

Experimental Trial

Recurrent Headache Increases Blood-Brain Barrier Permeability and VEGF Expression in Rats

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Background: The blood-brain barrier (BBB) is an important anatomical structure of the central nervous system (CNS) that limits the penetration of a variety of substances from the blood into the parenchyma. Dysfunction of the BBB is involved in various CNS disorders, including stroke, inflammation, and pain. However, the evidence concerning its role in migraine is insufficient.

Objective: This study will investigate whether recurrent headache increases BBB permeability and vascular endothelial growth factor (VEGF) expression in a rat model.

Study Design: This study used an experimental design.

Setting: The research took place in the Laboratory Research Center at The First Affiliated Hospital of Chongqing Medical University.

Methods: Eighty male Sprague-Dawley rats were randomly divided into 3 groups: inflammatory soup (IS), control (PBS), and treatment (IS+Sumatriptan) groups. Recurrent headache was induced by episodic IS stimulation: 20 μ L of IS were pumped into the dura 3 times per week in rats. The control group was administered 20 μ L of PBS. The rats in the treatment group were simultaneously treated with sumatriptan (300 μ g/kg, intraperitoneal) at the same time that IS was applied to the dura. Mechanical nociceptive thresholds were examined by electronic von Frey filaments with rigid tips. BBB permeability changes were measured with Evans blue (EB). The expression of VEGF was measured by double labeling and Western blotting.

Results: After 4 IS applications, the mechanical nociceptive thresholds significantly decreased. In addition, the mechanical hypersensitivity persisted for 4 hours after 9 applications. Only after 9 applications did the BBB permeability increase, as demonstrated by the EB tracer. The BBB disruption was accompanied by an elevation in VEGF expression. Sumatriptan treatment significantly reduced the mechanical hypersensitivity induced by IS stimulations and decreased the BBB disruption and VEGF expression.

Limitations: Potential mechanisms that underlie the relationship between BBB and VEGF were not examined in this study.

Conclusions: The present study showed that repeated IS stimulations induced long-lasting allodynia, increased BBB permeability, and upregulated VEGF expression, all of which could be attenuated by early sumatriptan treatment.

Key words: Migraine, inflammatory soup, blood-brain barrier, vascular endothelial growth factor, sumatriptan

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Migraine is an episodic, typically unilateral, disabling, throbbing headache accompanied by nausea, vomiting, photophobia, and

phonophobia (1). The mechanism underlying migraine is complex and unclear. The activation and sensitization of peripheral trigeminal neurons that innervate the

meninges, which subsequently produce the activation and sensitization of the central trigeminovascular neurons, play an important role in a migraine pain attack (2). Based on this pathological process, a migraine animal model was generated by administration of inflammatory soup (IS) over the dura. Previous studies have focused on one-time IS applications (3,4). In 2007, Oshinsky et al employed repeated inflammatory dural stimulation in freely moving rats to mimic the repeated activation of dural afferents, and they found more than 8 IS applications resulted in chronic trigeminal hypersensitivity, which reflects the features of patients with recurrent headache (5). Therefore, this model was considered a rat model of recurrent headache, involving repeated administration of IS to the dura.

The blood-brain barrier (BBB) is a dynamic and functional structure that separates the systemic circulation from the central nervous system (CNS). The BBB plays a crucial role in maintaining proper neuronal function (6), and disruption of the BBB has been reported in pain disorders including peripheral inflammatory pain and neuropathic pain. The mechanism involves an intense release of signaling molecules from peripheral and central neurons and from blood cells, which can generate significant effects on the CNS barriers including substance P, calcitonin gene-related peptide, and interleukin-1 β (7). Despite these findings, the evidence concerning migraine is insufficient. Some clinical evidence suggests the disruption of BBB in migraineurs by magnetic resonance imaging (MRI) or computed tomography (CT) scans (8,9). In addition, white matter lesions (WMLs), which are associated with the function of the BBB, are independent risk factors for migraine, and this risk is higher in those with higher attack frequency or longer migraine history (10-13). Therefore, a clear understanding of the permeability of the BBB in recurrent migraine will provide new insight into its underlying molecular mechanisms; such understanding can lead to more effective therapeutic approaches for migraine management and prevention of potential brain damage.

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and vascular permeability mediator that is mostly expressed in endothelial cells and neurons and enhances BBB permeability after stroke, cerebral cold injury, and trauma (14-16). Hypoxia, growth factors, hormones, and inflammatory cytokines induce VEGF production (17-19). Previous studies have demonstrated that VEGF participates in the modulation of nociception during chronic pain, including neuro-

pathic pain and peripheral inflammatory pain (20-22). Clinically, the VEGF haplotype AGC has been found to be more frequent in patients with migraine than in controls, suggesting that VEGF haplotypes are associated with susceptibility to migraine (23).

The aim of this study was to investigate the effects of recurrent headache on BBB permeability and VEGF expression in awake rats and the possible impact of sumatriptan.

METHODS

Experimental Animals

All experiments were conducted in male Sprague-Dawley rats weighing 200-300 g that were randomly divided into 3 groups: inflammatory soup (IS), control (PBS), and treatment (IS+Sumatriptan) groups. All animals were purchased from the laboratory animal center of Chongqing Medical University (certificate: SCXK [YU] 2007-0001); housed under optimal conditions for hygiene, temperature, and photoperiods (12:12 h light/dark cycle); and allowed food and water ad libitum, according to the institutional guidelines for the care and use of laboratory animals. All protocols were approved by the ethics committee of Chongqing Medical University.

The Model of Recurrent Headache

The model of recurrent headache associated with migraine was induced as previously described (5). Briefly, rats were anesthetized with 10% chloral hydrate (0.4 mL/100 g, intraperitoneal) and then placed in a stereotactic apparatus. A 2-mm craniotomy was performed above the junction of the superior sagittal and transverse sinuses to expose the dura; and a plastic cap with a sterile, stainless steel cannula was inserted, then affixed to the bone with small screws and dental cement. The cannula was sealed with an obturator cap. The dura was protected from damage throughout the whole procedure. After the surgery, the animals were allowed to recover for one week. The IS was composed of 1 mM histamine (Sigma, St. Louis, MO), 1 mM serotonin (Sigma, St. Louis, MO), 1 mM bradykinin (Sigma, St. Louis, MO), and 0.1 mM prostaglandin E2 (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS) pH 7.4 (3). During the IS application, the obturator cap of the cannula was moved, and PE50 tubing was connected to an infusion pump (Zhenghua Biologic Apparatus Facilities Co. Ltd., Huaibei, China) that steadily delivered 20 μ L of IS onto the dura for 5 minutes. Similar to the

protocol of Oshinsky et al (2007), the rats were administered IS 3 times per week. The rats were subjected to mechanical nociceptive threshold testing, detection of BBB integrity, and VEGF expression evaluations at 1, 2, and 3 weeks (i.e., 3, 6, and 9 IS applications). Rats in the control group were administered 20 μ L of PBS.

Drugs and Treatment

Sumatriptan succinate (Sigma, St. Louis, MO) was dissolved in saline and administered at 300 μ g/kg via intraperitoneal injection at the same time IS was applied. Similar doses and treatment paradigms have been shown to be effective for inhibiting transmission of pain signaling in inflammation-induced pain models (24). Based upon our preliminary study, BBB permeability increased after 9 IS applications. Therefore, the rats in the treatment group and the control group were analyzed following 9 IS and PBS applications, respectively.

Mechanical Nociceptive Threshold

Mechanical nociceptive thresholds were measured via electronic von Frey filaments with rigid tips (IITC Life Science, Inc., Woodland Hills, CA). The pressure value was automatically read so as not to change the monofilaments of different force values to test, which is more convenient than von Frey monofilaments. During the test, a tip was placed on a probe tip, and the pressure probe was applied to the test animals. The reaction unit subsequently displayed and stored the readings in grams. In the current study, bilateral periorbital regions of the face located over the rostral portion of the eye were chosen to test mechanical allodynia. Positive responses were indicated by withdrawal of the head, and the results were recorded as previously described (5).

Evans Blue Detection of BBB Permeability

To accurately assess and quantify BBB permeability, rats were injected with Evans blue (EB) via the femoral vein. EB (2%, 4 mL/kg) in normal saline was injected into the femoral vein and allowed to circulate for 60 minutes. The animals were anesthetized and then perfused transcardially with PBS until a colorless perfusion fluid was obtained from the right atrium. The brains were dissected quickly, snap-frozen, sectioned into 10- μ m-thick slices, and then analyzed via a confocal-laser scanning fluorescence microscope. For quantification of the EB tracer, the brain tissues were weighed, homogenized in 3 mL of N,N-dimethylformamide, incubated for 18 hours at 55°C, and centrifuged at 10,000 rpm for 20

minutes. The supernatants were analyzed with spectrophotometry at 620 nm.

Immunofluorescence Staining

Under anesthesia, the rats were perfused via the ascending aorta with 150 mL of PBS, followed by 300 mL of 4% paraformaldehyde. The brains were removed, postfixed overnight at 4°C, and then stored in 30% phosphate-buffered sucrose solution, pH 7.4. After being incubated overnight at 4°C, the brains were quickly frozen in isopentane (-80°C) and stored at -80°C until sectioning. Sagittal sections (10 μ m) were cut on a cryostat. Double-fluorescence labeling was performed on a subset of sections. After washing 3 times with PBS, the sections were permeabilized with 0.3% Triton X-100 in PBS for 15 minutes and then incubated with 5% normal goat serum in PBS for 30 minutes followed by incubation overnight with rabbit polyclonal VEGF antibody (1:100, Santa Cruz Biotechnology, Inc.), and a 1.5-hour incubation with anti-rabbit Alexa Fluor 555 (1:100, Beyotime, Beijing, China). Then, the sections were incubated overnight with mouse anti-NeuN Alexa Fluor 488-conjugated monoclonal antibody (1:100, Millipore, Temecula, CA). The sections were visualized using a confocal-laser scanning fluorescence microscope.

Western Blot Analysis

The brain tissues were snap-frozen in liquid nitrogen and stored at -80°C. Tissues were homogenized in a lysis buffer (20 mM Tris pH7.5, 150 mM NaCl, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM EDTA, 1% Na₃VO₄, 0.5 μ g/mL leupeptin, and 1 mM PMSF), and the homogenate was then centrifuged (10000 \times g, 5 min, 4°C). Protein samples (50 μ g) were separated on 10% SDS-polyacrylamide gels for electrophoresis, transferred onto PVDF membranes, and then blocked with 5% skimmed milk in Tris-buffered solution plus Tween-20 (TBST) at 37°C for 2 hours. The membranes were incubated overnight with rabbit anti-VEGF polyclonal antibody (1:500, Santa Cruz Biotechnology, Inc.) at 4°C. After washing in TBST (3 \times 10 minutes), the membranes were incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:500, Zhongshan Biotech Co., Beijing, China) for one hour. Finally, the proteins were visualized via enhanced chemiluminescence (ECL) using a Western blotting Luminol reagent (Beyotime, Beijing, China). β -actin was used as an internal standard. Finally, the data were analyzed using Bio-Rad software.

Statistical Analysis

All data are presented as mean \pm standard deviation (SD). The statistical significance of the nociceptive thresholds presented in Fig. 1A was tested by repeated-measures ANOVA, Evans blue detection of BBB permeability and Western blot analysis using one-way ANOVA, followed by the Dunnett test for multiple comparisons. $P < 0.05$ was considered significant. The statistical analyses were performed using SPSS Version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

IS-induced Mechanical Hypersensitivity

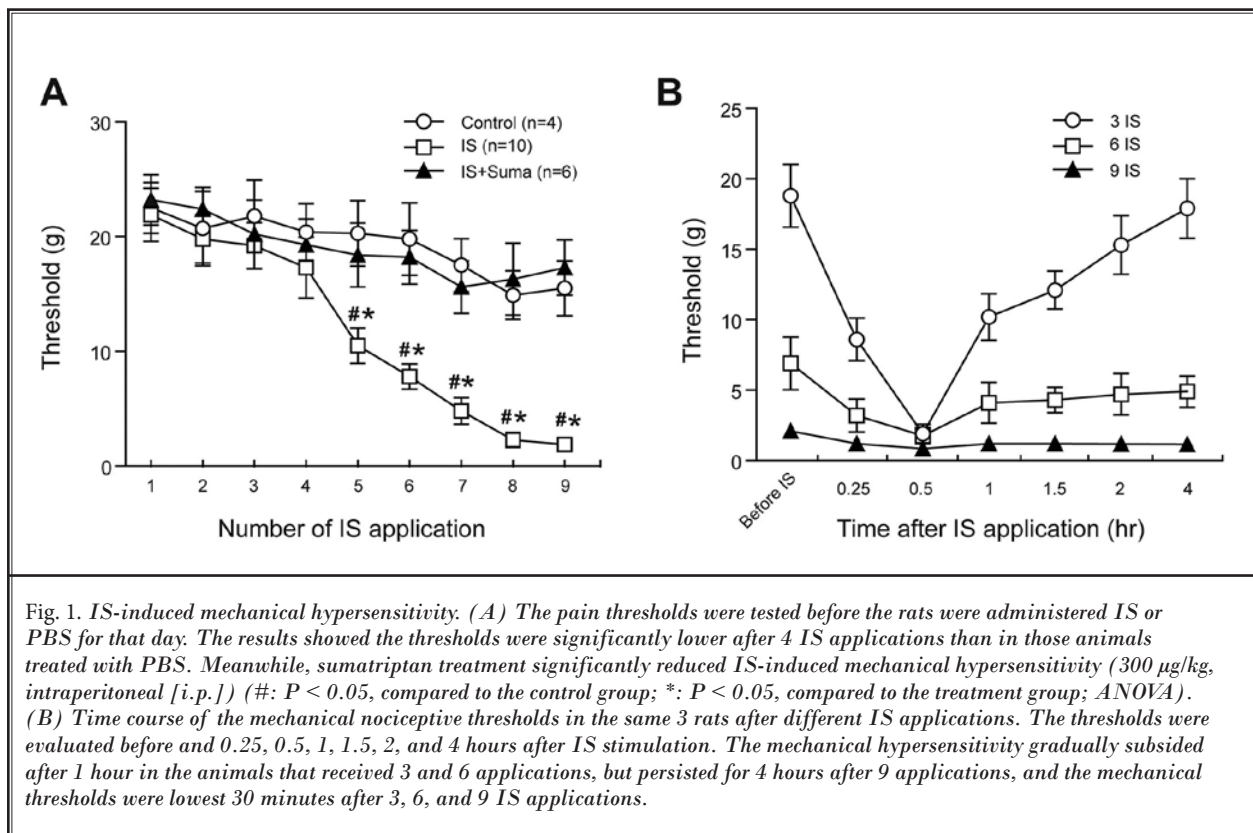
In the first week (1-3 applications) after surgery, responses to the mechanical nociceptive stimulation were not significantly different between the IS group and the control group. After 4 IS administrations, the thresholds of the IS group were significantly reduced compared with those of the control group ($P < 0.05$, Fig. 1A). Meanwhile, as the number of IS stimulations

increased, there was a tendency toward a decrease in pain thresholds. However, the thresholds of rats treated with sumatriptan remained at a level comparable to that observed in the control group ($P > 0.05$, Fig. 1A).

To determine the duration of mechanical allodynia after 3, 6, and 9 IS stimulations, the threshold changes were measured in the same 3 rats before and 0.25, 0.5, 1, 1.5, 2, and 4 hours after IS application. As Fig. 1B shows, the pain thresholds were lowest 30 minutes after 3, 6, and 9 IS applications. However, the mechanical threshold reductions induced by 3 and 6 applications gradually subsided after one hour. The mechanical hypersensitivity persisted for 4 hours after 9 applications.

BBB Permeability Changes Following Repeated IS Stimulation

The effects of repeated IS stimulations on BBB permeability were assessed at 30 minutes by intravenously administering EB. EB binds to albumin in the blood, producing a molecular complex of sufficient molecular size so that it does not cross the intact BBB. As shown



in Fig. 2A, EB extravasation was abundantly present around the blood vessels and entered the parenchyma in the frontal cortex of the bilateral hemispheres after 9 applications. We investigated several brain regions, including the brainstem, cerebellum, parietal cortex,

temporal cortex and occipital cortex, and little EB extravasation was measured. In contrast, EB was not detected after 3 stimulations, and the EB extravasation covered the lining of the vessels but did not enter the parenchyma after 6 stimulations.

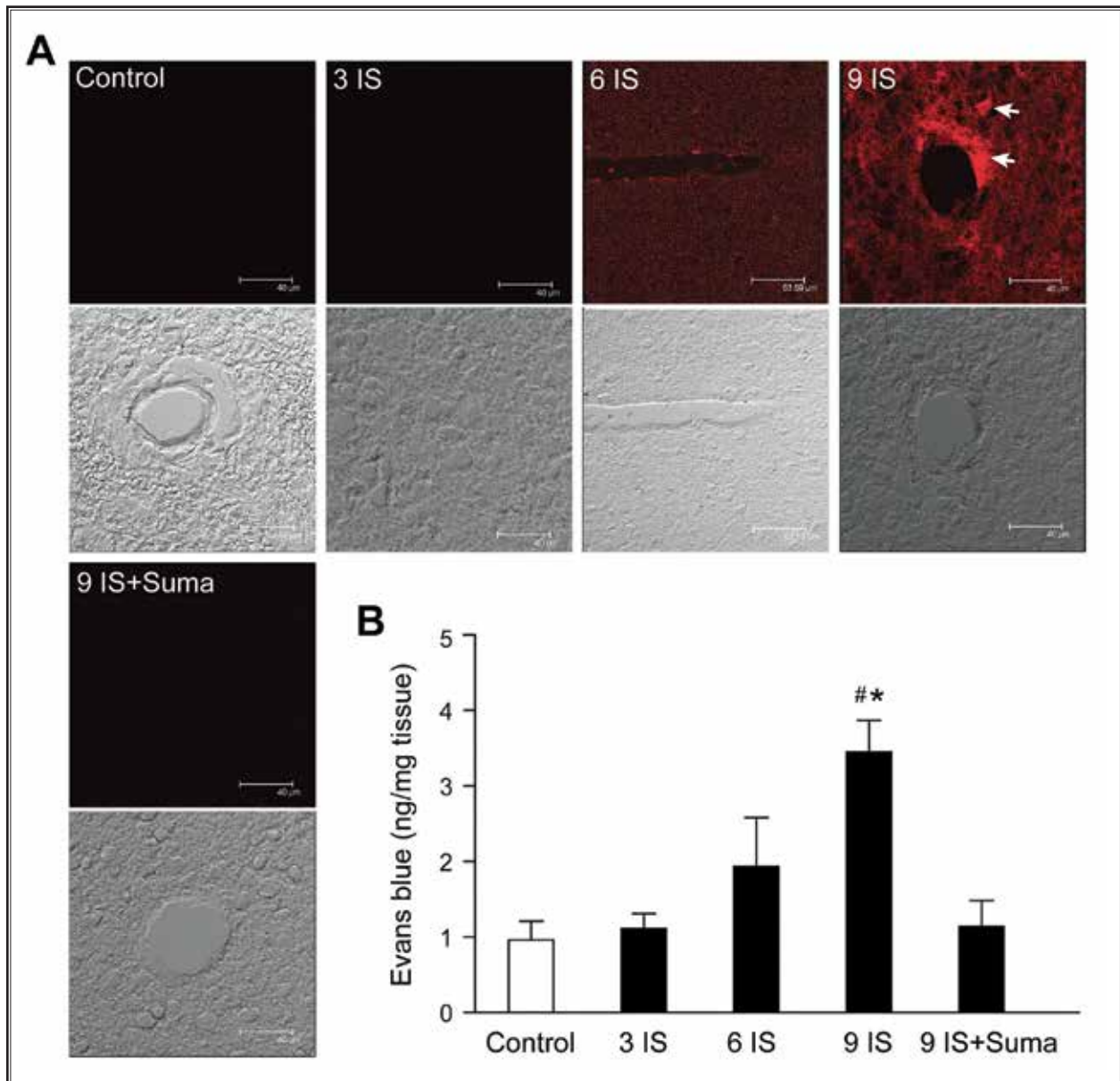


Fig. 2. Increased BBB permeability following repeated IS stimulations. (A) Representative brain sections of EB extravasation among the different groups. EB (arrows) was readily visualized in the frontal cortex after 9 IS stimulations, and EB was blocked by sumatriptan treatment. (B) Quantification of the amount of EB in the brain tissues. There were statistically significant differences among the different groups (#: $P < 0.05$, compared to the control group; *: $P < 0.05$, compared to the treatment group; one-way ANOVA followed by the Dunnett test; $n = 5$).

To investigate the effects of antimigraine drugs on BBB modulation, sumatriptan was simultaneously administered each time IS was pumped onto the dura. The rats were euthanized 30 minutes after 9 IS applications, and little EB accumulation was observed, demonstrating that sumatriptan reduced the BBB permeability (Fig. 2A).

The quantitative analysis results are presented in Fig. 2B. The levels of EB content significantly increased in the rats 30 minutes after 9 IS applications compared with those of the control group. The EB content of the brain tissue was 3.45 ± 0.42 ng/mg of tissue in the 9 IS group and 0.96 ± 0.25 ng/mg of tissue in the control group ($P = 0.001$). Sumatriptan decreased EB extravasation (1.14 ± 0.34 ng/mg of tissue vs 3.45 ± 0.42 ng/mg of tissue, $P = 0.002$). In addition, the levels of EB were not significantly different among the 3 IS, 6 IS, and control groups (1.11 ± 0.20 ng/mg of tissue vs 1.93 ± 0.65 ng/mg of tissue vs 0.96 ± 0.25 ng/mg of tissue, respectively; $P = 0.058$).

Changes in VEGF Protein Expression

The expression of VEGF in the frontal cortex was evaluated 30 minutes after repetition of the IS stimulations. As shown in Fig. 3, there was a mild increase in VEGF expression after 3 IS applications, compared with that in the control animals. Additionally, VEGF expression was markedly increased after 9 applications. The fluorescence results showed that VEGF was widely expressed in neurons, and inhibition of nociception by sumatriptan reduced the expression of VEGF.

The expression of VEGF protein was quantified by Western blot analysis. VEGF expression increased as the number of IS stimulations increased. However, this difference was not statistically significant among the control, 3 IS, and 6 IS groups. A significant upregulation of VEGF expression was detected after 9 applications compared with that in the control group. Furthermore, sumatriptan reduced the expression of VEGF ($P < 0.05$; Fig. 4).

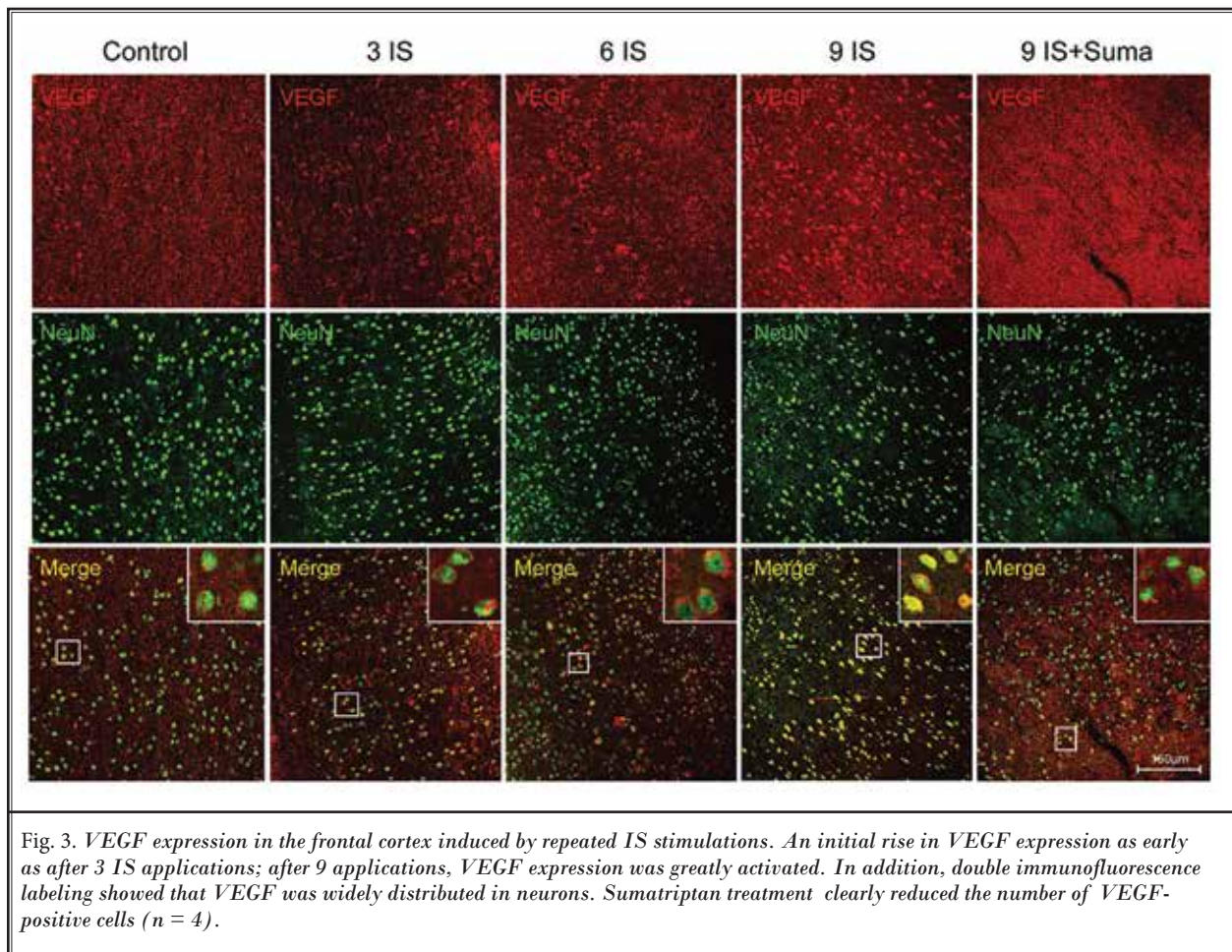
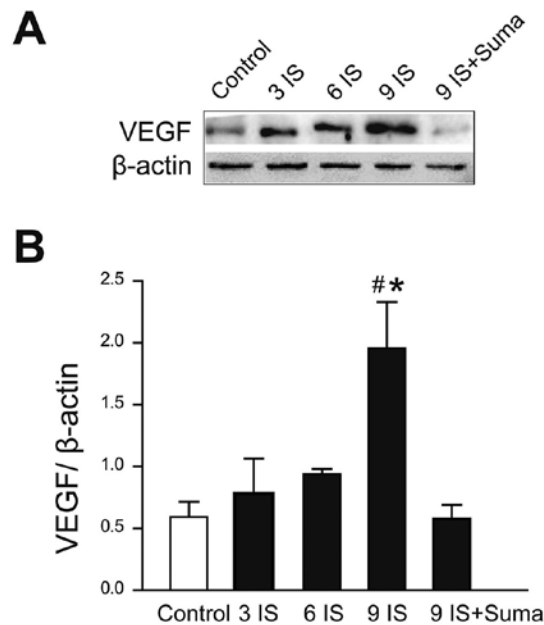


Fig. 3. VEGF expression in the frontal cortex induced by repeated IS stimulations. An initial rise in VEGF expression as early as after 3 IS applications; after 9 applications, VEGF expression was greatly activated. In addition, double immunofluorescence labeling showed that VEGF was widely distributed in neurons. Sumatriptan treatment clearly reduced the number of VEGF-positive cells ($n = 4$).

Fig. 4. IS stimulation upregulated the expression of VEGF protein. (A) Representative Western blot of VEGF expression levels among the different groups. (B) Densitometry analysis of the bands for VEGF expression. Compared with the control group, there was a slight increase in the 3 and 6 IS groups, but this change did not reach statistical significance. After 9 IS applications, the VEGF expression significantly increased. After treatment with sumatriptan, VEGF expression was greatly attenuated (#; $P < 0.05$, compared to the control group; *, $P < 0.05$, compared to the treatment group; one-way ANOVA followed by the Dunnett test; $n = 5$).



DISCUSSION

In the present study, using an established animal model of migraine, we observed that recurrent headaches induced by 9 episodic dural IS stimulations increased BBB permeability in awake rats. BBB disruption was associated with an elevation in VEGF expression. The mechanical thresholds were reduced after 4 IS stimulations, which was consistent with the findings of a previous study (5). Controlling pain with the anti-migraine drug sumatriptan effectively prevented both BBB disruption and VEGF expression.

Pain is a complex phenomenon that involves both the peripheral and central immune systems and the responses of the central nervous system (CNS), as well as the activation of the hypothalamic-pituitary-adrenal (HPA) axis (25). The pain pathway is classically thought of as a chain of neurons that extend from the periphery into the cerebral cortex, wherein one neuron relays action potentials to the next neuron. Although the pathogenesis of migraine is unclear, neurogenic inflammation that activates the trigeminovascular system is thought to be involved in the mechanism of migraine (2). This system includes the peripherally afferent terminals of the trigeminal nerve innervating cerebral vessels, as well as dura and pia mater. The cell bodies of trigeminal neurons extend their axonal projections to the caudal brainstem and the rostral regions of the cer-

vical spinal cord in the region of the trigeminal nucleus caudalis (TNC) (4). The pain signals from the afferent projections of the trigeminal nerve are transmitted to the TNC via the trigeminothalamic tract to higher CNS centers during migraine attacks. The peripheral mechanism of migraine is mainly the activation of the peripheral trigeminal nerve vascular system. The responses of the CNS to pain include the production and release of proinflammatory cytokines and growth factors, which further exaggerate pain transmission. These peripheral and CNS events may induce alterations in BBB permeability (26,27).

Traditionally, migraine has been considered a benign disorder without long-term consequences for the brain. However, in recent years, a growing body of evidence has reported that migraine is associated with an increased risk of structural brain lesions, including WMLs and clinically silent infarct-like lesions (10,11,28). The mechanism is not clear but may be attributed to BBB damage. In some ways, the conclusion of our study also supports this hypothesis.

In the current study, the time point of 30 minutes after IS application was chosen to measure BBB permeability and VEGF expression because the mechanical threshold was lowest at 30 minutes after stimulation. We employed exogenous EB, which binds to albumin, to assess and quantify BBB permeability. Albumin is

normally confined to the luminal side of endothelial cells, and its extravasation represents BBB damage (29). Neither 3 nor 6 IS stimulations induced albumin leakage into the brain parenchyma, but albumin extravasation occurred after 9 stimulations over 3 weeks. This observation indicates that repeated IS stimulations that mimic recurrent headache, enhanced BBB permeability, and chronic and high frequency headaches may be associated with BBB changes. This experimental result is consistent with clinical evidence showing that higher frequency migraine attacks are associated with more vulnerable WMLs (10,30). In addition, we found that the levels of EB significantly increased in the frontal cortex, a finding that may relate to the participation of this region in the pathophysiological effects of pain processing.

VEGF is expressed in the developing brain during embryogenesis, together with its receptors (31,32). In the adult brain, VEGF is expressed mainly by neurons at low levels (33,34). However, VEGF is upregulated under hypoxic, inflammatory, and tumorous conditions and is involved in the BBB disruption under these pathological conditions (14,17,19,35). Previous studies have demonstrated that VEGF and its receptor mediate the pathogenesis of neuropathic pain, and anti-VEGF treatment increases mechanical withdrawal thresholds and thermal withdrawal latencies in rats after chronic constriction injury (21). The results of our study are consistent with those of previous studies in that VEGF expression was enhanced after repeated IS stimulations in neurons. This observation possibly indicates that the CNS responded via VEGF expression to chronic and high-frequency pain stimulations. Although the IS application was on a small area of the dura, the repeated stimulations produced a chronic state of trigeminal hypersensitivity and potentiated the CNS central response to external stimulation.

In the current study, in order to further demonstrate the changes in BBB permeability and VEGF expression mediated by nociceptive pain, we blocked the pain from the headache using an analgesic without anti-inflammatory properties, namely, sumatriptan. Sumatriptan, a relatively specific drug for acute migraine

attacks, is commonly used in clinical settings (36). Sumatriptan can inhibit the release of pronociceptive transmitters, including CGRP, via activation of 5-HT_{1B/1D} receptors on trigeminal afferents, thus terminating the throbbing headache and preventing the development of central sensitization when administered as early as the onset of migraine (37-39). In the present study, simultaneous administration of sumatriptan with IS stimulation inhibited transmission of noxious signaling, which may have been responsible for the prevention of VEGF overexpression and BBB leakage.

This study had several limitations. First, only one time point (30 minutes) after IS stimulation was assessed for BBB permeability. This provided a snapshot but not a temporal observation, and it was not clear if the BBB disruption continued for a longer period of time or if 30 minutes after stimulation was the peak time of the BBB disruption. Second, the IS was composed of histamine, serotonin, bradykinin, and prostaglandin E₂, and these cytokines themselves may have adverse effects on the BBB. However, administration of the effective anti-migraine drug sumatriptan, which has no anti-inflammatory properties, prevented mechanical threshold reductions, VEGF expression upregulation, and BBB leakage, indicating that the major actions on VEGF and BBB are mediated by nociceptive pain. Third, in the present study, we observed the phenomenon involving significant increases in the BBB permeability and VEGF expression after 9 IS stimulations; however, the potential mechanism was not identified.

CONCLUSIONS

In summary, repeated IS stimulations to the dura decreased mechanical thresholds and increased VEGF expression and BBB permeability in a rat model of migraine. This study demonstrates that chronic pain stimulations may lead to BBB disruption in an animal model, which explains some neuroimaging observations in migraine. Early intervention targeting pain control may not only alleviate feelings of pain, but also preserve BBB function and prevent potential brain lesions.

REFERENCES

- Headache Classification Subcommittee of the International Headache Society. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia* 2004; 24 Suppl 1:9-160.
- Williamson DJ, Hargreaves RJ. Neurogenic inflammation in the context of migraine. *Microsc Res Tech* 2001; 53:167-178.
- Burstein R, Yamamura H, Malick A, Strassman AM. Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. *J Neurophysiol* 1998; 79:964-982.
- Wieseler J, Ellis A, Sprunger D, Brown K, McFadden A, Mahoney J, Rezvani N, Maier SF, Watkins LR. A novel method for modeling facial allodynia associated with migraine in awake and freely moving rats. *J Neurosci Methods* 2010; 185:236-245.
- Oshinsky ML, Gomonchareonsiri S. Episodic dural stimulation in awake rats: A model for recurrent headache. *Headache* 2007; 47:1026-1036.
- Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis* 2010; 37:13-25.
- DosSantos MF, Holanda-Afonso RC, Lima RL, DaSilva AF, Moura-Neto V. The role of the blood-brain barrier in the development and treatment of migraine and other pain disorders. *Front Cell Neurosci* 2014; 8:302.
- Dreier JP, Jurkat-Rott K, Petzold GC, Tomkins O, Klingebiel R, Kopp UA, Lehmann-Horn F, Friedman A, Dichgans M. Opening of the blood-brain barrier preceding cortical edema in a severe attack of FHM type II. *Neurology* 2005; 64:2145-2147.
- Gentile S, Rainero I, Daniele D, Binello E, Valfre W, Pinessi L. Reversible MRI abnormalities in a patient with recurrent status migrainosus. *Cephalalgia* 2009; 29:687-690.
- Kruit MC, van Buchem MA, Hofman PA, Bakkens JT, Terwindt GM, Ferrari MD, Launer LJ. Migraine as a risk factor for subclinical brain lesions. *JAMA* 2004; 291:427-434.
- Kruit MC, van Buchem MA, Launer LJ, Terwindt GM, Ferrari MD. Migraine is associated with an increased risk of deep white matter lesions, subclinical posterior circulation infarcts and brain iron accumulation: The population-based MRI CAMERA study. *Cephalalgia* 2010; 30:129-136.
- Simpson JE, Wharton SB, Cooper J, Gellsthorpe C, Baxter L, Forster G, Shaw PJ, Savva G, Matthews FE, Brayne C, Ince PG. Alterations of the blood-brain barrier in cerebral white matter lesions in the ageing brain. *Neurosci Lett* 2010; 486:246-251.
- Tomimoto H, Akiguchi I, Suenaga T, Nishimura M, Wakita H, Nakamura S, Kimura J. Alterations of the blood-brain barrier and glial cells in white-matter lesions in cerebrovascular and Alzheimer's disease patients. *Stroke* 1996; 27:2069-2074.
- van Bruggen N, Thibodeaux H, Palmer JT, Lee WP, Fu L, Cairns B, Tumas D, Gerlai R, Williams SP, van Lookeren Campagne M, Ferrara N. VEGF antagonism reduces edema formation and tissue damage after ischemia/reperfusion injury in the mouse brain. *J Clin Invest* 1999; 104:1613-1620.
- Nag S. The blood-brain barrier and cerebral angiogenesis: Lessons from the cold-injury model. *Trends Mol Med* 2002; 8:38-44.
- Nag S, Takahashi JL, Kilty DW. Role of vascular endothelial growth factor in blood-brain barrier breakdown and angiogenesis in brain trauma. *J Neuro-pathol Exp Neurol* 1997; 56:912-921.
- Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: Role of the HIF system. *Nat Med* 2003; 9:677-684.
- Ferrara N. Vascular endothelial growth factor: Basic science and clinical progress. *Endocr Rev* 2004; 25:581-611.
- Nagy JA, Dvorak AM, Dvorak HF. VEGF-A and the induction of pathological angiogenesis. *Annu Rev Pathol* 2007; 2:251-275.
- Nesic O, Sundberg LM, Herrera JJ, Mokkapati VU, Lee J, Narayana PA. Vascular endothelial growth factor and spinal cord injury pain. *J Neurotrauma* 2010; 27:1793-1803.
- Lin J, Li G, Den X, Xu C, Liu S, Gao Y, Liu H, Zhang J, Li X, Liang S. VEGF and its receptor-2 involved in neuropathic pain transmission mediated by P2X(2)/X(3) receptor of primary sensory neurons. *Brain Res Bull* 2010; 83:284-291.
- Hamilton JL, Nagao M, Levine BR, Chen D, Olsen BR, Im HJ. Targeting VEGF and its receptors for the treatment of osteoarthritis and associated pain. *J Bone Miner Res* 2016; 31:911-924.
- Goncalves FM, Martins-Oliveira A, Speciali JG, Izidoro-Toledo TC, Luizon MR, Dach F, Tanus-Santos JE. Vascular endothelial growth factor genetic polymorphisms and haplotypes in women with migraine. *DNA Cell Biol* 2010; 29:357-362.
- Bingham S, Davey PT, Sammons M, Raval P, Overend P, Parsons AA. Inhibition of inflammation-induced thermal hypersensitivity by sumatriptan through activation of 5-HT(1B/1D) receptors. *Exp Neurol* 2001; 167:65-73.
- Wolka AM, Huber JD, Davis TP. Pain and the blood-brain barrier: Obstacles to drug delivery. *Adv Drug Deliv Rev* 2003; 55:987-1006.
- Watkins LR, Maier SF. The pain of being sick: Implications of immune-to-brain communication for understanding pain. *Annu Rev Psychol* 2000; 51:29-57.
- Watkins LR, Maier SF. Immune regulation of central nervous system functions: From sickness responses to pathological pain. *J Intern Med* 2005; 257:139-155.
- Palm-Meinders IH, Koppen H, Terwindt GM, Launer LJ, Konishi J, Moonen JM, Bakkens JT, Hofman PA, van Lew B, Middelkoop HA, van Buchem MA, Ferrari MD, Kruit MC. Structural brain changes in migraine. *JAMA* 2012; 308:1889-1897.
- Brightman MW, Klatzo I, Olsson Y, Reese TS. The blood-brain barrier to proteins under normal and pathological conditions. *J Neurol Sci* 1970; 10:215-239.
- Schmitz N, Admiraal-Behloul F, Arkin EB, Kruit MC, Schoonman GG, Ferrari MD, van Buchem MA. Attack frequency and disease duration as indicators for brain damage in migraine. *Headache* 2008; 48:1044-1055.
- Breier G, Albrecht U, Sterrer S, Risau W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. *Development* 1992; 114:521-532.
- Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Ullrich A. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* 1993; 72:835-846.
- Alvarez JA, Baird A, Tatum A, Daucher J, Chorsky R, Gonzalez AM, Stopa EG. Localization of basic fibroblast growth factor and vascular endothelial growth factor in human glial neoplasms. *Mod*

- Pathol* 1992; 5:303-307.
34. Monacci WT, Merrill MJ, Oldfield EH. Expression of vascular permeability factor/vascular endothelial growth factor in normal rat tissues. *Am J Physiol* 1993; 264:C995-1002.
35. Machein MR, Plate KH. VEGF in brain tumors. *J Neurooncol* 2000; 50:109-120.
36. Ferrari MD, Roon KI, Lipton RB, Goadsby PJ. Oral triptans (serotonin 5-HT_{1B/1D} agonists) in acute migraine treatment: A meta-analysis of 53 trials. *Lancet* 2001; 358:1668-1675.
37. Vanmolkot F, Van der Schueren B, de Hoon J. Sumatriptan causes parallel decrease in plasma CGRP concentration and migraine headache during nitroglycerin-induced migraine attack. *Cephalalgia* 2006; 26:1037-1038.
38. Suwattanasophon C, Phansuwan-Pujito P, Srikiatkachorn A. 5-HT_{1B/1D} serotonin receptor agonist attenuates nitroglycerin-evoked nitric oxide synthase expression in trigeminal pathway. *Cephalalgia* 2003; 23:825-832.
39. Burstein R, Jakubowski M. Analgesic triptan action in an animal model of intracranial pain: A race against the development of central sensitization. *Ann Neurol* 2004; 55:27-36.