sRAGE is Elevated in Septic Patients and Associated With Patients Outcome

Christian Bopp, M.D.,* Stefan Hofer, M.D.,* Jürgen Weitz, M.D.,† Angelika Bierhaus, M.D.,‡ Peter P. Nawroth, M.D.,‡ Eike Martin, M.D., F.A.N.Z.C.A.,* Markus W. Büchler, M.D.,† and Markus A. Weigand, M.D., D.E.A.A.*

*Department of Anesthesiology; †Department of Surgery; and ‡Department of Medicine I, University of Heidelberg, Heidelberg, Germany

Submitted for publication April 7, 2007

Background. (1) To evaluate in septic patients the plasma levels of soluble receptor for advanced glycation end products (sRAGE), a soluble splice variant of the full length receptor RAGE, which is involved in acute inflammation (2) to determine whether sRAGE could be used as a potential diagnostic and prognostic marker in sepsis in the surgical intensive care unit.

Materials and methods. An observational clinical noninterventional pilot study in a surgical intensive care unit with patients admitted to the intensive care unit over a 6-mo period with clinical evidence of severe sepsis or septic shock.

Results. Twenty-nine intensive care patients were enrolled in the study within the first 24 h after onset of severe sepsis or septic shock. Eight healthy volunteers served as controls. Plasma sRAGE concentrations were elevated in septic patients compared with healthy volunteers (1764 ± 138 versus 1026 ± 177 pg/mL, P < 0.05). Additionally, nonsurvivors after 28 days have had higher plasma sRAGE concentrations than survivors (2302 ± 189 versus 1326 ± 112 pg/mL, P < 0.001). Receiver operating characteristic curve analysis of plasma sRAGE concentrations of septic patients showed a specificity of 75% and a sensitivity of 84.6% with 1596 pg/mL as cutoff.

Conclusions. This is the first study showing elevated plasma sRAGE concentrations in septic patients. It is noteworthy that nonsurvivors had higher plasma sRAGE concentrations than survivors, suggesting that sRAGE is related to severity and outcome of septic patients. Further clinical studies are required to investigate the usefulness of sRAGE as a new sepsis marker. © 2008 Elsevier Inc. All rights reserved.

Key Words: sepsis; severe sepsis; septic shock; clinical trial; sepsis markers; sRAGE.

INTRODUCTION

Sepsis remains an important clinical and economic challenge for intensive care units throughout the world. Severe complications such as multi-organ failure with high mortality and the lack of specific diagnostic tools characterize the clinical situation in sepsis [1].

The receptor for advanced glycation end products (RAGE) is a member of the Ig superfamily and a multi-ligand receptor interacting with a diverse class of ligands [2]. RAGE has been shown to be involved in the pathogenesis of several chronic diseases [2].

Recently, we clarified the role of RAGE in experimental sepsis, showing that RAGE-dependent activation of nuclear factor-kappa B (NF-κB) plays a central role in modulating mortality after cecal ligation and puncture (CLP) [3].

RAGE has secretory isoforms referred to as soluble RAGE (sRAGE), which comprise the extracellular ligand-binding domain but are lacking the cytosolic and transmembrane domains. It is noteworthy that sRAGE has the same ligand binding specificity and therefore competes with cell-bound RAGE and serves as a decoy abrogating cellular activation. In a sepsis model of CLP-induced sepsis the administration of exogenous sRAGE did slightly improve survival [3].

In humans, endogenous sRAGE (esRAGE) is produced by alternative splicing of RAGE mRNA. esRAGE, however, does not represent the entire pool of sRAGE present in the bloodstream and it is speculated that sRAGE can also be cleaved proteolytically from
the membranous RAGE. However, little is known about the functional role of endogenous levels of sRAGE/esRAGE in healthy patients. In nondiabetic patients sRAGE was elevated in parallel with serum advanced glycation endproduct (AGE) levels [4]. In contrast, Koyama et al. found that esRAGE was inversely associated with carotid or femoral atherosclerosis and seems to be a protective factor for the metabolic syndrome and atherosclerosis [5].

Indeed, there is a lack of knowledge regarding the role of sRAGE/esRAGE in sepsis. In view of these data, the aim of this work was to set up a pilot study to investigate whether total pool of sRAGE is increased in plasma of septic patients. Furthermore, we assessed the ability of sRAGE to predict mortality in patients with severe sepsis or septic shock.

**MATERIALS AND METHODS**

This observational clinical study was conducted in the surgical intensive care unit of the University Hospital of Heidelberg, Germany, after the study protocol was approved by the local ethical committee in accordance with the Helsinki Declaration of 1975.

Eight healthy volunteers served as controls. Twenty-nine consecutive patients of the surgical intensive care unit were enrolled in the study within the first 24 h after onset of severe sepsis or septic shock. Patients were classified according to the Sepsis Consensus Conference on 1992 [6], and clinical data, diagnosis, treatment modalities, and blood samples were collected. All patients were mechanically ventilated and were cared for by the intensive care unit staff. The severity of a patient’s illness was estimated using the APACHE II score. Patients with acute primary central nervous system disorders (e.g., meningitis or cerebrovascular accident), acute metabolic disorders, chronic renal disorders, and acute primary liver disease were excluded from the study.

At enrollment, blood samples were taken and sRAGE antigen was detected in plasma by enzyme-linked immunosorbent assay (R and D Systems, Wiesbaden, Germany). At the same time, APACHE II score, mean arterial pressure, arterial oxygen partial pressure/fraction of inspired oxygen ratio, temperature, pH, lactate, and creatinine were documented. A follow-up at 28 d was performed to distinguish between survivors and nonsurvivors. After enrollment of patients, data were blinded to avoid potential bias.

Data are expressed as mean and SEM. A stepwise multivariate linear regression model was calculated to detect independent associations of age, gender, APACHE II score, mean arterial pressure, arterial oxygen partial pressure/fraction of inspired oxygen ratio, temperature, pH, lactate, and creatinine with sRAGE. Comparison of means was performed using Student’s t-test after testing normal distribution. Receiver operating characteristic (ROC) curves were computed. SPSS 13.0 software was used for statistical analysis (SPSS Inc., Chicago, IL).

**RESULTS**

**sRAGE Measurement in Plasma of Healthy Volunteers and Patients**

To test if sRAGE concentrations of septic patients differ from healthy controls, blood samples of eight controls and 29 septic patients were performed. Plasma sRAGE concentrations were higher in septic patients than in healthy volunteers (1764 ± 138 versus 1026 ± 177 pg/mL, P < 0.05) (Fig. 1).

**sRAGE Measurement in Plasma of Survivors and Nonsurvivors**

To determine whether sRAGE may serve as an early marker of septic patients outcome, we divided the septic patients according to 28 d mortality in survivors (n = 16) and nonsurvivors (n = 13) and compared sRAGE concentrations of both groups. Within 24 h of the onset of sepsis, plasma sRAGE concentrations of nonsurvivors were significantly elevated compared with survivors (2302 ± 189 versus 1326 ± 112 pg/mL, P < 0.001) (Fig. 2).

**Patient Characteristics**

In the present study, APACHE II score, mean arterial pressure, arterial oxygen partial pressure/fraction of inspired oxygen ratio, temperature, pH, lactate, and creatinine did not significantly distinguish between survivors and nonsurvivors (Table 1).

![FIG. 1. Circulating sRAGE in patients with severe sepsis or septic shock and healthy controls. Controls (n = 8) and septic patients (n = 29). Values are means ± SEM; #P < 0.05 for controls versus septic patients.](image1.png)

![FIG. 2. Circulating sRAGE in controls, survivors, and nonsurvivors. Controls (n = 8), survivors (n = 16), and nonsurvivors (n = 13) of sepsis at day 28. Values are means ± SEM; *P < 0.05 for survivors versus controls; #P < 0.001 for survivors versus nonsurvivors.](image2.png)
**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Survivor (n = 16)</th>
<th>Nonsurvivor (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APACHE II</strong></td>
<td>25.5 ± 5.8</td>
<td>26.9 ± 5.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.4 ± 18.4</td>
<td>58.9 ± 12.7</td>
</tr>
<tr>
<td>Temperatur (°C)</td>
<td>38.1 ± 1.7</td>
<td>38.2 ± 1.4</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>68.1 ± 18.3</td>
<td>71.1 ± 12.7</td>
</tr>
<tr>
<td>PaO₂/FiO₂</td>
<td>139.8 ± 51.1</td>
<td>115.7 ± 51.7</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>4.2 ± 4.0</td>
<td>3.4 ± 2.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>2.6 ± 1.3</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>sRAGE (pg/mL)</td>
<td>1326 ± 136</td>
<td>2302 ± 189*</td>
</tr>
</tbody>
</table>

*Note. Values are mean ± SEM.

**Cutoff Value of sRAGE in Septic Patients**

To determine a cutoff value of sRAGE in plasma of septic patients (n = 29) we performed a ROC analysis of sRAGE concentrations of septic patients. In our cohort study, the cutoff of sRAGE was 1569 pg/mL with a specificity of 75% and a sensitivity of 84.6% (Fig. 3).

**DISCUSSION**

To the best of our knowledge, this is the first manuscript reporting elevated plasma sRAGE levels in septic patients. We demonstrate elevated sRAGE levels in sepsis. Furthermore, we found an increased sRAGE concentration in plasma of nonsurvivors compared with survivors of sepsis. After all, larger clinical studies are indicated to review the potential role of sRAGE as a potential new sepsis marker.

In the United States, sepsis is the main cause of death in non-cardiologic intensive care units and linked with increasing costs for patient care. The overall mortality is about 50% in patients with septic shock [1]. Even high engagement in sepsis research led only to slight improvements in new, effective sepsis treatment strategies. Despite several old as well as recently found molecules such as C-reactive protein, procalcitonin, soluble intercellular adhesion molecule (ICAM), interleukin-6, von Willebrand factor, or N-terminal pro-brain natriuretic peptide, the lack of specific and valid pathophysiologic sepsis markers may delay early, aggressive, and effective sepsis therapy [7–9]. Most of the actually known parameters can only reflect some subsystems or time-dependent aspects of sepsis, characterized by a summary of disorders involving bacterial, fungi, or virus infections [7].

Gibot et al. found that triggering receptor expressed on myeloid cells-1 (TREM-1) can identify patients with infection. Additionally, TREM-1 may have a predictive value for patients outcome [9]. However, TREM-1 was not correlated with the severity of sepsis [10]. Recently, Scherpereel et al. demonstrated that endocan, a circulating proteoglycan, is elevated in septic patients and may be associated with a patient's outcome [11]. However, endocan was only measured at admission. Thus, this data should be interpreted as preliminary. In septic patients circulating ICAM-1, one of the most important ligands for leukocyte integrins was higher in nonsurvivors than in survivors at day 0 [12]. In our study, we found an increased sRAGE concentration in plasma of nonsurvivors compared with survivors of sepsis. In conclusion, larger clinical trials should check the potential role of sRAGE as a new sepsis marker. However, since specificity and sensitivity is less than that for procalcitonin, clinical application as a marker seems to be limited.

The pattern that recognition receptor RAGE displays a central role in the innate immune system with an impact in perpetuation of the immune response [3]. Engagement of RAGE by its ligands results in a sustained NF-κB-activation in all cell types studied thus far. Increased NF-κB activation has been demonstrated to be a predictor of outcome in septic patients [13]. Since RAGE expression itself is controlled by NF-κB [14], conditions inducing NF-κB trigger RAGE expression and consecutively lead to sustained inflammation in situations such as septicemia, in which RAGE-ligands are abundantly present.

Recently, the role of RAGE in the innate immune response in mice was shown in a model of polymicrobial septic peritonitis, induced by CLP [3], in which

FIG. 3. ROC curve of sRAGE concentrations of survivors and nonsurvivors. To determine a cutoff value of sRAGE in plasma of patients with severe sepsis and septic shock (n = 29), we performed a ROC analysis of sRAGE concentrations. A sRAGE concentration of 1569 pg/mL was with a specificity of 75% and a sensitivity of 84.6% the cutoff value.
80% of the RAGE−/− mice survived. In contrast, RAGE-bearing wild-type mice were more susceptible to septic shock, as demonstrated by survival of 20% of the control group, indicating that RAGE deficiency seems to protect mice from lethal multibacterial peritonitis.

In a model of hemorrhagic shock and resuscitation, Raman and coworkers demonstrated that activation of RAGE-dependent signaling is a key factor leading to gut mucosal barrier dysfunction [15]. Hemorrhagic shock and resuscitation in mice induced bacterial translocation, ileal mucosal hyperpermeability, and elevated plasma Interleukin-6 levels in wild-type but not RAGE−/− mice.

These data indicate that RAGE is involved in experimental sepsis but no data in human sepsis are available. In our study, sRAGE was measured within the first 24 h after the onset of sepsis. In fact, our study was conceived as a pilot study explaining the number of patients and the single measurement. Nevertheless, this study shows that sRAGE is elevated in septic patients compared with healthy controls. Restrictively, we have selected healthy humans, not non-septic ICU patients, as controls for this pilot study. Certainly, mechanically ventilated controls or non-septic patients are necessary in additional larger studies.

Additionally, our data suggest that high plasma sRAGE levels are associated with patient outcome. A likely explanation of the elevated sRAGE levels is that increased RAGE expression is involved in human sepsis.

Actually, little is known about the function of sRAGE/esRAGE in patients. Humpert et al. demonstrated that plasma sRAGE levels might represent an early marker of microvascular dysfunction and diabetic nephropathy in type 2 diabetes [16]. Furthermore, the finding of Yamagishi et al. suggests that circulating esRAGE levels may reflect tissue RAGE expression and may be elevated in parallel with serum AGE levels as a counter-system against AGE-elicited tissue damage [4]. Since RAGE is a cell surface receptor that belongs to the immunoglobulin superfamily such as ICAM-1, which is also elevated in septic patients at day 0 [12], sRAGE/esRAGE might represent a marker of cellular damage.

Elevated sRAGE levels in our study might represent the acute inflammation status of the patients as splice variants of RAGE or split off variants of the cell surface RAGE. Interestingly, on the first day the levels of total sRAGE could distinguish between survivors and non-survivors. The enzyme-linked immunosorbent assay used in our study quantifies concentrations of total sRAGE in plasma. In detail, this kit could not differentiate between native secretory RAGE isoforms and soluble RAGE that results, e.g., from the cleavage of the cell-surface receptor by metalloproteinases [17].

In a mouse model of hemorrhagic shock and reperfusion, sRAGE treated wild-type mice were protected from the development of systemic inflammation [15]. It is noteworthy that mice treated with exogenous sRAGE show no significantly improved survival after CLP [3]. In contrast, we see higher sRAGE levels in plasma in nonsurvivors compared with survivors. This apparent discrepancy could be in part explained by different species, different time of measurement, and different kind of sepsis. Furthermore, exogenously administered sRAGE can be different from potential structural and functional modified sRAGE in patients with severe sepsis or septic shock. sRAGE was found to be released outside from the cells, to bind AGE ligands, and to be capable of neutralizing AGE actions on endothelial cells in culture [18]. In detail, sRAGE was shown to bind proinflammatory AGEs in a saturable and dose-dependent manner [19]. In our study, we did not test if plasma sRAGE was already active and capable of binding AGEs. One explanation is that the integrity of sRAGE could be modified in acute inflammation. Additionally, the concentrations of sRAGE might not be enough to scavenge AGEs or other RAGE ligands. Thus, it will be interesting in the future to define the share of esRAGE to total sRAGE to finally clarify its functional significance.

In conclusion, we demonstrate for the first time that sRAGE is elevated in septic patients. Furthermore, in our patients sRAGE is suitable to distinguish on the first day between survivors and nonsurvivors in case of 28 d mortality. Further larger clinical studies with repeated measurements of sRAGE in patients with different sources of sepsis are necessary to support the potential clinical evidence of sRAGE and to clarify the functional role of sRAGE/esRAGE in sepsis and its putative role as a new sepsis marker.

ACKNOWLEDGMENTS

The authors thank C. Rosenhagen for excellent practical help.

REFERENCES


