion

Although our cross-sectional analysis and correlations do not imply causality, the inter-relationships between sRAGE levels and cTnI in patients with NSTE-ACS is biologically plausible. We speculate that sRAGE is responsive to stimuli in the local environment. In the myocardial injury context, increased proteolysis at the cell surface or major secretion by activated inflammatory cells results in higher levels of circulating sRAGE. Conceivably, high levels of sRAGE are part of a counter-regulatory mechanism elicited by vascular/myocardial injury and aimed at RAGE signalling suppression.

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Conflicts of interest

There are no conflicts of interest.

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