Beneficial effects of edaravone on the expression of serum matrix metalloproteinase-9 after cerebral hemorrhage

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ABSTRACT

الأهداف: لاستكشاف آثار الادرافوني للتعبير عن مصفوفة مصل الفلزي9- (MMP-9) الخلفي لنزيف فى المخ، وتحليل العلاقة بين هذا التعبير والعجز العصبي.

الطريقة: تم تضمين مجموعة من 160 مريضاً مصابين بنزيف العقد القاعدية والمسجلين في 4 مستشفيات خلال فترة من أبريل 2009م إلى يوليو2011 م، وتنقسم إلى مجموعة معالجة ومجموعة شاهدة (ن = 80). تم علاج جميع المرضى الذين يعانون من ورم دموي عدواني صغير باستئصاله، وكانت تدار مجموعة العلاج في وقت واحد مع الاندرافوني. تم قياس مستويات مصل MMP-9 بالنقر المزدوج على الأجسام المضاد باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (تقنية الألايزا). تم تحديد مجموعتين من مقياس المعاهد الوطنية لصحة السكتة الدماغية (NIHSS) ومقياس غلاسكو كوما قبل وبعد العلاج.

النتائج: اختلفت إجمالي المعدلات الشاملة للعلاج (86.3%) ومجموعات التحكم (75.0%) بشكل كبير ($p<0.05$). كانت مستويات مصل MMP-9 لمجموعتين مشابهة ($p>0.05$) وقبل العلاج انخفضت بشكل ملحوظ ($p<0.05$) بعد العلاج، وتلك المجموعتين اختلفت أيضا بشكل ملحوظ ($p<0.05$). و كانت درجات NIHSS لمجموعتين مشابهة ($p>0.05$) و انخفضت قبل العلاج بشكل كبير ($p<0.05$) بعد العلاج، واختلفت تلك المجموعتين اختلافاً كبيراً ($p<0.05$). وكشف تحليل الارتباط بيرسون أن مستوى مصل MMP-9 ارتبط بشكل كبير مع النتيجة NIHSS قبل العلاج (R=0.491).

الخاتمة: يتم علاج الورم الدموي العدواني الصغير والادرافوني بشكل فعال في نزيف بالدماغ مباشرة من خلال خفض مستوى المصل MMP-9.

Objective: To explore the effects of edaravone on the expression of matrix metalloproteinase-9 (MMP-9) posterior to cerebral hemorrhage, and to analyze the relationship between this expression and neurological deficit.

Methods: A total of 160 basal ganglia hemorrhage patients enrolled in Dongfeng Hospital, Hubei University of Medicine, Shiyan, China between April 2009 and July 2011 were included and divided into a treatment group and a control group (n=80). All patients were treated with minimally invasive hematoma evacuation, and the treatment group was administered with edaravone simultaneously. Serum MMP-9 levels were measured by double-antibody sandwich enzyme-linked immunosorbent assay. The National Institutes of Health Stroke Scale (NIHSS) and Glasgow Coma Scale scores of the 2 groups were determined before and after treatment.

Results: The overall effective rates of the treatment (86.3%) and control (75.0%) groups differed significantly ($p<0.05$). The serum MMP-9 levels of the 2 groups that were similar ($p>0.05$) before treatment significantly decreased ($p<0.05$) after treatment, and those of the 2 groups also differed significantly ($p<0.05$). The NIHSS scores of the 2 groups that were similar ($p>0.05$) before treatment also significantly decreased ($p<0.05$) after treatment, and those of the 2 groups differed significantly ($p<0.05$). Pearson's correlation analysis revealed that the level of serum MMP-9 was significantly correlated with the NIHSS score before treatment (R=0.491).

Conclusion: Combined minimally invasive hematoma evacuation and edaravone effectively treated cerebral hemorrhage by directly lowering the level of serum MMP-9.

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Cerebral hemorrhage, as a common acute cerebrovascular disease, is more prone to occurring recently due to overwhelming working pressure and changes of environmental factors. A basal ganglia hemorrhage, which is a type of hypertensive intracerebral hemorrhage,[1,2] should be treated as soon as possible by effectively clearing hematomas pressing normal cerebral tissues.[3] However, traditional conservative treatment undesirably leads to mortality rates of 35-52%, thus requiring surgical protocols.[4] Minimally invasive hematoma evacuation can decrease intracranial pressure by directly and rapidly relieving the hematoma pressure to cerebral tissues. Particularly, burr hole surgery, as a minor traumatic and easily operating surgical protocol, is suitable for basal ganglia hemorrhage patients under critical conditions. Edaravone, which is a new potent drug, can block the peroxidation of lipid mainly by scavenging free radicals.[5] In addition, edaravone can protect cerebral tissue and ischemic neurons around the hematoma from damage, thus improving the clinical treatment of cerebral hemorrhage by mitigating edema.[6,7] Matrix metalloproteinases (MMPs) are a family of endopeptidases that are able to degrade extracellular matrix proteins, of which MMP-9 participates in the breakdown of the blood-brain barrier during the formation of cerebral edema after hemorrhage, as suggested by previous animal and clinical studies.[8,9] However, relevant studies in clinical practice remain insufficient. Therefore, the aim of this study was to evaluate the effects of edaravone on the expression of MMP-9 after cerebral hemorrhage. We also wanted to analyze the relationship between its expression and the neurological deficits of patients by determining the changes of MMP-9 levels before and after minimally invasive hematoma evacuation.

Methods.

Study subjects. A total of 160 basal ganglia hemorrhage patients enrolled in Dongfeng Hospital, Hubei University of Medicine, Shiyan, China between April 2009 and July 2011 were included. The Ethics Committee of Dongfeng Hospital Affiliated to Hubei University of Medicine approved the study. Written consent was obtained before examination and treatment for enrolled patients according to the Declaration of Helsinki and relevant laws in China. All treatments were performed based on the patients' best interests.

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Inclusion criteria. Patients were selected in accordance with the diagnostic criteria stipulated by the 4th National Conference on Cerebrovascular Disease (1995),[10] and verified by cranial CT. Patients were no younger than 30 years old and no older than 80 years old. Basal ganglia hemorrhage patients who were subjected to first onset within 24 hours. Exclusion criteria included patients who had taken anticoagulant drugs or edaravone, and patients with severe heart, liver, and kidney diseases. The patients were divided into a treatment group and a control group according to the treatment methods (n=80), without significant differences in their gender, age, height, body weight, cerebral hemorrhage risk factors, Glasgow Coma Scale (GCSs), National Institute of Health stroke scale (NIHSSs) and hematoma volume ($p>0.05$) (**Table 1**).

Treatment methods. All patients were subject to minimally invasive hematoma evacuation. After regular skin shaving, the puncture site that bled the most while being located closest to the skull was determined by CT, and a proper YL-1 type puncture needle was selected according to the length from puncture site to hematoma center. The patients were then locally anesthetized by 2% lidocaine. After puncturing the skull with an electric drill, the puncture needle was replaced with a blunt plastic one that was slowly pushed into the hematoma center. Then, the semi-liquid hematoma was slowly sucked by a 5 ml syringe after removing the needle, connecting a side pipe, and tightly closing the lid. In case of suction issues, the hematoma was smashed by a specific needle and rinsed with normal saline (same amount). Another 3-5 ml of normal saline containing 10-20 thousands IU urokinase (Tianjin Biochemical

Table 1 - Demographic details of basal ganglia hemorrhage patients.

Item	Control (n=80)	Treatment (n=80)	χ^2 or t	*P*
Gender (M/F)	42/38	41/39	0.065	0.783
Age (years old)	56.33 ± 6.44	56.83 ± 6.91	0.125	0.673
Height (cm)	171.25 ± 10.63	171.68 ± 9.32	0.136	0.653
Body weight (kg)	66.25 ± 3.62	66.45 ± 4.18	0.098	0.712
History of smoking (n)	41	43	0.158	0.612
History of drinking (n)	45	46	0.056	0.813
GCS	11.25 ± 3.63	11.35 ± 4.12	0.158	0.612
NIHSS	13.25 ± 4.12	13.33 ± 5.12	0.369	0.432
Hematoma volume (ml)	150.25 ± 0.45	151.36 ± 10.88	0.263	0.684

GCS - Glasgow Coma Scale,
NIHSS - National Institute of Health Stroke Scale

Table 2 - Comparison of the therapeutic effects (n) of edaravone between the treatment and control groups of basal ganglia hemorrhage patients.

Group	Case No.	Recovery	Markedly effective	Effective	Ineffective	Overall effective rate
Treatment	80	11	23	35	11	86.3%
Control	80	7	13	40	20	75.0%
χ^2						4.086
P						0.021

Pharmaceutical Co., Ltd., National Medicine Permit No. H12020492, Tianjin, China) was injected into the puncture hole and the drainage pipe was closed when the drainage fluid became obviously light-colored. The pipe was reopened after 2-4 hours. Then rinsing, suction, and drainage was performed according to CT results (2-4 times/d) until over 70% of the hematoma had disappeared, after which the drainage pipe was closed for another 24 hours. In case of any abnormality, the needle was immediately removed while suturing and bandaging to prevent CSF leakage. On average, rinsing, liquefying, and drainage was performed 5.2 times. The needle was retained for 2-15 days, with an average of 9.5 days. Blood pressure was maintained stable to prevent edema and infection, and to support treatment. The treatment group was then administered with edaravone (Xi'an Lijun Pharmaceutical Co., Ltd., National Medicine Permit No. H20120042, Xi'an, China) for 2 consecutive weeks (bid) as a course of treatment. They were treated with 2 courses in total.

Prognostic therapeutic effects. Therapeutic effect criteria. The patients were evaluated according to their symptoms and previous studies. Basic recovery - decrease of neurological deficit score by 91-100%; markedly effective - decrease of neurological deficit score by 46-90%; effective - decrease of neurological deficit score by 18-45%; ineffective - decrease of neurological deficit score by <17%. Overall effective rate = (recovery rate + markedly effective rate + effective rate) × 100%.

Determination of MMP-9 levels of fasting venous blood samples. Fasting venous bloods (4 ml) were collected before and after treatment, centrifuged at 3000 r/min at low temperature after anticoagulation, and stored at -80°C after separating the supernatant. Serum MMP-9 levels were measured by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) according to the instructions of the kit purchased from Chengdu Jingbo Biotechnology Co., Ltd. (produced by Shanghai Senxiong Biotechnology Co., Ltd., Shanghai, China).

Determination of scores. The NIHSS and GCS scores of the 2 groups were determined before and after treatment.

Statistical analysis. All data were analyzed by SAT 12.0 (Stanford University, California, USA). The numerical data were compared by $\chi 2$ test, and the measurable data were compared by analysis of variance and t test. Correlations were analyzed by Pearson's correlation analysis. $P<0.05$ was considered statistically significant.

Results.

Comparison between therapeutic effects. The overall effective rate of the treatment group (86.3%) was significantly different to that of the control group (75%) after treatment ($p<0.05$) (**Table 2**).

Changes of serum MMP-9 level. The serum MMP-9 levels of the 2 groups, which were similar before treatment ($p>0.05$), became significantly different ($p<0.05$) with evident decreases in both ($p<0.05$) (**Table 3 and Figure 1**).

Comparison between NIHSS scores. The NIHSS scores of the 2 groups, which were similar before treatment ($p>0.05$), became significantly different ($p<0.05$) after treatment, with evident decreases in both ($p<0.05$) (**Table 4**).

Correlation analysis. Pearson's correlation analysis revealed that the MMP-9 level was significantly correlated with NIHSS score (R=0.491) before treatment ($p=0.009$) (**Figure 2**).

Discussion.

Cerebral hemorrhage, an extremely dangerous and common brain complication among the elderly, mainly results from the spontaneous rupture of intracerebral blood vessels induced by hypertension. The prognostic factors of hypertensive intracerebral

Table 3 - Serum MMP-9 levels (ng/ml, mean ± SD) among basal ganglia hemorrhage patients.

Group	Case (n)	Before	After	t	*P*
Treatment	80	66.52 ± 20.16	38.26 ± 4.12	26.321	0.000
Control	80	65.15 ± 18.62	51.99 ± 6.25	12.258	0.000
t		0.362	14.251		
P		0.288	0.000		

MMP - Matrix metalloproteinases

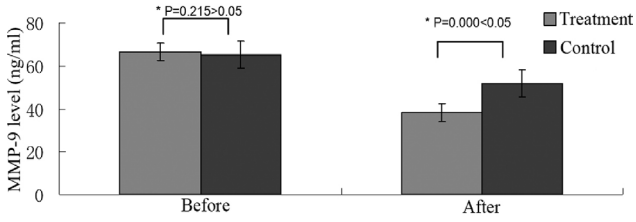

Figure 1 - Comparison of serum MMP-9 levels among basal ganglia hemorrhage patients. MMP - matrix metalloproteinase

Table 4 - National Institute of Health Stroke Scale scores (mean ± sd) among basal ganglia hemorrhage patients.

Group	Case (n)	Before	After	t	*P*
Treatment	80	13.33 ± 5.12	5.26 ± 0.52	14.632	0.000
Control	80	13.25 ± 4.37	9.52 ± 0.48	8.632	0.000
t		0.369	7.521		
P		0.215	0.000		

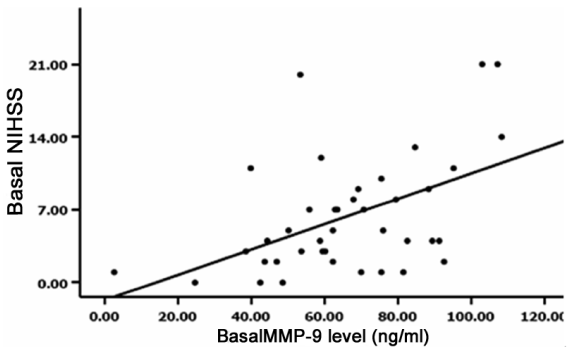

Figure 2 - Relationship between NIHSS score and MMP-9 levels among basal ganglia hemorrhage patients. NIHSS - National Institute of Health Stroke Scale, MMP - matrix metalloproteinase

hemorrhage include the position, and size of hematoma, and the degree of edema.[9] Six hours after hematoma formation, surrounding cerebral tissues are bound to undergo irreversible injuries, which may be induced by enlarged hematoma, edema, and hydrocephalus. Therefore, a hematoma should be evacuated as soon as possible to alleviate damage to intracerebral nerves, and to protect other tissues from pressure.[11]

Hypertensive intracerebral hemorrhage should be treated by decreasing intracranial pressure via water removal, mitigating edema, monitoring blood pressure, relieving hematoma-induced secondary injuries, and preventing complications. Moderate and major cerebral hemorrhage patients are mainly treated with minimally invasive surgery, which is secure and facile with satisfactory effects, with lower costs, and lower morality rates. This method outweighs conservative internal medical protocols, and craniotomy.[12]

Edaravone, a novel and potent hydroxyl radical-scavenging agent and antioxidant, mainly comprises 3-methyl-1-phenyl-2-pyrazoline-5-one as a lipophilic group, with a blood-brain barrier-penetrating rate of 60%. Since edaravone does not affect blood coagulation, it does not increase the risk of hemorrhage during treatment. Ning et al[6] reported that the morality rates of edaravone-treatment (15.6%) and control (48.4%) groups differed significantly, which indicated regular drugs plus edaravone were able to reduce the morality rates of patients with acute massive brain infarction at the middle cerebral artery, and to augment their survival rates. In this study, the overall effective rates of the treatment and control groups were 86.3% and 75% after treatment, with a statistically significant difference ($p<0.05$). The NIHSS scores of the 2 groups that were similar ($p>0.05$) before treatment also significantly decreased ($p<0.05$) after treatment, and the scores of the 2 groups differed significantly ($p<0.05$), suggesting that combined edaravone and regular minimally invasive surgery functioned better than individual surgical treatment.

The MMPs are a family of Zn^{2+}-depending proteases that only work at neutral pH and participate in the degradation or reconstruction of extracellular matrix in vivo. In the CNS, the MMPs participate in inflammatory reactions of neurological diseases by destruction of the hemato-encephalic barrier after degrading matrix constituents.[13] The MMP-9, which is synthesized by vascular endothelial cells, monocyte macrophages, and neutrophils, and so forth, is excreted as an inactive zymogen. After being activated by hydrolysis of plasmin, MMP-9 is bound to break the hemato-encephalic barrier and result in vasogenic cerebral edema by damaging extracellular matrix and basilar membrane. Recently, the effects of MMP-9 on the early diagnosis of hypertensive cerebral hemorrhage as well as the determination of focus volume and edema have been confirmed, which offers new insight into relevant clinical treatment.[9] However, this study only involved patients with basal ganglia hemorrhage. Whether all the hypertensive cerebral hemorrhage patients can be treated by the method herein still needs further studies.

Tejima et al[14] found that poly ADP ribose polymerase (PARP) inhibitors could reduce the expressions of MMP-9 and NF-κB as well as the infiltration of neutrophils. Zhang et al[15] prepared a series of 1-hydroxy-2-pyridine inhibitors based on the MMP-9 inhibitor hydroxamate and demonstrated that they functioned well in an animal model. In addition, they found that the thiirane-type gelatinase inhibitor SB-3CT managed to inhibit the activity of MMP-9, indicating MMP-9 was a promising target for anti-cerebral infarction drugs, and SB-3CT derivatives were potentially therapeutically

effective. The serum MMP-9 levels of the 2 groups that were similar before treatment significantly decreased (p<0.05) after treatment, and those of the 2 groups also differed significantly (p<0.05). After analyzing the samples of cerebral hemorrhage patients 6 hours after their deaths, Koh et al[16] reported that the MMP-9 levels dramatically increased, being mainly distributed surrounding blood vessels and accompanied by the infiltration of neutrophils and active microglias.

In this study, the MMP-9 level and NIHSS score (R=0.491) were significantly correlated before treatment (p<0.05), suggesting that the MMP-9 level was able to mark the degree of neurological deficits.[17] Castellanos et al[18] found that the plasma MMP-9 levels of hemorrhagic transformation patients were higher than those of other cerebral infarction patients, revealing that the elevated plasma MMP-9 level was an independent index marking hemorrhagic transformation. Ishikawa et al[8] reported that edaravone mitigated the edema of acute cerebral infarction patients by reducing the expression of vascular endothelial growth factor (VEGF) in astrocytes under hypoxia inducible factor (HIF)-1a-inhibited hypoxic conditions. Kelly et al[13] reported that the early enhanced oxidation reactions of cerebral infarction patients were associated with MMP-9 expression, but they also claimed that the results required further demonstration. Niyaz et al[11] established a rat cerebral infarction model to verify that MCI-186 decreased cell apoptosis by enhancing intracellular signaling transduction in addition to scavenging free radicals. Therefore, it is of great significance to explore the changes of other serum cytokines in the treatment of cerebral hemorrhage.

Although this study has verified the beneficial effects of edaravone on cerebral hemorrhage, the detailed mechanism has not been thoroughly investigated. Its therapeutic effects on different types of cerebral hemorrhage should be further evaluated. In the meantime, the effects of edaravone on MMP-9 expression are still ongoing in our group.

In summary, combined minimally invasive surgery and edaravone can effectively treat cerebral hemorrhage by directly lowering the level of serum MMP-9. Since relevant clinical studies remain scarce, the findings in this study are valuable and useful for further studies with larger sample size.

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