Effect of cervical sympathetic block on cerebral vasospasm after subarachnoid hemorrhage in rabbits[1]

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ABSTRACT

PURPOSE: Cerebral vasospasm (CVS) is a major complication after subarachnoid hemorrhage (SAH) induced by the rupture of intracranial aneurysms. The aim of the present study was to investigate the effect and mechanism of cervical sympathetic block on cerebral vasospasm of the rabbits after SAH**.**

METHODS: After successful modeling of cervical sympathetic block, 18 healthy male white rabbits were randomly divided into three groups (n=6), ie, sham operation group (Group A), SAH group (Group B) and SAH with cervical sympathetic block group (Group C). Models of delayed CVS were established by puncturing cisterna magna twice with an injection of autologous arterial blood in Groups B and C. A sham injection of blood through cisterna magna was made in Group A. 0.5 ml saline was injected each time through a catheter for cervical sympathetic block after the first injection of blood three times a day for 3 d in Group B (bilateral alternating). 0.5 ml of 0.25% bupivacaine was injected each time through a catheter for cervical sympathetic block after the first injection of blood three times a day for 7 d in Group B. 2 ml venous blood and cerebrospinal fluid were obtained before (T1), 30 min (T2) and 7 d (T3) after the first injection of blood, respectively, and conserved in a low temperature refrigerator. Basilar artery value at T1, T2 and T3 was measured via cerebral angiography. The degree of damage to nervous system at T1 and T3 was recorded.

RESULTS: There was no significant difference in diameter of basilar artery at T1 among three groups. The diameters of basilar artery at T2 and T3 of Groups B and C were all smaller than that in Group A, which was smaller than Group C, with a significant difference. There was no significant difference in NO and NOS in plasma and cerebrospinal fluid among three groups. The NO and NOS contents at T2 and T3 of Groups B and C were all lower than Group A; Group C was higher than Group B, with a significant difference. The nerve function at T3 of Groups B and C were all lower than Group A and that of Group C higher than Group B, with a significant difference.

CONCLUSION: Cervical sympathetic block can relieve cerebral vasospasm after subarachnoid hemorrhage and increase NO content and NOS activity in plasma and cerebrospinal fluid to promote neural functional recovery.

Key words: Sympathectomy. Subarachnoid Hemorrhage. Vasospasm, Intracranial. Rabbits.

Introduction

Cerebral vasospasm (CVS) is a major complication after subarachnoid hemorrhage (SAH) induced by rupture of intracranial aneurysms[1]. Over 50% patients with SAH have symptomatic CVS (accounting for 40%) that is the main cause of disability and mortality. Over 20% patients without treatment were left with permanent neurologic impairment or even died[2-5]. Despite extensive clinical and experimental studies, the pathogenesis of cerebra vasospasm is still controversial and poorly understood. The cause of vasospasm is presently considered to be multifactorial. In the presence of CVS, current management consists of hypertension, high blood volume and blood high dilution (3H) therapy and neuroradiological procedures[6-13]. The results of these treatment options have been hampered by some technical limitations and medical complications. Therefore, understanding of the pathological and physical mechanism and exploration of effective therapy have become the hot topic in current research. Cervical sympathetic block is a safe and effective therapy[14-15] with little side effect. Cervical sympathetic block can significantly dilate cerebral vessels, increase cerebral blood velocity, regulate the imbalance with endothim (ET) and calcitonin gene related protein (CGRP) and excessive expression of neurons' heat shock protein 70 after global cerebral ischemia-reperfusion injury in rabbits, improve the immune function of erythrocyte of patients with cerebral infarction, regulate the content of substance causing systolic and diastolic vessels in serum such like NO and NOS, and promote neural functional recovery. At present, there still exists argument on alleviative role of cervical sympathetic block on CVS after SAH and the role of cervical sympathetic block in regulating substances causing systolic and diastolic vessels to protect the brain. Wang *et al*.[16] found that the upper thoracic epidural external sympathetic block with 0.5% lidocaine dilated the cerebral vessels of rabbits after SAH, increased cerebral blood flow and relieved the nervous dysfunction, which was effective to CVS after SAH. However, Treggiari *et al*.[17] studied effect of the transaction of sympathetic nerves after SAH on vascular smooth muscles and the mechanism by recording the response of carotid artery in vitro to norepinephrine, 5-hydroxytryptamine and endothelin. The results showed that one week after SAH, the supersensitivity of carotid artery and cerebral artery caused by SAH induced CVS. Neither the contractile response of cervical arteries to epinephrine and 5-hydroxytryptamine nor CVS mediated by ET-1 could be relieved or prevented by the transaction of sympathetic nerves. The inconsistent result may be related with the opportunity of transaction and blockage of cervical sympathetic nerves. The aim of the present study was to investigate the effect and mechanism of cervical sympathetic block on CVS of rabbits after SAH.

Methods

All procedures were approved by Animal Research Committee of People's Hospital of Guizhou Province, China. Healthy SD New Zealand rabbits (male, weighing 2-2.5kg, aged 6-7 months) were provided by the Animal Laboratory of Third Military Medical University, Chongqing.

(1) Establishment of experimental models. The model of cervical sympathetic block: 3% pentobarbital sodium (1 m1/kg) was injected though ear vein of healthy rabbits. After anesthesia, an incision was made at the neck, placing catheters on both sides respectively, for a blunt separation along cervical artery to cervical sympathetic. One end of the catheter was placed and fixed near the cervical sympathetic; the other end along the spine through the skin of nape was fixed to expose a 2 cm long section for a local anesthesia. The rabbits with no infected incision were prepared for experiments after being fed for one week.

The double-hemorrhage model of SAH was used in this experiment[18]. After successful modeling of cervical sympathetic block, 18 healthy male rabbits were randomly divided into three groups (n=6), ie, sham operation group (Group A), SAH group (Group B) and SAH with cervical sympathetic block group (Group C). Models of delayed CVS were established by puncturing cisterna magna twice with an injection of autologous arterial blood (0.8 ml/kg, at interval of 48 hours) in Groups B and C. A sham injection of blood through cisterna magna (equivalent normal saline at the same time) was made in Group A. Normal saline for 0.5ml was injected each time through a catheter for cervical sympathetic ganglia block after the first injection of blood three times a day for three days in Group B (bilateral alternating). Bupivacaine at 0.25% for 0.5ml was injected each time through a catheter for cervical sympathetic ganglia block after the first injection of blood three times a day for seven days in Group C (bilateral alternating).

Pentobarbital sodium (1ml/kg) at 3% was injected through ear vein at T1, T2 and T3 to induce anesthesia. Then, the rabbits were placed in a supine position, and a 5-F catheter (Terumo Corp., Japan) was inserted selectively into the aortic arch through a femoral artery by the Seldinger method. Angiograms of the basilar artery were obtained by injection of 5ml Omnipaque contrast medium for two seconds at a pressure of 50 psi. The speed of digital image acquisition was six frames per second. The angiograms were transferred to an analytic processing system, and the diameter of the basilar artery was measured at five points (at

the midpoint of the basilar artery, at 1mm central and peripheral from the midpoint, and at 2mm central and peripheral from the midpoint). The mean diameter at these five points was then determined. All angiograms were obtained by one investigator and the diameters of the basilar artery were measured by a colleague working in a blinded fashion. Angiograms were performed at T1, T2 and T3.

Venous blood and cerebrospinal fluid for 2ml at T1, T2 and T3 were obtained respectively and conserved in a lower temperature refrigerator (-80ºC). The degree of nervous system damage at T3 was recorded.

(2) Neurological score. The discrimination of changes in neurological state was conducted with Otsuji' method 19 and neurological score at T3 was graded (Chart 1).

CHART 1 - Classification standard for neurological state of experimental animals.

Grade	Symptoms of nerve damage
Grade I	None
Grade II	Mild or not obvious (suspicious) symptoms of nerve damage
Grade III	Moderate symptoms of nerve damage
Grade IV	Severe symptoms of nerve damage

Statistical analysis

Statistical software SAS was used for analysis. All measurement data were presented as mean ±standard deviation. Group *t*-test was used for comparison among groups, variance analysis of repeated measurement data for intra-group comparison and *Chi-square* test for enumeration data. $p<0.05$ indicates a significant difference.

Results

There were no significant differences in each group in terms of basilar artery diameter, NO content and NOS activity in the serum and cerebrospinal fluid at T1 ($p>0.05$). The rabbit double-hemorrhage models were established successfully. In Group B and Group C, the basilar artery vasospasm was readily apparent at T2 and T3 (Figure 1 from **a** to **f**). There was no significant difference in diameter of basilar artery at T1 among three groups. Basilar artery vasospasm at different degrees was seen at T2 and T3 in all groups (Figure 1 from **a** to **b**).

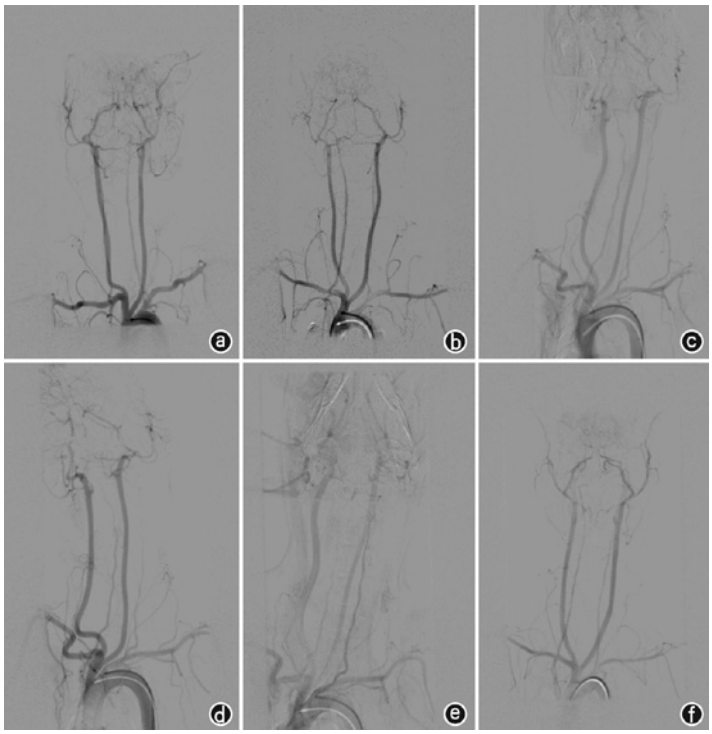

FIGURE 1 - The changes of the basilar artery vasospasm. Before SAH, there showed no basilar artery vasospasm in Group A (**a**), Group B (**b**) and Group C (**c**). At day 7 after SAH, various degrees of the basilar artery vasospasm was found in Group A (**d**), Group B (**e**) and Group C (**f**), with more obvious vasospasm in Group A than that in Groups B and C.

The diameter of basilar artery at T2 and T3 in Groups B and C were all smaller than that in Group A. While the diameter of basilar artery at T2 and T3 in Group B was smaller than that in Group C, with a significant difference ($p<0.01<$).Cervical sympathetic block significantly decreased the narrowing of the basilar artery after SAH (Table 1).

There was no significant difference in NO content and NOS activity in serum and cerebrospinal fluid at T1 among three groups. NO content and NOS activity at T2 and T3 were lower than that at T1 in all groups; while NO content and NOS activity in Group C was lower than that in Group A but higher than that in Group B, with a significant difference ($p<0.01$) (Table 1).

TABLE 1 - Changes of basilar artery diameter, NO content and NOS activity in serum and cerebrospinal fluid at different time points.

Index	Group	T1	T2	T3
Diameter of basilar artery (mm)	A	0.7±0.02	0.58±0.04*	0.67±0.03
	B	0.7±0.02	0.15±0.05*#	0.34±0.06*#
	C	0.7±0.03	0.42±0.06*#△	0.57±0.06*#△
NO in serum (μmmol/L)	A	54.97±4.17	40.07±6.97*	57.08±4.65
	B	54.45±4.33	21.08±5.98*#	28.38±5.82*#
	C	54.73±8.74	50.58±6.26*#△	55.52±8.25*#△
NO in cerebrospinal fluid (μmmol/L)	A	69.77±2.52	67.22±3.22	69.25±2.62
	B	69.82±3.11	25.4±4.58*#	25.42±4.47*#
	C	69.8±2.58	36.65±6.07*#△	40.33±4.86*#△
NOS in serum (U/ml)	A	54.87±4.09	55.17±33.31	53.25±3.77
	B	55.03±4.61	21.02±3.13*#	22.38±1.90*#
	C	54.98±3.03	36.83±3.33*#△	39.8±4.01*#△
NOS in cerebrospinal fluid (U/ml)	A	57.45±4.83	51.42±5.47*	50.12±5.67*
	B	57.65±4.36	21.37±3.43*#	22.5±3.18*#
	C	56.9±4.47	35.42±4.63*#△	38.83±4.15*#△

Notes: Compared with T_1: *p<0.01; Compared with group A: #p<0.01; Compared with group B: △p<0.01.

The neurological function at T3 in Groups B and C were all lower than that in Group A; while the neurological function at T3 in Group C was higher than that in Group B, with significant difference (Table 2).

TABLE 2 - Neurological function of rabbits in all groups post treatment.

Groups	I	II	III	IV
A	6	0	0	0
B	0*	0	2*	4*
C	0*#	2*#	3*#	1*#

Notes: Compared with Group A: *p<0.01; Compared with Group B: #p<0.01.

The animals in our experiment showed no obvious abnormal symptoms associated with the adverse effects of cervical sympathetic block. This results demonstrated that cervical sympathetic block had therapeutic efficacy in the experimental CVS.

Discussion

The present study shows that a noninvasive procedure of temporary chemical sympathectomy contributes to prevention and treatment of CVS. The rationale for this procedure is that the blockage of sympathetic nerve activity or reversal of overactivity may dilate intracerebral vessels. Nitric oxide (NO) plays an important role in the regulation of hemostasis. It is believed that vasospasm is resulted from the decreased availability of NO and the reduced endothelial and neuronal NOS-mediated relaxation of large conductive cerebral arteries[20-22]. In this study, the NO content and NOS activity in the serum and cerebrospinal fluid of Groups B and C after SAH were significantly lower than that before SAH. The explanation for decreased level of NO in the present study is that the presence of blood around the major cerebral arteries caused morphological changes in the vessels, including the endothelial cells[23]. This endothelial injury impairs the equilibrium between NO and prostacylin whose balance is critical for the maintenance of vascular tone[24,25]. Previous reports have indicated that CVS is observed as early as 30 minutes (the acute phase)[26] after SAH, and reaches a peak at days 3-7 (the chronic phase)[27]. Among several animal models of CVS, the double-hemorrhage model was considered to resemble more closely the human pathological features of CVS[27]. Therefore, we adopted to successfully establish this model of CVS. In the double-hemorrhage model, it has been reported that there are a variety of morphological changes in the arteries in spasm, such as endothelial cell vacuolization, disruption of the internal elastic lamina, and so on. In the present study, it is reasonable to consider that the inhibitory effect of cervical sympathetic block on the experimental CVS after SAH may have contributed to the increase in NO content and NOS activity in the serum and cerebrospinal fluid. It may be due to that postganglionic fiber of cervical sympathetic ganglia regulated and excreted NOS. It has proved that use of sympathetic ganglion inhibitor quaternary ammonium at stellate ganglion can increase the excretion of NOS at nerve endings[28-30].

Conclusion

Cervical sympathetic block can relieve cerebral vasospasm and increase NO content and NOS activity in plasma and cerebrospinal fluid to promote neural function recovery after subarachnoid hemorrhage in rabbits.

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