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Increased levels of soluble receptor for advanced glycation end products (sRAGE) and high mobility group box 1 (HMGB1) are associated with death in patients with acute respiratory distress syndrome

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ABSTRACT

Objectives: Receptor for advanced glycation end products (RAGE) plays a role in inflammatory reactions. Soluble RAGE (sRAGE) level is elevated in patients with acute respiratory distress syndrome (ARDS). However, which clinical parameters and inflammatory biomarkers including sRAGE are associated with death in ARDS patients remain unknown.

Design and methods: We examined whether sRAGE level was independently associated with death in 20 ARDS patients with severe infection.

Results: Compared with age- and sex-matched control subjects, blood pressure levels were lower and KL-6, high mobility group box 1 (HMGB1), interleukin-6 and sRAGE levels were higher in ARDS patients. In multivariate analysis, sRAGE was associated with death in ARDS patients, but severity of illness was not. HMGB1 was a sole independent correlate of sRAGE.

Conclusions: This study demonstrated that sRAGE was independently associated with death in ARDS patients. Our present results suggest active involvement of HMGB1–RAGE axis in poor prognosis of ARDS.

Introduction

Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury, is a major cause of rapid-onset respiratory failure in critically ill patients, which could account for the high disability rate in these subjects [1,2]. A variety of injurious insults could elicit diffuse alveolar damage, which is followed by alveolar edema and interstitial fibrosis [3]. Although the etiology of ARDS is multifactorial, uncontrolled inflammation has recently been considered to play a central role in the pathogenesis of this devastating disorder [4,5].

There is a growing body of evidence that receptor for advanced glycation end products (RAGE) is mainly involved in inflammatory reactions in various disorders such as atherosclerotic cardiovascular disease, rheumatoid arthritis, and acute lung injury [6–9]. Further, high mobility group box-1 (HMGB1), one of the ligands for RAGE, has been implicated as a putative danger signal in a variety of inflammatory conditions as well [10–12]. HMGB1–RAGE interaction promotes chemotaxis and maturation of immune cells, enhances the expression of adhesion molecules in endothelial cells, and stimulates the production of cytokines by various types of cells, thereby evoking local and systemic inflammation [10–12].

Recently, HMGB1 was found to be a late mediator of endotoxin-induced acute lung injury in mice [13], and serum levels of HMGB1 were increased in septic shock patients and positively associated with sepsis-related organ failure assessment score [14]. In addition, blockade of the HMGB1 and RAGE axis was reported to ameliorate acute pulmonary inflammatory responses after endotoxin instillation, including lung edema with neutrophil accumulation and increased production of cytokines [13–16].

Moreover, soluble RAGE (sRAGE) has been identified in human plasma [17–19]. RAGE is expressed in alveolar type 1 cells and most abundant in the lung [20,21]. In endotoxin-induced acute lung injury mouse models, sRAGE levels in bronchoalveolar lavage (BAL) fluid and serum were elevated and correlated with the severity of lung damage [20,21]. These observations suggest that serum level of sRAGE is a biomarker of severity and clinical outcomes of ARDS subjects. Therefore, in this study, we examined whether sRAGE level was independently associated with the severity of lung injury and/or death in 20 ARDS patients with severe infection. We further examined

Abbreviations: ARDS, acute respiratory distress syndrome; RAGE, receptor for advanced glycation end products; HMGB1, high mobility group box 1; sRAGE, soluble RAGE; BAL, bronchoalveolar lavage; BP, blood pressure; APACHE, Acute Physiology and Chronic Health Evaluation; ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6; SD, standard deviation.

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which clinical parameters and inflammatory variables could be independent correlates of elevated sRAGE level in ARDS subjects.

Patients and methods

Twenty ARDS patients with severe infection (12 males and 8 females, mean age; 64.6 years (pneumonia $n=12$, pyelonephritis $n=2$, peritonitis $n=2$, pancreatitis $n=1$, cholangitis $n=1$, colitis $n=1$, unknown origin $n=1$) and 20 age-matched healthy volunteers (11 males and 9 females, mean age; 63.9 years) were included in this study. Research Ethics Committee of our hospital approved this study protocol, and informed consent was obtained from all the alert subjects or first-degree relatives of drowsy patients. The criteria of American European Consensus Conference were used for the diagnosis of ARDS [22]. Patients with malignancy, ischemic heart disease, alcohol abuse, tuberculosis, collagen disease and liver disease were excluded from the study.

At admission, blood pressure (BP) was measured in supine position and blood was drawn from the antecubital vein for determinations of biochemical variables. Severity of ARDS was evaluated clinically by both Acute Physiology and Chronic Health Evaluation (APACHE) II score and lung injury score [23,24]. Serum level of KL-6, a lung epithelial cell injury marker [25], was measured by a commercially available kit (electrochemiluminescence immunoassay) (Eizai, Tokyo, Japan). Serum HMGB1 level was measured with an enzyme-linked immunosorbent assay (ELISA) as described previously [26]. Validated inter-assay and intra-assay coefficients of variation were <10%, and detection limit of HMGB1 was 0.3 ng/mL. Interleukin-6 (IL-6) and sRAGE levels were determined with commercially available ELISA kits (R&D systems, Minneapolis, MN, USA) [27,28]. Inter-assay and intra-assay coefficient of variations of sRAGE were 7.7% and 5.7%, respectively [28]. Serum endotoxin level was determined by a turbidimetric time assay using an ET-2000 toxinometer (Wako, Tokyo, Japan). Each sample was stored at −80 °C and analyzed in a single analytical run. All patients received intensive therapy for ARDS such as mechanical ventilation, direct hemoperfusion with polymyxin B-immobilized fiber, vasopressor agents and antibiotics, and followed until death or 28 days. Data are expressed as mean ± standard deviation (SD). Mann–Whitney's *U* test was performed for comparison of the parameters between ARDS patients and healthy volunteers and between survivors and non-survivors. Univariate and multivariate stepwise regression analysis was performed for correlates of death and sRAGE levels. Statistical significance was defined as $p<0.05$. All statistical analyses were performed with the use of the SPSS system (SPSS Japan Inc., IBM company, Tokyo, Japan).

Table 1
Characteristics of the patients.

Characteristics	ARDS	Control	*p*-Value
Age (years)	64.7 ± 11.3	63.9 ± 5.5	–
Sex (M/F)	12/8	11/9	–
SBP (mm Hg)	111.4 ± 8.7	129.7 ± 4.2	$p<0.01$
DBP (mm Hg)	68.7 ± 7.1	75.9 ± 3.1	$p<0.01$
Heart rate (bpm)	95.4 ± 7.7	73.7 ± 4.0	$p<0.01$
APACHE II	20.2 ± 2.0	–	–
Lung injury score	2.24 ± 0.45	–	–
KL-6 (U/mL)	404.0 ± 103.0	151.5 ± 44.0	$p<0.01$
HMGB1 (ng/mL)	2.3 ± 0.94	n.d.	–
IL-6 (pg/mL)	1227.5 ± 863.6	2.4 ± 0.8	$p<0.01$
sRAGE (pg/mL)	2013.7 ± 414.2	410.1 ± 188.7	$p<0.01$
Endotoxin (pg/mL)	1.4 ± 0.4	n.d.	–

SBP, systolic blood pressure; DBP, diastolic blood pressure; bpm, beats/minutes, HMGB1, high mobility group box-1; IL-6, interleukin-6; sRAGE, soluble RAGE.
n.d., Not detectable.

Table 2
Characteristics of survivors and non-survivors of ARDS patients.

Characteristics	Survivors	Non-survivors	*p*-Value
Age (years)	67.2 ± 10.5	62.1 ± 12.1	–
Sex (M/F)	5/5	7/3	–
SBP (mm Hg)	114.0 ± 9.3	108.8 ± 7.6	0.17
DBP (mm Hg)	69.6 ± 7.5	67.8 ± 6.9	0.38
Heart rate (bpm)	94.6 ± 5.7	96.2 ± 9.6	0.76
APACHE II	19.9 ± 2.2	20.5 ± 1.9	0.52
Lung injury score	2.1 ± 0.4	2.4 ± 0.5	0.25
KL-6 (U/mL)	395.0 ± 73.1	413.0 ± 130.4	0.97
HMGB1 (ng/mL)	1.8 ± 0.9	2.7 ± 0.8	**0.012**
IL-6 (pg/mL)	934.0 ± 879.0	1521.0 ± 781.4	0.69
sRAGE (pg/mL)	1796.8 ± 418.5	2230.5 ± 287.3	**0.002**
Endotoxin (pg/mL)	1.4 ± 0.3	1.4 ± 0.5	0.68

Results

Table 1 shows the clinical and laboratory data of ARDS patients and age- and sex-matched healthy volunteers. Systolic BP (SBP) and diastolic BP (DBP) were significantly lower and heart rate was higher in ARDS patients than those in healthy volunteers ($p<0.01$). Serum levels of KL-6, HMGB1, IL-6, HMGB1, sRAGE, and endotoxin were significantly elevated in ARDS patients compared with healthy volunteers. APACHE II score and lung injury score in ARDS subjects were 20.2 and 2.24, respectively. As shown in Table 2, serum levels of HMGB1 and sRAGE were significantly higher in non-survivors of ARDS patients compared with survivors, although there were no significant differences of other clinical parameters and inflammatory biomarkers between the two groups. In the univariate analysis, HMGB1 ($p<0.05$) and sRAGE ($p<0.01$) were positively associated with death in ARDS patients (Table 3). Because the parameters could be closely correlated with each other, to determine independent determinants of death, multiple stepwise regression analysis was performed. This analysis showed that sRAGE was independently correlated to death in ARDS patients ($R^2=0.289$) (Table 3). Further, multiple stepwise regression analysis revealed that HMGB1 was a sole independent correlate of sRAGE in ARDS patients ($R^2=0.813$) (Table 4).

Discussion

In this study, we demonstrated for the first time that sRAGE and HMGB1 levels were correlated with each other and HMGB1 was a sole independent correlate of sRAGE in ARDS patients with severe infection. Furthermore, we found here that baseline sRAGE level was elevated in non-survivors compared with survivors and

Table 3
Univariate and multivariate analyses for determinants of death.

	Univariate			Multivariate	
Characteristics	β	SE	*p*-Value	β	*p*-Value
Age	0.232	0.010	0.326		
Sex	0.204	0.235	0.388		
SBP	0.307	0.013	0.188		
DBP	-0.131	0.017	0.582		
Heart rate	0.106	0.016	0.656		
APACHEII	0.153	0.059	0.520		
Lung injury score	0.287	0.259	0.221		
KL-6	0.089	0.001	0.708		
IL-6	0.349	0.000	0.132		
sRAGE	0.537	0.000	**0.015**	**0.537**	**0.015**
HMGB1	0.506	0.111	**0.023**		
Endotoxin	-0.025	0.294	0.917		

β: Regression coefficients. Female = 0, male = 1.
SE: standard error.
$R^2=0.289$.