

Original Article

Morphometric and ultrastructural analysis of the effect of bromocriptine and cyclosporine on the vasospastic femoral artery of rats

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Abstract: Vasospasm is the main causes of mortality and morbidity in patients with subarachnoid hemorrhage (SAH). The arterial narrowing mechanism that develops after SAH is not yet fully understood but many studies showed that hypotension, neurogenic reflexes, clots in the subarachnoidal space, spasmogenic agents, humoral and cellular immunity play a role in the etiology. In this study we investigate the effects of Bromocriptine and Cyclosporine A in vasospasm secondary to SAH on rat femoral artery from ultrastructural and morphometric perspectives. 120 male Sprague-Dawley rats divided into 12 groups: Vasospasm (V), control (K), surgical control (CK) groups, vasospasm+Bromocriptine and/or Cyclosporine-A groups (VCyA, VBr, VBr+CyA), Bromocriptine and/or Cyclosporine-A control groups (CK, BK, Br+CyAK), Bromocriptine and/or Cyclosporine-A surgical control groups (BCK, CyCK, Br+CyACK). In order to create SAH model, 0, 1 cm³ blood injected into silastic sheath wrapped rat femoral artery. Bromocriptine (2 mg/kg/d) and Cyclosporine A (10 mg/kg/d) combinations applied to control, surgical control and vasospastic models. Light microscopy, transmission electron microscopy and scanning electron microscopy used during this study. Statistical evaluation of the morphometric measurement data concerning vascular wall thickness and luminal cross-sectional areas of all groups were performed using Mann-Whitney U, Wilcoxon-signed rank, and Student-t tests. Cyclosporine A, whose effects in the prevention of vasospasm have been demonstrated in previous studies. In this study we discovered that Bromocriptine demonstrated strong effects similar to Cyclosporine-A. Bromocriptine and Cyclosporine A markedly prevent the development of chronic morphologic vasospasm following SAH. The combined use of both drugs does not change this preventive effect.

Keywords: Bromocriptine, cyclosporine-a, subarachnoid hemorrhage, vasospasm

Introduction

Vasospasm is among the most predominant causes of mortality and morbidity in patients who present subarachnoid hemorrhage (SAH) secondary to aneurysmal bleeding. Vasospasm can present itself along with head traumas, tumors, and arteriovenous malformation (AVM). Although pathogenesis of cerebral vasospasm is not fully understood, it constitutes a serious problem for neurosurgery [1].

Cerebral vasospasm emerges after SAH. SAH can be defined as the pathological narrowing of basal cerebral vessels caused by a collection of blood, blood metabolites, and some chemi-

cal substances within cisterna. Although the arterial narrowing mechanism that develops after subarachnoidal bleeding is not yet fully understood, degradation of erythrocytes, hypotension, neurogenic reflexes, clots in the subarachnoidal space, and spasmogenic agents released from degradation products (i.e., PGE₂, PGF₂, arachidonic acid, serotonin, tromboxan A₂, epinephrine, norepinephrine, histamine, angiotensin, thrombin, plasmin, fibrin degradation products, and potassium) are known to play a role in the etiology of arterial narrowing [2-4]. In addition to other mechanisms, responsiveness to cellular immunity, followed by humoral immunity and eicosanoid reactions,

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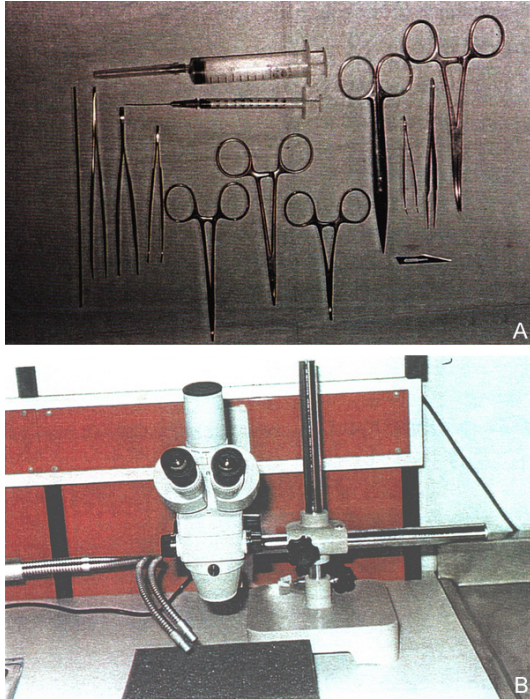


Figure 1. A. Microsurgical instruments. B. Surgical microscope.

have been demonstrated to have a role in the development of cerebral vasospasm [5-13]. According to this autoimmune theory, it is accepted that erythrocytes appearing after minor bleedings behave like an autoantigen and lead to the formation of an immune complex [10, 11].

Bromocriptine is a dopamine type 2 agonist and prevents secretion of prolactin (PRL). Bromocriptin, which is frequently used in hypophyseal, endocrinologic disorders and Parkinson's disease, has a strong immunosuppressive effect. It prevents delayed type hypersensitivity, primary antibody response, T-lymphocyte-related macrophage activation, and proliferation of T- and B-lymphocytes [14-18].

Cyclosporine A has a strong anti-immunosuppressive activity, and it is especially used in transplantation and for the treatment of autoimmune diseases. It inhibits T-cell-dependent antibody formation, lymphokine production, and release of interleukin 2 including T-cell growth factor (TCGF). It blocks resting lymphocytes at G0 or G1 cell phases of the T-cell cycle. It also inhibits the release of antigen-stimulated lymphokine from active T-cells [19-24].

Many studies have demonstrated that the combined use of Bromocriptine and Cyclosporine A is very effective for organ transplantation and the treatment of autoimmune diseases [9, 15, 18, 25-32]. However, currently there is not a literature study on its effects on cerebral vasospasm.

In this study, a chronic vasospasm model is constructed with a rat femoral artery, and the effect of Bromocriptine and Cyclosporine A on chronic morphological vasospasm is analyzed from ultrastructural and morphometric perspectives.

Material and method

This study was performed in the Laboratories of Department of Pharmacology, Marmara University Faculty of Medicine. Light microscopic examinations were conducted in the Pathology Department of Marmara University Faculty of Medicine using transmission electron microscopy (TEM), and scanning electron microscopic (SEM) examinations were performed in the Department of Histology and Embryology.

In this study 120 male Sprague-Dawley rats each weighing 180-220 g were used. Microsurgical instruments (**Figure 1A**) and surgical microscope (Nikon; **Figure 1B**) were used. A vasospasm model called the "Rat Femoral Artery Vasospasm Model" proposed by Okada et al. was utilized [33].

Rats were divided into two groups. Vasospasm was induced in the right femoral arteries of rats, and these rats were treated with either Cyclosporine A, Bromocriptine, or a combination therapy (Bromocriptine and Cyclosporine A). The left femoral arteries of the rats constituted as control and surgical control groups (**Table 1**).

Group designations

Vasospasm Group (V) (n=30): Vasospasm was induced in the right femoral arteries.

Vasospasm+Cyclosporine A Group (VCyA) (n=30): Vasospasm was induced in the right femoral arteries and Cyclosporin A was applied.

Vasospasm+Bromocriptine Group (VBr) (n=30): Vasospasm was induced in the right femoral arteries, and Bromocriptine was applied.

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Table 1. Group designations

Vasospasm Group (V) (n=30)	Vasospasm was induced in the right femoral arteries
Vasospasm+Cyclosporine A Group (VCyA) (n=30)	Vasospasm was induced in the right femoral arteries and Cyclosporine A was applied
Vasospasm+Bromocriptine Group (VBr) (n=30)	Vasospasm was induced in the right femoral arteries, and Bromocriptine was applied
Vasospasm+Bromocriptine+Cyclosporine A Group (VBr+CyA) (n=30)	Vasospasm was induced in the right femoral arteries, and Bromocriptine+Cyclosporine A was applied
Control Group (K) (n=15)	Left femoral arteries were left intact
Surgical Control Group (CK) (n=15)	A silastic sheath was wrapped around the left femoral arteries; blood samples were not obtained; and only physiologic saline was used
Bromocriptine+Cyclosporine A Control Group (Br+CyAK) (n=15)	Left femoral arteries were left intact and Bromocriptine+Cyclosporine A was applied
Bromocriptine Control Group (BK) (n=15)	Left femoral arteries were left intact, and Bromocriptine was applied
Bromocriptine Surgical Control Group (BCK) (n=15)	A silastic sheath was wrapped around the left femoral arteries; blood samples were not given; and only physiologic saline and Bromocriptine were applied
Cyclosporine A Control Group (CyAK) (n=15)	Left femoral arteries were left intact, and only Cyclosporine A was applied
Cyclosporine A Surgical Control Group (CyCK) (n=15)	A silastic sheath was wrapped around the left femoral arteries. Instead of blood samples, only physiologic saline and Cyclosporine A were applied
Cyclosporine A+Bromocriptine Surgical Control Group (Br+CyACK) (n=15)	A silastic sheath was wrapped around the left femoral arteries; instead of blood samples, only physiologic saline and Cyclosporine A+Bromocriptine were applied

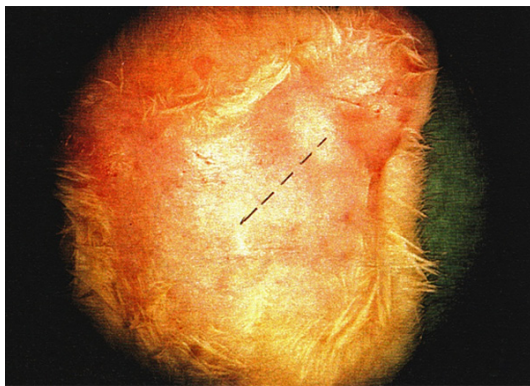


Figure 2. Incision.

Vasospasm+Bromocriptine+Cyclosporine A Group (VBr+CyA) (n=30): Vasospasm was induced in the right femoral arteries, and Bromocriptine+Cyclosporine A was applied.

Control Group (K) (n=15): Left femoral arteries were left intact.

Surgical Control Group (CK) (n=15): A silastic sheath was wrapped around the left femoral arteries; blood samples were not obtained; and only physiologic saline was used.

Bromocriptine+Cyclosporine A Control Group (Br+CyAK) (n=15): Left femoral arteries were left intact and Bromocriptine+Cyclosporine A was applied.

Bromocriptine Control Group (BK) (n=15): Left femoral arteries were left intact, and Bromocriptine was applied.

Bromocriptine Surgical Control Group (BCK) (n=15): A silastic sheath was wrapped around the left femoral arteries; blood samples were not given; and only physiologic saline and Bromocriptine were applied.

Cyclosporine A Control Group (CyAK) (n=15): Left femoral arteries were left intact, and only Cyclosporine A was applied.

Cyclosporine A Surgical Control Group (CyCK) (n=15): A silastic sheath was wrapped around the left femoral arteries. Instead of blood samples, only physiologic saline and Cyclosporine A were applied.

Cyclosporine A+Bromocriptine Surgical Control Group (Br+CyACK) (n=15): A silastic sheath was wrapped around the left femoral arteries; instead of blood samples, only physiologic saline and Cyclosporine A+Bromocriptine were applied.

Rats were anesthetized with intraperitoneal 2 mg/kg ketamine HCl and laid supine on mushroom blocks.

The inguinal region of the rats were shaved and cleansed with a sterile povidone (PVD) iodine

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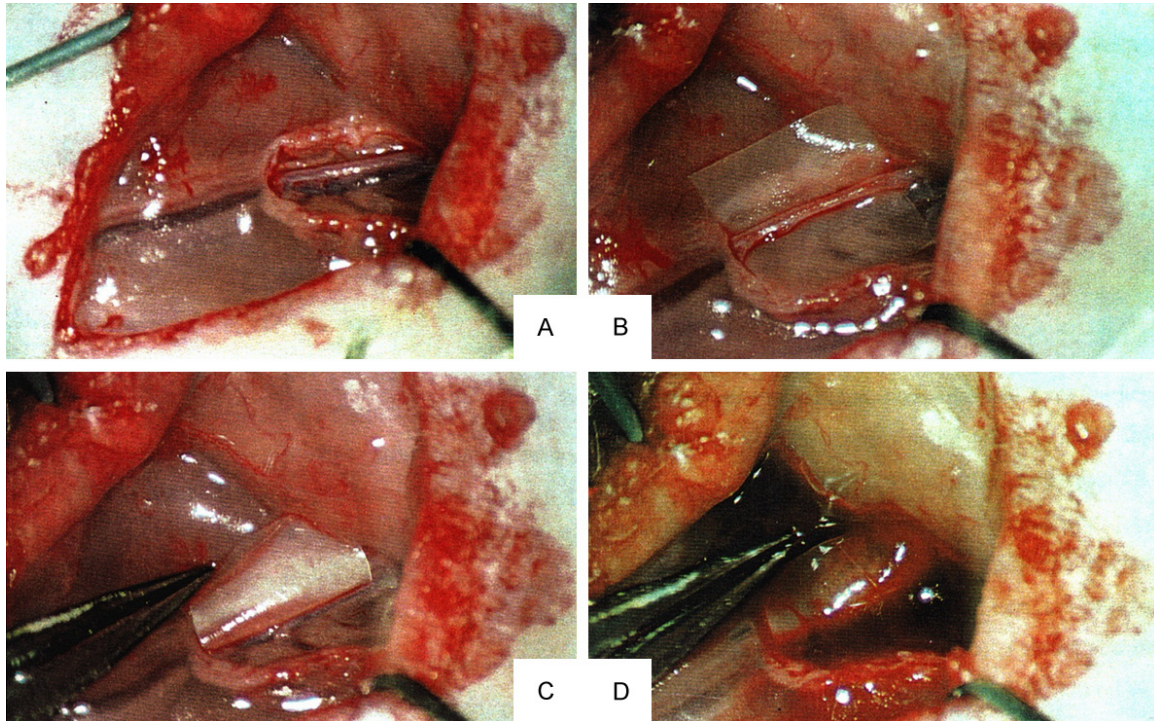


Figure 3. A. The femoral neurovascular bundle. B. Silastic sheath and the femoral artery. C. Wrapping silastic sheath around the femoral artery. D. Blood injection into the silastic sheath wrapped around the femoral artery in order to create a subarachnoid hemorrhage model.

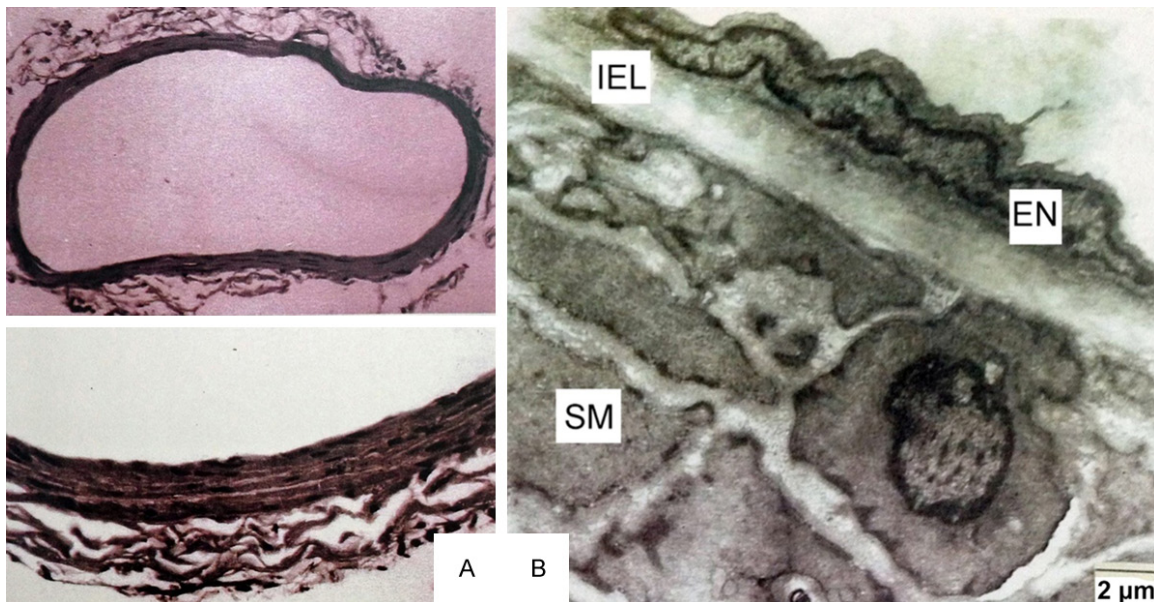


Figure 4. A. Control group, light microscopic examination. B. Control group, TEM examination the of femoral artery. (En: Endotelium, IEL: Internal elastic lamina, SM: Muscle).

solution. Through a longitudinal 2 cm skin incision (**Figure 2**), the femoral neurovascular bundle was explored under a surgical microscope. The femoral artery was dissected away from

the adjacent vein and nerve without traumatizing the femoral artery (**Figure 3A**). A silastic sheath was wrapped and sutured around a 1.5 cm segment of the femoral artery (**Figure 3B**

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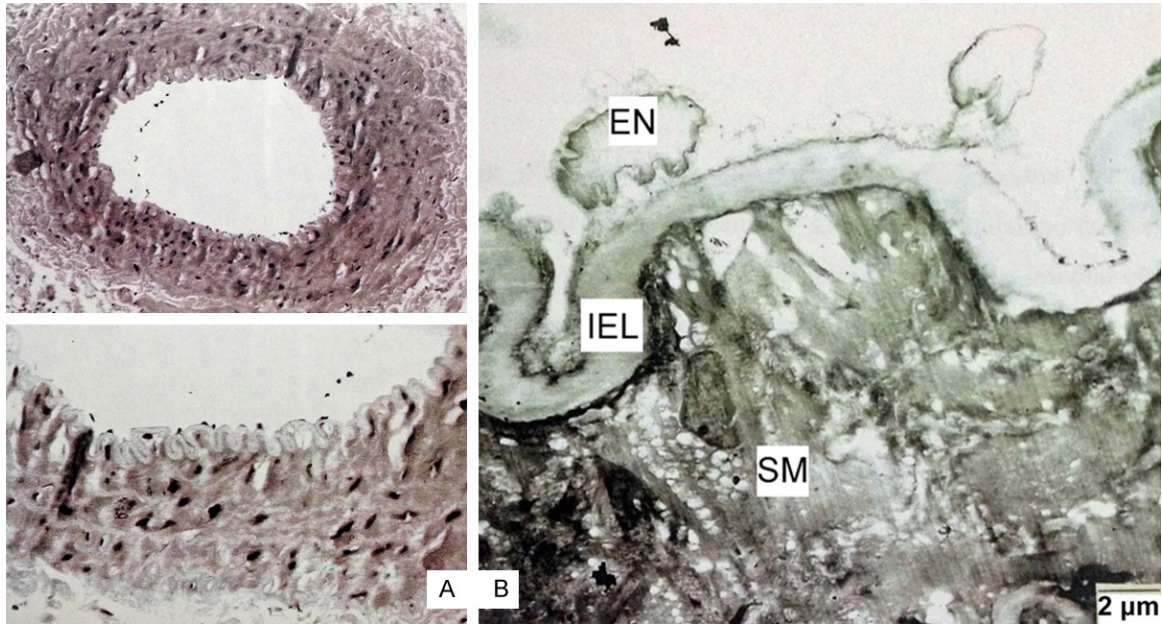


Figure 5. A. Vasospasm group, light microscopic examination. B. Vasospasm group TEM examination the of femoral artery. (En: Endotelium, IEL: Internal elastic lamina, SM: Muscle).

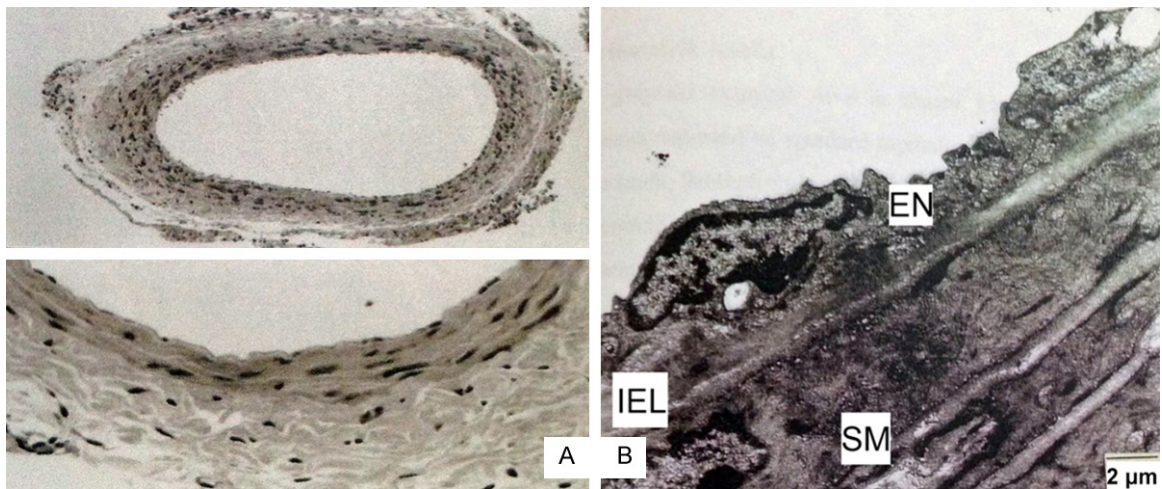


Figure 6. A. Bromocriptine+Cyclosporine A applied group, light microscopic examination. B. Bromocriptine+Cyclosporine A applied group, TEM (EN: Endotelium, IEL: Internal elastic lamina, SM: Muscle).

and 3C). Blood drawn from inside the cardiac chambers was used as a source of whole blood. With an insulin injector, 0.1 cm³ of blood samples were percutaneously drawn from inside the cardiac chambers and injected into the silastic sheath wrapped around the femoral artery so as to create a subarachnoid hemorrhage model (Figure 3D).

After hemostatic control, layers were closed in compliance with surgical principles. Rats were

awoken under normal room temperature in cages that each contained only one rat. The cages were warmed to an appropriate ambient temperature, and the rats were fed with standard rat food for 7 days.

In each of the groups treated with only Bromocriptine or Cyclosporine A+Bromocriptine, a SAH model was constructed, and starting from the day of operation for 7 days through intraperitoneal route, the VBr group received

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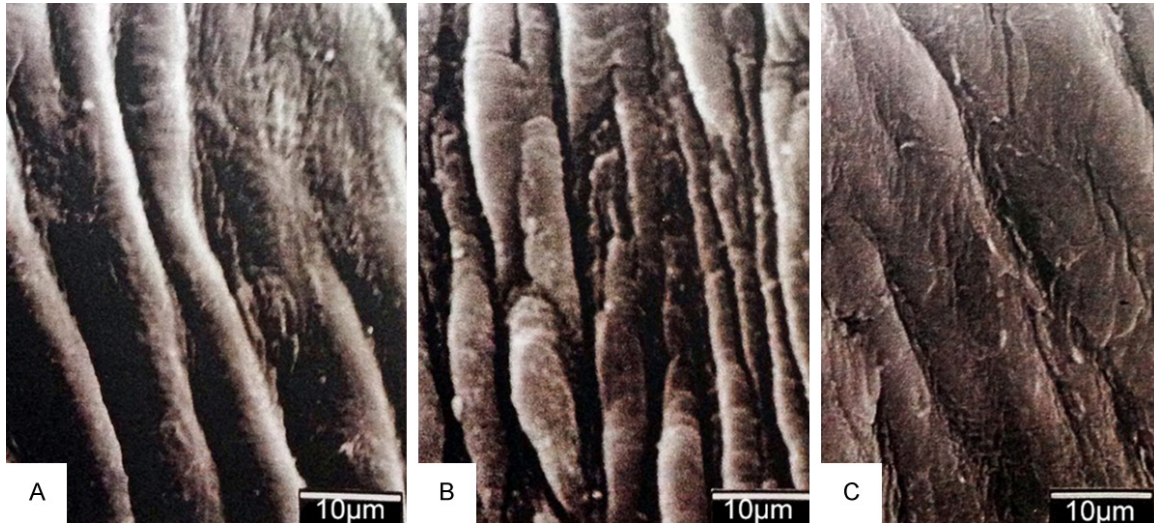


Figure 7. A. Control group, SEM examination the of femoral artery. B. Vasospasm group SEM examination the of femoral artery. C. Bromocriptine+Cyclosporine A applied group, SEM.

Table 2. Lumen widths

	Average \pm Std. Deviation	P
Vasospasm Group (V)	907.16 \pm 124.81	P<0.001
Vasospasm+Bromocriptine Group (VBr)	4205.93 \pm 124.81	P<0.001
Vasospasm+Cyclosporine A Group (VCyA)	4181.03 \pm 625.98	P<0.001
Vasospasm+Bromocriptine+Cyclosporine A Group (VBr+CyA)	4283.83 \pm 697.44	P<0.001
Control Group (K)	5744.00 \pm 470.54	P>0.05
Bromocriptine+Cyclosporine A Control Group (Br+CyAK)	5773.33 \pm 341.92	P>0.05
Surgical Control Group (CK)	5573.33 \pm 306.65	P>0.05
Cyclosporine A+Bromocriptine Surgical Control Group (Br+CyACK)	5638.33 \pm 376.42	P>0.05

Table 3. Vessel wall thicknesses

	Average \pm Std. Deviation	P
Vasospasm Group (V)	94.33 \pm 13.28	P<0.001
Vasospasm+Bromocriptine Group (VBr)	49.47 \pm 8.26	P<0.001
Vasospasm+Cyclosporine A Group (VCyA)	49.33 \pm 8.13	P<0.001
Vasospasm+Bromocriptine+Cyclosporine A Group (VBr+CyA)	47.33 \pm 8.26	P<0.001
Control Group (K)	36.06 \pm 5.81	P>0.05
Bromocriptine+Cyclosporine A Control Group (Br+CyAK)	47.33 \pm 8.26	P>0.05
Surgical Control Group (CK)	38.06 \pm 4.85	P>0.05
Cyclosporine A+Bromocriptine Surgical Control Group (Br+CyACK)	37.06 \pm 7.00	P>0.05

Bromocriptine (2 mg/kg/d), the VCyA group received Cyclosporine A (10 mg/kg/d), and the VBr+CyA group received Bromocriptine (2 mg/kg/d)+Cyclosporine (10 mg/kg/d).

The left femoral arteries of the rats in the control group were left intact, while in the surgical control group the left femoral arteries of the

rats were wrapped with a silastic sheath, and only physiologic saline was instilled; blood samples were not used. To assess the effect of Bromocriptine and Cyclosporine A on normal vessels and vessels wrapped only with a silastic sheath, control and surgical control groups received Bromocriptine (2 mg/kg/d)+Cyclosporine A (10 mg/kg/d) through a intraperitone-

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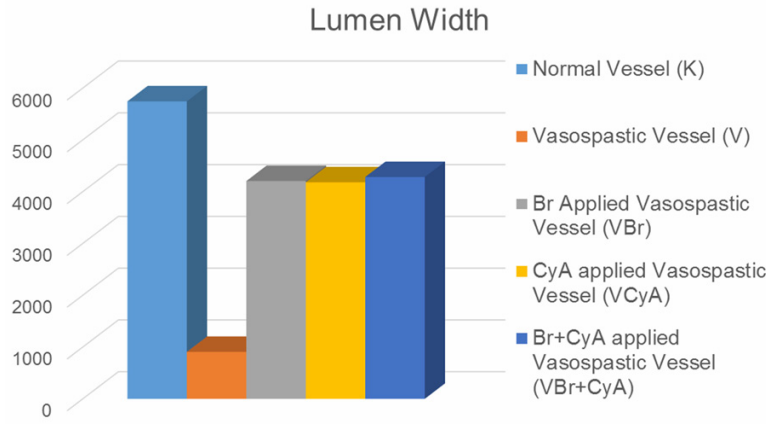


Figure 8. Comparison of lumen widths between normal vessel, vasospastic vessel and Br or CyA or both applied vasospastic vessel.

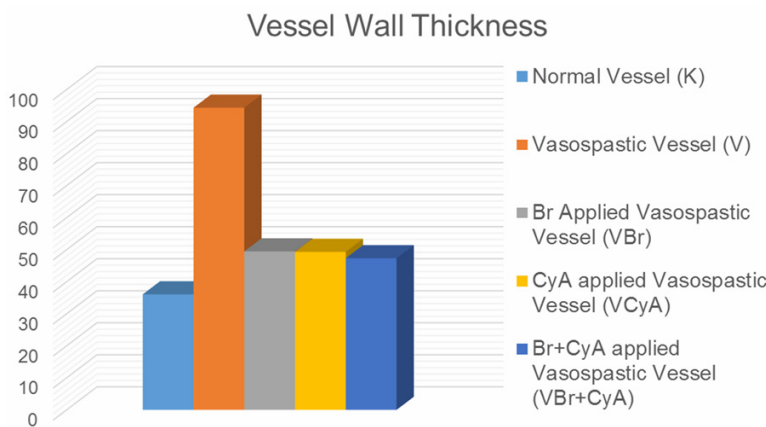


Figure 9. Comparison of vessel wall thicknesses between normal vessel, vasospastic vessel and Br or CyA or both applied vasospastic vessel.

al route for 7 days. The rats were observed for 7 days. None of the rats died. Loss of weight and infection were not observed.

At the end of 7 days, the rats were anesthetized with percutaneously administered intraperitoneal ketamine HCl (2 mg/kg/d) anesthesia and laid in the supine position on mushroom blocks. Previous incisions were opened, and the silastic sheath around the femoral artery was explored. Meanwhile, the skin over the sternum was shaved, and following cleansing of the surgical field with PVD iodine, the sternum was dissected away from its attachment to the ribs using dissection scissors, and the thoracic cavity was explored. The pericardium was opened, and the left ventricular cavity was punctured. The serum set was connected to the puncture catheter. A mixture of 100 ml 0.03

M phosphate buffer (pH 7.4), 200 ml 4% paraformaldehyde and 1% glutaraldehyde solution was injected under physiologic arterial pressure into the left ventricle. The injected solution was circulated through the entire vascular system and then drained from the opened right atrium. Then, a 1.5 cm segment of the intact left femoral arteries was excised and harvested for light microscopic, TEM, and SEM examinations. In the surgical control groups, the left femoral arteries of the rats were wrapped with a silastic sheath, and physiologic saline was applied instead of blood. The 1.5 cm segments of the femoral arteries were excised to be subjected to light microscopic, TEM, and SEM examinations. In the vasospasm group, 1.5 cm segments of vasospasm-induced right femoral arteries were excised for further light microscopic, TEM, and SEM examinations. In vasospasm-induced groups where only Bromocriptine (VBr Group), Cyclosporine A (VCyA Group), or Bromocriptine+Cyclosporine A (VBr+CyA Group) were applied, 1.5 cm segments of the right femoral arteries of the rats were excised to be subjected to light microscopic, TEM, and SEM examinations.

Light microscopic examinations were performed in the laboratories of the Department of Pathology, Marmara University, Faculty of Medicine. The femoral artery specimens were obtained and immersed in buffered 10% formaldehyde solution. They were transferred to cassettes and placed in a tissue monitorization device. In this device, specimens were fixated with formol and dehydrated with a graded alcohol solution. They were also treated with a xylene solution followed by paraffine. All these procedures lasted for 24 hours. After these processes were performed, tissue samples were embedded in paraffine blocks and frozen.

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Five-micron sections were cut with a microtome, and these cut sections were deparaffinized at 600°C in an incubator. Deparaffinization was preceded with xylene treatment that was repeated 3 times. Then the solution was reacted with graded alcohol for rehydration, rinsed with water, and stained with hematoxylin-eosin. Prepared slides were examined under a microscope (Olympus BX7, Japan) at 100X magnification, and their photos were taken for morphometric analyses. Luminal cross-sectional areas and the number of squares within a lumen were calculated and taken as unit values. The same units were also used for the measurements of vascular wall thicknesses. The morphometric analyses were performed, and the tissue samples were compared with regards to vascular lumen thickness and luminal cross-sectional areas.

For the TEM examinations, tissue samples were immersed in a buffered 2.5% glutaraldehyde solution and placed in a buffered 1% osmium tetroxide solution and dehydrated with graded alcohol. Then the specimens were embedded in Epon 812 and examined under a JEOL 100C TEM (JEOL Ltd., Tokyo, Japan). For SEM examinations, tissue samples were immersed and left for 3 hours in 2.5% glutaraldehyde buffered with cacodylate. Then these cacodylate-buffered samples were post-fixed for one hour in 1% osmium tetroxide and dehydrated in alcohol and amyl acetate. The specimens were dried with CO₂ at critical-point, coated with gold in the evaporator, and examined under a JEOL JSM 5200 SEM (JEOL Ltd., Tokyo, Japan).

Statistical evaluation

Statistical evaluation of the morphometric measurement data concerning vascular wall thickness and luminal cross-sectional areas of all groups were performed using Mann-Whitney U, Wilcoxon-signed rank, and Student-t tests.

Histological changes

In the control group, light microscopic examination of the femoral artery vessels had a thin and smooth endothelium, a thin and unfolded internal elastic lamina, and concentrically arrayed smooth muscle cells (**Figure 4A**). In the surgical control group, the vascular structure did not demonstrate a significant difference when compared with the control group. In the

control group, during TEM examination the femoral artery, development of normal organelles of endothelial cells were observed. Also observed was a continuum of endothelial cells, a thin internal elastic lamina, and normal smooth muscle cells (**Figure 4B**). In the SEM examination, a flat and unfolded endothelium, a thin vessel wall, and a dilated lumen were observed (**Figure 7A**).

In the vasospasm group, in the light microscopic examination of the femoral artery, a prominent decrease in lumen diameter and a marked increase in the vascular wall thickness were detected. A disruption in the endothelial integrity was also observed as well as folding of the internal elastic lamina and vacuolization in the muscular layer (**Figure 5A**).

TEM examination of the vasospasm-induced group showed distortion of the endothelial cells, vacuolization, and a decrease in cytoplasmic density. It also showed marked thickening and folding of the internal elastic lamina, sub-endothelial deposition, vacuolar degeneration, and myonecrosis (**Figure 5B**).

The SEM examination of the vasospasm-induced group showed increased and impaired endothelial foldings, craters, and a conspicuous thickening of the vascular wall (**Figure 7B**).

During the light microscopic examination of the femoral artery in the vasospasm-induced group treated with Bromocriptine, just like in the control group, the vessels had a thin and smooth endothelial layer, thin and patchy areas of folded internal elastic lamina, and concentrically aligned smooth muscle cells.

In the vasospasm-induced group treated with Cyclosporine A, light microscopic examination of the femoral artery, similar findings to those seen in the control group were detected.

In the vasospasm-induced group treated with Bromocriptine+Cyclosporine A, light microscopic analysis of the femoral artery did not reveal different findings compared to those of only Bromocriptine, or only Cyclosporine-A, applied groups (**Figure 6A**). The vascular structure in the surgical control group was not significantly different from that of the control group.

TEM examination of the femoral artery in the vasospasm-induced Br+CyA Group showed

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results similar to those seen in the control group, including physiologic development of the endothelial cell organelles, integrity and continuum of the endothelial cells, thin internal elastic lamina, and normal smooth muscle cells (**Figure 6B**). During the SEM examination the following were observed: a flat and mildly folded endothelium, a thin vessel wall, and enlarged lumens (**Figure 7C**).

Morphometric analysis

The mean values (\pm SD) of lumen diameters and vascular wall thicknesses were compared between the groups. Changes were not detected in normal vessel walls and lumens after application of Bromocriptine or Cyclosporine A. Besides, the silastic sheath wrapped around the normal vessels did not lead to changes in the vessel wall or lumen. Also, after application of Cyclosporine A and Bromocriptine onto the silastic sheath wrapped vessel, the vessel wall and lumen did not demonstrate any alteration ($P>0.05$).

The control group and the vasospasm-induced groups were compared, and statistically significant decreases in lumen diameter increases in vascular wall thickness were detected ($P<0.001$). When the vasospasm group was compared with the VCyA, VBr and VBr+CyA groups, the vasospastic artery groups exposed to these drugs showed a decrease in vascular wall thickness and an increase in lumen diameters. The decreases in vascular wall thickness and increases in the lumen of vasospastic arteries in the Bromocriptine or Cyclosporine A groups did not significantly differ between these groups ($P>0.05$). A significant difference was not detected when both drugs were applied singly or in combination (**Tables 2, 3**); (**Figures 8, 9**).

Discussion

Inflammation and cellular and humoral immunity have been known to play a role in the pathogenesis of vasospasm developed after SAH. In 1981, Pellettieri et al. reported the presence of increased amounts of an immune complex circulating in the blood of patients with SAH and subsequently proposed the autoimmune theory [34]. The autoimmune theory, which asserts that red blood cells released and fragmented during minor bleedings behave like

autoantigens and induce formation of immune complexes, was supported by Ostergaard et al., and the association between emerging immune complexes with vasospasm has been demonstrated [8].

Following the introduction of autoimmune theory, studies investigating the use of agents with immunosuppressive activity in the prevention of vasospasm were performed.

Favourable outcomes on the vasospasm-inhibitory effects of Cyclosporine A were obtained in experimental studies and have been used in the prevention of graft rejection in organ transplantation and in the treatment of autoimmune diseases, including endogenous uveitis, psoriasis, atopic dermatitis, and nephrotic syndrome thanks to its potent immunosuppressive effects.

Cyclosporine A exerts its effects by blocking the development of cell-mediated reactions, formation of T-cell dependent antibodies, production and release of lymphokines (incl. interleukin-2), resting lymphocytes at G0 or G1 phases of the cell cycle, and inhibition of release of antigen-stimulated lymphokines from active T-cells [19, 21-24, 35].

The favourable effect of Cyclosporine A on vasospasm was firstly reported by Peterson et al. in the year 1990 and has been subsequently demonstrated by many researchers [6, 12, 23, 36, 37].

Cyclosporine A and Bromocriptine are used in combination in the treatment of autoimmune diseases and transplantation studies. Their combined use increases their immunosuppressive effects [25, 31, 32, 34].

We have not encountered any literature study that has investigated the vasospasm-preventive effect of Bromocriptine, which also has a potent immunosuppressive activity.

Bromocriptine demonstrates its immunosuppressive effect by inhibiting the development of hypersensitivity, primary antibody response, T-lymphocyte dependent macrophage activation, and proliferation of T and B lymphocytes. Bromocriptine (2-Bromo-x-ergocryptine) also has potent dopaminergic effects induced by the activation of dopamine receptors. D2 re-

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ceptors that are affected by Bromocriptine exert their effects by activating the adenylate kinase enzyme. Because of its potent dopaminergic effect, Bromocriptine inhibits the secretion of prolactin, and it is frequently used in the treatment of prolactinoma, galactorrhea, and amenorrhea. Still, due to its dopaminergic effect, it is used as an adjunct to other treatment modalities in Parkinson's disease and acromegaly.

When used in combination with Cyclosporine A in the treatment of organ transplantation and autoimmune diseases, Bromocriptine reinforces the immunosuppressive effect. Besides, effectiveness of Bromocriptine against vasospasm has been demonstrated in many studies as well as in our investigation [6, 12, 23, 37]. Does the Bromocriptine+Cyclosporine A combination also more effectively prevent the development of vasospasm?

We performed this study to find the answer to this question using the "Rat Femoral Artery Vasospasm Model" proposed by Okada et al. [33]. Rats are the most frequently preferred experimental animals in neuroanatomic, neurophysiologic, and neuropharmacologic studies because they are cost-effective, easily available, and they are easy to maintain. In the study where this model was developed, whole blood and washed red and white blood cells were applied singly around the femoral artery adventitia, and the presence of maximum vasospasm was demonstrated at the end of the 7th day of their application. In the light and electron microscopic examinations, morphologic changes were seen along the vascular wall that resembled changes occurring during the vasospasm of cerebral arteries following subarachnoid bleeding [33].

Cerebral arteries differ from systemic arteries in many ways. These differences involve endothelial permeability, response to vasoactive agonists, and the nature of the adventitial matrix [38]. The response of cerebral arteries to various traumatic events (e.g., subarachnoid bleeding) is never the same with the response elicited by systemic arteries. Clinical and experimental studies have demonstrated that vasospasm is dependent on the volume of blood in the subarachnoid space and duration of contact between blood and the vascular wall. Within this context, the tendency of cere-

bral vessels to develop vasospasm is simply associated with the persistence of blood around the vessel following subarachnoid bleeding. This delayed arterial constriction represents the typical individual response of various arteries to the periadventitial presence of blood [39-41]. Outcomes of subarachnoid bleeding concerning vasospasm and those of chronic constriction of rat femoral artery treated with periadventitial blood are comparable in degree, specificity, natural course, and histological appearance [33].

For the investigation of pathologic mechanisms of vasospasm and its potential treatment modalities, this model appears to be simple and applicable.

In the study, a 0.1 ml autologous blood sample was used for a duration of 7 days. In many SAH models, the role of coagulated blood and the impact of its exposure time on ultrastructural changes of the vascular wall have been demonstrated [33, 40, 42-47]. A silastic sheath was used to prolong the exposure time between the whole blood sample and to increase the amount of whole blood coming in contact with the adventitia. Any effect of silastic material on the construction of the vasospasm model has not been reported in the literature [33, 40]. In our study, any statistically significant ultrastructural and morphological differences were not detected between control and surgical control groups.

The effects of Bromocriptine, and Cyclosporine A on vasospasm (when used at doses with maximum effectiveness) were investigated using ultrastructural and morphometric methods. Since in the vasospasm model used, maximum vasospasm does not become manifest before 7 days, in this study vascular wall samples were obtained after 7 days of drug application.

Among the basic criteria of vasospasm, including the cross-sectional area of the lumen and the vascular wall and wall thickness, only the first two parameters were used for morphometric evaluation of outcomes. Statistical analysis was performed using the Student-t, Mann-Whitney U, and Wilcoxon signed-rank tests.

Cyclosporine A, whose favourable effects in the prevention of vasospasm have been demon-

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strated in previous studies, also markedly precluded the development of chronic morphologic vasospasm in our study. We could not detect any change in the amplitude of this preventive effect when Bromocriptine, whose impact in the prevention of vasospasm has not been investigated so far, is used in combination with Cyclosporine A.

However most importantly, when we investigated if Cyclosporine A+Bromocriptine might be more effective, an unexpected and pleasing development became manifest: In the prevention of a morphological vasospasm, Bromocriptine demonstrated strong effects similar to Cyclosporine-A. Any difference was not detected between Bromocriptine and cyclosporine A for the prevention of vasospasm. The mechanism of action of this effect is beyond the scope of this study. However potent immunosuppressive and dopaminergic effects of Bromocriptine have been already recognized. Its success in the prevention of vasospasm may be associated with one or both of these mechanisms. Presence of dopamine-specific receptors in the vascular beds of cerebral vessels has been demonstrated in experimental and human studies using classical pharmacologic methods.

Bromocriptine is a semi-synthetic derivative of ergocriptine that is included in the ergotoxine fraction of ergot alkaloids. Vasodilatory effects of ergotoxine are already known. They demonstrate partial agonistic effectiveness on alpha-receptors via their blockage of vascular x-adrenergic receptors. Their blocking efficacy becomes dominant in constricted vessels, and vasodilation occurs under these conditions.

Our study is promising. Investigations concerning the strong effect of Bromocriptine on cerebral vasospasm, which we revealed in this study, should be continued with experimental and clinical studies that also address its mechanism of action. These investigations are necessary and inevitable so as to demonstrate the extent of beneficial effects of this drug against vasospasm in human beings.

Conclusion

Bromocriptine and Cyclosporine A markedly prevent the development of chronic morphologic vasospasm following SAH. With respect to

the prevention of vasospasm, Bromocriptine and Cyclosporine do not differ-both of them are equally effective. The combined use of both drugs does not change this preventive effect.

Disclosure of conflict of interest

None.

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References

- [1] Kassell NF, Torner JC, Haley EC Jr, Jane JA, Adams HP, Kongable GL. The International Cooperative Study on the Timing of Aneurysm Surgery. Part 1: Overall management results. *J Neurosurg* 1990; 73: 18-36.
- [2] Chehrazhi BB, Giri S, Joy RM. Prostaglandins and vasoactive amines in cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Stroke* 1989; 20: 217-24.
- [3] Kwan AL, Lin CL, Wu CS, Chen EF, Howng SL, Kassell NF, Lee KS. Delayed administration of the K⁺ channel activator cromakalim attenuates cerebral vasospasm after experimental subarachnoid hemorrhage. *Acta Neurochir (Wien)* 2000; 142: 193-7.
- [4] Takeuchi H, Tanabe M, Okamoto H, Yamazaki M. Effects of thromboxane synthetase inhibitor (RS-5186) on experimentally-induced cerebral vasospasm. *Neurol Res* 1999; 21: 513-6.
- [5] German JW, Lin CL, Wu CS, Chen EF, Howng SL, Kassell NF, Lee KS. Systemic complement depletion inhibits experimental cerebral vasospasm. *Neurosurgery* 1996; 39: 141-5; discussion 145-6.
- [6] Handa Y, Hayashi M, Takeuchi H, Kobayashi H, Kawano H, Kabuto M. Effect of cyclosporine on the development of cerebral vasospasm in a primate model. *Neurosurgery* 1991; 28: 380-5; discussion 385-6.
- [7] Kasuya H and Shimizu T. Activated complement components C3a and C4a in cerebrospinal fluid and plasma following subarachnoid hemorrhage. *J Neurosurg* 1989; 71: 741-6.
- [8] Ostergaard JR, Kristensen BO, Svehag SE, Teisner B, Miletic T. Immune complexes and complement activation following rupture of intracranial saccular aneurysms. *J Neurosurg* 1987; 66: 891-7.
- [9] Palestine AG, Muellenberg-Coulombre CG, Kim MK, Gelato MC, Nussenblatt RB. Bromocriptine and low dose cyclosporine in the treatment of experimental autoimmune uveitis in the rat. *J Clin Invest* 1987; 79: 1078-81.

Medical treatment of vasospasm

- [10] Pellettieri L, Nilsson B, Carlsson CA, Nilsson U. Serum immunocomplexes in patients with subarachnoid hemorrhage. *Neurosurgery* 1986; 19: 767-71.
- [11] Peterson JW, Kwun BD, Hackett JD, Zervas NT. The role of inflammation in experimental cerebral vasospasm. *J Neurosurg* 1990; 72: 767-74.
- [12] Ryba M, Pastuszko M, Iwanska K, Bidzinski J, Dziewiecki C. Cyclosporine A prevents neurological deterioration of patients with SAH—a preliminary report. *Acta Neurochir (Wien)* 1991; 112: 25-7.
- [13] Tsementzis SA, Chao SW, Hitchcock ER, Gill JS, Beevers DG. Oligoclonal immunoglobulin G in acute subarachnoid hemorrhage and stroke. *Neurology* 1986; 36: 395-7.
- [14] Bernton E, Bryant H, Holaday J, Dave J. Prolactin and prolactin secretagogues reverse immunosuppression in mice treated with cysteamine, glucocorticoids, or cyclosporin-A. *Brain Behav Immun* 1992; 6: 394-408.
- [15] Hiestand PC, Mekler P, Nordmann R, Grieder A, Permmongkol C. Prolactin as a modulator of lymphocyte responsiveness provides a possible mechanism of action for cyclosporine. *Proc Natl Acad Sci U S A* 1986; 83: 2599-603.
- [16] Jara LJ, Lavalle C, Fraga A, Gómez-Sánchez C, Silveira LH, Martínez-Osuna P, Germain BF, Espinoza LR. Prolactin, immunoregulation, and autoimmune diseases. *Semin Arthritis Rheum* 1991; 20: 273-84.
- [17] Morkawa K, Oseko F and Morikawa S. Immunosuppressive property of bromocriptine on human B lymphocyte function in vitro. *Clin Exp Immunol* 1993; 93: 200-5.
- [18] Morikawa K, Oseko F and Morikawa S. Immunosuppressive activity of bromocriptine on human T lymphocyte function in vitro. *Clin Exp Immunol* 1994; 95: 514-8.
- [19] Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB. Cyclosporine: a new immunosuppressive agent for organ transplantation. *Ann Intern Med* 1984; 101: 667-82.
- [20] de la Monte SM, Bour C, Radhakrishnan VV, Jupiter JB, Smith RJ, Hedley-Whyte ET. Effects of cyclosporin A and predegeneration on survival and regeneration of peripheral nerve allografts in rabbits. *Surg Neurol* 1988; 29: 95-100.
- [21] Howard MA 3rd, Dacey, RG Jr and Winn HR. Brain xenografts: the effect of cyclosporin A on graft survival. *J Neurosurg* 1988; 69: 121-6.
- [22] Kahan BD. Individualization of cyclosporine therapy using pharmacokinetic and pharmacodynamic parameters. *Transplantation* 1985; 40: 457-76.
- [23] Manno EM, Gress DR, Ogilvy CS, Stone CM, Zervas NT. The safety and efficacy of cyclosporine A in the prevention of vasospasm in patients with Fisher grade 3 subarachnoid hemorrhages: a pilot study. *Neurosurgery* 1997; 40: 289-93.
- [24] Nagata K, Sasaki T, Iwama J, Mori T, Iwamoto S, Nirei H, Hamada K, Kirino T. Failure of FK-506, a new immunosuppressant, to prevent cerebral vasospasm in a canine two-hemorrhage model. *J Neurosurg* 1993; 79: 710-5.
- [25] Cardon SB, Larson DF and Russell DH. Rapid elevation of rat serum prolactin concentration by cyclosporine, a novel immunosuppressive drug. *Biochem Biophys Res Commun* 1984; 120: 614-8.
- [26] Carrier M, Wild J, Pelletier LC, Copeland JG. Bromocriptine as an adjuvant to cyclosporine immunosuppression after heart transplantation. *Ann Thorac Surg* 1990; 49: 129-32.
- [27] Clodi M, Kotzmann H, Riedl M, Schmidt A, Barnas U, Mühlbacher F, Mustafa G, Hörl WH, Waldhäusl W, Mayer G, Luger A. The long-acting dopamine agonist bromocriptine mesylate as additive immunosuppressive drug after kidney transplantation. *Nephrol Dial Transplant* 1997; 12: 748-52.
- [28] Compton CC, Rizk I, Regauer S, Burd E, Holaday J, Kenner J. The effect of bromocriptine-induced hypoprolactinemia on xenogeneic and allogeneic skin graft survival in a mouse model. *J Burn Care Rehabil* 1994; 15: 393-400.
- [29] Morinaga K, Hayashi S, Matsumoto Y, Omiya N, Mikami J, Ueda M, Sato H, Inoue Y, Okawara S. [Hyponatremia and cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage]. *No To Shinkei* 1992; 44: 629-32.
- [30] Neidhart M. Synergism between long-acting bromocriptine microcapsules and cyclosporine A in the prevention of various autoimmune diseases in rats. *Experientia* 1996; 52: 892-9.
- [31] Russell DH, Larson DF, Cardon SB, Copeland JG. Cyclosporine inhibits prolactin induction of ornithine decarboxylase in rat tissues. *Mol Cell Endocrinol* 1984; 35: 159-66.
- [32] Wilner ML, Ettenger RB, Koyle MA, Rosenthal JT. The effect of hypoprolactinemia alone and in combination with cyclosporine on allograft rejection. *Transplantation* 1990; 49: 264-7.
- [33] Okada T, Harada T, Bark DH, Mayberg MR. A rat femoral artery model for vasospasm. *Neurosurgery* 1990; 27: 349-56.
- [34] Pellettieri L, Carlson CA and Lindholm L. Is the vasospasm following subarachnoidal hemorrhage an immunoreactive disease? *Experientia* 1981; 37: 1170-1.
- [35] Cook DA. The pharmacology of cerebral vasospasm. *Pharmacology* 1984; 29: 1-16.

Medical treatment of vasospasm

- [36] Peterson JW, Nishizawa S, Hackett JD, Bun T, Teramura A, Zervas NT. Cyclosporine A reduces cerebral vasospasm after subarachnoid hemorrhage in dogs. *Stroke* 1990; 21: 133-7.
- [37] Yanamoto H, Kikuchi H and Okamoto S. Effects of protease inhibitor and immunosuppressant on cerebral vasospasm after subarachnoid hemorrhage in rabbits. *Surg Neurol* 1994; 42: 382-7.
- [38] JA R. Architecture of the wall, in: SR G, editor. *Handbook of physiology*. Philadelphia: bethesda; 1980. pp. 1-31.
- [39] Clowes AW, Reidy MA and Clowes MM. Mechanisms of stenosis after arterial injury. *Lab Invest* 1983; 49: 208-15.
- [40] Mayberg MR, Okada T and Bark DH. The significance of morphological changes in cerebral arteries after subarachnoid hemorrhage. *J Neurosurg* 1990;72: 626-33.
- [41] Ross R. George Lyman Duff Memorial Lecture. Atherosclerosis: a problem of the biology of arterial wall cells and their interactions with blood components. *Arteriosclerosis* 1981; 1: 293-311.
- [42] Brismar J and Sundbarg G. Subarachnoid hemorrhage of unknown origin: prognosis and prognostic factors. *J Neurosurg* 1985; 63: 349-54.
- [43] Findlay JM, Macdonald RL, Weir BK, Grace MG. Surgical manipulation of primate cerebral arteries in established vasospasm. *J Neurosurg* 1991; 75: 425-32.
- [44] Kassell NF, Sasaki T, Colohan AR, Nazar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. *Stroke* 1985; 16: 562-72.
- [45] Mayberg MR, Okada T and Bark DH. Morphologic changes in cerebral arteries after subarachnoid hemorrhage. *Neurosurg Clin N Am* 1990; 1: 417-32.
- [46] Mayberg MR, Houser OW and Sundt TM Jr. Ultrastructural changes in feline arterial endothelium following subarachnoid hemorrhage. *J Neurosurg* 1978; 48: 49-57.
- [47] Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Johshita H, Nazar GB, Torner JC. Effect of subarachnoid hemorrhage on endothelium-dependent vasodilation. *J Neurosurg* 1987; 66: 915-23.