Am J Transl Res 2022;14(4):2617-2624
www.ajtr.org /ISSN:1943-8141/AJTR0139501

Original Article

Effect of nimodipine combined with fasudil on vascular endothelial function and inflammatory factors in patients with cerebral vasospasm induced by aneurysmal subarachnoid hemorrhage

Hui Ye, Zaiming Liu, Long Zhou, Qiang Cai

Department of Neurosurgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China

Received September 29, 2021; Accepted December 12, 2021; Epub April 15, 2022; Published April 30, 2022

Abstract: Objective: Aneurysmal subarachnoid hemorrhage (aSAH) is an acute disease with rapid progression and critical condition. The most common complication of aSAH is cerebral vasospasm (CVS). Patients are predisposed to severe cerebral ischemia, brain injury, or even death if effective measures are not taken in time to relieve symptoms. This study mainly determines the effect of nimodipine (NM) combined with fasudil on vascular endothelial function (VEF) and inflammatory factors (IFs) in patients with aSAH induced CVS. Methods: The clinical data of 77 patients with aSAH induced CVS treated in the Renmin Hospital of Wuhan University from March 2019 to June 2020 were analyzed retrospectively. Based on different drug therapies, patients receiving NM monotherapy were assigned to the control group (n=32), while those treated with NM combined with fasudil were included in the observation group (n=45), both received two consecutive weeks of treatment. The two arms were compared regarding the following items: clinical efficacy, average blood flow velocities (BFVs) of anterior, posterior and middle cerebral arteries, serum IFs, levels of vascular endothelial growth factor (VEGF), ET-1 and CGRP, cognitive function (Montreal Cognitive Assessment Scale, MOCA), activities of daily living (Bathel index), and adverse reactions. Results: The overall response rate of the observation group was significantly higher than that of the control group ($P<0.05$). After treatment, the BFVs of the anterior, posterior and middle cerebral arteries in the observation group decreased significantly compared with the control group ($P<0.05$). ET-1 and VEGF decreased in both groups, while CGRP increased, with more significant changes in the observation group ($P<0.05$). Serum IFs reduced in both arms, with more evident reductions in the observation group ($P<0.05$). The MOCA score and Barthel index increased statistically in both arms and were higher in the observation group compared with the control group ($P<0.05$). There was no significant difference in the total incidence of adverse reactions between the observation group and the control group ($P>0.05$). Conclusions: NM combined with fasudil in the treatment of aSAH induced CVS can effectively improve the VEF, alleviate IFs, and enhance the cognitive function and quality of life of patients, which is worth popularizing in clinic.

Keywords: Nimodipine, fasudil, aneurysmal subarachnoid hemorrhage, cerebral vasospasm, vascular endothelial function, cognitive function

Introduction

Cerebral vasospasm (CVS), one of the common and serious complications of aneurysmal subarachnoid hemorrhage (aSAH), is also one of the important factors causing death in aSAH patients [1, 2]. Clinical studies have found that aSAH is related to cerebral edema and hematoma. Moreover, some patients with CVS are prone to brain damage and cerebral ischemia, which will seriously affect the prognosis of patients if not treated in time [3, 4]. Currently, there is no specific treatment plan for CVS after aSAH, so clinical treatment is usually carried out in time after the occurrence of aSAH to prevent CVS and other serious complications [5]. Therefore, how to implement an effective treatment plan is an urgent problem to be solved in clinic.

Nimodipine (NM), as a second generation dihydropyridine L-type calcium channel blocker, has the effects of cerebral vasodilation and anti-ischemia, and can promote the improvement of

patients' cognitive function and neurological function. It is often used clinically to prevent CVS after SAH [6, 7]. However, the curative effect of NM monotherapy is not ideal, so it is particularly important to explore an appropriate combination therapy. Different from traditional calcium channel blockers, fasudil is a new drug with extensive pharmacological effects. It mainly antagonizes intracellular calcium ions, but does not depend on extracellular calcium ions, with remarkable vasodilating function, as well as potent vasodilating effects that will not be offset by propranolol, atropine and other drugs [8, 9]. Previous studies [10] have compared the efficacy of NM and fasudil monotherapy, and found that fasudil was superior to NM in curative effect, but few scholars had analyzed the effects of their combination on vascular endothelial function (VEF) and inflammatory factors (IFs) in patients with aSAH induced CVS.

Consequently, in this study, the effects of NM combined with fasudil on VEF and IF levels of patients with aSAH induced CVS were investigated, with a hope to provide more options for the treatment of CVS induced by aSAH.

Materials and methods

Clinical data

The clinical data of 77 patients (41 males and 36 females) with aSAH induced CVS admitted to the Renmin Hospital of Wuhan University from March 2019 to June 2020 were analyzed retrospectively. Patients were treated with either NM monotherapy or NM + fasudil and divided into a control group (n=32) and an observation group (n=45), respectively.

Inclusion criteria: (1) Patients with aSAH induced CVS confirmed by diagnostic criteria [11] and CT; (2) Patients with an age between 50-65 years; (3) Patients not allergic to the drugs used in the study. Exclusion criteria: (1) Patients with severe organ dysfunction; (2) Patients with drug allergies; (3) Patients with malignant tumors; (4) Patients with aSAH caused by blood diseases or tumors; (5) Patients with blood diseases or immune system diseases.

All patients signed the informed consent form to give their consent for participation. This experiment was approved by the Ethical Committee of Renmin Hospital of Wuhan University (WDRY2020-K120) and conformed to the *Declaration of Helsinki*.

Treatment methods

After intracranial aneurysm occlusion, all patients were treated with routine therapies such as keeping respiratory tract unobstructed, regulating blood pressure, dehydrating to lower down intracranial pressure, correcting shock and hemostasis, and preventing infection and complications. Patients in the control group were given 50 mL NM (Jumpcan Pharmaceutical, NMPA Approval Number H20030306), which was added into 150 mL 0.9% NaCl for intravenous drip once a day for 14 days. Patients in the observation group were additionally given 30 mg fasudil (Easton Biopharmaceuticals, NMPA Approval Number H201132-49) in 150 mL 5% glucose injection by intravenous infusion 3 times a day for 14 days.

Outcome measures

(1) The therapeutic effects, which were divided into markedly effective (signs and symptoms basically disappeared with no fresh infarct as indicated by brain CT or MRI; patients were able to take care of themselves), effective (signs and symptoms ameliorated obviously without fresh infarct as indicated by brain CT or MRI; patients were basically able to take care of themselves), and ineffective (signs and symptoms did not change, brain CT or MRI found fresh infarct, and patients could not take care of themselves), were evaluated in both arms. Overall response rate = (markedly effective + effective) cases/total cases ×100%. (2) The mean blood flow velocities (BFVs) of anterior, posterior and middle cerebral arteries before and after treatment were measured by color Doppler ultrasound. (3) Before and after treatment, serum levels of IFs including hypersensitive C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured by ELISA. The ELISA kits specific for hs-CRP (Cat. No. SP11244), TNF-α (Cat. No. SP12250) and IL-6 (Cat. No. SP10234) were all purchased from Wuhan Saipei Biotechnology Co, Ltd. (4) Vascular endothelial growth factor (VEGF) was detected by ELISA, and endothelin-1 (ET-1) and CGRP were determined by radioimmunoassay. (5) Before and after treatment, the Montreal Cognitive Assessment

Table 1. Baseline data

Variables	Observation group (n=45)	Control group (n=32)	χ^2	P
Gender			0.893	0.345
Male	26 (57.78)	15 (46.88)		
Female	19 (42.22)	17 (53.13)		
Age (years)			0.687	0.407
≤58	24 (53.33)	14 (43.75)		
>58	21 (46.67)	18 (56.25)		
BMI (kg/m^2)			0.009	0.923
≤23	23 (51.11)	16 (50.00)		
>23	22 (48.89)	16 (50.00)		
History of smoking			0.351	0.554
Yes	35 (77.78)	23 (71.88)		
No	10 (22.22)	9 (28.13)		
Underlying diseases			0.252	0.616
Hypertension	27 (60.00)	21 (65.63)		
Diabetes mellitus	18 (40.00)	11 (34.38)		
Hunt-Hess classification			0.131	0.936
Class I	10 (22.22)	8 (25.00)		
Class II	20 (44.44)	13 (40.63)		
Class III	15 (33.34)	11 (34.37)		

Table 2. Comparison of therapeutic effects between the two groups

Curative effect	Observation group (n=45)	Control group (n=32)	t	P
Markedly effective	24 (53.33)	13 (40.63)	-	-
Effective	19 (42.22)	12 (37.50)	-	-
Ineffective	2 (4.44)	7 (21.87)	-	-
Effective rate of treatment	43 (95.56)	25 (78.13)	5.505	0.019

Scale (MoCA), including 11 items in 8 cognitive domains of attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations and orientation, was used to compare the cognitive function of patients. (6) The patients' activities of daily living were evaluated using the Bathel index, with a total score of 100. The higher the score, the better the activities of daily living. (7) Adverse reactions, including nausea and vomiting, rash, blood pressure drop and gastrointestinal symptoms, were recorded and counted in both arms.

Statistical methods

The statistical software used in this study was SPSS180. Continuous variables were expressed as mean ± standard deviation. The intergroup comparison was performed by independent samples t-test, and the intragroup comparison before and after treatment was conducted by paired t-test. χ^2 test was used for comparison of categorical variables. P<0.05 indicated statistically significant difference.

Results

Comparison of general information

The baseline data such as gender, age and operation type were similar in the two arms (P>0.05), indicating comparability, as shown in **Table 1**.

Comparison of therapeutic effects

The therapeutic effects were evaluated after treatment. The overall response rate was 95.56% in the observation group (24 cases with markedly effective, 19 with effective and 2 patients with ineffective treatment), which was notably higher than 78.13% in the control group (13 cases with markedly effective, 12 with effective and 7 patients with ineffective treatment) (P<0.05), as shown in **Table 2**.

Comparison of BFVs before and after treatment

The mean BFVs of anterior cerebral, posterior and middle cerebral arteries showed no evident difference between the two arms before treatment (P>0.05). After treatment, the above indexes reduced significantly in both arms (P<0.05), especially in the observation group (P<0.05), as shown in **Figure 1**.

Comparison of serum levels of IFs before and after treatment

Serum hs-CRP, TNF-α and IL-6 differed insignificantly between the two arms before treatment

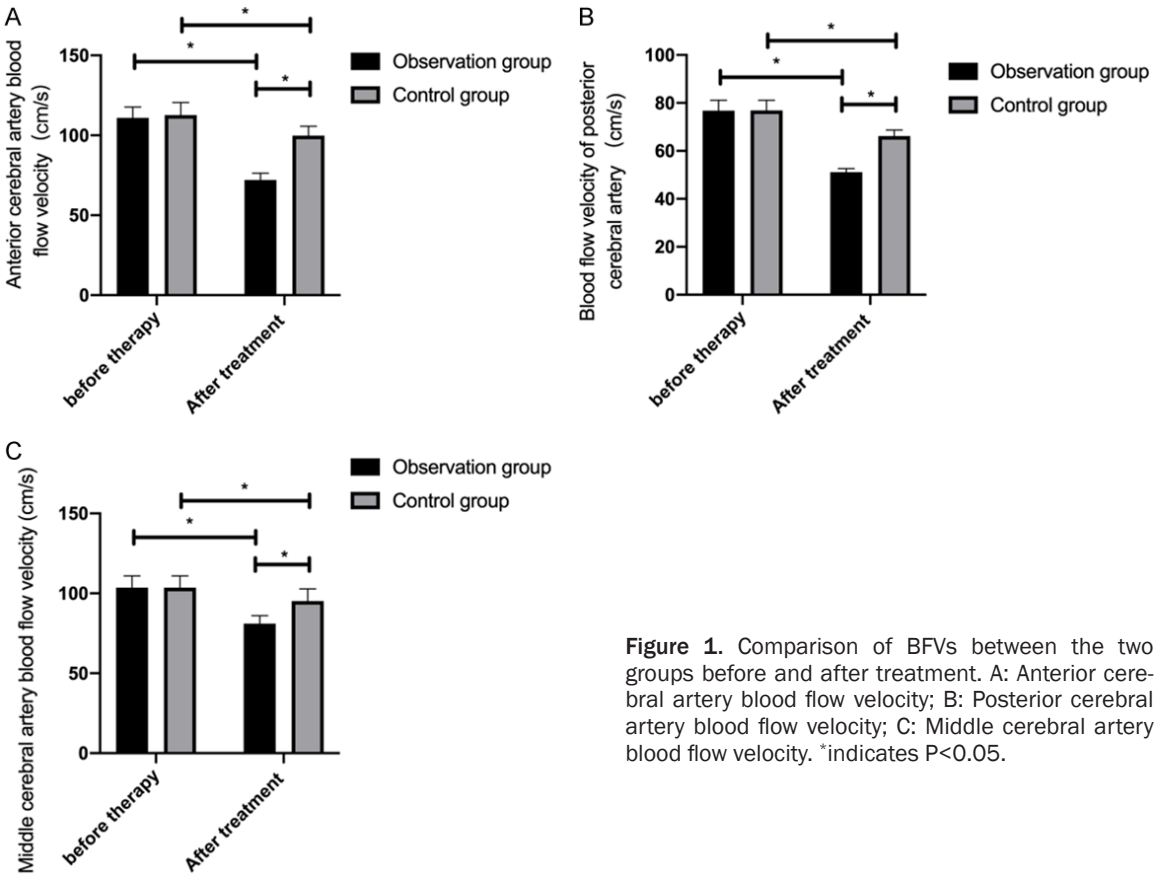

Figure 1. Comparison of BFVs between the two groups before and after treatment. A: Anterior cerebral artery blood flow velocity; B: Posterior cerebral artery blood flow velocity; C: Middle cerebral artery blood flow velocity. *indicates P<0.05.

(P<0.05). After treatment, the above indexes improved significantly in both arms (P<0.05), and the improvement was more distinct in the observation group (P<0.05), as shown in **Figure 2**.

Expression of vascular endothelial related factors before and after treatment

The serum VEGF, ET-1 and CGRP levels showed no significant differences between the two arms before treatment (P<0.05). After treatment, ET-1 and VEGF declined while CGRP increased significantly in both arms (P<0.05), and the changes of the above indexes were more significant in the observation group (P<0.05), as shown in **Figure 3**.

Comparison of MoCA and Bathel index scores before and after treatment

There were no significant differences in MoCA and Bathel index scores between the two arms before treatment (P<0.05). After treatment, the MoCA score and Bathel index rose obviously in both arms (P<0.05), and the increases were more obvious in the observation group (P< 0.05), as shown in **Figure 4**.

Comparison of adverse reactions

The number of patients with nausea and vomiting, rash, blood pressure drop and digestive tract symptoms was 2, 1, 1 and 1 respectively in the observation group, while it was 1, 1, 1 and 0 respectively in the control group. The incidence of adverse reactions was not significantly different between the observation group (11.11%) and the control group (9.38%) (P>0.05), as shown in **Table 3**.

Discussion

SAH is caused by the rupture of diseased blood vessels on the surface or bottom of the brain, resulting in direct blood flow into the subarachnoid space. The main presentations were nausea, severe headache, meningeal irritation and vomiting [12]. In general clinical treatment, it is believed that the main reason for the disease is

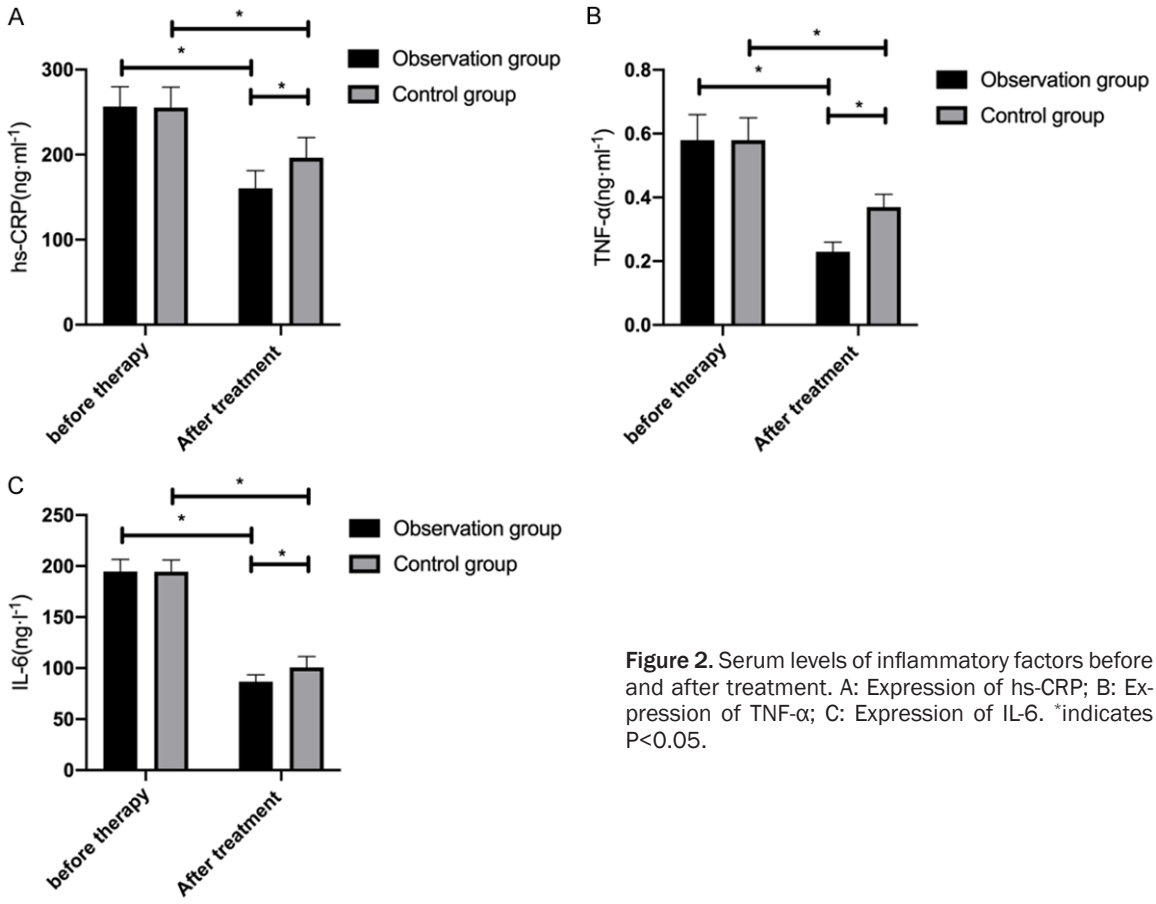

Figure 2. Serum levels of inflammatory factors before and after treatment. A: Expression of hs-CRP; B: Expression of TNF-α; C: Expression of IL-6. *indicates $P<0.05$.

that the blood clot in the place of ruptured aneurysm causes varying degrees of mechanical compression on the blood vessels around it, which leads to different degrees of pathological changes in the blood vessel wall [13]. Among them, the mechanical stimulation caused by blood clots will cause severe contraction of the pia mater blood vessels, followed by a diffuse vasospasm of cerebral vessels, which will lead to severe loss of blood oxygen in the brain and the damage of brain cells to varying degrees [14].

With the advances in endovascular interventional therapy in recent years, the incidence of CVS after SAH is obviously reduced, but there is still a lack of specific treatment methods [15]. In order to find more possible treatment schemes for patients with CVS after aSAH, we analyzed the efficacy and safety of NM combined with fasudil for the disease. First, we found a significantly higher overall response rate in the observation group compared with the control group after two weeks of treatment, which suggested that the curative effect of NM combined with fasudil was far superior to NM monotherapy. NM is a calcium antagonist with high lipid solubility, which can penetrate the blood-brain barrier and directly act on the cerebrovascular, thereby stabilizing the cell function and alleviating the cerebral vasoconstriction caused by different vascular substances [16]. However, NM monotherapy has been found to have some limitations, so it is of great significance to search for combination therapies. As a protein kinase inhibitor, fasudil can effectively inhibit the phosphorylation process of protein light chains after entering the human body, reduce the sensitivity of vascular smooth muscle to the increase of intracellular Ca^{2+} concentration, relieve smooth muscle spasm, and dilate blood vessels, thereby effectively inhibiting CVS. It can also protect damaged tissues by promoting the formation of nitric oxide and increasing the expression of endothelial nitric oxide synthase. Moreover, it can reduce the generation of inflammatory mediators and effectively suppress apoptosis [17, 18]. More

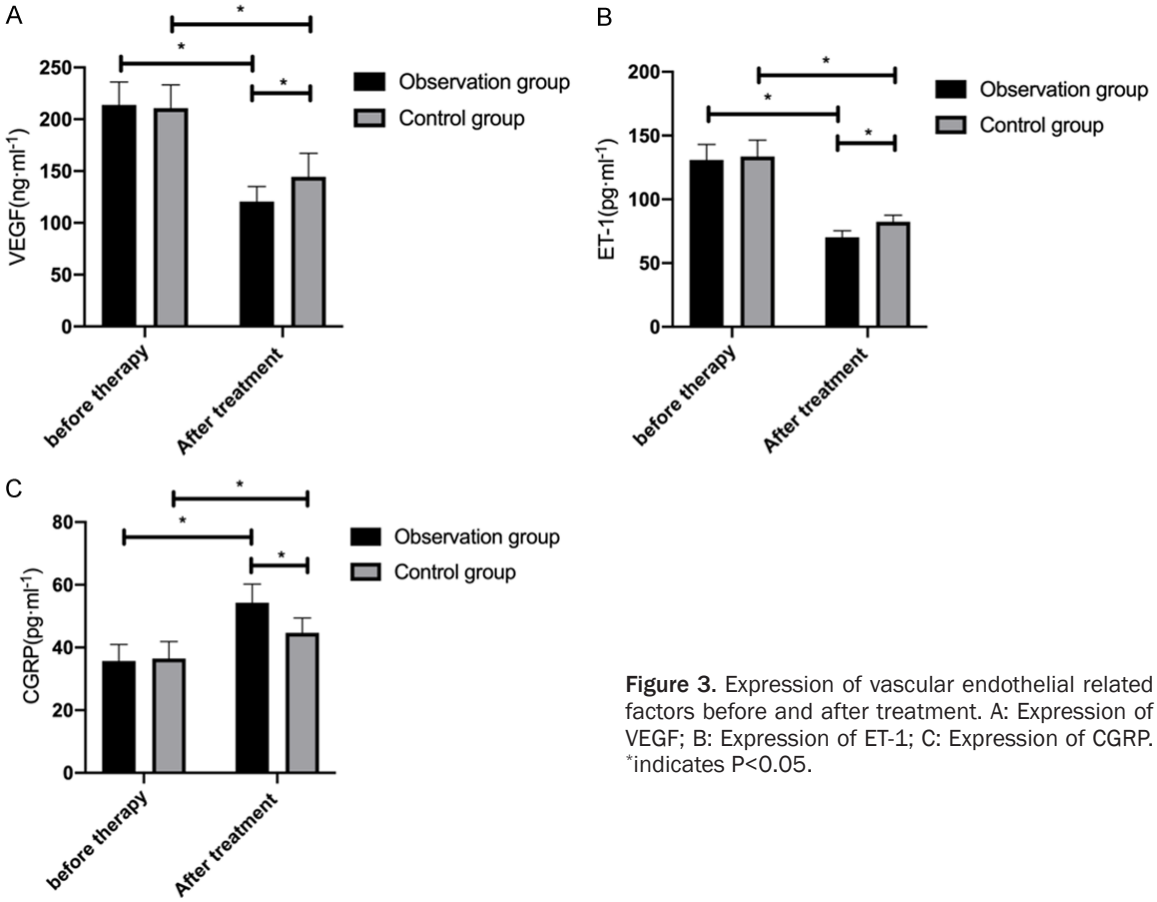

Figure 3. Expression of vascular endothelial related factors before and after treatment. A: Expression of VEGF; B: Expression of ET-1; C: Expression of CGRP. *indicates P<0.05.

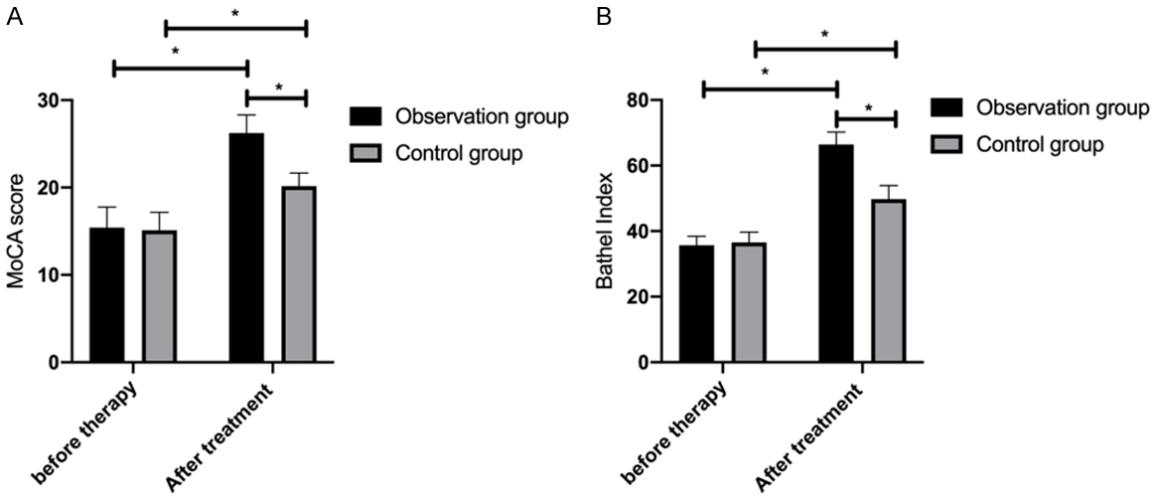

Figure 4. MoCA score and Bathel index of the two groups before and after treatment. A: Comparison of MoCA score; B: Comparison of Bathel index. *indicates P<0.05.

importantly, fasudil can effectively dilate cerebral parenchymal arteries without significantly affecting systemic blood pressure, so it can be used as an effective drug to prevent and relieve CVS [19]. Then we found that the cerebral artery BFVs of patients treated with the combined therapy improved more obviously compared with the control group, suggesting that

Table 3. Comparison of incidence of adverse reactions between the two groups

Adverse reactions	Observation group (n=45)	Control group (n=32)	χ^2	P
Nausea and vomiting	2 (4.44)	1 (3.13)	-	-
Rash	1 (2.22)	1 (3.13)	-	-
Blood pressure drops	1 (2.22)	1 (3.13)	-	-
Gastrointestinal symptoms	1 (2.22)	0 (0)	-	-
Total incidence	5 (11.11)	3 (9.38)	0.141	0.707

the combined medication could effectively improve the circulation of cerebral arteries and their surrounding tissues, and help to reduce the volume of hematoma and its surrounding low density area, which was consistent with previous reports.

Previous research [20] has pointed out that vascular endothelial injury and inflammatory reaction are important causes of CVS after aSAH. The main reason is that after receiving physical and chemical stimulation, vascular endothelial cells can exert the biological effects of regulating vascular tension, inhibiting thrombosis and controlling vascular growth through synthesis and secretion of various vasoactive substances [21]. Injury of vascular endothelial cell function can trigger abnormal contraction or spasm of blood vessels, promote the formation of thrombus, and further induce ischemic injury [22]. After the episode of aSAH, the vascular outer wall is exposed to oxygenated hemoglobin and other components released by erythrocyte lysate, which causes a series of inflammatory cascade reactions including recruitment, infiltration and activation of white blood cells [23]. ET-1, CGRP and VEGF are common indicators of vascular endothelial function [24]. Subsequently, we compared ET-1, CGRP, VEGF and inflammation-related factors between the two arms. The results showed that compared with NM monotherapy, fasudil combined with NM had a significantly better protective effect on VEF of patients with aSAH induced CVS, with a better regulating effect on inflammatory reaction. Fasudil and NM work together through two different mechanisms, which can significantly improve the therapeutic effect of patients with CVS induced by aSAH.

To further evaluate patient outcomes, we assessed patients' cognitive function and activities of daily living before and after treatment. The results showed that the observation group was significantly superior to the control group in both cognitive function and activities of daily living after treatment, which suggested that the combination of fasudil and NM could effectively improve the prognosis of patients. Finally, we compared the incidence of adverse reactions, and determined no evident difference between the two arms, which suggested that the combination of fasudil and NM was reliable and safe. In a previous study [24] comparing fasudil and NM monotherapy, it was found that fasudil did not produce serious adverse reactions, which supported our conclusions.

To sum up, fasudil combined with NM is more effective and safer than NM monotherapy in treating patients with aSAH induced CVS, and can clearly improve the prognosis of patients, which is worth promoting clinically. However, this study still has some limitations. For example, we are not clear about the efficacy of fasudil combined with NM when compared with other drugs. In addition, the specific mechanism of better efficacy of the combination therapy needs to be further explored. In future studies, we will further carry out large-sample clinical trials and basic experiments to provide more supporting data for our conclusions.

Disclosure of conflict of interest

None.

Address correspondence to: Qiang Cai, Department of Neurosurgery, Renmin Hospital of Wuhan University, No. 238 Jiefang Road, Wuchang District, Wuhan 430060, Hubei, China. Tel: +86-15072358160; E-mail: caiqiangno@yeah.net

References

[1] Li K, Barras CD, Chandra RV, Kok HK, Maingard JT, Carter NS, Russell JH, Lai L, Brooks M and Asadi H. A review of the management of cerebral vasospasm after aneurysmal subarachnoid hemorrhage. World Neurosurg 2019; 126: 513-527.

[2] Ciurea AV, Palade C, Voinescu D and Nica DA. Subarachnoid hemorrhage and cerebral vasospasm-literature review. J Med Life 2013; 6: 120-125.

[3] Connolly ES Jr, Rabinstein AA, Carhuapoma JR, Derdeyn CP, Dion J, Higashida RT, Hoh BL, Kirkness CJ, Naidech AM, Ogilvy CS, Patel AB, Thompson BG and Vespa P; American Heart Association Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; Council on Cardiovascular Surgery and Anesthesia; Council on Clinical Cardiology. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2012; 43: 1711-1737.

[4] Soliman R and Zohry G. Effect of magnesium sulphate and milrinone on cerebral vasospasm after aneurysmal subarachnoid hemorrhage: a randomized study. Braz J Anesthesiol 2019; 69: 64-71.

[5] Boulouis G, Labeyrie MA, Raymond J, Rodriguez-Regent C, Lukaszewicz AC, Bresson D, Ben Hassen W, Trystram D, Meder JF, Oppenheim C and Naggara O. Treatment of cerebral vasospasm following aneurysmal subarachnoid haemorrhage: a systematic review and meta-analysis. Eur Radiol 2017; 27: 3333-3342.

[6] Carlson AP, Hanggi D, Macdonald RL and Shuttleworth CW. Nimodipine reappraised: an old drug with a future. Curr Neuropharmacol 2020; 18: 65-82.

[7] Mahmoud SH, Ji X and Isse FA. Nimodipine pharmacokinetic variability in various patient populations. Drugs R D 2020; 20: 307-318.

[8] Zhang Y and Wu S. Effects of fasudil on pulmonary hypertension in clinical practice. Pulm Pharmacol Ther 2017; 46: 54-63.

[9] Ji E, Wang J, Wang L, Pan Z and Gao W. Pharmacological effects of fasudil on flap survival in a rodent model. J Surg Res 2020; 255: 575-582.

[10] Zhao J, Zhou D, Guo J, Ren Z, Zhou L, Wang S, Zhang Y, Xu B, Zhao K, Wang R, Mao Y, Xu B and Zhang X; Fasudil Aneurysmal Subarachnoid Hemorrhage Study Group. Efficacy and safety of fasudil in patients with subarachnoid hemorrhage: final results of a randomized trial of fasudil versus nimodipine. Neurol Med Chir (Tokyo) 2011; 51: 679-683.

[11] Bacon JD, Slade E, Smith AL, Allareddy G, Duan R, Fraser JF and Hatton KW. Potentially harmful ionizing radiation exposure from diagnostic tests and medical procedures in patients with aneurysmal subarachnoid hemorrhage. World Neurosurg 2020; 140: e153-e160.

[12] Petridis AK, Kamp MA, Cornelius JF, Beez T, Beseoglu K, Turowski B and Steiger HJ. Aneurysmal subarachnoid hemorrhage. Dtsch Arztebl Int 2017; 114: 226-236.

[13] Long B, Koyfman A and Runyon MS. Subarachnoid hemorrhage: updates in diagnosis and management. Emerg Med Clin North Am 2017; 35: 803-824.

[14] Rabinstein AA and Lanzino G. Aneurysmal subarachnoid hemorrhage: unanswered questions. Neurosurg Clin N Am 2018; 29: 255-262.

[15] Athiraman U, Jayaraman K, Liu M, Giri T, Yuan J and Zipfel GJ. Role of endothelial nitric oxide synthase in isoflurane conditioning-induced neurovascular protection in subarachnoid hemorrhage. J Am Heart Assoc 2020; 9: e017477.

[16] Knapik-Kowalczuk J, Tu W, Chmiel K, Rams-Baron M and Paluch M. Co-stabilization of amorphous pharmaceuticals-the case of nifedipine and nimodipine. Mol Pharm 2018; 15: 2455-2465.

[17] Gao Y, Yan Y, Fang Q, Zhang N, Kumar G, Zhang J, Song LJ, Yu J, Zhao L, Zhang HT and Ma CG. The Rho kinase inhibitor fasudil attenuates Abeta1-42-induced apoptosis via the ASK1/JNK signal pathway in primary cultures of hippocampal neurons. Metab Brain Dis 2019; 34: 1787-1801.

[18] Zhang X, Liu X, Zhou W, Yang M, Ding Y, Wang Q and Hu R. Fasudil increases temozolomide sensitivity and suppresses temozolomide-resistant glioma growth via inhibiting ROCK2/ABCG2. Cell Death Dis 2018; 9: 190.

[19] Dai K, Wang Y, Tai S, Ni H, Lian H, Yu Y, Liao W, Zheng C, Chen Q, Kuver A and Li J. Fasudil exerts a cardio-protective effect on mice with coxsackievirus B3-induced acute viral myocarditis. Cardiovasc Ther 2018; 36: e12477.

[20] Ugidos-Rodriguez S, Matallana-Gonzalez MC and Sanchez-Mata MC. Lactose malabsorption and intolerance: a review. Food Funct 2018; 9: 4056-4068.

[21] Pyne-Geithman GJ, Caudell DN, Cooper M, Clark JF and Shutter LA. Dopamine D2-receptor-mediated increase in vascular and endothelial NOS activity ameliorates cerebral vasospasm after subarachnoid hemorrhage in vitro. Neurocrit Care 2009; 10: 225-231.

[22] Shome S, Dasgupta PS and Basu S. Dopamine regulates mobilization of mesenchymal stem cells during wound angiogenesis. PLoS One 2012; 7: e31682.

[23] He Z, Ostrowski RP, Sun X, Ma Q, Tang J and Zhang JH. Targeting C/EBP homologous protein with siRNA attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. Exp Neurol 2012; 238: 218-224.

[24] Sun P, Zhu K, Wang C, Liu WW, Peng DG and Zhao X. Prophylactic effects of alkaloids from Ba lotus seeds on L-NNA-induced hypertension in mice. Chin J Nat Med 2016; 14: 835-843.