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Original Article

The efficacy of flunarizine combined with nimodipine in patients with subarachnoid hemorrhage-induced cerebral vasospasms and the combination's effects on the vascular endothelial functions and the inflammatory factors

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Abstract: Objective: This study sought to investigate the therapeutic effect of flunarizine, in combination with nimodipine, on patients with subarachnoid hemorrhage-induced cerebral vasospasms (CVS) as well as to determine the combination's effects on the vascular endothelial function and the inflammatory response. *Methods:* A total of 80 patients diagnosed with subarachnoid hemorrhage-induced CVS in the Inner Mongolia People's Hospital from March 2016 to September 2018 were enrolled and randomly divided into an observation group (n=40) and a control group (n=40). The control group was treated with nimodipine tablets, while the observation group was administrated flunarizine hydrochloride capsules and nimodipine tablets. The two groups' CVS indexes, vascular endothelial functions, inflammatory factors, and vascular endothelial growth factors (VEGF) were evaluated at different time points, and the correlations of their CVS with the changes in their vascular endothelial function-related factors and inflammatory factors were analyzed. *Results:* There were no significant differences in gender, age, history of smoking and drinking, or common medical diseases between the two groups ($P>0.05$). No statistically significant differences between the two groups regarding their CVS indexes were found before the treatment. However, the CVS index level was significantly reduced in the observation group compared to the control group at 1 week, 1 month, and 3 months after the treatment ($P<0.05$). At 3 months after the treatment, the endothelin-1 (ET-1), high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) levels in the observation group were significantly decreased, but the nitric oxide (NO), VEGF, and bFGF levels were elevated compared to the levels in the control group ($P<0.05$). Moreover, the CVS index had a positive correlation with the changes in the vascular endothelial function-related factor ET-1 and the inflammatory factors, but it had a negative correlation with the changes in NO ($P<0.05$). *Conclusion:* Flunarizine combined with nimodipine effectively relieve subarachnoid hemorrhage-induced CVS by attenuating the inflammatory response and improving the vascular endothelial function.

Keywords: Flunarizine, nimodipine, subarachnoid hemorrhage, cerebral vasospasm, vascular endothelial function, inflammatory factors

Introduction

Subarachnoid hemorrhage is a common disease of the nerve and cerebral vessels, and its major pathogeneses include intracranial aneurysm, cerebral vascular malformation, the abnormal development of the basilar vascular network, vasculitis, benign/malignant tumors, and coagulation disorders [1]. Subarachnoid hemorrhage is the third most common condition after ischemic stroke and cerebral hemorrhage seen in patients in neurology departments [2]. After onset, it is easily complicated with cerebral vasospasm (CVS), which occurs within several hours to 2 weeks after the subarachnoid hemorrhage, and more than 40% of patients may have significant neurological deficits, leading to body dysfunction, and signifi-

cantly increasing the fatality and disability rates of subarachnoid hemorrhage [3]. It is therefore of great importance to actively perform the symptomatic and supportive treatment to prevent secondary CVS, thereby improving the prognosis of patients with subarachnoid hemorrhage.

Nimodipine, a calcium ion antagonist commonly used in the past, is the preferred therapeutic drug for CVS currently. However, its long-term administration frequently leads to many adverse reactions [4] and even impedes in the drug sensitivity as well as the clinical effect. Flunarizine hydrochloride is a new kind of highly-selective calcium channel antagonist, an ammoniac compound, which has been recognized as a class 4 calcium channel antagonist by the World Health Organization [5]. It can significantly inhibit cerebral vasoconstriction, reduce intracranial pressure and protect brain tissues [6]. In this study, we investigate the efficacy of flunarizine combined with nimodipine in patients with subarachnoid hemorrhage-induced cerebral vasospasms and evaluate the combination's role in the vascular endothelial function and the inflammatory factors.

Patients and methods

General data

Eighty patients with subarachnoid hemorrhage-induced CVS treated in the Inner Mongolia People's Hospital from March 2016 to September 2018 were enrolled. The informed consents were signed, and the study was approved by the Medical Ethics Committee of Xiangyang Central Hospital before the patients enrolled. The diagnoses were based on the criteria for CVS published by the Chinese Medical Association in 2015, combined with the clinical manifestations, head imaging examinations, and head-neck transcranial Doppler ultrasonography. Inclusion criteria: patients with stable vital signs, aged 40-70 years old, and with an educational level of primary school or above. Exclusion criteria: patients with systemic infections, heart failure, acute and chronic hepatic and renal dysfunction, ischemic stroke, allergies to drugs, a history of mental disease, language dysfunction complications after onset or immune-related diseases. The enrolled patients were randomly divided into an observation group (n=40) and a control group (n=40).

Treatment

All the patients enrolled in the study received therapy, including hemostasis, anti-fibrinolysis, reduction of intracranial pressure, sedation and analgesia, enhancement of nutritional support, maintenance of water-electrolyte and acid-base balance, oxygen inhalation, ventilator-assisted ventilation if necessary, and other symptomatic and supportive treatment. The blood glucose and blood pressure levels in the patients complicated with diabetes mellitus and hypertension were regulated, and calcium channel blockers were avoided. The patients in the control group were given nimodipine tablets (NMPN H41023238, Zhengzhou Yonghe Pharmaceutical) 4 times a day (60 mg/time), and the patients in the observation group were given flunarizine hydrochloride capsules (NMPN H10930003, Xi'an Janssen Pharmaceutical) (first dose: 10 mg at night, then 5 mg every night) plus nimodipine tablets. The above drugs were orally administered to the conscious patients, but they were administered nasally to the unconscious patients. Both groups received the treatment for 4 consecutive weeks as 1 course of treatment, and 3 courses of treatment was considered 1 treatment cycle.

The observation indexes

The cerebral vasospasms were measured using a DWL Doppler Box T color Doppler diagnostic apparatus (Germany). The vascular endothelial growth factor (VEGF), the basic fibroblast growth factor (bFGF), the hs-CRP, IL-6, TNF-α, ET-1, and NO were all measured from serum samples centrifuged from venous blood samples taken after an overnight fast and stored at -40°C using cytokine-array biochip kits (Randox, Belfast, U.K.) and analyzed using the Randox Evidence Investigator. The variation trends in the cerebral vasospasm (CVS) indexes were compared between the two groups during the treatment (before treatment, and at 1 week, 1 month, and 3 months post treatment), and the vascular endothelial function, inflammatory factor, and vascular endothelial growth factor (VEGF) levels were tested at 3 months after the treatment. The effective rate of CVS treatment was recorded in both groups at different time points after the treatment (at 1 week, 1 month, and 3 months), and the correlations of the CVS indexes with the changes in the vascular endo-

thelial function-related factors and the inflammatory factors were analyzed.

Evaluation criteria

The CVS index (normal reference value in adults: 1.3-2.1) is the ratio of the bilateral middle cerebral artery flow velocity to the mean flow velocity of the extracranial internal carotid artery. The measurement depths of the bilateral middle cerebral artery flow velocity and the vertebral artery were 50-60 mm and 45-55 mm, respectively, and the internal carotid artery flow velocity was based on the extracranial segment. Cerebral blood flow = internal carotid artery blood flow + vertebral artery blood flow. The diagnostic criteria for CVS are as follows: a CVS index ≥3 indicates CVS, a CVS index ≥6 indicates severe CVS, and a CVS index =2.1-3.0 indicates increased cerebral blood flow velocity. The effective rate of CVS treatment was evaluated as follows: remarkably effective: the CVS index returned to normal after the treatment, effective: the CVS index returned to 2.1-3.0 after the treatment, and ineffective: the CVS index had no changes or even increased after the treatment. The vascular growth function was mainly determined by the changes in the angiogenesis-related cytokines, such as VEGF and basic fibroblast growth factor (bFGF). The changes in the inflammatory factors, including the hs-CRP (reference ranges for normal values: <10 mg/L), IL-6 (reference ranges for normal values: 0.37-0.46 ηg/L) and the TNF-α (reference ranges for normal values: 5-100 ηg/L) levels were also determined. Moreover, the vascular endothelial function was mainly determined by the changes in the ET-1 (reference ranges for normal values: 43.50-58.38 ηg/L) and the NO (reference ranges for normal values: 13.8-34.6 μmol/L) levels.

Statistical processing

Statistical analysis was performed using SPSS16.0 (SPSS Inc., Chicago, IL, USA). The data in this study were presented as the mean ± standard deviation. *t* tests were used for the intergroup comparisons, and chi-square tests were used for the enumeration data. The continuous data from multiple groups were analyzed using using one-way ANOVA, with Tukey's post hoc tests. *P*-values <0.05 were considered statistically significant.

Results

Comparison of the general information

The observation group included 24 males and 16 females and the patients ranged in age from 40-70 years old with an average age of (54.4±1.2) years old. In terms of educational level, there were 11 patients who completed senior high school or above and 29 patients who completed junior high school or below. Also, 19 patients were smokers and 11 were alcoholics, and there were 21 patients who also suffered from diabetes mellitus, 17 patients who also had hyperlipidemia, and 17 patients who also had chronic obstructive pulmonary disease. The control group consisted of 23 males and 17 females, and the patients ranged in age from 40-69 years old with an average age of (54.3±1.3) years old. There were 10 patients who completed senior high school or above and 30 patients who completed junior high school or below. Also, 18 of the patients were smokers and 12 were alcoholics. There were 20 patients who also suffered from diabetes mellitus, 16 who had hyperlipidemia, and 18 who had chronic obstructive pulmonary disease. Our results showed that there were no significant differences in terms of gender, age, history of smoking and drinking, or common medical diseases between the two groups (*P*>0.05) (**Table 1**).

Variation trend of the CVS indexes in both groups during the treatment

Before the treatment and at 1 week, 1 month, and 3 months after the treatment, the CVS indexes were (5.5±0.4), (3.3±0.3), (2.1±0.2), and (1.6±0.1) in the observation group and (5.6±0.4), (4.6±0.3), (4.1±0.2), and (3.1±0.2) in the control group. No statistically significant differences between the two groups regarding their CVS indexes were found before the treatment (t=1.118, P=0.267>0.05). However, the levels were significantly reduced in the observation group compared with the control group at 1 week, 1 month, and 3 months after the treatment (t=19.379, 44.721 and 42.416, P< 0.05) (**Figure 1**).

Comparison of effective rates of the CVS treatment in the two groups

At 1 week, 1 month, and 3 months post treatment, there were 25 cases (62.5%), 29 cases

Table 1. Comparisons of the general data in the two groups ($\bar{x} \pm s$)

	Conservation group	Control group	P
Gender (Male/Female)	24/16	23/17	0.8203
Age	54.4±1.2	54.3±1.3	0.7217
Educational level			0.7994
senior high school	11	10	
junior high school	29	30	
smokers	19	18	0.8226
alcoholics	11	12	0.8049
complicated with diabetes mellitus	21	20	0.8230
complicated with hyperlipidemia	17	16	0.8203
complicated with chronic obstructive pulmonary disease	17	18	0.8217

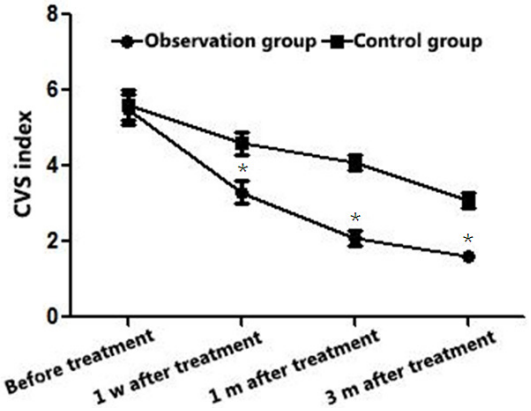

Figure 1. Variation trend of the CVS index in both groups during the treatment. *P<0.05, compared to control group.

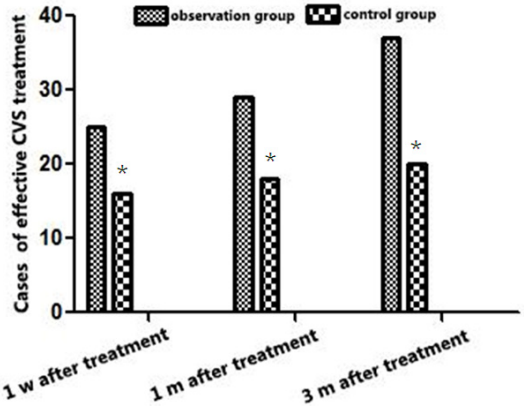

Figure 2. Changes in the effective rate of CVS treatment in both groups at different time points after the treatment. *P<0.05, compared to control group.

(72.5%) and 37 cases (92.5%) of effective CVS treatment, respectively, in the observation group, and 16 cases (40.0%), 18 cases (45.0%) and 20 cases (50.0%) of effective CVS treatment, respectively, in the control group. It can be noted that at 1 week, 1 month, and 3 months after the treatment, the flunarizine hydrochloride capsules significantly increased the effective rates of the CVS treatment compared to the control group (χ^2=4.053, 6.241 and 15.622, *P*=0.044, 0.012 and 0.000<0.05) (**Figure 2**).

Comparisons of the vascular endothelial functions and inflammatory factors in the two groups

At 3 months after the treatment with flunarizine hydrochloride, the ET-1, hs-CRP, IL-6, and TNF-α levels were significantly decreased but the NO level was significantly elevated compared to the corresponding levels in the control group (**Table 2**).

Comparison of the VEGF levels in the two groups at 3 months after the treatment

At 3 months after the treatment, the observation group had significantly higher VEGF and bFGF levels than the control group (*P*<0.05) (**Table 3**).

Correlation analysis between the CVS indexes and the changes in the vascular endothelial function-related factors

The CVS index had a positive correlation with the changes in ET-1 (*P*<0.05), but it had a negative correlation with the changes in NO (*P*< 0.05) (**Figures 3**, **4**).

Correlation analysis between the CVS indexes and the changes in the inflammatory factors

The CVS index was positively correlated with the changes in the inflammatory factors, hs-CRP, IL-6, and TNF-α (*P*<0.05) (**Figures 5**-**7**).

Table 2. Comparisons of the inflammatory factors and vascular endothelial function-related indexes in the two groups ($\bar{x} \pm s$)

	ET-1 (ηg/L)	NO (μmol/L)	hs-CRP (mg/L)	IL-6 (ηg/L)	TNF-α (ηg/L)
Observation group	43.1±3.1	59.1±9.3	4.9±0.5	0.5±0.1	61.3±2.3
Control group	79.8±8.0	30.2±3.3	15.9±1.8	1.7±0.2	131.3±5.9
t	27.054	18.523	37.240	33.941	69.913
P	0.000	0.000	0.000	0.000	0.000

Table 3. Comparison of the VEGF levels in the two groups at 3 months after the treatment (pg/mg, $\bar{x} \pm s$)

	VEGF	bFGF
Observation group	81.9±9.7	40.9±5.3
Control group	59.7±5.7	21.4±2.6
t	12.480	20.891
P	0.000	0.000

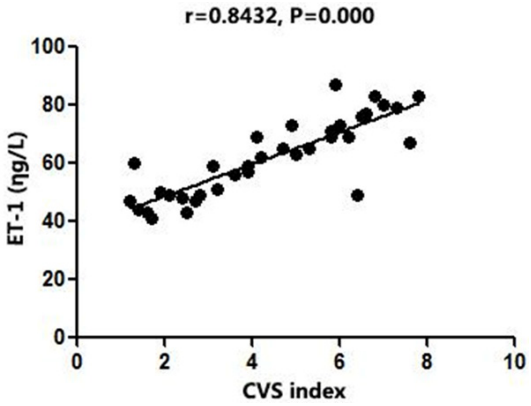

Figure 3. Correlation analysis between the CVS index and the changes in ET-1.

Discussion

Subarachnoid hemorrhage is a relatively critical acute nervous system disease, and its common pathogeneses include intracranial aneurysms, arteriovenous malformation, and coagulation disorders [7]. The intracranial pressure frequently rises when the blood enters the subarachnoid space, and the patients suffer from projectile vomiting, severe headache, blurred vision, and cervical rigidity [8], in which, CVS is the most common complication, leading to a poor prognosis or even threatening a patient's life if no timely treatment is adopted [9]. At present, the most effective examination method for subarachnoid hemorrhage-induced CVS is the transcranial Doppler ultrasonography, which diagnoses CVS by measuring the cerebral blood flow velocity [10]. The transcranial Doppler ultrasonography meets the requirements for early detection and targeted intervention to reduce the intracranial pressure and relieve CVS. It ensures the cerebral arterial blood flow [11], increases the cerebral oxygen supply, and improves patient prognosis [12]. The most commonly-used drug to dilate the cerebral vessels in the past was nimodipine, but its long-term application may easily produce drug resistance, thus affecting the therapeutic effect. In addition to the symptomatic and supportive treatments, the patients with subarachnoid hemorrhage-induced CVS in this study were treated with nimodipine in the control group and nimodipine combined with flunarizine in the observation group. It was found that the combined treatment of flunarizine and nimodipine decreased the CVS index and increased the effective rate of CVS treatment, compared to the exclusive use nimodipine, indicating that nimodipine combined with flunarizine can dilate the cerebral vessels and alleviate the CVS manifestations. Moreover, the combination therapy with flunarizine effectively relieved the inflammatory response by reducing the ET-1, hs-CRP, IL-6, TNF-α, VEGF, and bFGF levels, and it further facilitated the vascular endothelial regeneration in patients with subarachnoid hemorrhage-induced CVS. Finally, according to the correlation analysis between the CVS index and the changes in the vascular endothelial function-related factors and the inflammatory factors, the CVS index had a positive correlation with the changes in ET-1, but it had a negative correlation with the changes in NO, and it was also positively correlated with the changes in the inflammatory factors.

Nimodipine is the most frequently-used calcium channel antagonist for CVS, but its long-term application can easily produce drug resistance [13]. Flunarizine hydrochloride, a novel calcium channel blocker, effectively inhibits the opening of the calcium channel, thereby lowering the cellular calcium ion level [14], inhibiting the production of oxygen (hydroxyl) free radicals, and reducing the calcium ion overload after ischemia and oxygen [15]. Meanwhile, flu-

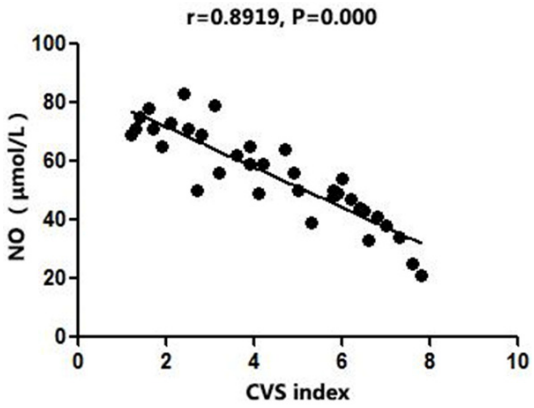

Figure 4. Correlation analysis between the CVS index and the changes in NO.

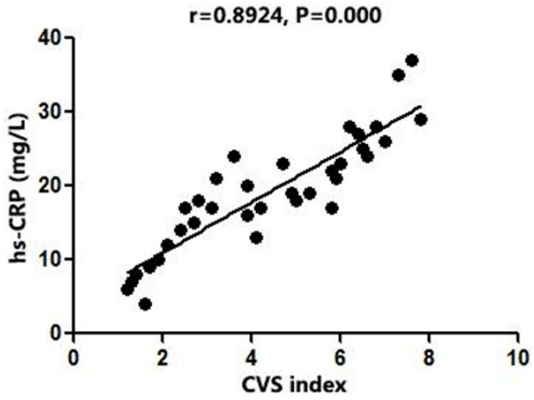

Figure 5. Correlation analysis between the CVS index and the changes in hs-CRP.

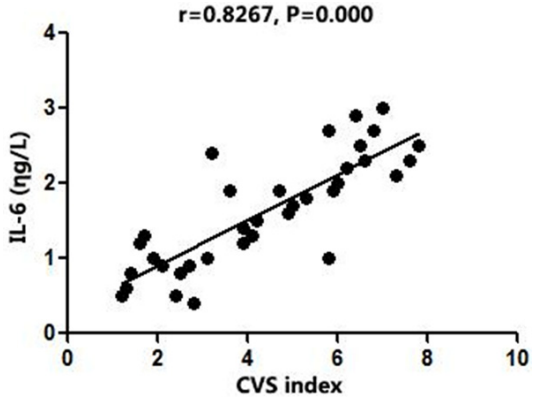

Figure 6. Correlation analysis between the CVS index and the changes in IL-6.

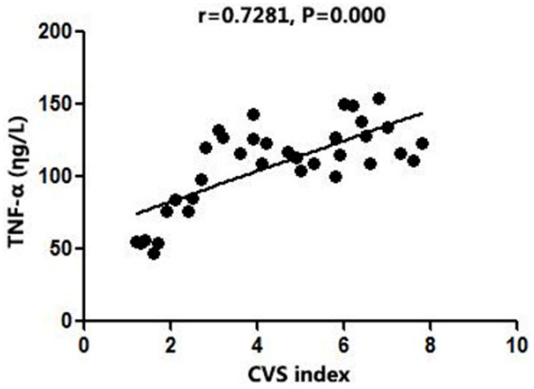

Figure 7. Correlation analysis between the CVS index and the changes in TNF-α.

narizine hydrochloride can also suppress the contraction of cerebral vascular smooth muscle and reduce the incidence of delayed ischemic neurological deficit in patients with subarachnoid hemorrhage [16]. In subarachnoid hemorrhage-induced CVS, flunarizine hydrochloride, a difluorinated piperazine-derived ammoniac compound [17], can selectively act on the intracranial artery [18] without affecting the cardiac and peripheral vascular functions [19], and it has strong lipophilicity and can traverse the blood-brain barrier, so its concentration in the central nervous system is higher after administration [20]. Our data indicate that flunarizine combined with nimodipine leads to the improvement of CVS, the inflammatory response, and the vascular endothelial functions in patients with subarachnoid hemorrhage-induced CVS. However, our study cohort was small, so the clinical effect of the combined use of flunarizine and nimodipine requires further validation with a large number of patients. Also, the safety as well as the mechanism remain to be further determined.

Conclusion

In conclusion, our data demonstrate that flunarizine combined with nimodipine effectively relieves subarachnoid hemorrhage-induced CVS by mitigating the body's inflammatory response and improving the vascular endothelium function.

Disclosure of conflict of interest

None.

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