Original article

High-mobility group box-1 and receptor for advanced glycation end products in preterm infants with brain injury

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Background: **High-mobility group box-1 (HMGB1) protein acts as an important pro-inflammatory mediator, which is capable of activating inflammation and tissue repair. HMGB1 can bind to its receptor such as advanced glycation end products (RAGE). RAGE, in turn, can promote the production of pro-inflammatory cytokines. Soluble RAGE (sRAGE) is a truncated form of the receptor comprising the extracellular domain of RAGE and can inhibit RAGE-activation. The objective of this study was to investigate whether HMGB1 and RAGE are involved in the development of brain injury in preterm infants.**

Methods: **In total, 108 infants ≤34 weeks gestation at birth were divided into 3 groups according to cranial altrasound scan: mild brain damage (*n*=33), severe brain damage (*n*=8) and no brain damage (*n*=67). All the placentas were submitted for pathologic evaluation. Histological chorioamnionitis (HCA) was defined as neutrophil infiltration of amniotic membranes, umbilical cord or chorionic plate. Expressions of HMGB1 and RAGE proteins were assessed by immunohistochemical analysis. The concentration of HMGB1 and sRAGE in umbilical cord blood were measured by enzyme-linked immunosorbent assay.**

Results: **The frequency of HCA was 30.12%. HCA was associated with elevated concentrations of HMGB1 and decreased sRAGE in umbilical cord blood. The severe brain injury group demonstrated higher cord blood HMGB1 concentrations (*P*<0.001) and lower sRAGE concentrations (*P*<0.001) than both other groups. Brain injury in the premature infants was linked to intense staining for HMGB1/RAGE, particularly in inflammatory cells.**

Conclusions: **Changes of cord blood HMGB1 and sRAGE of premature infants had direct relationship with the degree of inflammation and severity of brain damage. Monitoring sRAGE and HMGB1 levels may be helpful to predict intrauterine infection and brain injury in premature infants.**

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Key words: **brain injury;**
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preterm infants;
RAGE;
sRAGE

Introduction

Despite great advances in perinatal-neonatal care, brain damage still accounts for significant morbidity and mortality in preterm infants. However, the potential causes of brain damage are diverse, and the underlying mechanism is complicated. Several pathogenetic pathways can lead to brain damage in premature infants.[1] Among these pathways, intrauterine infection and subsequent inflammation have attracted more attention in recent years.[2,3] This mechanism mainly involves the release of cytokines and chemokines, as well as the activation of microvascular endothelial cells and leukocytes.[4] Clinical and laboratory studies[5-7] have found some pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, which are involved in the immunopathogenesis of brain damage in premature infants. However, such research is still not enough to disclose the inner mechanism.

High-mobility group box1 (HMGB1) has recently been characterized as an important extracellular cytokine and can initiate inflammation and tissue repair. HMGB1 is now also considered to be an alarmin.[8,9] As an important pro-inflammatory mediator, HMGB1 can be released either passively by necrotic cells or actively secreted by living inflammatory cells.[10-13] HMGB1 exerts its biological effects through specific receptors including Toll-Like receptor (TLR)-2, 4 and 9[14,15] as well as receptor for advanced glycation end products

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(RAGE).[16,17] Binding of HMGB1 to RAGE can induce the production of pro-inflammatory cytokines and chemokines, which can lead to inflammation[18,19] and tissue repair.[20,21] More recently, HMGB1 has been implicated as a late mediator of several infectious and inflammatory disorders, such as sepsis.[22-24] Furthermore, some researchers find that blockade of HMGB1 activity can reduce mortality in animal models of endotoxemia and sepsis.[23,24] However, its role in premature brain damage has never been specifically assessed.

RAGE is a transmembrane receptor and can bind several endogenous ligands including advanced glycation end-products(AGEs), S100 proteins and HMGB1.[25] The RAGE-ligand interaction can promote the production of reactive oxygen species and activate a variety of transcription factors, including nuclear factor kappa B (NFκB) and mitogen-activated protein kinase. This further enhances expression of genes for cytokines, growth factors, adhesion molecules, and pro-inflammatory mediators.[26] Therefore, the RAGE-ligand interaction plays an important role in promoting and continuing the host inflammatory response. RAGE can be either membrane-bound to propagate signaling or in a soluble form (sRAGE). sRAGE encodes the ligand-binding domain but lacks the signal-transducing cytoplasmatic domain of the receptor, so it might function as a decoy due to its ability to bind RAGE ligands and abrogate RAGE signaling.

In order to investigate whether HMGB1 and RAGE participate in the mechanisms of infection/inflammation-induced premature brain damage, a prospective laboratory experiment was designed as follow. First, we detected HMGB1 and sRAGE levels in cord blood serum of premature infants and determine whether histological chorioamnionitis or brain damage is associated with changes in the cord blood serum concentration of HMGB1 and sRAGE. Second, we immunolocalized HMGB1 and RAGE in the fetal membranes to investigate if these two proteins are expressed more intensively in placentas from preterm infants with brain damage.

Methods

Study population

The original sample pool consists of 189 infants ≤34 weeks gestation at birth. Infants were excluded if: congenital malformations, genetic or chromosomal disorders, placenta without pathological examination, without umbilical cord blood, multiple pregnancy, these factors were supposed to affect the final results. Infants that died were also excluded because the given days and/or their outcomes could not be followed. Finally, 108 infants were enrolled in this prospective exploratory study. Patients were enrolled with written informed consent of the parent(s) and recruited from the Affiliated Hospital of Jiangsu University (Zhenjiang, China) between April 2011 and July 2013. All studies were approved by the ethics committee of the Affiliated Hospital of Jiangsu University and the registration number was 2011002.

Definitions and study procedures

Clinical data of maternal and infants were collected through medical records from enrollment to hospital discharge. Data were collected on the specifics of birth, development of morbidities, and treatments. Diagnosis of brain injury required head ultrasonography with radiographic confirmation. Gestational age was estimated by menstrual history and ultrasound examinations performed before 14 weeks of gestation. Diagnosis of maternal chorioamnionitis relied on histological pathological examination. Premature rupture of membranes (PROM) was diagnosed as membrane of natural fracture occurred before the onset of spontaneous labor by visualization of amniotic fluid loss. Maternal steroids included two doses of dexamethasone, which was administered parenterally to induce lung maturation.

All preterm infants accepted cranial ultrasonography examination as soon as possible after birth, at least once a week until discharge to the local hospital, and again at 40 weeks post menstrual age. Intraventricular hemorrhage (IVH) was divided into 4 grades based on Papile:[27] grade I, hemorrhage restricted to the germinal matrix; grade II, IVH without ventricular dilatation; grade III, IVH with ventricular dilatation; and grade IV, parenchymal hemorrhage. Periventricular leukomalacia (PVL) was graded as described previously:[28] grade I: periventricular areas of increased echogenicity present for 7 days or more; grade II: periventricular areas of increased echogenicity evolving into small localized fronto-parietal cysts; grade III: periventricular areas of increased echogenicity evolving into extensive periventricular cystic lesions involving the occipital and fronto-parietal white matter; and grade IV: areas of increased echogenicity in the deep white matter evolving into extensive subcortical cysts. Brain injury degree of preterm infants was categorized as follow:[29] mild brain damage (grade I-II of IVH or I-II of PVL), severe brain damage (grade III-IV of IVH or III-IV of PVL), and no brain damage (cranial altrasound scans results were normal).

Histological examinations

Details of the placenta histopathology protocol have been published previously.[30] Briefly, the chorion amnion, the chorionic plate, and the umbilical cord were examined microscopically by a placental pathologist who was blinded to gestational age at delivery and all clinical data. Histological chorioamnionitis (HCA) was defined as neutrophil infiltration of amniotic membranes, umbilical cord or chorionic plate according to the criteria previously described.[31] The acute inflammation of amnion and chorion-decidua was defined as the presence of at least one focus of more than 5 neutrophils; the chorionic plate acute inflammation was defined as the presence of more than one focus of at least 10 neutrophils; funisitis was defined as the presence of neutrophil infiltration into the umbilical vessel walls or Wharton's jelly.

Enzyme-linked immunosorbent assay (ELISA)

Umbilical vein blood was obtained from all infants and centrifuged at 2000 rpm (revolutions per minute) for 10 min within 2 hours after birth. Serum was aliquoted and stored at -70°C before analysis. Serum HMGB1 and sRAGE were measured with commercially available ELISA kits according to the manufacturer's recommendations. Immunoassay kits for HMGB1 and sRAGE levels were purchased from Alpha Diagnostic International (Shino-Test, Kanagawa, Japan) and RayBiotech Inc (Norcross, GA, USA) respectively. The inter- and intra-assay coefficients of variations were <10% for all analytes. All samples were run in duplicate and mean values were used in the analysis.

Immunohistochemistry

Five-micron sagittal serial paraffin sections were deparaffinized in xylene and rehydrated with graded ethanol to potassium-PBS solution with pH value of 7.2. Following antigen retrieval with citrate buffer, the sections were pretreated with 1% hydrogen peroxide for 15 minutes. Primary antibody to rabbit anti-HMGB1 (1:1000, Shino-Test, Kanagawa, Japan) or goat anti-RAGE polyclonal antibodies (1:1000, R&D Systems, Minneapolis, MN, USA) was added, and the sections were incubated overnight at 4ºC. The secondary antibody, biotinylated mouse/anti-rabbit IgG for HMGB1, biotinylated mouse/anti-goat IgG for RAGE was applied for 30 min, followed by the avidin-biotin-peroxidase complex (Elite ABC Kit, Beijing Zhongshan Biotechnology Co, China) for 30 min at room temperature following the manufacturer's instructions. The immunoreaction was visualized by incubating the sections for 4 min in a 0.1% 3,3'-diaminobenzidine and 0.02% hydrogen peroxide solution. The sections were lightly counterstained with haematoxylin. The cells with yellow brown particle deposition in nucleus (HMGB1) or cell cytoplasm (RAGE) were judged to be positive. The analysis of HMGB1 and RAGE expression was performed with HMIAS-2000 high resolution color pathological imaging system. Five fields were randomly selected in each slide under a light microscope (×200) for average absorption optical density (A value).

Statistical analysis

Data were expressed as mean±standard deviation (SD) or median (lower quartile, upper quartile). The difference among groups was assessed by AVONA and Student's *t*-test. If the analysis of variance was significant, post hoc testing of differences between groups was performed using the least significant difference multiple comparison test or Dunn's multiple comparison test after Kruskal-Wallis test (nonparametric analysis of variance). Categorical and nominal values were compared by Chi-square test or Fisher's exact test. The Spearman or Pearson correlation coefficient test was used to evaluate associations between two quantitative variables. Further, receiver operating characteristic curve (ROC) was computed, and area under the curve (AUC) was used to evaluate how well the biomarkers can be used to diagnose brain injury in premature. Test characteristics (sensitivity, specificity) were also calculated to determine the accuracy of HMGB1 and sRAGE in predicting brain injury in premature infants. For all tests, $P<0.05$ was considered significant. Statistical evaluation was performed using SPSS 16 for Windows (SPSS Inc., Chicago, USA).

Results

Patient characteristics

The major characteristics of the 33 infants with mild brain injury, the 8 infants with severe brain injury, and the 67 healthy control subjects are summarized in Table 1. Among them, IVH was diagnosed in 29 cases, including 15 grade I, 8 grade II, 5 grade III and one grade IV. PVL was diagnosed in 12 cases, including 7 grade I, 3 grade II and 2 grade III. There was no case of PVL grade IV. Infants in the two brain injury group had a lower birth weight and small gestational age compared with patients in the control group ($P<0.05$). The gender distribution and cesarean birth of patients in each group showed no significant difference among the three groups ($P>0.05$). Compared with the other two groups, mothers of infants with severe brain injury were more likely to have clinical chorioamnionitis ($P<0.05$), more prevalent of preterm premature rupture of membranes and longer interval between rupture of membranes and birth ($P<0.05$). The severe brain injury group also had a lower

Table 1. Clinical characteristics of the study population

Characteristics	Status of brain injury in preterm infants			*P* value
	Control (*n*=67)	Mild (*n*=33)	Severe (*n*=8)	
Gestational age (wk), mean±SD*	32.7±1.1	30.0±1.8	30.3±1.1	0.001
Birth weight (g), mean±SD*	1926.0±319.8	1615.0±218.9	1611.9±177.2	0.001
Male†, *n*	38	17	6	0.469
Cesarean birth†, *n*	35	22	5	0.368
5 min Apgar‡	9 (6-9)	9 (5-9)	7 (5-9)	0.023
NRDS†, *n*	16	7	4	0.271
PROM†, *n*	21	8	6	0.022
Interval PROM-birth (d)‡	0 (0-5)	1 (0-7)	5 (4-20)	0.001
Chorioamnionitis†, *n*	12	8	5	0.018
Prenatal steroids†, *n*	23	7	4	0.206
Surfactant therapy†, *n*	16	5	3	0.350
Mechanical ventilation†, *n*	17	6	2	0.711

PROM: premature rupture of membranes; NRDS: neonatal respiratory distress syndrome; SD: standard deviation. *: Analyzed by One-way analysis of variance; †: Analyzed by Chi square tests; ‡: Data presented as median with 5th and 95th percentiles in brackets as analyzed by Kruskal-Wallis analysis of variance.

5-min Apgar score (P<0.01). But treatment with prenatal betamethasone or surfactant, mechanical ventilation, and NRDS were the same among the three groups (P>0.05).

Cord blood levels of HMGB1 and sRAGE

The frequency of HCA was 30.12%. HCA was associated with elevated concentrations of HMGB1 and decreased sRAGE in umbilical cord blood. Infants with HCA had a higher median HMGB1 concentration (165.32 μg/L) in cord blood than those without HCA (64.0 μg/L) (P<0.01, Table 2). Infants with HCA had a lower median sRAGE concentration (289.38 pg/mL) in cord blood than those without HCA (591.40 pg/mL) (P<0.01, Table 2).

The levels of cord blood HMGB1 were 349.98 μg/L for 8 infants with severe brain injury, 61.46 μg/L for 33 infants with mild brain injury and 53.91 μg/L for 67 controls, P<0.001 for pairwise comparison of each two groups (Table 3). The severe brain injury group demonstrated higher HMGB1 concentrations in cord blood serum than both other groups.

The sRAGE concentrations were significantly lower in severe brain injury group (162.62 pg/mL) compared with the mild brain injury group (191.85 pg/mL) and the control group (288.16 pg/mL), P<0.001 for pairwise comparison (Table 3). The severe brain injury group demonstrated lower HMGB1 concentrations in cord blood serum than the other two groups.

Table 2. HMGB1 and sRAGE levels in umbilical cord blood in infants with or without HCA

Variables	Infants without HCA (*n*=83)	Infants with HCA (*n*=25)	*P* value
HMGB1(μg/L)	64.0 (53.13-81.0)	165.32 (59.74-394.28)	0.013
sRAGE (pg/ml)	591.40 (212.93-681.27)	289.38 (163.30-447.40)	0.008

Data presented as median with 5th and 95th percentiles in brackets as analyzed by nonparametric tests. HMGB1: high-mobility group box1, sRAGE: soluble advanced glycation end products; HCA: histological chorioamnionitis.

Numbers of neutrophilic infiltrates in placenta

The numbers of neutrophilic infiltrates in placenta were 44/20× for infants with severe brain injury, 8/20× for infants with mild brain injury and 6/20× for controls, P<0.05 for pairwise comparison of each of the two groups (Table 3).

Correlation between the intensity of neutrophilic infiltrates in placenta and levels of HMGB1 and sRAGE

A significant positive correlation was observed between the intensity of neutrophilic infiltrates and HMGB1 (r=0.579, P<0.001), while a significant negative correlation was observed between the intensity of neutrophilic infiltrates and sRAGE (r=-0.415, P<0.001).

Performance of HMGB1 and sRAGE level to predict brain injury in premature infants

ROC analysis of the performance of HMGB1 level to predict brain injury in premature infants had an AUC of

Table 3. Results of cord blood analysis and histological examinations

Variables	Status of brain injury in preterm infants			*P* value
	Control (*n*=67)	Mild (*n*=33)	Severe (*n*=8)	
HMGB1 (μg/L)	53.91 (22.93-221.54)	61.46 (23.53-494.02)	349.98 (190.49-561.27)	0.001
sRAGE (pg/ml)	288.16 (147.55-1479.31)	191.85 (146.24-743.84)	162.62 (127.61-288.16)	0.001
Neutrophilic (n/HP)	6 (0-23)	8 (0-47.6)	44 (23-50)	0.001

Data presented as median with 5th and 95th percentiles in brackets as analyzed by Kruskal-Wallis analysis of variance. HMGB1: high-mobility group box1; sRAGE: soluble advanced glycation end products; HP: high power.

0.675 [95% confidence interval (CI): 0.561-0.789]. An HMGB1 cutoff point of 185.52 ug/L had a sensitivity of 46.3% and specificity of 95.5%. ROC analysis of sRAGE revealed an AUC of 0.668 (95% CI: 0.563-0.773). A cutoff point of 192.80 pg/ml resulted in a sensitivity of 53.7% and a specificity of 77.6%.

Immunostaining of HMGB1 and RAGE in human placenta

HMGB1 and RAGE proteins were all readily identified in the placenta tissue. Both proteins could express in the syncytiotrophoblasts, cytotrophoblasts, vascular muscle cells, endothelial cells, neutrophils, lymphocytes, macrophages and placental mesenchymal cells.

HMGB1 was mainly expressed in the nuclei of virtually all cells in the placenta tissue. In the control groups, we observed that the HMGB1 signal mainly appeared in syncytiotrophoblasts, cytotrophoblasts, and placental mesenchymal cells. No HMGB1-positivity inflammatory cells was observed (Fig. 1A). While within the placenta villus of severe brain damage groups, HMGB1 signal was not only expressed in syncytiotrophoblasts, cytotrophoblasts, and placental mesenchymal cells, but also abundantly expressed in neutrophils, lymphocytes and macrophages (Fig. 1B). In mild brain injury groups (Fig. 1C), HMGB1-positivity inflammatory cells were also observed but were less than those in severe brain injury group. Our histological scoring analysis (Fig. 1D) indicated that in severe brain injury samples HMGB1 immunostaining intensity was the strongest in placenta villus compared with other two groups (one way ANOVA, $P<0.001$).

Immunohistochemistry with antibodies against RAGE on the placental slides were also performed. In the control group, only a few syncytiotrophoblasts and cytotrophoblasts with RAGE-positivity were found (Fig. 2A). In the severe brain injury group, RAGE was highly expressed in the cytoplasm of almost all cells in the placental tissue (Fig. 2B); RAGE was also abundantly expressed in the brain injury group (Fig. 2C) but its intensity was less than that in the severe brain injury group. Our histological scoring analysis (Fig. 2D) indicated that in severe brain injury samples RAGE immunostaining intensity was the strongest in placenta villus compared with other two groups (one way ANOVA, $P<0.001$).

Discussion

This study explored the presence of a HMGB1/RAGE axis in cord blood and placenta in the context of intrauterine infection and brain damage. We found that (1) brain injury in preterm infants was associated with

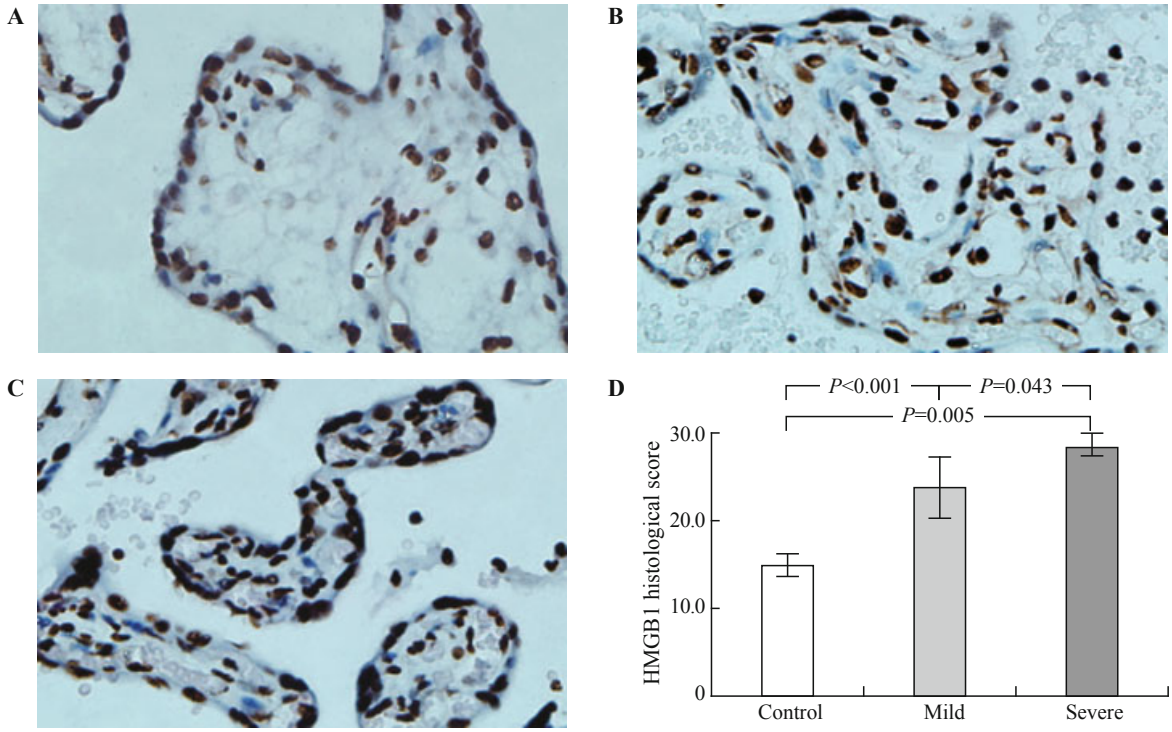

Fig. 1. Immunohistochemical staining of high-mobility group box1 (HMGB1) protein in placenta tissue of healthy infants (**A**), infants with severe brain injury (**B**) and infants with mild brain injury (**C**) (original magnification × 400). The results of the histological scoring analysis (mean±SE) are shown in **D**. Statistical analysis: one-way ANOVA followed by Student-Newman-Keuls tests.

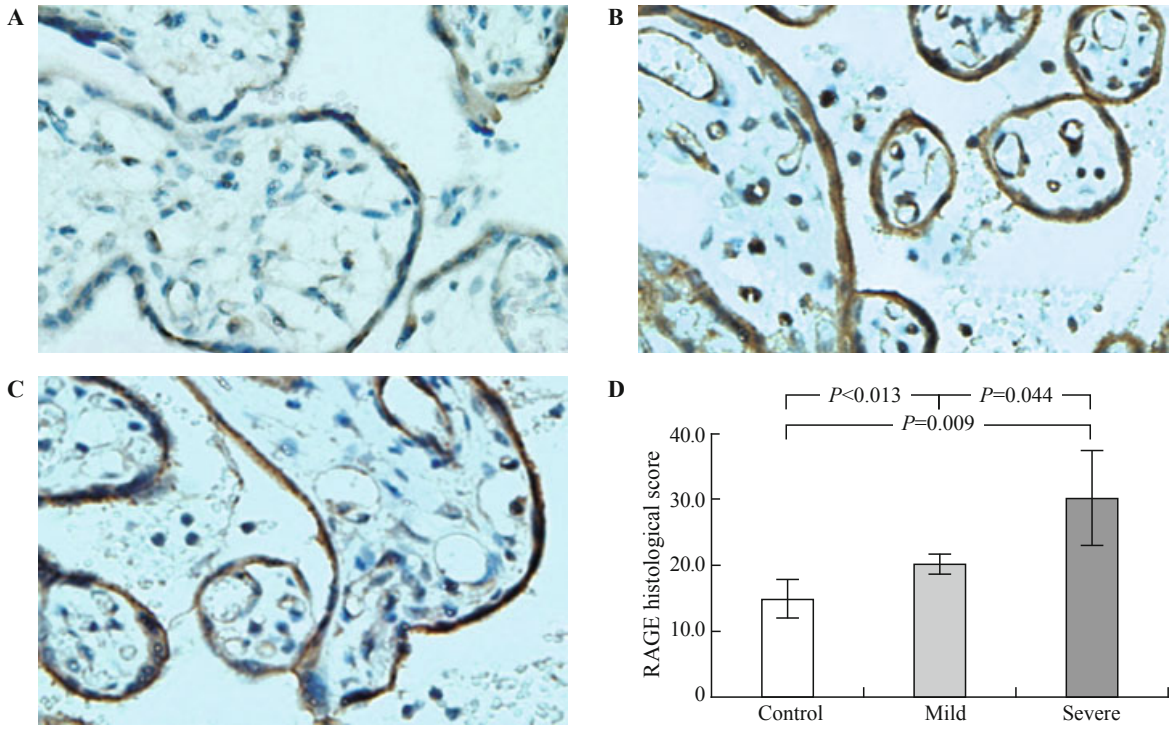

Fig. 2. Immunohistochemical staining of advanced glycation end product (RAGE) protein in placenta tissue of healthy premature infants (**A**), infants with severe brain injury infants (**B**), and infants with mild brain injury (**C**) (original magnification × 400). The results of the histological scoring analysis (mean±SE) are shown in **D**. Statistical analysis: one-way ANOVA followed by Student-Newman-Keuls tests.

a lower gestational age and a lower birth weight, and other variables, such as chorioamnionitis, premature rupture of the membranes, 5 min Apgar score was also identified; (2) elevated HMGB1 and decreased sRAGE in umbilical cord blood were closely related to brain injury of preterm infants after intrauterine infection; (3) changes of HMGB1/sRAGE in cord blood of premature infants had direct relationship with the degree of inflammation and severity of brain damage; (4) monitoring sRAGE and HMGB1 levels may be helpful in predicting intrauterine infection and brain injury in premature infants.

Intrauterine infection and inflammation have been recognized as key features of both preterm labor and brain damage. Maternal inflammation (chorioamnionitis) is often followed by a systemic fetal inflammatory response characterized by elevated levels of pro-inflammatory cytokines in the fetal circulation. Uncontrolled inflammatory response which manifested by excessive accumulation of pro-inflammatory cytokines may contribute to the pathogenesis of brain damage in premature infants. Recently, Hagberg et al[32] reported that IL-18 in umbilical blood was shown to correlate with brain injury in preterm infants and IL-18 deficiency in mice decreases central nervous system vulnerability. Subsequently, Ambalavanan et al[33] confirmed that blood cytokines, including IFN-γ, IL-1, IL-18 and TNF-α, were likely associated with brain injury but may not be clinically useful as biomarkers for white matter damage. The results of our study showed that histological chorioamnionitis was associated with elevated concentrations of HMGB1 and decreased sRAGE in umbilical cord blood, and intense staining for HMGB1/RAGE proteins were mainly expressed in inflammatory cells, which indicated that RAGE and HMGB1 may be induced by intrauterine infection.

HMGB1, the major component of the nuclear protein group, can be released by activated innate immune cells after stimulation. HMGB1 is not the initiator of an acute inflammatory course, but it is a late mediator of several inflammatory diseases.[34-36] HMGB1 has been reported to signal through several receptors including RAGE, TLR2 and TLR4.[14-17] Upon binding to its receptors, HMGB1 stimulates immune cells to produce various pro-inflammatory cytokines. These pro-inflammatory cytokines can induce excessive cellular/tissue damage in inflammatory lesions. It is well known that RAGE activation induces increased vascular permeability, expression of tissue factor in promoting coagulation, neutrophils adhesion in the vessel walls, and expression of matrix metalloproteases, On the other hand, sRAGE suppresses the inflammatory events and enhances wound repair.[29,37] In our study, we found that brain damage in preterm infants was directly correlated with the change

of cord blood HMGB1 and sRAGE levels. Compared with the normal groups, significant increase of HMGB1 and decrease of sRAGE levels were observed in brain damage infants. The more severe the brain damage was, the lower the cord blood sRAGE levels and the higher HMGB1 levels would be. Our results again confirmed that premature brain damage was associated with intrauterine infection and inflammation. In our study, we also noticed that a significant direct correlation between HMGB1, sRAGE and the neutrophilic infiltrates in placentas. It indicated that HMGB1 and sRAGE may have participated in the pathogenesis of brain injury after intrauterine infection. In mouse modle of lipopolysaccharide-induced inflammation and preterm birth, Buhimschi CS et al[38] found that a specific increase in HMGB1 and RAGE immunostaining in the brain of fetuses exposed to inflammation in utero, as compared with normal control group. Thus, from previously published data, coupled with our results presented here, we conclude that RAGE and HMGB1 may be important mediators of brain injury in fetuses delivered in the setting of inflammation induced preterm birth.

Prognostic and diagnostic biomarkers of brain injury may enable the clinicians to supplement clinical and laboratory assessment. In our study, changes of cord blood HMGB1 and sRAGE levels of preterm infants were associated with brain injury. This confirms and extends the role of HMGB1 and sRAGE in prediction of premature brain damage. Moreover, the levels of these two proteins could predict the severity of brain injury in preterm infants. This suggests that quantification of HMGB1 and sRAGE may be a useful prognostic marker to predict and distinguish mild or severe brain injury in premature infants. However, more studies are needed to confirm the present findings.

Due to the immunohistochemical studies confirm the overall level of RAGE, immunochemical examination of RAGE can not distinguish between RAGE and sRAGE. We compared HMGB1 and RAGE immunostaining in the placentas from brain injury infants and the control group. We determined that HMGB1 and RAGE were expressed in a variety of placenta cells. Unlike RAGE, which localized primarily on the cellular surface, HMGB1 had a primarily intranuclear location. This is consistent with other previous reports. Furthermore, we observed that these two proteins were expressed more stronger in placenta in brain injury infants than those of the control group, They were not only expressed in syncytiotrophoblasts, cytotrophoblasts, and placental mesenchymal cells, but also expressed abundantly in neutrophils, lymphocytes and macrophages. This result suggests that the HMGB1/RAGE pathway may play an important role in the initiation of brain damage in premature infants. RAGE is a multiligand receptor. sRAGE, due to its capacity to block RAGE signals, can reduce inflammatory response. In our study, we found that the cord blood sRAGE level was the lowest in severe brain injury group while the expression of RAGE in placenta among these groups was the highest. The decrease of sRAGE protein would be accompanied by an increase in membrace-bound RAGE. This supports the possibility that sRAGE is likely to play a protective role in the induction of preterm brain injury by intrauterine inflammation. A loss of sRAGE or imbalance of sRAGE/RAGE may propagate intrauterine inflammation that contributes to the injury.

The present study was a cross-sectional study that could not elucidate the causal relationship between levels of sRAGE, HMGB1 in infants and brain injury. Longitudinal studies will help to clarify whether activation of HMGB1-RAGE is mechanistically linked to the pathological and physiological progression of brain injury in preterm infants. It would be interesting to re-analyze the data of chorioamnionitis, HMGB1, RAGE and brain injury, or do more analysis to connect them. Therefore, further studies on larger cohorts of patients are warranted to confirm our results and to clarify the role played by HMGB1 and RAGE in the immunopathogenesis and the evolution of brain injury in premature infants.

In conclusion, this study provides initial evidence that changes of HMGB1 and sRAGE in cord blood of premature infants had direct relationship with the degree of inflammation and severity of brain damage. Monitoring sRAGE and HMGB1 levels may be helpful in predicting intrauterine infection and brain injury in premature infants.

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Ethical approval: This study was approved by the ethics committee of the Affiliated Hospital of Jiangsu University.

Competing interest: None of the authors has conflict of interest.

Contributors: Lu HY contributed to the research design, manuscript writing, revising and final approval; Ma JL and Shan JY contributed to research performing, data analysis; Zhang J, Wang QX and Zhang Q contributed to the data acquisition and analysis, critical manuscript revision. All authors were involved in revising it critically for important intellectual content and approved the final version.

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