Receptor for Advanced Glycation End-Product Antagonist Reduces Blood–Brain Barrier Damage After Intracerebral Hemorrhage

Fan Yang, MD; Zhe Wang, MD; John H. Zhang, MD, PhD; Jiping Tang, MD; Xin Liu, PhD; Liang Tan, MD; Qing-Yuan Huang, MD; Hua Feng, MD, PhD

Background and Purpose—To determine whether the receptor for advanced glycation end-products (RAGE) plays a role in early brain injury from intracerebral hemorrhage (ICH), RAGE expression and activation after injury were examined in a rat model of ICH with or without administration of a RAGE-specific antagonist (FPS-ZM1).

Methods—Autologous arterial blood was injected into the basal ganglia of rats to induce ICH. The motor function of the rats was examined, and water content was detected after euthanization. Blood–brain barrier permeability was determined by Evans blue staining and colloidal gold nanoparticle tracers. Nerve fiber injury in white matter was determined by diffusion tensor imaging analysis, and the expression of target genes was analyzed by Western blotting and quantitative reverse transcription polymerase chain reaction. FPS-ZM1 was administered by intraperitoneal injection.

Results—Expression of RAGE and its ligand high-mobility group protein B1 were increased at 12 hours after ICH, along with blood–brain barrier permeability and perihematomal nerve fiber injury. RAGE and nuclear factor-κB p65 upregulation were also observed when FeCl₂ was infused into the basal ganglia at 24 hours. FPS-ZM1 administration resulted in significant improvements of blood–brain barrier damage, brain edema, motor dysfunction, and nerve fiber injury, and the expression of RAGE, nuclear factor-κB p65, proinflammatory mediators interleukin 1β, interleukin-6, interleukin-8R, cyclooxygenase-2, inducible nitric oxide synthase, and matrix metallopeptidase-9 was attenuated. Moreover, decreases in claudin-5 and occludin expression were partially recovered. FPS-ZM1 also reversed FeCl₂-induced RAGE and nuclear factor-κB p65 upregulation.

Conclusions—RAGE signaling is involved in blood–brain barrier and white matter fiber damage after ICH, the initiation of which is associated with iron. RAGE antagonists represent a novel therapeutic intervention to prevent early brain injury after ICH. (Stroke. 2015;46:1328-1336. DOI: 10.1161/STROKEAHA.114.008336.)

Key Words: advanced glycosylation end-product receptor ■ brain edema ■ cerebral hemorrhage ■ FPS-ZM1 ■ inflammation

Intracerebral hemorrhage (ICH) and subsequent early brain injury (EBI) are associated with high mortality worldwide. Patients with EBI develop blood–brain barrier (BBB) damage and angioedema in the early stages of injury. Although potential therapies targeting EBI after ICH have been proposed, there are no effective ways to manage these lesions. At the site of ICH, degradation of hemoglobin releases iron, which can augment brain edema and atrophy. Iron has been identified as an independent risk factor in the pathogenesis of ICH-induced inflammatory pathways and can activate c-Jun N-terminal kinase signaling and Wnt signaling in posthemorrhagic chronic hydrocephalus.

The receptor for advanced glycation end-products (RAGE) is a member of the immunoglobulin superfamily, which promotes inflammatory responses on binding of its ligands, including amyloid-β fibrils, S100 proteins, and high-mobility group box-1 protein (HMGB1). RAGE activation is also involved in BBB damage in a variety of human brain disorders, including multiple sclerosis, diabetes mellitus, and Alzheimer disease. Indeed, inflammatory responses, increased BBB permeability, and brain edema are significantly reduced after brain trauma in RAGE knockout rats. Furthermore, intravenous injection of anti-HMGB1 monoclonal antibodies attenuates ischemic BBB damage. However, the role of RAGE in EBI after ICH is unknown. Therefore, we investigated the activity of RAGE signaling in a rat ICH model and evaluated the effects of a high-affinity RAGE-specific inhibitor (FPS-ZM1) on BBB dysfunction and nerve fiber injury.
Materials and Methods

Rat ICH Model

A total of 192 male Sprague-Dawley rats (275–300 g) were obtained from the Laboratory Animal Center, Third Military Medical University, Chongqing, China. Rats were housed under specific pathogen-free conditions and had free access to food and water. Experimental protocols were approved by the Medical Ethical Committee of the Third Military Medical University.

The rat ICH model was established as described by Teng et al. Briefly, animals were anesthetized with an intraperitoneal injection of chloral hydrate (100 mg/kg body weight) and placed on a stereotactic apparatus. A hole was drilled in the skull, and a needle was inserted into the left basal ganglia under stereotactic guidance (coordinates: 0.2-mm anterior, 5.5-mm ventral, and 3.5-mm lateral to the midline), and 100 μL of autologous arterial blood was infused slowly (5 μL/min) with a microinfusion pump. After the infusion was complete, the needle was left in place for 20 minutes before withdrawal. Control animals received an infusion of 100 μL of normal saline. Bone wax was placed around the Burr hole, and the skin incision was closed with sutures in both the experimental and control groups. All procedures were conducted under aseptic conditions to avoid infection. The rats received various concentrations of FPS-ZM1 (1, 5, or 10 mg/kg IP; Merck, White House Station, NJ) or an equal volume of saline at 5 μL/min with a microinfusion pump. After the infusion was complete, the needle was left in place for 20 minutes before withdrawal. Control animals received an infusion of 100 μL of normal saline. Bone wax was placed around the Burr hole, and the skin incision was closed with sutures in both the experimental and control groups. All procedures were conducted under aseptic conditions to avoid infection. The rats received various concentrations of FPS-ZM1 (1, 5, or 10 mg/kg IP; Merck, White House Station, NJ) or an equal volume of saline at 5 μL/min and 6 hours after ICH. The physiological parameters of rats were measured 1 hour before and 6 hours after the ICH procedure.

To measure the effects of FeCl3 (Sigma-Aldrich, St. Louis, MO) on RAGE expression, 50 μL of 0.5 mmol/L FeCl3 (experimental group) or 50 μL of normal saline (control group) was slowly infused into the basal ganglia of rats (5 μL/min) with a microinfusion pump. All the rats were then given 100 mg/kg of the iron chelator deferoxamine (Sigma-Aldrich) or 5 mg/kg of FPS-ZM1 by intraperitoneal injection. The rats were then given 100 mg/kg of the iron chelator deferoxamine (Sigma-Aldrich) or 5 mg/kg of FPS-ZM1 by intraperitoneal injection. Three separate experiments were conducted (Figure 1 in the online-only Data Supplement) in 2 models.

Western Blotting

Total protein was extracted from brain tissues surrounding the hemorrhagic region (Figure IIA in the online-only Data Supplement) at 24 hours after ICH, and the protein concentrations were measured using a bicinchoninic acid protein assay. Tissue samples were taken from 6 rats in each group, one sample from each brain. The following antibodies were used: anti-RAGE (1:1000), anti-HMGB1 (1:1000), anti-occludin-1 (1:1000), anti-claudin-5 (1:1000), anti-claudin-5 (1000), antinuclear factor (NF)-κB p65 (1:500), antimatrix metalloproteinase (MMP)-9 (1:500) and anti-MMP2 (1:500) from Abcam (Cambridge, United Kingdom), and anti-β-actin (1:1000; Santa Cruz Biotechnologies, Dallas, TX). The immuno-reactive bands were visualized using enhanced chemiluminescence (Amersham Biosciences, Arlington Heights, IL) according to the manufacturer’s instructions. The expression bands of target proteins were detected on a bioimaging system (VersaDoc MP 4000; Bio-Rad, Hercules, CA), and the densitometric values were analyzed by ImageJ software. The housekeeping protein β-actin was used as an internal control.

Immunohistochemistry

After 24 hours of ICH, brain tissue samples were removed, fixed in 4% formaldehyde, cryoprotected in a 30% sucrose solution, sectioned into 20-μm thick sections, and then stained with primary antibodies at 4°C overnight. The primary antibodies used were anti-CD31 (1:200), anti-RAGE (1:300), or anti-Ilbα (1:200; Abcam). After washing, samples were probed with the appropriate secondary antibodies (Jackson ImmunoResearch, West Grove, PA). Micrographs were randomly selected and captured under a fluorescent microscope and analyzed using MagnaFire SP 2.1B software (Olympus, Melville, NY).

Functional Behavioral Test

The rotarod test was conducted to evaluate the motor function of rats as described by Ahn et al. All the animals were trained for the test 1 day before surgery. Only rats with a latency of >60 s before falling off were used for analysis. The rotarod test was performed 1 hour before surgery and at 12, 24, 48, and 72 hours after surgery. Six animals were used for each test at each time point. The animals were placed on the rod (diameter, 10 cm; constant speed, 8 rpm), and the time until they fell (or ≤180 s) was recorded. Each animal participated in a total of 5 trials, and the average time of the top 3 latency values was calculated.
Analysis of the Brain Water Content

Brain water content was examined in rats 24 hours after ICH was instituted. Animals were anesthetized with an intraperitoneal injection of chloral hydrate (100 mg/kg body weight), and their brains were immediately removed. The cerebrum was divided into 5 parts: ipsilateral and contralateral cortex, ipsilateral and contralateral basal ganglia, and cerebellum. Each part was weighed on an electric analytic balance to obtain the wet weight, and then they were dried at 100°C for 24 hours to obtain the dry weight. The brain water content was calculated using the formula:

\[
\text{Brain water content (\%)} = \left( \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \right) \times 100
\]

Evans Blue Staining

BBB permeability was evaluated using Evans blue staining at 24 and 72 hours after ICH as described by Ma et al. Animals were anesthetized with an intraperitoneal injection of chloral hydrate (100 mg/kg body weight), and then they were dried at 100°C for 24 hours to obtain the dry weight. The brain water content was calculated using the formula:

Transmission Electron Microscopy Analysis

After 24 hours of ICH, the animals were given 3 mL/kg of colloidal gold nanoparticles (30-nm diameter; Shengtaier, Chengdu, China). Fifty minutes later, the anesthetized rats were transcardially perfused with PBS and 4% formaldehyde. Brain tissues stained with Evans blue near the hemorrhagic region were removed and postfixed with 2% glutaraldehyde and 2% formaldehyde for 15 minutes. Tissues were minced into pieces of 1×1×1 mm and stored overnight at 4°C in 2% glutaraldehyde and 2% formaldehyde. After dehydration, samples were impregnated with epoxy resin and sectioned. Sections were double-stained with lead citrate and uranyl acetate. Images were obtained using an H-7100 transmission electron microscope (Hitachi, Tokyo, Japan).

Diffusion Tensor Imaging

Diffusion tensor imaging enables the visualization and characterization of white matter fibers in 3 dimensions and was conducted as reported by Yeo et al. At 24 or 72 hours after ICH, the animals underwent a 7.0-T nuclear MRI examination. Animals were anesthetized by chloral hydrate (100 mg/kg body weight), and the samples were incubated in a 50°C-water bath for 48 hours. After 30 minutes of centrifugation (12000g at 4°C), the supernatant was collected and the absorbance of the sample was measured at 620 nm using a spectrophotometer.
mg/kg), and isoflurane (2 mL/min) was used for the maintenance of anesthesia. The respiratory rate was maintained at $\approx 30 \times $ per minute, and body temperature was maintained at 37±0.5°C. The heart rate was also monitored using model 1030 MR-compatible Small Animal Monitoring and Gating System. The parameters were set as follows: segments=4; bandwidth=2×10^6 Hz; $\beta$ value=0, 1000 s/mm^2, repetition time/echo time=3000/25 ms, thickness/gap=1/0 mm, field of view=30×30 mm, matrix=128×128, and number of excitations=4. Three-dimensional reconstructions were created to analyze white matter tracts using the Diffusion Toolkit software (Harvard Medical School, Boston). Brain tissues near the hemorrhagic region were scanned by MRI-View3D software (ShockWatch, Graham, TX), and the fractional anisotropy (FA) and apparent diffusion coefficient (ADC) values were calculated. FA was calculated using the 3 diagonal elements of the diagonalized diffusion tensor in the regions of interest. ADC reveals water diffusion within each magnetic resonance voxel, and FA reveals both how well cellular structures are aligned within the fiber tracts and fiber tract structural integrity.20

**Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction**

Twelve hours after ICH, the total RNA was extracted from brain tissues near the hemorrhagic region (Figure IIA in the online-only Data Supplement). Total RNA was reverse transcribed using the SuperScript III kit (Invitrogen, Carlsbad, CA), and quantitative reverse transcription polymerase chain reaction was performed using a polymerase chain reaction instrument (Opticon CFD-3200; MJ Research, Waltham, MA). Polymerase chain reaction amplification was performed using the specific primers (Table I in the online-only Data Supplement). $\beta$-actin was used as the internal control, and the relative expression of amplified RNA samples was calculated using the $2^{-\Delta\Delta CT}$ method.

**Statistical Analysis**

Data were analyzed by SPSS 13.0 software (SPSS Inc, Chicago, IL). The data are presented as mean±SD. Statistical significance among different groups was determined using 1-way ANOVA. Multifactorial comparison was conducted using a multifactor ANOVA with Student–Newman–Keuls multiple comparison tests. A repeated measures ANOVA was used for the comparison of continuous data. A $P$ value of $<0.05$ was considered as statistically significant.

**Results**

**Increased Expression of RAGE and HMGB1 After ICH**

To understand the activity of RAGE in rodent brains affected by ICH, the protein expression of RAGE in brain tissues surrounding the hemorrhagic region was evaluated by Western blotting at different time points after ICH. As shown in Figure 1A, the level of RAGE was elevated at 12 hours and was sustained for 72 hours after ICH.

The protein expression of HMGB1 in the ipsilateral hemisphere of the brain was significantly increased 24 hours after ICH compared with the control group (0.441±0.079 versus 0.317±0.026; $P<0.05$; Figure 1B). The expression of HMGB1 was also higher in the ipsilateral than the contralateral hemisphere of the brain after ICH (0.441±0.079 versus 0.342±0.079; $P<0.05$). Immunocytochemistry revealed that RAGE was colocalized with Iba1, a microglia marker, but not CD31, the surface biomarker for vascular endothelial cells (Figure 1C).

**FPS-ZM1 Suppressed the Effects of ICH on Expression of RAGE, NF-κB p65, MMP-9, Claudin-5, and Ocludin**

To illustrate the molecular mechanism involved in FPS-ZM1-mediated protection after ICH, the expressions of...
several potential downstream effectors of RAGE were determined. As revealed by Figure 2A, FPS-ZM1 significantly reduced RAGE levels in brain tissues near the hemorrhagic region after 24 hours of ICH compared with ICH alone (0.81±0.41 versus 1.28±0.17; P<0.05). In addition, FPS-ZM1 significantly downregulated the expression of NF-κB p65 (1.09±0.39 versus 1.85±0.57) and MMP-9 (0.70±0.13 versus 0.96±0.18) compared with ICH alone (P values, <0.05) but not MMP-2 (Figure 2B and 2C). FPS-ZM1 upregulated the expression of the tight junction proteins claudin-5 (0.32±0.10 versus 0.17±0.07) and occludin (0.40±0.15 versus 0.24±0.03) compared with ICH alone (P values, <0.05) but not zonula occludens-1.

FPS-ZM1 Suppressed the Expression of Inflammatory Cytokines in Rats After ICH

To determine the potential anti-inflammatory activities of FPS-ZM1 in rats after ICH, the mRNA expression of cytokines and cytokine factors was examined. At 24 hours after ICH, FPS-ZM1 significantly reduced the levels of proinflammatory mediators, including interleukin (IL)-1β, IL-6, IL-8R, cyclooxygenase-2, and inducible nitric oxide synthase in the ipsilateral hemisphere (IL-1β, 0.14±0.07 versus 1.42±0.64, P<0.01; IL-6, 0.33±0.13 versus 1.00±0.07, P<0.01; IL-8R, 0.42±0.20 versus 1.06±0.38, P<0.05; cyclooxygenase-2, 0.20±0.07 versus 1.11±0.49, P<0.01; inducible nitric oxide synthase, 0.24±0.08 versus 2.83±1.08, P<0.01; Figure 3). Because vascular endothelial growth factor (VEGF) contributes to increased blood vessel permeability in hypoxic-ischemic brain damage,21 we also examined the expression of VEGF splice forms, which are abundantly expressed in the human body. The results show that FPS-ZM1 reduced the mRNA level of VEGF-A165 in rats compared with ICH alone (0.04±0.02 versus 1.14±0.51; P<0.01) but not other VEGF splice forms (VEGF-A121 or VEGF-A189; Figure 3).

FPS-ZM1 Preserved the BBB, Reduced Brain Edema, and Improved Motor Function in ICH Rats

No significant differences were found between the basic physiological parameters of the experimental groups (Table II in the online-only Data Supplement). As RAGE was upregulated in brain tissues after ICH, we investigated the effects of FPS-ZM1 in ICH-induced brain damage. BBB permeability was evaluated using Evans blue staining. As shown in Figure 4A, Evans blue permeability was increased at 24 and 72 hours after ICH, and FPS-ZM1 dose dependently reduced Evans blue permeability. Compared with the ICH group, the administration of 5 mg/kg of FPS-ZM1 significantly reduced the permeability (24 hours, 1.23±0.47 versus 1.91±0.78 μg/g; P<0.05; 72 hours, 0.61±0.42 versus 1.51±0.81 μg/g; P<0.01). Similar results were obtained when using colloidal gold nanoparticles as tracers. The accumulation of gold

Figure 4. The effects of FPS-ZM1 on blood–brain barrier permeability, brain edema, and motor function in rats after intracerebral hemorrhage (ICH). A, Blood–brain permeability was evaluated using Evans blue staining at 24 and 72 hours after ICH. B, Blood–brain permeability was evaluated using colloidal gold nanoparticles staining followed by transmission electron microscopy analysis at 24 hours after ICH. The boxed area was enlarged in the same micrograph. Scale bar, 1 μm (n=5 for electron microscopy analysis and n=6 for other experiments for each time point; #P<0.05). C, Motor function was analyzed by the rotarod test, and the ratio of walking time was calculated. D, Brain water content in ipsilateral cortex (Ipsi-CX), contralateral cortex (Cont-CX), ipsilateral basal ganglia (Ipsi-BG), contralateral basal ganglia (Cont-BG), and the cerebellum (Cerebel) at 24 hours after ICH.
nanoparticles surrounding blood vessels was found 24 hours after ICH, and barely any gold nanoparticles could be found in the FPS-ZM1 (5 mg/kg) treatment group (Figure 4B). The rotarod test demonstrated that FPS-ZM1 (5 mg/kg) remarkably improved motor function compared with ICH alone after 48 and 72 hours (48 hours, 0.75±0.05 versus 0.64±0.05; P<0.05; 72 hours, 0.88±0.03 versus 0.72±0.07; P<0.05; Figure 4C).

The brain water content was analyzed in the ipsilateral and contralateral cortices and basal ganglia and the cerebellum. Saline injection caused brain edema in sham-operated animals. We found that FPS-ZM1 (5 mg/kg) treatment reduced the brain water content in ipsilateral basal ganglia compared with ICH alone after 24 hours (80.29±0.82 versus 81.66±0.69%; P<0.05; Figure 4D).

**FPS-ZM1 Reduces White Matter Fiber Damage Induced by ICH**

Using diffusion tensor imaging techniques, we found a significant loss and fragmentation of nerve fiber bundles in the ipsilateral hemisphere of the brain at 24 or 72 hours after ICH (Figure 5A). In the ICH group, the ADC values in brain tissues near the hemorrhage were significantly increased compared with sham at 24 hours (10.71±1.59 versus 7.38±0.70 mm²/s; P<0.05) but returned to sham level values at 72 hours (Figure 5B), indicating that brain edema occurred at 24 hours but resolved at 72 hours after ICH. The FA values were greatly reduced at 24 and 72 hours after ICH compared with the sham group (24 hours, 0.21±0.14 versus 0.49±0.14, P<0.05; 72 hours, 0.23±0.09 versus 0.51±0.18, P<0.05; Figure 5C), demonstrating nerve fiber damage. However, 5 mg/kg of FPS-ZM1 administration was protective because it dramatically reduced ADC values compared with ICH alone at 24 hours after ICH (8.11±1.55 versus 10.71±1.59 mm²/s; P<0.05; Figure 5B) and elevated FA values after 72 hours (0.42±0.17 versus 0.23±0.09; P<0.05; Figure 5C).

**FPS-ZM1 Reversed FeCl₂-Induced RAGE Upregulation**

Iron ions are the main metabolic products after ICH, and their accumulation leads to brain injury after cerebral hemorrhage. We found that injection of FeCl₂ into the basal ganglia
increased RAGE expression at 24 hours after ICH compared with the control (control, 0.73±0.39; 0.5 mmol/L FeCl$_2$, 1.65±0.26; 1 mmol/L FeCl$_2$; 1.37±0.44; $P<0.05$; Figures 6A). In contrast, the administration of either the iron chelator deferoxamine or FPS-ZM1 efficiently inhibited the FeCl$_2$-induced upregulation of RAGE (control, 2.22±0.92; deferoxamine, 1.23±0.55; FPS-ZM1, 0.91±0.58; $P<0.05$) and NF-kB p65 (control, 2.01±0.53; deferoxamine, 0.63±0.25; FPS-ZM1, 0.55±0.32; $P<0.05$; Figure 6B and 6C).

### Discussion

Inflammatory responses that cause increased BBB permeability and brain edema are hallmarks of ICH-induced secondary brain injury. However, the involvement of RAGE and its association with these pathogenic events remain unclear. The evidence shown here implicates the RAGE singling pathway in the development of EBI after ICH, thus presenting a possible therapeutic target.

The BBB disruption and white matter fiber damage from EBI lead to early brain edema and sensorimotor dysfunction and ultimately result in high morbidity and mortality. A significant contributor to EBI is mechanical damage, which causes hematoma formation and expansion within the brain parenchyma. The edema and disruption of white matter tracts around the hematoma promote apoptosis and necrosis, and inunudation of inflammatory cells. Together, inflammation, red cell lysis, and thrombin production disrupt the BBB. Although it is known that tumor necrosis factor-α, IL-1β, and toll-like 4 receptor signaling pathways are involved, not all the inflammatory pathways contributing to neuronal injury and brain edema from ICH are known.

This study shows that RAGE is associated with ICH, and its expression in the area surrounding the hematoma increases with brain edema in a time-dependent manner. RAGE is a key regulator in the inflammatory response involved in neurodegenerative diseases, such as Alzheimer disease, where it interacts with the Aβ peptide to inhibit transport across the BBB. In addition, RAGE is involved in inflammation and edema after traumatic brain injury and ischemia. Consistent with these reports, we found that FPS-ZM1 administration suppressed RAGE and NF-kB p65 expression and brain edema, and improved motor function 24 hours after ICH. The inhibition of RAGE expression by FPS-ZM1 was likely because of a positive-feedback mechanism because RAGE activated by Aβ promotes the generation of reactive oxygen species and NF-kB activation, and increases the expression of RAGE itself. Furthermore, the expression of several proinflammatory cytokines, including IL-1β, inducible nitric oxide synthase, and VEGF-A165, was suppressed, whereas tight junction proteins were upregulated, by the RAGE antagonist, which would be secondary effects because of neuroprotection. The increased levels of the tight junction proteins claudin-5 and occludin, which are involved in the physical attachments between vascular endothelial cells, and greatly reduced MMP-9, which degrades tight junction proteins and basal lamina, helped preserve the BBB. These results suggest that FPS-ZM1 may also be able to reverse ICH-induced brain edema by inhibiting inflammation.

White matter damage induced by inflammatory mediators, edema, and mechanical force is an important consideration in ICH. We evaluated white matter fibers in rats after ICH using diffusion tensor imaging analysis and observed white matter fiber bending and reduced FA values in the basal ganglia, indicative of fiber degeneration. These changes were accompanied by motor impairment, providing further evidence for functional damage. FPS-ZM1 administration reduced the ADC value, an indicator that is also increased with vasogenic edema or accumulation of cellular debris from
neuronal injury, and increased the FA value at 72 hours after ICH. These results imply that FPS-ZM1 protects nerve fibers and reduces brain edema after ICH. Such phenomena may partially explain the improved motor function in rats after FPS-ZM1 therapy. However, further study is needed to correlate FA values and motor function.

Cerebral ischemia and hemorrhage-induced neurotoxicity can result from iron overload. The source of iron in ischemia is primarily not only from iron transport and storage proteins but also from the lysis of red blood cells. After ICH, erythrocytes with hemoglobin in the intracerebral hematoma are lysed, which can lead to a sustained release (≤1 month) of iron. Both hemoglobin and iron cause BBB disruption. Moreover, the accumulation of iron leads to auto-oxidation, oxidative stress, and brain edema after ICH. The data from this study suggest that iron stimulates RAGE signaling, which is a possible mechanism for the observed edema and BBB permeability. Consequently, we think that RAGE may represent an important target for inhibiting iron toxicity after ICH. In support of this, the iron infusion-induced increase in RAGE expression was blocked by FPS-ZM1 and deferoxamine.

Conclusions

The findings presented here indicate that the RAGE–NF-kB signaling pathway plays an essential role in EBI of ICH. Inhibition of RAGE activity by FPS-ZM1 significantly reduced brain edema, preserved BBB integrity, and improved motor function in rats after ICH. It is noteworthy that as a next-generation RAGE inhibitor, FPS-ZM1 possesses the advantages of high permeability, high affinity, and no toxicity. Furthermore, it can effectively prevent white matter fiber damage and relieve brain edema after ICH. Above all, we suggest that it could be developed in the future as a disease-modifying agent for patients diagnosed with ICH.

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Disclosures

References


Disclosures


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References


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**Supplemental Figure I.** Experimental procedure.

**Supplemental Figure II.** Gross examination and staining of cerebral hemorrhage. (A) Gross examination of cerebral hemorrhage. The tissues near the hemorrhage used for Western blotting and qRT-PCR are indicated. (B–D) Hematoxylin and eosin staining (C: 100×; D: 400× of boxed region in C).
**Supplementary Table I.** Forward and reverse primers used for PCR.

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**Supplementary Table II.** Basic physiologic parameters in different experimental groups.

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<td>137.0 ± 2.2</td>
<td>134.0 ± 3.6</td>
<td>133.0 ± 2.4</td>
</tr>
<tr>
<td>Ca</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Cl</td>
<td>104.0 ± 1.6</td>
<td>99.5 ± 4.8</td>
<td>99.3 ± 1.9</td>
</tr>
<tr>
<td>Lac</td>
<td>1.7 ± 0.2</td>
<td>2.1 ± 0.5</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>WBC</td>
<td>10.8 ± 1.8</td>
<td>12.8 ± 1.5</td>
<td>12.0 ± 2.2</td>
</tr>
<tr>
<td>RBC</td>
<td>8.8 ± 3.9</td>
<td>11.3 ± 1.2</td>
<td>10.0 ± 1.7</td>
</tr>
<tr>
<td>Hb</td>
<td>156.0 ± 9.9</td>
<td>165.2 ± 5.8</td>
<td>156 ± 7.8</td>
</tr>
<tr>
<td>PLT</td>
<td>572.8 ± 70.8</td>
<td>657.2 ± 52.5</td>
<td>594.2 ± 43.7</td>
</tr>
<tr>
<td>Neu (%)</td>
<td>21.2 ± 1.7</td>
<td>19.6 ± 1.91</td>
<td>20.8 ± 1.3</td>
</tr>
<tr>
<td>Mon (%)</td>
<td>2.2 ± 0.9</td>
<td>4.2 ± 1.4</td>
<td>3.9 ± 1.2</td>
</tr>
</tbody>
</table>

Abbreviations: Ca, calcium ion; Cl, chloride ion; CTHb, total hemoglobin
concentration; FPS-ZM1, receptor for advanced glycation end-product-specific inhibitor; Glu, glucose; Hb, hemoglobin; ICH, intracerebral hemorrhage; K, potassium ion; Lac, lactic acid; Mon, monocytes; Na, sodium ion, Neu, neutrophils; PCO₂, partial pressure of carbon dioxide; PH, acidity and alkalinity; PLT, platelet; PO₂, oxygen partial pressure; RBC, red blood cell count; SO₂, arterial oxygen saturation; WBC, white blood cell count.