The correlation between accumulation of amyloid beta with enhanced neuroinflammation and cognitive impairment after intraventricular hemorrhage

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OBJECTIVE Intraventricular hemorrhage (IVH) is found in approximately 40% of intracerebral hemorrhages and is associated with increased mortality and poor functional outcome. Cognitive impairment is one of the complications and occurs due to various pathological changes. Amyloid beta (A β) accumulation and neuroinflammation, and the Alzheimer disease–like pathology, may contribute to cognitive impairment. Iron, the degradation product of hemoglobin, correlates with A β . In this study, the authors investigated the correlation between A β accumulation with enhanced neuroinflammation and cognitive impairment in a rat model of IVH.

METHODS Nine male Sprague-Dawley rats underwent an intraventricular injection of autologous blood. Another 9 rats served as controls. Cognitive function was assessed by the Morris water maze and T-maze rewarded alternation tests. Biomarkers of A β accumulation, neuroinflammation, and c-Jun N-terminal kinase (JNK) activation were examined.

RESULTS Cognitive function was impaired in the autologous blood injection group compared with the control group. In the blood injection group, A β accumulation was observed, with a co-located correlation between iron storage protein ferritin and A β . Beta-site amyloid precursor protein cleaving enzyme–1 (BACE1) activity was elevated. Microgliosis and astrogliosis were observed in hippocampal CA1, CA2, CA3, and dentate gyrus areas, with elevated proinflammatory cytokines tumor necrosis factor– α and interleukin-1. Protein levels of phosphorylated JNK were increased after blood injection.

CONCLUSIONS A β accumulation and enhanced neuroinflammation have a role in cognitive impairment after IVH. A potential therapeutic method requires further investigation.

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KEYWORDS intraventricular hemorrhage; amyloid beta; beta-site amyloid precursor protein cleaving enzyme–1; BACE1; neuroinflammation; ferritin; c-Jun N-terminal kinase; JNK; vascular disorders

INTRAVENTRICULAR hemorrhage (IVH) is found in approximately 40% of intracerebral hemorrhages and is associated with increased mortality and poor functional outcome.^{12,13,17} The degradation of hemoglobin after IVH could cause iron overload in the brain, which plays an important role in brain injury.⁶

Cognitive impairment is one of the complications of intracerebral hemorrhage. Various pathological changes may contribute to the progression of this impairment.^{21,30} Amyloid beta (A β) is one of the hallmarks for Alzheimer

disease (AD), and its accumulation is well recognized to impair memory.²⁹ Neuroinflammation, characterized by activation of glial cells and release of proinflammatory cytokines, is activated by A β accumulation and also acts as an important contributor in the pathogenesis of AD. These inflammatory responses may cause neuronal death and blockade of neurogenesis, thus leading to cognitive impairment.^{18,19,27,31}

A β is a key protein in AD pathology, with other underappreciated functions like antimicrobial activity.²³ Iron

ABBREVIATIONS A β = amyloid beta; AD = Alzheimer disease; APOE = apolipoprotein E; BACE1 = beta-site amyloid precursor protein cleaving enzyme–1; β APP = amyloid precursor protein beta; DG = dentate gyrus; ELISA = enzyme-linked immunosorbent assay; GFAP = glial fibrillary acidic protein; Iba1 = ionized calcium binding adapter molecule–1; IL-1 = interleukin-1; IVH = intraventricular hemorrhage; JNK = c-Jun N-terminal kinase; MWM = Morris water maze; P-JNK = phosphorylated JNK; NeuN = neuron-specific nuclear protein; RFU = relative fluorescence units; T-JNK = total JNK; TNF α = tumor necrosis factor– α .

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overload correlates with A β accumulation in patients with AD⁹ and in the APP/PS1 transgenic mouse model,³ as well as in the normal aging process.²⁸ Moreover, iron enhances the neurotoxicity of A β by generating reactive oxygen species.¹ The correlation between iron overload after IVH and AD-like pathological changes, however, has not been investigated. Therefore, our study uses an intraventricular autologous blood injection model to investigate A β accumulation along with neuroinflammation, and the effect of these phenomena on cognitive impairment after IVH.

Methods

All experiments were approved by the Animal Care and Use Committee of Huashan Hospital, Fudan University. Eighteen 10-week-old male Sprague-Dawley rats, weighing between 300 and 350 g, were used in the experiment. All the animals were raised and kept in a specific pathogen-free room with free access to food and water, and were maintained in a 12 hours light/12 hours dark cycle.

Animal Grouping and Surgical Protocols

Rats were randomly divided into the IVH group (n = 9) and the normal control group (n = 9) before surgical procedures. The investigators were blinded to the animal group assignment during behavioral tests and sampling. For the IVH group, the intraventricular autologous blood injection model was used (Fig. 1A and B). Rats underwent surgical procedures following intraperitoneal injection of 1.5% pentobarbital sodium at a dose of 0.2 ml per 100 g, and the anesthetic effect was checked by tail pinch. Local anesthesia by 1% lidocaine was supplemented if the rat twitched while its scalp was incised. All surgical procedures were performed using standard sterile techniques. The right femoral artery was catheterized for arterial blood sampling. After that, the rats were positioned in a stereotactic frame and received injection into the right ventricle. A 1- to 2-mm burr hole was drilled 0.9 mm posterior to the bregma and 1.4 mm lateral to the midline.²⁵ The rats received a 200- μ l injection of autologous arterial blood into the right ventricle over 10 minutes through a microinjection syringe at a depth of 4.0 mm. The needle was held for an additional 60 seconds to prevent reflux. After closure of the operation site, the rats were allowed to recover and return to their home cages with free access to food and water.

Behavioral Tests

All behavioral tests were performed at 1 month after intraventricular blood injection (Fig. 1A and C). The tests were conducted in an isolated room during the same period of time, from 2 PM to 6 PM. Animals were divided into an IVH group and a control group and were assessed in all behavioral tests.

T-Maze Rewarded Alternation Test

The T-maze rewarded alternation test was conducted according to previous studies, with slight modification.^{10,26} The apparatus consisted of a start arm (50 cm long, 16 cm wide, and 30 cm high) and 2 goal arms (50 cm long, 10

cm wide, and 30 cm high). There was a guillotine door at the beginning of each goal arm and a food well (4 cm in diameter and 1 cm deep) at the end.

Rats were fasted 2 days ahead of the test, with a normal water supply. During the test, rats remained fasting but were given a 1-hour feeding after the T-maze test every day to maintain body weight. Before the test, rats were fed small amounts of cheese pellets to habituate the reward. All rats were given 4 days of habituation, from day 1 to day 4. The habituation was performed by putting the entire home cage of the rats into the maze for 3 minutes, with cheese pellets in the food wells, and allowing the animals to freely explore and eat. The habituation procedure was repeated 4 times each day, with a 10-minute interval between procedures. After habituation was completed, we allowed each rat to run from the start arm to find and eat a cheese pellet in one goal arm, with the other one blocked by a guillotine door. We repeated this 4 times total, randomly blocking one arm, but equal numbers of left or right arms were blocked. At day 5, each rat was tested in 18 continuous trials with a 30-second interval between trials. At each trial, the rat was allowed to find and eat a cheese pellet in one randomly chosen goal arm; then we put the rat in the start arm facing away from the goal arms and allowed it to explore the two goal arms. If the rat entered the other goal arm, a reward was given and a correct choice was scored; if the rat entered the goal arm chosen previously, we made sure it ultimately discovered that the food well was empty, and an incorrect choice was scored. Apparatus arms were wiped using 10% alcohol to remove odor after each trial. The percentage of correct choices for each rat was calculated.

Morris Water Maze

Morris water maze (MWM) testing was performed after the T-maze test on the same day. The MWM procedure was performed as described previously.^{25,35} The apparatus was a large black circular tank (150 cm in diameter and 60 cm high) filled with nontoxic white-dyed water. The water temperature was set at 25°C. A white escape platform (12 cm in diameter) was placed in the middle of one quadrant and 2 cm below the water surface. Four visual cues were placed on the tank wall above the water surface. Rats were released from 4 starting points and allowed a maximum of 120 seconds to find and mount the escape platform. If the rat did not find the platform in 120 seconds, the researcher put the rat on the platform and waited 30 seconds before removing it from the maze. There was a 4-minute rest interval between trials in a dry and warmed cage. Each rat had 4 trials every day, 1 from each starting point. Tests were performed on 5 consecutive days. The time that elapsed before each rat found the platform in each trial was recorded and the mean latency was calculated.

Brain Sampling

After behavioral tests, the rats were allowed to survive for 2 weeks to alleviate the stress experienced in the behavioral tests (Fig. 1A). Before sampling, all rats were intraperitoneally injected with 1.5% pentobarbital sodium at a dose of 0.3 ml per 100 g for deep anesthesia. Ice-cold

FIG. 1. **A:** Timeline of animal modeling, behavioral tests, and planned death. **B:** Diagram of intraventricular blood injection model. **C:** Timeline of MWM and T-maze rewarded alternation tests. Figure is available in color online only.

saline (approximately 4°C) was used for transcardiac perfusion. The brain was removed carefully in an icebox and divided into anterior and posterior parts along the coronal plane just through the injection position. The anterior part was kept at −80°C, and the posterior part was fixed in 10% formalin for further staining. Bilateral hippocampus (approximately 1 mm thickness toward the coronal plane—cortex and basal ganglia were separated) was then dissected from the anterior part under a stereomicroscope for Western blot and enzyme-linked immunosorbent assay (ELISA) analysis.

Immunofluorescent Staining

Brain tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 4-μm thicknesses, and then stained with anti-amyloid precursor protein beta (βAPP), anti-feritin, anti-ionized calcium binding adapter molecule-1 (Iba1), anti-glial fibrillary acidic protein (GFAP) antibodies (rabbit polyclone, Abcam HK Ltd.), anti-Aβ, and anti-neuron-specific nuclear protein (NeuN) antibodies (mouse polyclone, Abcam HK Ltd.). The secondary antibody was goat anti-rabbit or anti-mouse immunoglobulin. Nuclei were visualized with DAPI. Slides were photographed for red (Alexa Fluor 594) and green (Alexa Fluor 488) fluorescence with a fluorescent microscope (E400; Nikon). Iba1-labeled and GFAP-labeled cells were counted by ImageJ version 1.5 (NIH) in the area of CA1, CA2, CA3, and dentate gyrus (DG), respectively. Each area in 3–5 sections was chosen for calculation, which was performed by 2 independent observers who were blinded to the rat's group assignment.

Western Blot Analysis

The bilateral hippocampal tissue was prepared for further analysis. All the nuclear and cytoplasmic proteins (100 μg) of the hippocampus tissue were size-fractionated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to Immobilon-P membranes (Millipore). The blotted membranes were incubated with primary antibodies against interleukin-1 (IL-1), tumor necrosis factor-α (TNFα), glyceraldehyde-3-phosphate de-

hydrogenase (Abcam HK Ltd.), c-Jun N-terminal kinases (JNKs), and phosphorylated JNK (P-JNK) (Cell Signaling Technology, Inc.), followed by incubation with a horseradish peroxidase–conjugated secondary antibody (Jackson). The immunoreactivity was detected using an enhanced chemiluminescence reaction system (Amersham Pharmacia Biotech).

Beta-Secretase Activity Assay

The level of beta-secretase (more precisely, beta-site amyloid precursor protein cleaving enzyme-1 [BACE1]) in rats' hippocampus tissue was detected using the ELISA kit (Abcam HK Ltd.) according to the operating instructions. Hippocampus tissue (10 mg) was homogenized in ice-cold extraction buffer and cleared by centrifugation (12,000 rpm for 10 minutes). A total of 50 μl of sample loaded into a black 96-well microplate, and the fluorogenic substrate, was added and incubated in the dark at 37°C for 2 hours. Fluorescence was read at excitation and emission wavelengths of 355 and 495 nm, respectively, by using an FLx800 fluorometer (Biotek). BACE1 activity is expressed as relative fluorescence units (RFU) per milligram of protein.

Statistical Analysis

All the data are presented as either the mean ± SEM or the mean ± SD. Data were analyzed using independent samples t-tests and 1-way ANOVA. For the MWM test, we used repeated multivariate ANOVA (MANOVA), with assessment days as the repeated variable within subjects. A *p* value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 18 and GraphPad Prism 5 for Windows.

Results

Impaired Cognitive Function in the IVH Rat Group

Cognitive function was assessed by the MWM and T-maze rewarded alternation tests. In MWM tests, MANOVA analysis demonstrated that IVH is the main cause of longer escape latency (*p* = 0.03). Further comparison

FIG. 2. Graphs showing MWM and T-maze rewarded alternation tests. **A:** Escape latency of IVH and control groups from day 1 to day 5 (mean $\pm$ SEM); * $p < 0.05$ compared with control group. **B:** Percent alternation of IVH and control groups on day 5 (mean $\pm$ SEM); * $p < 0.05$ compared with control group. Figure is available in color online only.

showed that rats that had received intraventricular blood injection had significantly longer escape latency on days 3 and 4 compared with the control group (32.44 ± 6.01 seconds vs 14.50 ± 2.57 seconds, $p = 0.013$; 21.72 ± 4.04 seconds vs 13.21 ± 1.83 seconds, $p = 0.049$ [mean $\pm$ SEM]), whereas there was no statistical difference in days 2 and 5 (Fig. 2A). In T-maze tests, 4 control rats exhibited anxiety during the test and did not move in 2 minutes. After putting the rats back in their home cage and comforting them for a while, finally 2 of 4 rats completed the 18 trials. We found that the odds were significantly lower for rats in the IVH group to make alternation moves than for rats in the control group to do so ($70.37\% \pm 5.16\%$ vs $87.30\% \pm 3.37\%$, $p = 0.011$ [mean $\pm$ SEM]; Fig. 2B).

IVH Causes Accumulation of A β and Elevated BACE1 Activity Around Hippocampus

Immunofluorescent staining demonstrated ferritin deposition and A β accumulation in the DG area surrounded by neurons (Fig. 3A), as well as the injection site (Fig. 3B) and periventricular area (Fig. 3C) after intraventricular blood injection. Notably, the A β and ferritin had a co-located correlation. The noteworthy spatial correlation can

be observed in all 3 areas mentioned above. Moreover, some of the ferritin and A β were found surrounding or close to the nucleus stained by DAPI.

Furthermore, we investigated whether aberrant β APP distribution or BACE1 activity plays a role in A β accumulation by using immunofluorescent staining and ELISA, respectively. We observed that β APP distributed only in the periventricular area but not in the hippocampus in IVH rats, with ferritin deposition and ventricle enlargement around the injection site (Fig. 4A). The staining of specimens obtained in control rats demonstrated similar β APP distribution (Fig. 4B). The activity of BACE1, the major beta-secretase to cleave β APP and produce A β ,^{5,7} was significantly increased in the hippocampal area of the IVH group compared with that in the control group (175.90 ± 1.06 vs 157.50 ± 7.19 RFU, $p = 0.016$ [mean $\pm$ SEM]; Fig. 4C).

Enhanced Neuroinflammation in Hippocampus

To verify the enhanced neuroinflammation in the hippocampus, the cell numbers of microglia and astrocytes were counted. When we compared the hippocampus on the blood injection side of the IVH group with the hip-

FIG. 3. Ferritin and A β immunofluorescent staining of the hippocampus (A), injection site (B), and lateral ventricle (C), showing the co-located correlation between ferritin and A β after IVH. Arrowheads: ferritin and A β double-labeling cells. Bar = 100 μ m.

FIG. 4. Ferritin and β APP immunofluorescent staining of the injection site (A) and control rat (B), showing similar distribution of β APP between IVH and control rat groups. Dotted line: injection site. Bar = 100 μ m. Bar graph of β APP cleaving enzyme-1 activity (C), showing elevated BACE1 activity in the IVH group (mean $\pm$ SEM); * p < 0.05 compared with control group. RFU = relative fluorescence units.

pocampus of the control group, we observed a significant increase in microglia cell counts (data in Fig. 5A and C: 25.50 ± 6.50 vs 10.00 ± 0.89 , $p = 0.0091$; 29.67 ± 6.17 vs 16.20 ± 2.13 , $p = 0.022$; 24.75 ± 5.98 vs 11.40 ± 1.81 , $p = 0.025$; and 28.40 ± 6.76 vs 11.40 ± 0.93 , $p = 0.019$ [mean $\pm$ SEM]) in hippocampal CA1, CA2, CA3, and DG, respectively, and in the injection site (Fig. 5B). There was also a significant increase in astrocyte cell counts (data in Fig. 6A and C: 41.00 ± 7.02 vs 11.25 ± 1.80 , $p = 0.0025$; 39.33 ± 4.70 vs 29.08 ± 2.93 , $p = 0.0013$; 25.50 ± 6.51 vs 8.00 ± 2.35 , $p = 0.022$; and 31.67 ± 6.64 vs 13.50 ± 2.22 , $p = 0.032$ [mean $\pm$ SEM]) in the same areas of the hippocampus and in the injection site (Fig. 6B).

Furthermore, we examined the inflammatory cytokines secreted by microglia. Western blot analysis demonstrated that IL-1 and TNF α levels were significantly elevated in the hippocampus of the IVH group compared with that of the control group ($p = 0.017$ and $p = 0.0038$, Fig. 7A–C). In addition, the P-JNK/total JNK (T-JNK) ratios were significantly higher in the IVH group compared with the control group ($p = 0.048$ and $p = 0.0021$, Fig. 7D), thus implying activation of the JNK pathway.

Discussion

IVH has drawn massive attention due to its high mortality and morbidity rates.^{13,17} Previous studies reported cognitive impairment in experimental rat models, but the mechanism remains unclear.²⁵ In the present study, we focused on A β , a hallmark for AD, whose accumulation and accompanying neuroinflammation is a possible mechanism for cognitive impairment in a rat model of IVH from intraventricular autologous blood injection.

The hippocampus is a complex brain region that par-

ticipates in cognitive function and also acts as the focus of AD.¹⁵ Hippocampal brain tissue loss has been confirmed after IVH,⁶ and because of its position adjacent to the temporal limb of the lateral ventricle anatomically, the hippocampus became our major research site. We performed cognitive tests 1 month after blood injection. The reason for our choosing this time point is that cell death in the hippocampus was considered complete at this time and no further performance changes would be expected later.²⁵ The MWM and T-maze rewarded alternation tests were performed to assess spatial memory and working memory, respectively, in IVH rats. Both mazes showed cognitive impairment in the IVH group. Although other brain regions are involved, the hippocampus is the pivotal site for both mazes. It is believed that the T-maze alteration test is probably better for detecting hippocampal dysfunction.^{10,35}

Iron accumulation could be detected by MRI from the 1st day after IVH to 4 weeks. Ferritin, the iron storage protein, was found to be increased from day 1 to 4 weeks,⁶ and even up to approximately 2 months in the hippocampus, periventricular area, and injection site when rats in the IVH group were euthanized in the present study. Despite a correlation in quantity, it is notable that a spatial correlation with amyloid plaque and iron existed, and even formed an iron-amyloid complex in cortical tissue of the APP/PS1 transgenic mouse model.³² Similarly, co-localization of iron and A β plaque was observed in neuroimaging of mildly cognitively impaired patients.³³ Together with our findings, the aforementioned articles prove that iron or storage protein ferritin has a spatial correlation with A β , implying that iron may be involved in the accumulation of A β . The role of iron in neuronal atrophy, cognitive impairment, or even AD provides us with a poten-

FIG. 5. Iba1 and NeuN immunofluorescent staining in the CA1, CA2, CA3, and DG areas (**A**) and injection site (**B**) showing microgliosis in the CA1, CA2, CA3, and DG areas and in the injection site after IVH. *Dotted line*: injection site. Bar = 100 μ m. Bar graphs showing Iba1-positive cell counts in the CA1, CA2, CA3, and DG areas (**C**) (mean $\pm$ SEM). * $p < 0.05$, ** $p < 0.01$ compared with control group. Figure is available in color online only.

FIG. 6. GFAP and NeuN immunofluorescent staining in the CA1, CA2, CA3, and DG areas (**A**) and injection site (**B**) showing astro-gliosis in the CA1, CA2, CA3, and DG areas and in the injection site after IVH. *Dotted line*: injection site. Bar = 100 μ m. Bar graphs showing GFAP-positive cell counts in the CA1, CA2, CA3, and DG areas (**C**) (mean $\pm$ SEM). * $p < 0.05$, ** $p < 0.01$ compared with control group. Figure is available in color online only.

FIG. 7. Western blot analysis of IL-1, TNF α , P-JNK, and T-JNK in the hippocampus of IVH and control groups. **A:** Representative bands of IL-1, TNF α , P-JNK, and T-JNK. **B:** Bar graph showing the relative density of IL-1 (mean $\pm$ SD). * p < 0.05 compared with control group. **C:** Bar graph showing the relative density of TNF α (mean $\pm$ SD). ** p < 0.01 compared with control group. **D:** Bar graph showing the ratio of P-JNK/T-JNK (mean $\pm$ SD). * p < 0.05, ** p < 0.01 compared with control group. GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

tial therapeutic target. Iron chelators like deferoxamine⁴⁰ and M30²⁴ could inhibit amyloid deposits and alleviate cognitive impairment.

APP can be cleaved by beta- and gamma-secretase and can generate A β in the amyloidogenic pathway.⁷ We found that the activity of BACE1, the major beta-secretase in the brain, is significantly increased in IVH rats, implying that aberrant BACE1 activity may be a factor for A β accumulation after IVH. Elevated BACE1 levels were also found in patients with AD, making BACE1 inhibitor a promising therapeutic method.^{22,38} Additionally, a recent study demonstrated that there is an imbalance between amyloidogenic and nonamyloidogenic pathways in aged rats, which may be related to cognitive impairment and needs further investigation.⁵

In the present study, we observed neuroinflammation in the hippocampal area, including microgliosis and astrogliosis, as well as increased levels of proinflammatory cytokines IL-1 and TNF α . Microglia, as part of the innate immune system, can be activated by A β and can produce proinflammatory cytokines, and they are also involved in A β clearance via phagocytosis.^{11,16} However, the phagocytosis is very limited in human AD, and increased cytokine concentrations may affect microglial phagocytosis by downregulation of the expression of A β phagocytosis receptors.¹⁸ Microglial alternation from the M1 (proinflammation) phenotype to the M2 (antiinflammation) phenotype may have a protective effect.⁴¹ Astrocytes were also observed around A β plaque next to activated microglia in a previous study. Astrocytes not only release cytokines and exacerbate the inflammatory response but also are involved in A β internalization and degradation.¹⁸

In our study, because A β and ferritin were found in glia-like cells, we may infer that gliosis and inflammatory response were induced by A β accumulation. However, focal glial activation may occur before A β accumulation, which could precede the deposition of A β plaque in the APP transgenic mouse model.²⁰ The relationship between A β accumulation and neuroinflammation is like the “chicken or egg” dilemma and is still under debate.

JNKs are a family of stress-responsive kinases activated by exposure to various stimuli, including cytokines, oxidative stress, and A β .³⁹ Activation of JNKs can be observed in a variety of cerebral diseases, from cerebral ischemia or intracerebral hemorrhage to neurodegenerative diseases like AD.^{4,36,39} The activated JNK signaling pathway not only regulates neuronal death through apoptosis but also has a role in neuroinflammation.^{8,14,37} In the present study, we found that the P-JNK/T-JNK ratio was significantly increased in the IVH group compared with the control group, thus suggesting a potential role for JNKs in the occurrence of cognitive impairment after IVH.

It is believed that iron could enhance the neurotoxicity of A β by generating reactive oxygen species and aggravating oxidative stress. Plenty of studies have noticed a correlation of iron overload with A β accumulation in patients with AD or in animal models. To our knowledge, no previous study has investigated the possible mechanism, especially for the IVH model. Recently, the apolipoprotein E (APOE) ϵ 4 allele has attracted increasing attention in AD-related research. APOE has been demonstrated to promote A β accumulation by competing for the same clearance pathways³⁴ and to increase cerebral iron retention by impairing lipoprotein transport due to its low affinity to high-

density lipoprotein.² That can partly explain the correlation of spontaneous iron overload and A β accumulation in AD. However, because iron overload in our model resulted from hemoglobin degradation after IVH, this mechanism may not be applicable. Further experiments on APOE knockout animals may help to clarify this. Besides, iron and/or ferritin could activate the microglia and contribute to inflammation. Previous studies have reported that glial activation may precede A β accumulation, which means that inflammation could also be a possible link between iron overload and A β accumulation.

There are several limitations of this study—one is the fact that we observed the results in rats within 2 months. However, because A β accumulation increased with age, the long-term effect needs to be studied in the future. Meanwhile, changes in more brain regions than the hippocampus alone need to be taken into consideration in cognitive research. We observed the correlation of ferritin overload with A β accumulation and neuroinflammation, but the mechanism is still unclear. Simultaneously, quantification of Western blotting only provides us with a correlation of the activated JNK signaling pathway in vivo with A β accumulation and neuroinflammation. Application to transgenic animals and other intervention methods, like JNK pathway inhibitor, are needed to verify this possible mechanism in the future. Multiple therapeutic methods, like iron chelator, BACE1 inhibitor, or antiinflammatory agents, are possible and promising in alleviating AD-like pathological changes and improving cognitive function. Application of these drugs in animal models needs further investigation and, hopefully, will benefit patients with cognitive impairment after IVH.

Conclusions

Despite the limitations of this study, our overall findings demonstrated that A β accumulation and neuroinflammation have a role in cognitive impairment in this rat model of IVH from autologous arterial blood injection. Iron overload may be involved in A β accumulation. Further investigations are needed to find potential therapeutic agents to alleviate AD-like pathological changes after IVH.

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Disclosures

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Author Contributions

Conception and design: Chen, Zou. Acquisition of data: Jiang, Zhu. Analysis and interpretation of data: Jiang, Zhu, Shi. Drafting the article: Jiang, Zhu. Critically revising the article: Chen, Zhao. Reviewed submitted version of manuscript: Chen, Zhao. Statistical analysis: Shi, Wu. Administrative/technical/material support: Wu.

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