LIVER TRANSPLANTATION 21:847–854, 2015

ORIGINAL ARTICLE

Circulating Levels of Soluble Receptor for Advanced Glycation End Products and Ligands of the Receptor for Advanced Glycation End Products in Patients With Acute Liver Failure

Giuseppina Basta,[1] Serena Del Turco,[1] Teresa Navarra,[1] William M. Lee,[2] and Acute Liver Failure Study Group

[1]*Institute of Clinical Physiology, National Research Council, Pisa, Italy; and* [2]*Division of Digestive and Liver Diseases, Internal Medicine, Southwestern Medical Center, University of Texas, Dallas, TX*

Animal studies suggest that receptor for advanced glycation end products (RAGE)–dependent mechanisms contribute to acetaminophen-induced liver damage. We examined whether circulating levels of soluble receptor for advanced glycation end products (sRAGE) or RAGE ligands, including extracellular newly identified receptor for advanced glycation end products binding protein (EN-RAGE), high-mobility group box 1 (HMGB1), and Nϵ-(Carboxymethyl)lysine adducts (CML), could aid in prognostication after an acetaminophen overdose. Sixty well-characterized acetaminophen-related acute liver failure (ALF) patients (30 spontaneous survivors and 30 patients who underwent transplantation and/or died) who were enrolled in the National Institutes of Health–sponsored Acute Liver Failure Study Group, were matched by age, met standard criteria for encephalopathy, and had an international normalized ratio > 1.5 were retrospectively studied. HMGB1, EN-RAGE, CML, and sRAGE were detected by enzyme-linked immunosorbent assay methods in sera from ALF patients and 30 healthy controls. Levels of sRAGE, EN-RAGE, and HMGB1 (but not CML) were significantly greater ($P < 0.001$) in ALF patients versus normal controls. The levels of sRAGE, HMGB1, and EN-RAGE were significantly higher ($P = 0.03$, $P < 0.01$, and $P = 0.03$) in patients with a systemic inflammatory response syndrome (SIRS) score > 2 versus patients with a SIRS score ≤ 2. Nevertheless, only sRAGE levels were significantly higher in patients who underwent transplantation and/or died versus spontaneous survivors ($P < 0.001$), and they were positively associated with conventional markers of liver disease severity.

Additional Supporting Information may be found in the online version of this article.

Abbreviations: ALF, acute liver failure; ALFSG, Acute Liver Failure Study Group; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BiLE, bilirubin, lactate, and etiology; CI, confidence interval; CML, Nε-(carboxymethyl)lysine adducts; COMA, encephalopathy grade; ELISA, enzyme-linked immunosorbent assay; EN-RAGE, extracellular newly identified receptor for advanced glycation end products binding protein; HE, hepatic encephalopathy; HMGB1, high-mobility group box 1; INR, international normalized ratio; LT, liver transplantation; MAPK, mitogen-activated protein kinase; MELD, Model for End-Stage Liver Disease; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa B; RAGE, receptor for advanced glycation end products; SD, standard deviation; SIRS, systemic inflammatory response syndrome; sRAGE, soluble receptor for advanced glycation end products.

Potential conflict of interest: Nothing to report.

The project was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (U01-DK-58369).

Giuseppina Basta, principal investigator, participated in the design of the study and in the statistical plan and drafted the article. Serena Del Turco was involved in data analysis and in writing the article. Teresa Navarra participated in performing the experiments. William M. Lee participated in the design and coordination of the study and supervised all aspects of the study.

The members of the Acute Liver Failure Study Group are provided in the supporting information.

Address reprint requests to Giuseppina Basta, D.Biol, Institute of Clinical Physiology, National Research Council, Via Moruzzi, 1, 56124 Pisa, Italy. Telephone: 0039-050-315 2216; FAX: 0039-050-315 2166; E-mail: lapina@ifc.cnr.it

DOI 10.1002/lt.24129
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

Multivariate logistic regression identified an encephalopathy grade > 2 as an independent predictor of an adverse outcome on admission (odds ratio, 13; 95% confidence interval, 2.3-73; $P < 0.001$). The RAGE-ligand axis may interfere with liver regeneration and should be a promising objective for further research. ***Liver Transpl 21:847-854, 2015***. © **2015 AASLD.**

Received December 17, 2014; accepted March 20, 2015.

Acute liver failure (ALF) is a rare but serious condition occurring in individuals without preexisting liver disease and characterized by sudden severe liver dysfunction associated with coagulopathy and hepatic encephalopathy (HE).[1,2] Acetaminophen overdoses are the number 1 cause of ALF in the United States; they account for approximately 50% of all cases of ALF and have a 30% mortality rate.[2,3] Although the majority of patients recover spontaneously after an acetaminophen overdose, many develop severe ALF. Despite significant advances in medicinal therapy, the only effective treatment for severe ALF due to acetaminophen remains emergency liver transplantation (LT).[4-6] The decision to transplant is complex, particularly in this patient population. Accurate and early identification of those patients who survive spontaneously is, therefore, vital for using LT effectively and preventing needless transplants. Therefore, prognostication in ALF patients remains extremely interesting to investigate. Current models such as the King's College Hospital criteria[7] are not satisfactory, and improved strategies for predicting outcomes in ALF are needed.[8,9]

Spontaneous recovery from ALF depends, in part, on the capacity of the liver to regenerate after acute injury,[10] and amplification of proinflammatory mediators in the regenerating tissue is also recognized as playing an important role in limiting liver regeneration.[11] A key pathway in this process appears to involve the receptor for advanced glycation end products (RAGE),[12-14] a cell-surface multiligand pattern recognition receptor linked with amplification of the innate inflammatory response to cell death.[15] Engagement of membrane-bound RAGE with ligands such as high-mobility group box 1 (HMGB1) protein, extracellular newly identified receptor for advanced glycation end products binding protein (EN-RAGE), and Nε-(Carboxymethyl)lysine adducts (CML) sustains inflammatory responses and promotes apoptosis in the hepatic remnant after massive hepatectomy.[15] The blockade of this pathway by soluble receptor for advanced glycation end product (sRAGE), the extracellular ligand-binding domain of the receptor, which interrupts ligand-RAGE signaling, markedly reduced hepatic necrosis in animals with acetaminophen-induced liver injury.[14] In human plasma, there are also circulating isoforms of RAGE, collectively called sRAGE, which have the same ligand-binding specificity as membrane RAGE. The sRAGE isoform consists of a heterogeneous population with at least 2 different isoforms: (1) extracellular RAGE formed by ectodomain shedding of the membrane-associated receptor by the action of membrane-associated matrix metalloproteinases (MMPs)[16] and (2) an endogenous secreted form generated by alternative RNA splicing.[17] Spanning the ligand-binding domain, sRAGE probably acts as a decoy for ligands and thus competitively inhibits the engagement of cell-surface RAGE.[17] Circulating levels of sRAGE are elevated in patients with decreased renal function, and this may be due to increased levels of MMPs[18]; however, they are reduced in chronic diseases, including coronary artery disease, essential hypertension, chronic obstructive lung disease, and heart failure.[17] Therefore, the balance of RAGE ligands, circulating sRAGE, and cell-surface RAGE expression is a complex, dynamic system with important pathophysiological implications.

In patients with acetaminophen-induced ALF who were enrolled in the National Institutes of Health–sponsored Acute Liver Failure Study Group (ALFSG), we explored whether circulating levels of sRAGE and RAGE ligands (EN-RAGE, HMGB1, and CML) were involved and could aid in prognostication in this group of patients.

PATIENTS AND METHODS

Patient Selection

The ALFSG was established in 1998 as a consortium of liver centers interested in better defining the causes and outcomes of ALF. To date, more than 2500 subjects have been enrolled prospectively at 23 tertiary centers within the United States, all of which have LT programs. All enrolled subjects met standard criteria for ALF: presence of coagulopathy [prothrombin time > 15 seconds or international normalized ratio (INR) ≥ 1.5] and any degree of HE occurring within 26 weeks of the onset of first symptoms in a patient without a previous underlying liver disease.[19,20] Because the subjects were encephalopathic by definition, written informed consent was obtained from their legal next of kin. Detailed demographic, clinical, laboratory, and outcome data as well as daily sera for 7 days were collected prospectively. All centers were in compliance with their local institutional review board requirements. A certificate of confidentiality was obtained from the National Institutes for Mental Health for the entire study.

The cohort of 60 well-characterized acetaminophen-related ALF patients from the registry were selected as consecutive patients meeting the aforementioned criteria; they were balanced to obtain roughly an equal number of survivors and patients who either died or underwent transplantation. Within this cohort, 30 patients who were spontaneous survivors and 30 who underwent transplantation and/or died (3 of whom underwent transplantation) of similar ages were selected. Patients in the ALFSG registry are enrolled at or near admission to the study site hospital and

are managed according to a relatively uniform protocol[21]; however, decisions concerning listing and transplantation are, of necessity, individual site decisions. In general, patients meeting the usual criteria are listed as United Network for Organ Sharing status 1 and are assumed to have a high 7-day mortality. The decision to transplant depended on subsequent organ availability, and the decision was made at the time an organ became available.

In addition, 30 age-matched healthy controls were included. Serum samples were tested for RAGE ligands as well as circulating sRAGE. The institutional ethics committee of the University Hospital of Pisa (Italy) approved the study (approval number 3653).

Laboratory Analysis

Blood Sample Collection

Serum samples, which were usually obtained on day 1 of admission to the study, were centrifuged at 4°C and stored at −80°C until analysis. All laboratory tests were performed in blinded fashion with respect to the identity of the samples. Routine biochemical analyses were determined by standard laboratory methods.

The Model for End-Stage Liver Disease (MELD) score and the bilirubin, lactate, and etiology (BiLE) score were derived as indicated elsewhere.[22,23]

Determination of Serum sRAGE Levels

Serum sRAGE levels were determined with a double-sandwich enzyme-linked immunosorbent assay (ELISA) kit (DuoSet ELISA development kit; R&D Systems, Minneapolis, MN) as previously described.[24] The intra-assay and interassay coefficients of variation were 5.9% and 8.2%, respectively. The lower limit of detection of sRAGE was 21.5 pg/mL.

Determination of Serum CML Levels

Serum CML levels were measured with an in-house competitive ELISA using the mouse F(ab')2 fragment anti-advanced glycation end product monoclonal antibody (clone 6D12; ICN Biochemical Division, Aurora, OH), as previously described.[25] The intra-assay and interassay coefficients of variation were 3.2% and 8.7%, respectively. The lower limit of detection of CML was 0.5 μg/mL.

Determination of Serum HMGB1 Levels

Serum HMGB1 levels were determined with a double-sandwich ELISA kit (IBL International GMBH, Hamburg, Germany) according to the manufacturer's description. The intra-assay and interassay coefficients of variation were <8% and 10%, respectively. The lower limit of detection of HMGB1 was 0.1 ng/mL.

Determination of Serum EN-RAGE Levels

The EN-RAGE concentration was measured with an in-house competitive ELISA assay as previously described.[26] The intra-assay and interassay coefficients of variation were <7.9% and <8.0%, respectively. The lower limit of detection of EN-RAGE was 0.05 ng/mL.

Statistical Analysis

Data with a normal distributions are presented as means and standard deviations (SDs). Variables with a skewed distribution are expressed as medians and interquartile ranges. Variables with a nonnormal distribution were logarithmically transformed before each analysis. Chi-square tests and Student *t* tests were used to compare categorical variables and continuous variables, respectively. A Pearson correlation analysis was performed to examine the relationship between sRAGE and the study's variables. Variables that were statistically significant ($P < 0.01$) in Table 1 formed a pool of potential independent predictors for which the sensitivity, specificity, diagnostic accuracy, and area under the receiver operating characteristic curve (AUROC) were assessed (Table 2). These predictors were then entered into a backward elimination variable selection procedure by multivariate logistic regression analysis. The predictors with a *P* value < 0.05 were retained. For all analyses, a 2-tailed *P* value < 0.05 was considered significant. Statistical analyses were performed with SPSS software for Windows (version 10.0; SPSS, Chicago, IL).

RESULTS

Serum levels of RAGE ligands and sRAGE were measured in 30 healthy subject controls and in 60 age-matched acetaminophen-related ALF patients. Circulating levels of sRAGE, EN-RAGE, and HMGB1 were found to be significantly higher ($P < 0.001$) in ALF patients versus normal controls, whereas CML values did not differ between the 2 groups (Table 3). The values of sRAGE were significantly higher in the transplanted and/or died group versus the spontaneous survivor group ($P < 0.001$). EN-RAGE levels tended to be higher in nonsurviving patients versus spontaneous survivors ($P = 0.05$), whereas HMGB1 and CML levels did not differ between the 2 groups (Table 1). Between the 2 groups, there were significant differences in plasma levels of prothrombin time, creatinine, aspartate aminotransferase (AST), and pH (Table 1). Furthermore, they differed significantly for the encephalopathy grade (COMA) and MELD score, and there were significant differences in the BiLE score, a novel predictor of poor outcome in ALF patients[23] (Table 1).

The Pearson correlation analysis highlighted that in all patients, sRAGE levels were positively associated with the MELD score ($r = 0.39$, $P = 0.002$), BiLE score ($r = 0.48$, $P = 0.004$), COMA ($r = 0.28$, $P = 0.03$), and systemic inflammatory response syndrome (SIRS) score ($r = 0.33$, $P < 0.01$). Furthermore, sRAGE was positively associated with conventional markers of disease severity such as AST ($r = 0.30$, $P = 0.02$), creatinine ($r = 0.48$, $P < 0.001$), and prothrombin time ($r = 0.26$, $P = 0.04$) as well as EN-RAGE ($r = 0.41$, $P = 0.001$) and HMGB1 levels ($r = 0.25$, $P = 0.05$). We

TABLE 1. Univariate Analysis of Variables on Admission in Spontaneous Survivors and Patients Who Died or Underwent Transplantation for ALF

	Spontaneous Survivors (n = 30)	Patients Who Died or Underwent Transplantation (n = 30)	*P* Value
Age, years	34.4 ± 14	38.9 ± 13.5	0.21
Male, n (%)	11 (37)	5 (17)	0.14
Prothrombin time, seconds	30.6 ± 19.3	45.9 ± 23.1	0.007
Platelets count, $\times 10^9$/L	136 ± 77	155 ± 93	0.39
Creatinine, mg/dL	1.1 ± 0.7	2.5 ± 1	< 0.001
Bilirubin, μmol/L	77.9 ± 61	79.7 ± 51	0.90
INR	2.4 (1.75-3.35)	4.4 (2.2-6.9)	0.01
AST, U/L	2569 (1031-5768)	7818 (3314-11,027)	<0.01
White blood cells, $\times 10^2$/L	9.2 ± 4.4	12.9 ± 10	0.08
COMA > 2, n (%)	13 (43)	26 (87)	0.001
pH	7.45 ± 0.1	7.36 ± 0.16	0.01
SIRS > 2, n (%)	4 (13)	9 (30)	0.18
BiLE score	2.8 (0.99-4.2)	5.84 (4.3-8.6)	0.01
MELD score	22.6 ± 10.6	35.6 ± 10.3	<0.001
sRAGE, pg/mL	2625 (1636-3813)	4864 (3538-6858)	<0.001
EN-RAGE, ng/mL	15.7 (11-28)	33.6 (12-42)	0.05
HMGB1, ng/mL	2.6 (1.0-7.4)	3.9 (1.6-8.7)	0.33
CML, μg/mL	6.2 ± 0.7	5.9 ± 0.7	0.16

NOTE: Data are given as mean ± SD, number (%), or median (interquartile range).

also found significantly higher levels of sRAGE, HMGB1, and EN-RAGE in patients with SIRS scores >2 (3 and 4 grades) versus patients with SIRS scores ≤2 (Fig. 1).

Variables that were statistically significant (with $P < 0.01$) in Table 1 formed a pool of potential independent predictors of outcome. As shown in Table 2, creatinine > 1.9 mg/dL displayed the best diagnostic accuracy (84%), whereas both COMA > 2 and sRAGE > 3021 pg/mL had major diagnostic sensitivity (87% and 83%, respectively). Finally, a multivariate logistic regression analysis with these predictors was then assessed. COMA > 2, with an odds ratio of 13 [confidence interval (CI), 2.3-73; $P < 0.001$], was the best predictor of outcome selected in this model.

In order to improve the prognostication of ALF patients, we emulated the study of Bechmann et al.,[27] in which a modified MELD score using levels of cell death marker cytokeratin 18/M65 in place of bilirubin better predicted the prognosis of ALF patients. Thus, we modified the MELD score with sRAGE in place of bilirubin (which in our ALF patients had no prognostic value) while retaining all other factors:

Modified MELD score = 10[0.957 × ln creatinine (mg/dL) + 0.378 × ln sRAGE (pg/mL) + 1.12 × ln INR + 0.643]

The sRAGE-modified MELD scores had a somewhat higher diagnostic accuracy (84% versus 80%) than unmodified MELD scores, but there was no effect on outcome in the multivariate logistic analysis.

DISCUSSION

In this study, we examined the levels and prognostic significance of sRAGE and RAGE ligands on admission in a carefully studied cohort of patients with acetaminophen-related ALF and compared these novel markers with other indicators of ALF prognosis. RAGE and RAGE ligands appear to be involved in the pathogenesis of severe liver injury in humans exposed

TABLE 2. AUROC and Diagnostic Accuracy for the Prediction of Poor Outcomes for ALF Patients

	AUROC (95% CI)	*P* Value	Cutoff Value	Sensitivity (%)	Specificity (%)	Diagnostic Accuracy (%)
Prothrombin time	0.70 (0.56-0.84)	0.001	>35.8 seconds	63	80	72
sRAGE	0.77 (0.64-0.90)	<0.001	>3021 pg/mL	83	67	75
MELD score	0.80 (0.56-0.84)	<0.001	>30.02	70	79	75
AST	0.73 (0.60-0.86)	0.001	>5768 U/L	67	77	72
Creatinine	0.84 (0.73-0.95)	<0.001	>1.9 mg/dL	77	90	84
COMA	0.78 (0.66-0.90)	<0.001	>2	87	57	72

TABLE 3. Comparison of sRAGE, EN-RAGE, HMGB1, and CML Values Between ALF Patients and Normal Controls

	Controls (n = 30)	All ALF Patients (n = 60)
sRAGE, pg/mL	735 (519-1150)	3629 (2165-5974)*
EN-RAGE, ng/mL	7.9 (5.5-13.2)	20.5 (12-37.1)*
HMGB1, ng/mL	0.3 (0.1-0.49)	3.0 (1.35-8.4)*
CML, μg/mL	6.0 ± 0.58	6.2 ± 0.71

NOTE: Data are given as mean ± SD or median (interquartile range).
*$P < 0.001$ by an unpaired t test performed on the log-transformed data.

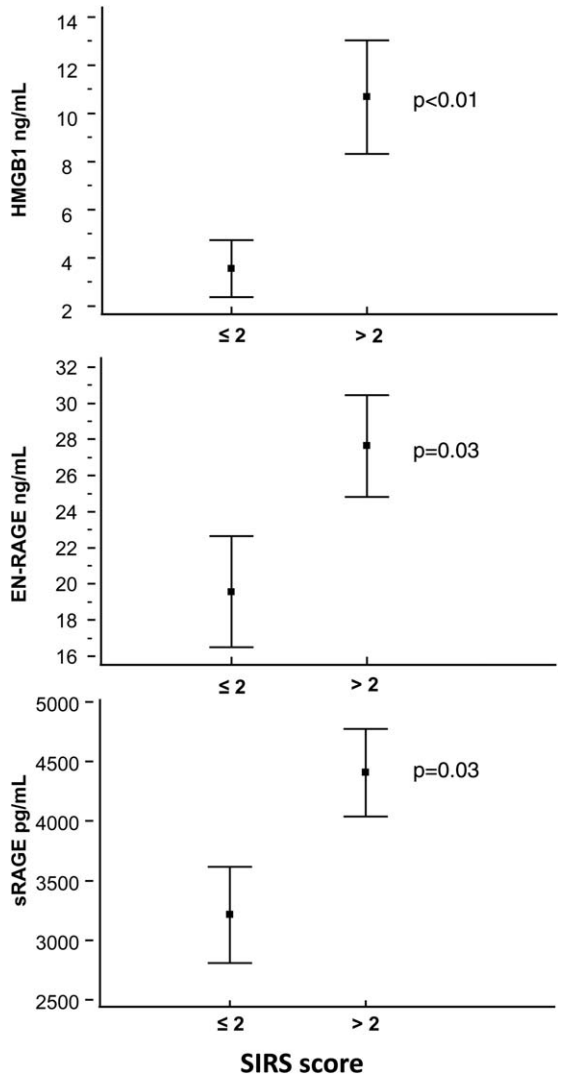

Figure 1. Serum levels of sRAGE, HMGB1, and EN-RAGE with respect to SIRS severity. Data are given as means and standard errors of the mean. *P* values are taken from an unpaired *t* test performed on the log-transformed data.

to excessive acetaminophen. We measured 3 RAGE ligands as well as sRAGE in the same cohort of patients to explore their interrelationship. EN-RAGE, HMGB1, and sRAGE were all markedly elevated in this setting from 3 to 10 times the control values, whereas CML levels were not increased. We focused attention on sRAGE because it was the most increased in ALF patients. The reasons for these elevated levels might be secondary to decreased renal function or possibly up-regulation of sRAGE, which would provide "protection" against possible toxic effects of RAGE ligands. The close association that we found between sRAGE and creatinine levels indeed supports the first hypothesis. The relationship between sRAGE and compromised renal function has been noted previously by Kalousová et al.[18] and in a recent study on trauma patients in which the release of sRAGE in the bloodstream was associated with coagulation abnormalities and acute renal failure.[28] These findings notwithstanding, higher levels of sRAGE in ALF patients and particularly in those patients who died and/or underwent transplantation may still reflect increased tissue expression and release due to the development of an inflammatory response and coagulopathy. The positive correlation between sRAGE levels and SIRS severity in our study suggests that sRAGE may be implicated in inflammatory responses occurring in ALF patients. In support of our results, it has been shown that high admission plasma levels of sRAGE in nonsurvivors of sepsis versus survivors were associated with worse outcomes,[29] and this suggests that circulating sRAGE levels may reflect tissue RAGE expression and may be elevated in parallel with serum RAGE ligands as a counter-system against ligand-elicited tissue damage. Because RAGE is a cell-surface receptor that belongs to the immunoglobulin superfamily (eg, intercellular adhesion molecule 1) and whose values at admission were also more elevated in nonsurvivor septic patients[30] and nonsurvivor ALF patients[31] versus survivors, sRAGE might represent a marker of cellular damage. In this study, we have no serial data on sRAGE levels during recovery or with deterioration to allow us to more firmly establish an association between improving levels in the survivors and outcomes. However, we recently found that in patients with chronic liver disease undergoing LT, serum levels of sRAGE decreased by day 7, and this showed that the levels of this protein were not stable but changed after surgery.[32]

Because bilirubin levels had no prognostic value for ALF patients, we emulated the study of Bechmann et al.[27] and modified the MELD score through the substitution of bilirubin by sRAGE in the formula. This modification improved the diagnostic accuracy of the MELD score but not its predictive value for outcome. Although sRAGE levels alone or in combination with MELD scores were not independent predictors of mortality, high levels of sRAGE in ALF patients who died are an important signal of the severity of damage

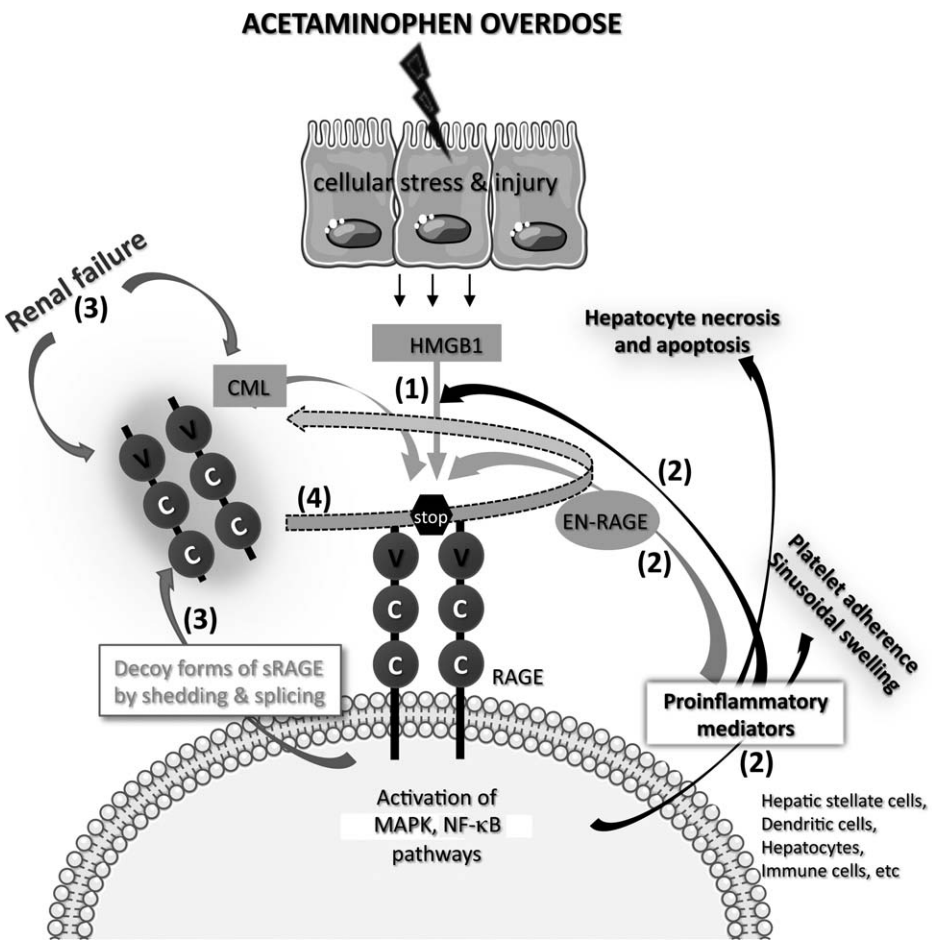

Figure 2. Schematic diagram of the RAGE-ligand release, inflammatory responses, and feedback mechanism in the RAGE ligand/RAGE/sRAGE system after an injury induced by an acetaminophen overdose. (1) Highly toxic metabolites produced by acetaminophen induce initial cell death, and this resulted in diverse "danger" molecules, including HMGB1, which, probably in conjunction with other inflammatory mediators, activate RAGE signaling on hepatic cells and/or dendritic cells and inflammatory cells. A ligand-RAGE interaction can lead to the activation of the MAPK pathways and the translocation of the transcription factor NF-κB from the cytosol to the nucleus, and this results in the up-regulation of genes involved in cellular inflammatory responses. (2) Then, these mediators, in an autocrine or paracrine manner, lead to receptor-triggered transcriptional pathways, which result in the production of further proinflammatory mediator substances to create full-scale tissue inflammation. (3) Although the exact mechanisms are still unclear, plausibly high levels of sRAGE are part of a counter-regulatory mechanism elicited by inflammation/injury and other pathological situations, such as renal failure, that enhance the expression of RAGE and its ligands and result in an increased accumulation of sRAGE as a negative feedback to RAGE interactions with its ligands (4).

to the liver and presumably of spontaneous survival in ALF patients.

Among RAGE ligands, the most remarkable indicator of liver impairment is HMGB1 protein, which has been shown to play a role in experimental models of both acetaminophen-induced hepatotoxicity[33-35] and ischemia/reperfusion injury.[36,37] In our patients, there was a positive correlation between HMGB1 and SIRS severity that is in line with the literature, which shows the highest levels of HMGB1 in patients with sepsis and especially in those with a poor prognosis.[38] In agreement with Craig et al.,[39] our data confirmed that HMGB1 levels at admission were significantly higher in ALF patients versus healthy control subjects, but the levels did not significantly differ between nonsurvivor patients and spontaneous survivors. Because of the crucial role attributed to HMGB1 in many experimental models of acute injury and sepsis, it is perhaps surprising that circulating HMGB1 levels did not predict outcomes for ALF patients. A plausible explanation is that HMGB1 is not sufficient to cause systemic dysfunction because recombinant HMGB1 fails to stimulate strong proinflammatory reactions.[40] Rather, it seems that HMGB1 forms complexes with other inflammatory mediators and amplifies the downstream effects of these mediators through interactions with a variety of receptors, including RAGE.[41]

In addition to this ligand, we found a good correlation between SIRS severity and the RAGE ligand EN-RAGE but no value of EN-RAGE in the prediction of outcomes. Similarly to HMGB1, the EN-RAGE levels at admission were found to be up-regulated in patients with SIRS features versus healthy subjects.[42]

Of interest, the third ligand studied—the CML adduct—did not differ between ALF patients and healthy subjects, and this result was unexpected. CML adducts form and accumulate in diverse settings, including the inflammatory milieu through the myeloperoxidase pathway.[43] Nevertheless, their accumulation in plasma is not an immediate process, and more time might be required for them to become detectable.

In order to bring out the global picture of these interrelationships and to highlight the mechanisms by which sRAGE may increase in this pathological context, we have schematically shown these possible dynamics in Fig. 2. Thus, we suppose that sRAGE may be released to counter the toxic effect of its ligands as a beneficial response to the inflammatory milieu of ALF; in addition, its circulating levels increase in the presence of decreased renal function (Fig. 2).

The chief limitation of this study is its retrospective nature. It will be necessary to perform a prospective evaluation of a larger patient set to draw unequivocal conclusions. However, it provides proof-of-concept that the RAGE-ligand axis may interfere with liver regeneration and should be a promising objective for further research.

ACKNOWLEDGMENT

The serum specimens from the Acute Liver Failure Study Group, reported here, were supplied by the National Institute of Diabetes and Digestive and Kidney Diseases Central Repositories.

REFERENCES

1. Vaquero J, Blei AT. Etiology and management of fulminant hepatic failure. Curr Gastroenterol Rep 2003;5:39-47.
2. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, et al.; for Acute Liver Failure Study Group. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 2005;42:1364-1372.
3. Lee WM. Acetaminophen-related acute liver failure in the United States. Hepatol Res 2008;38(suppl 1):S3-S8.
4. Lee WM. Acute liver failure in the United States. Semin Liver Dis 2003;23:217-226.
5. Squires RH Jr, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. J Pediatr 2006;148:652-658.
6. Craig DG, Lee A, Hayes PC, Simpson KJ. Review article: the current management of acute liver failure. Aliment Pharmacol Ther 2010;31:345-358.
7. Dhiman RK, Jain S, Maheshwari U, Bhalla A, Sharma N, Ahluwalia J, et al. Early indicators of prognosis in fulminant hepatic failure: an assessment of the Model for End-Stage Liver Disease (MELD) and King's College Hospital criteria. Liver Transpl 2007;13:814-821.
8. MacQuillan G. Predicting outcome in acute liver failure: are we there yet? Liver Transpl 2007;13:1209-1211.
9. Harbrecht BG. Predicting outcome in patients with acute liver failure: what works best? Crit Care Med 2012;40:1666-1667.
10. Blei AT. Selection for acute liver failure: have we got it right? Liver Transpl 2005;11(suppl 2):S30-S34.
11. Panis Y, McMullan DM, Emond JC. Progressive necrosis after hepatectomy and the pathophysiology of liver failure after massive resection. Surgery 1997;121:142-149.
12. Zeng S, Feirt N, Goldstein M, Guarrera J, Ippagunta N, Ekong U, et al. Blockade of receptor for advanced glycation end product (RAGE) attenuates ischemia and reperfusion injury to the liver in mice. Hepatology 2004;39:422-432.
13. Sternberg DI, Gowda R, Mehra D, Qu W, Weinberg A, Twaddell W, et al. Blockade of receptor for advanced glycation end product attenuates pulmonary reperfusion injury in mice. J Thorac Cardiovasc Surg 2008;136:1576-1585.
14. Ekong U, Zeng S, Dun H, Feirt N, Guo J, Ippagunta N, et al. Blockade of the receptor for advanced glycation end products attenuates acetaminophen-induced hepatotoxicity in mice. J Gastroenterol Hepatol 2006;21:682-688.
15. Basta G, Navarra T, De Simone P, Del Turco S, Gastaldelli A, Filipponi F. What is the role of the receptor for advanced glycation end products-ligand axis in liver injury? Liver Transpl 2011;17:633-640.
16. Zhang L, Bukulin M, Kojro E, Roth A, Metz VV, Fahrenholz F, et al. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. J Biol Chem 2008;283:35507-35516.
17. Basta G. Receptor for advanced glycation endproducts and atherosclerosis: From basic mechanisms to clinical implications. Atherosclerosis 2008;196:9-21.
18. Kalousová M, Hodková M, Kazderová M, Fialová J, Tesar V, Dusilová-Sulková S, Zima T. Soluble receptor for advanced glycation end products in patients with decreased renal function. Am J Kidney Dis 2006;47:406-411.
19. Ostapowicz G, Fontana RJ, Schiødt FV, Larson A, Davern TJ, Han SH, et al.; for U.S. Acute Liver Failure Study Group. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137:947-954.
20. Polson J, Lee WM; for American Association for the Study of Liver Disease. AASLD position paper: the management of acute liver failure. Hepatology 2005;41:1179-1197.
21. Stravitz RT, Kramer AH, Davern T, Shaikh AO, Caldwell SH, Mehta RL, et al.; for Acute Liver Failure Study Group. Intensive care of patients with acute liver failure: recommendations of the U.S. Acute Liver Failure Study Group. Crit Care Med 2007;35:2498-2508.
22. Wiesner RH, McDiarmid SV, Kamath PS, Edwards EB, Malinchoc M, Kremers WK, et al. MELD and PELD: application of survival models to liver allocation. Liver Transpl 2001;7:567-580.
23. Hadem J, Stiefel P, Bahr MJ, Tillmann HL, Rifai K, Klempnauer J, et al. Prognostic implications of lactate, bilirubin, and etiology in German patients with acute liver failure. Clin Gastroenterol Hepatol 2008;6:339-345.
24. Miniati M, Monti S, Basta G, Cocci F, Fornai E, Bottai M. Soluble receptor for advanced glycation end products in COPD: relationship with emphysema and chronic cor pulmonale: a case-control study. Respir Res 2011;12:37.
25. Basta G, Berti S, Cocci F, Lazzerini G, Parri S, Papa A, et al. Plasma N-epsilon-(carboxymethyl)lysine levels are associated with the extent of vessel injury after coronary arterial stenting. Coron Artery Dis 2008;19:299-305.
26. Basta G, Sironi AM, Lazzerini G, Del Turco S, Buzzigoli E, Casolaro A, et al. Circulating soluble receptor for advanced glycation end products is inversely associated

with glycemic control and S100A12 protein. J Clin Endocrinol Metab 2006;91:4628-4634.

27. Bechmann LP, Jochum C, Kocabayoglu P, Sowa JP, Kassalik M, Gieseler RK, et al. Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. J Hepatol 2010;53:639-647.
28. Cohen MJ, Carles M, Brohi K, Calfee CS, Rahn P, Call MS, et al. Early release of soluble receptor for advanced glycation endproducts after severe trauma in humans. J Trauma 2010;68:1273-1278.
29. Bopp C, Hofer S, Weitz J, Bierhaus A, Nawroth PP, Martin E, et al. sRAGE is elevated in septic patients and associated with patients outcome. J Surg Res 2008;147: 79-83.
30. Weigand MA, Schmidt H, Pourmahmoud M, Zhao Q, Martin E, Bardenheuer HJ. Circulating intercellular adhesion molecule-1 as an early predictor of hepatic failure in patients with septic shock. Crit Care Med 1999; 27:2656-2661.
31. Ohnishi A, Miyake Y, Matsushita H, Matsumoto K, Takaki A, Yasunaka T, et al. Serum levels of soluble adhesion molecules as prognostic factors for acute liver failure. Digestion 2012;86:122-128.
32. Navarra T, De Simone P, Del Turco S, Filipponi F, Basta G. Involvement of the receptor for advanced glycation end products in liver transplantation. Ann Hepatol 2015; 14:190-197.
33. Higuchi S, Yano A, Takai S, Tsuneyama K, Fukami T, Nakajima M, Yokoi T. Metabolic activation and inflammation reactions involved in carbamazepine-induced liver injury. Toxicol Sci 2012;130:4-16.
34. Martin-Murphy BV, Holt MP, Ju C. The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice. Toxicol Lett 2010;192:387-394.
35. Antoine DJ, Williams DP, Kipar A, Jenkins RE, Regan SL, Sathish JG, et al. High-mobility group box-1 protein and keratin-18, circulating serum proteins informative of acetaminophen-induced necrosis and apoptosis in vivo. Toxicol Sci 2009;112:521-531.
36. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005;201:1135-1143.
37. Watanabe T, Kubota S, Nagaya M, Ozaki S, Nagafuchi H, Akashi K, et al. The role of HMGB-1 on the development of necrosis during hepatic ischemia and hepatic ischemia/reperfusion injury in mice. J Surg Res 2005;124: 59-66.
38. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248-251.
39. Craig DG, Lee P, Pryde EA, Masterton GS, Hayes PC, Simpson KJ. Circulating apoptotic and necrotic cell death markers in patients with acute liver injury. Liver Int 2011;31:1127-1136.
40. Rouhiainen A, Tumova S, Valmu L, Kalkkinen N, Rauvala H. Pivotal advance: analysis of proinflammatory activity of highly purified eukaryotic recombinant HMGB1 (amphoterin). J Leukoc Biol 2007;81:49-58.
41. Bianchi ME. HMGB1 loves company. J Leukoc Biol 2009;86:573-576.
42. Achouiti A, Föll D, Vogl T, van Till JW, Laterre PF, Dugernier T, et al. S100A12 and soluble receptor for advanced glycation end products levels during human severe sepsis. Shock 2013;40:188-194.
43. Anderson MM, Requena JR, Crowley JR, Thorpe SR, Heinecke JW. The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. J Clin Invest 1999;104:103-113.