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Research Article

Morphometric Analysis Of Vasospastic Rat Femoral Artery Via Double Hemorrhage Model

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Summary

Objective: We employed a rat femoral artery model of experimental vasospasm in order to evaluate morphometric changes and a possible autoimmune response in a delayed secondary hemorrhage vasospasm.
Methods: We established a vasospasm model of rat femoral arteries by using Okada's model, and evaluated vasospasmic arteries in terms of morphometric changes at first week and first month. We evaluated the effect of secondary hemorrhage, which we performed at the end of the first month, at the end of the first week after secondary hemorrhage. For the evaluation of arteries, we performed hematoxylin and eosin staining. We measured vessel wall thickness and lümen areas and compared these across all the groups.
Results: We observed no significant difference between first week and first month results, in terms of vessel wall thickness and lumen area measurements. There was also no significant difference between the secondary hemorrhage results evaluated at first week (after secondary hemorrhage) and first month. However, we found a signficant difference when these results were compared to the femoral arteries of control group rats. Furthermore, we observed an excessive number of fibrous structures and amount of granulation after secondary hemorrhages.
Conclusions: It was not possible for us to investigate the response of the femoral artery to secondary delayed hemorrhage and a possibly-related autoimmune mechanism. This was due to the excessive fibrous structure and granulation around the vessel, which prevented treatment of the secondary hemorrhage with vessels. Furthermore, morphological changes caused the prevention of vessel vasospasm and vasoconstriction. Finally, we believe that Okada's vasospasm model is not suitable for secondary delayed hemorrhage studies.

Key words: Double hemorrhage, Subarachnoid hemorrhage, Rat femoral artery, Vasospasm model

İkili Kanama Modeli İle Rat Vazospastik Femoral Arterin Morfometrik Analizi

Özet

Amaç: Bu çalışmada, sıçanlarda deneysel vazospastik femoral arter modeli oluşturularak geç oluşan ikinci kanamaya verilen arter duvarı cevabındaki morfometrik değişiklikler ve muhtemel otoimmün cevabın incelenmesi hedeflenmiştir.
Yöntem ve Gereç: Bu çalışmada Okada'nın rat femoral arter vazospazm modeli kullanılarak femoral arterlerde vazospazm oluşturuldu. Vazospasmik hale getirilen femoral arterler birinci hafta ve birinci ay sonunda morfometrik olarak analiz edildi. Birinci ayın sonunda yapılan ikinci kanamanın etkisi de bir hafta (ikinci kanamadan) sonra analiz edildi. Analizler için

hematoksilen eosin boyaması yapıldı. Tüm guruplara damar duvar kalınlığı ve lümen çapı ölçümleri yapıldı ve karşılaştırıldı.
Bulgular: Sonuçlarımız, ilk bir ay sonunda damar duvar kalınlığı ve lümen çapı ölçümleri, birinci hafta sonuçlarıyla karşılaştırıldığında anlamlı bir fark olmadığını gösterdi. Birinci ay sonunda yapılan ikinci kanama sonrasında yapılan analizlerde ise, birinci ay sonunda kanama yapılan ve yapılmayan gruplar arasında anlamlı bir fark bulunmazken, kontrol grubuna göre sonuçlar anlamlıydı. Ayrıca ikinci kanama sonrası yapılan analizde damar çevresinde aşırı miktarda fibröz yapılar ve granülasyon saptanmıştır.
Sonuç: Yaptığımız çalışma sonrasında femoral arterin ikinci kanamaya verdiği tepki ve bununla ilgili olabileceğini düşündüğümüz otoimmun mekanizma ile olan ilişkisi araştırılamamıştır. Bunun sebebi ise, damar çevresindeki aşırı fibröz yapı ve granülasyonun damarın kanla tekrar muamelesine engel olması ve damarın vazospazm ve vazokonstrüksiyonunu engellemesi olarak düşünüldü. Sonuç olarak Okada'nın vazospazm modeli geç ikincil kanama çalışmaları için uygun bir model değildir.

Anahtar Kelimeler: İkincil kanama, Subaraknoid kanama, Sıçan femoral arteri, Vazospazm modeli

INTRODUCTION

One of the most serious consequences of aneurysmal rupture is cerebral vasospasm following subarachnoid hemorrhage (SAH), and it is the major cause of death, as well as cerebral ischemia, which results in many disabilities.[2,12].Although a relatively small percentage of all strokes are caused by spontaneous SAH, approximately 30,000 Americans are affected yearly, and it carries a high mortality rate, reported as being 33–45%.[6,10,27] In addition, 22% to 25% of cerebrovascular deaths are also caused by spontaneous SAH.[3,9]

Despite many advances, the pathophysiology of cerebral vasospasm remains unclear, as it is complex and multifactorial.[14,18,19] However, it is known that one of the most affected molecular mechanisms in SAH is inflammation; the presence of a blood clot in the subarachnoid space causes a series of cellular and molecular events, resulting in an acute inflammatory response in the cerebral vessels. Although the possible role of inflammation in the process of cerebral vasospasm has been hypothesized for some time, its function, the importance at the cellular level, and its molecular basis, have only recently been studied in greater detail.

Mortality and morbidity dependent on ischemia due to vasospasm develop in approximately 30% of patients after aneurysmal SAH.[13,15] It is vital to understand the pathogenesis of SAH, in order to reduce its post SAH symptoms and find a solution. Pathogenesis of vasospasm cannot easily be modeled in rats, as single SAH-induced rat models possess complex characteristics. In addition, the majority of single injection models reflect acute studies that investigate only initial constriction and do not provide mechanistic information with regard to the morbidity and mortality caused by cerebral ischemia. However, a double hemorrhage rat model replicates the time course of vasospasm and can reveal pathological or angiographical similarities to delayed femoral artery vasospasm. Several experimental studies inducing a double hemorrhage have been developed to demonstrate delayed cerebral vasospasm in rats, using different methodological approaches, and have revealed similar results in terms of vasospastic process and pathogenesis.[1,20,23,26,28] However, some methodological differences between these models are present in injected blood volumes, time course and injection area. Moreover, to our knowledge, no studies showing a morphometric analysis of rat femoral arteries have yet been conducted.

Therefore, we aimed to investigate the response of femoral artery to a double hemorrhage, and a possible autoimmune response preventing secondary vasospasm, by providing a morphometric analysis using Okada's[22] previously approved and confirmed 'Rat femoral artery vasospasm model'.

MATERIAL AND METHODS

Induction of vasospasm

We carried out a study at the Istanbul University, Experimental Medicine Research Institute, and all experimental protocols were approved by Istanbul University School of Medicine Animal Care and Research Committee. We performed all surgical procedures under intraperitoneal injection of 50 mg/kg pentorbital sodium anesthesia.

Our general purpose was to induce experimental vasospasm in rats using a vasospasm model that was previously established by Okada et al.,[22] and to evaluate the diameter of large femoral arteries at various time points post vasospasm and secondary delayed vasospasm. We used 35 male Wistar rats weighing 225g to 240g, and held their rectal temperature constant at 37°C via a heating pad. We exposed the right femoral artery and covered it with a silastic cuff, and then injected 0.1 ml of autologous whole blood (Figure 1).[16]

We used five different study groups, each containing 7 rats, as shown in Table 1: control group-1 (C), in which we injected saline into the silastic cuff surrounding femoral artery instead of blood; the week-1 vasospasm group-2 (V1W) in which we injected blood between femoral artery and silastic cuff, then femoral artery was removed and analyzed at end of the first week. Month 1 vasospasm group-3 (V1M), which we performed the same procedure, but femoral artery was removed at first month and analyzed. Group 4 (V1M+B) describe the delayed secondary hemorrhage group, which we performed second vasospasm to the one month before vasospasm created artery, and injected same amount of blood again, then delayed vasospasm created artery was removed and analyzed at first week. Group 5 (V1M+S) describe the delayed secondary hemorrhage's control group, which we performed second vasospasm to the one month before vasospasm created artery, but instead of blood same amount of saline was injected at second procedure, then femoral artery was removed and analyzed at the first week. The control animals underwent the same basic procedure as the experimental animals, except that saline was injected into the intracuff space rather than the blood.

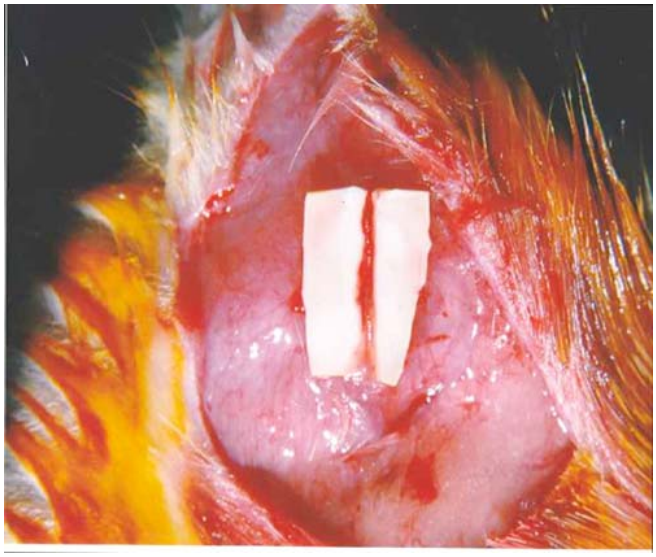

Figure 1: *Exposure of right femoral artery. We covered the artery with a silastic cuff, and then injected 0.1 ml of autologous whole blood was into this cuff.*

Table 1: Study groups of control and experimental rats.

Groups	Name	N	Details
Group 1	Control, normal artery (C)	7	Saline was injected into the silastic cuff surrounding femoral artery instead of blood
Group 2	Vasospasm + 1. week (V1W)	7	Blood was injected between femoral artery and silastic cuff, then femoral artery was removed at end of the first week
Group 3	Vasospasm + 1. month (V1M)	7	Blood was injected between femoral artery and silastic cuff, then femoral artery was removed one month later
Group 4	Vasospasm + 1. month + Delayed secondary vasospasm (V1M+B)	7	Secondary delayed vasospasm performed to the one month before vasospasm created artery. Same amount of blood injected at second procedure and analyzed at first week.
Group 5	Vasospasm + 1. month + Saline group (V1M+S)	7	Secondary delayed vasospasms' control group which we performed to the one month before vasospasm created group, but same amount of saline injected instead of blood, then femoral artery was removed and analyzed at first week.

Histological assessment

We examined all animals undergoing the vasospasm procedure, including the control groups, at different time points to evaluate histological changes in the femoral arteries. We perfused these animals with 50ml of 0.1 mol/l phosphate-buffered solution (pH 7.4 at 37°C), followed by 200 ml of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 mol/l phosphate-buffered solution. We conducted all perfusions at a flow rate of 5.5ml/min and these were complete after all the solution had drained from the right atrium. Following perfusion, we immersed the removed femoral artery tissues overnight at 4°C in identical fixative. We post fixed and paraffinized the specimens in 10% formaldehyde solution, and cut cross-sections of arteries at a thickness of 0.5mm with an ultramicrotome, which we then mounted on glass slides, and stained. For routine histology, we used hematoxylin and eosin for staining, in order to observe the lumen area and vessel diameter, placed the sections in 10 mM citrate buffer (pH 6) and boiled them for 5 min in a microwave. We subsequently incubated the sections at 37°C for 30 min in a destaining buffer.

We viewed and photographed the sections using a standard transmission light microscope (Olympus BX7, Japan) with 100X magnification.

Statistical analysis

Two independent observers evaluated morphometric analysis, including vessel lumen area and vessel wall thickness, in a double-blind manner. We analyzed the images using Adobe Photoshop CS5®, and compared image data between experimental animal and controls, using an unpaired Mann Whitney U-test for the histochemistry results, expressed as mean ± standard deviation. We performed statistical analysis using the GraphPrism program package, version 4 (GraphPad Software, San Diego, CA, USA) and set significance at $p<0.05$.

RESULTS

Histological observations

We evaluated all arteries under light microscopy. The control rat femoral arteries showed a thin vessel wall and median layer with smooth endothelia, and thin and unfolded internal elastic lamina including concentric smooth muscle cells (Figure 2A). Conversely, the V1W group showed a significant reduction in the lumen area diameter, as well as a significant increase in vessel wall thickness. Endothelial integrity was interrupted, internal elastic lamina was partially folded and smooth muscle cells were vacuolated. In addition, we observed inflammatory cell infiltration and rare capillary proliferation (Figure 2B). The V1M group showed the same changes as the V1W group, with the exception of the inflammatory cell infiltration, which we did not find (Figure 2C).

The V1M+S group also showed the same morphological changes in the vessel wall and lumen area diameter differentiations, along with the endothelial changes. Further, we also observed myxoid degeneration in the median layer, rare perivascular inflammatory cells, granulation layer with extensive fibrosis, and capillary proliferation. Intracellular and extracellular hemosiderin pigments were also present (Figure 2D). Finally, the V1M+B group showed the same changes as the V1M group, and inflammatory cell invasion was obvious (Figure 2E).

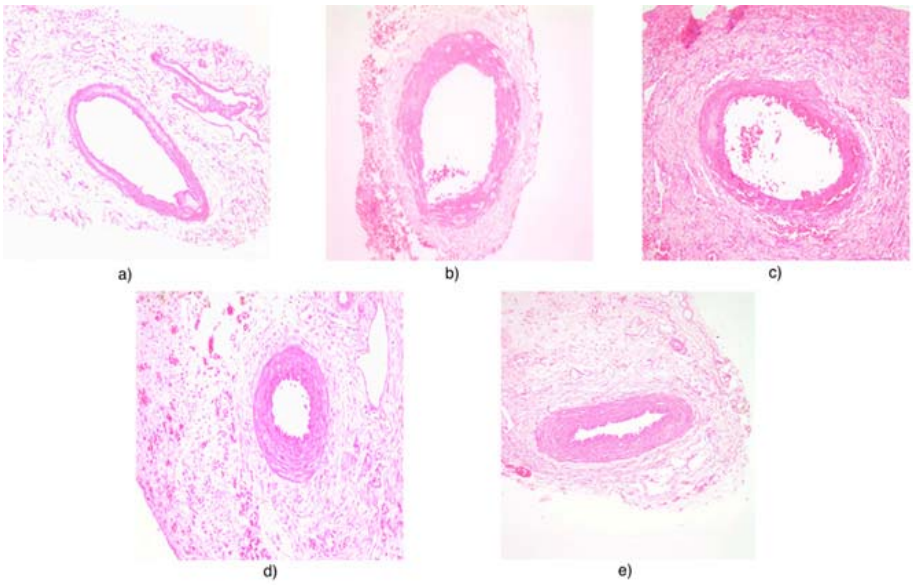

Figure 2: *Histological observations of femoral arteries. Sections were stained with hematoxylin and eosin and photographed at 40X magnication. a) Control artery; thin vessel wall with normally appearing internal elastic lamina and smooth muscle layer. b) Week 1 vasospasm artery (V1W); decreased lumen area with increase in wall thickness and interrupted internal elastic laminae. c) First month vasospasm artery (V1M); decreased lumen area with increase in wall thickness and interrupted internal elastic laminae. d) First month vasospasm artery + saline injected artery (V1M+S); decreased lumen area with increase in wall thickness, interrupted internal elastic laminae and fibrosis. e) First month vasospasm artery + secondary injected artery (V1M+B); decreased lumen area with increase in wall thickness, interrupted internal elastic laminae and fibrosis.*

Morphometric analysis

In the control group, the lumen area diameter varied between 22.04mm and 58.32mm, with a mean of 36.46mm ± 12.25mm, where the mean vessel wall thickness was 4.86mm ± 1.02mm. We found a significant difference in lumen area diameter in the V1W (p=0.007), V1M (p=0.0041), V1M+S (p=0.0012) and V1M+B (p=0.007) groups, compared to the control group. Vessel wall thickness measurements also showed significant changes in the V1W (p=0.006), V1M (p=0.0111), and V1M+S (p=0.0175) groups compared to the control group, but the V1M+B group did not show such changes (p>0.05) (Figure 3A).

We compared lumen diameter and vessel wall thickness measurements between the V1W group and the other groups (Figure 3B), and lumen diameters showed significant change in the V1M group only. However, there were no significant changes in vessel wall thickness measurements between the V1W group and the other study groups.

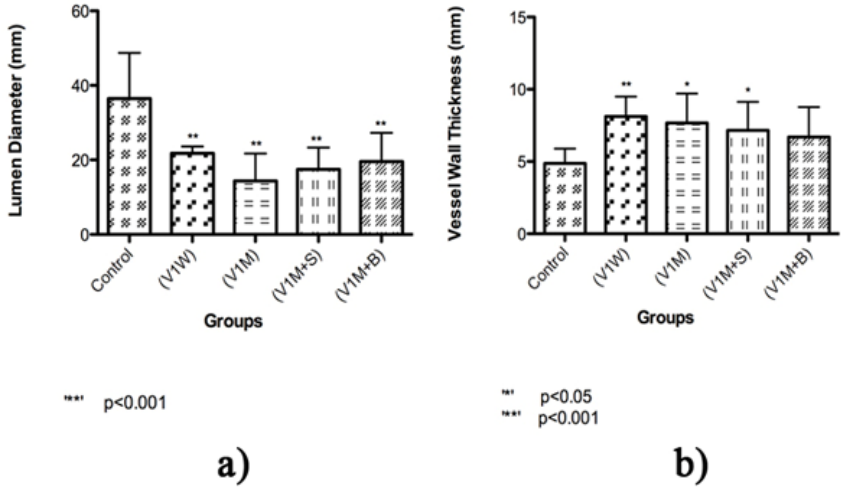

Figure 3: *Morphometric analysis of arteries. a) Lumen diameter, b) Vessel wall thickness.*

DISCUSSION

Our primary aim was to reveal the response of the rat femoral artery to secondary late hemorrhage by using Okada's[22] 'Rat femoral artery vasospasm model'. Rats are the most widely used laboratory animals in neuroanatomical, neurophysiological and neuropharmalogical SAH studies, as they are easy to maintain and handle. This model previously indicated that treatment of either blood or isolated erythrocytes and leucocytes, separate from the adventitia of the femoral artery, would cause maximum vasospasm level at the end of day 7. This created a viable avenue for the study of vasospasm in the rat femoral artery and thus enabled further analysis of the results of double hemorrhage.

Cerebral arteries differ from systemic arteries in various ways, including endothelial permeability, response to vasoactive agonists, and the nature of adventitial matrix.[24] The response of

cerebral arteries to various factors (such as SAH) is not the same as systemic arteries, but clinical and experimental studies have shown that it is related to both blood volume and the time that the vessel wall and blood interact. One can imply that the predisposition of the cerebral arteries for developing vasospasm is simply related to the persistence of blood with the vessel wall and its periphery, and this is the typical response of late arterial vasoconstriction to the periadvential blood.[5,18,25] Therefore characteristics of post-SAH vasospasm and periadvential autologous blood-injected rat femoral artery show similarities in terms of the degree of vasoconstriction, specificity, angiography, and histopathology.[22] The role of coagulated blood and its exposure duration on ultrastructural effects on the vessel wall has been shown in various studies.[4,8,12,18,19,21] We used a silastic cuff to increase the exposure duration of blood in the adventitial environment; silastic cuff has no effect on the vasospasm process[18,22].

The primary aim of this study was to create a vasospastic artery and to return it to normal at the end of the first month without treatment. This artery would then again be exposed to the autologous whole blood, and we wanted to evaluate the response of this artery to a double hemorrhage in terms of hypersensitivity and desensitivity by means of morphometric analyses of the vessel wall and vessel lumen area. Previous clinical studies of the vasospasm-time relationship have indicated that vasospasm starts a couple of hours after SAH and disappears 3 to 4 weeks later.[7] Maspes and Marini[17] found a post-SAH vasospasm incidence of 6 to 7%, but Kalström et al.[11] found no significant difference between vessel wall diameters after 3 to 4 days, a slight decrease after 6-12 days, and a complete return to normal by week 4. Conversely, Okada et al.,[22] found that maximum vasoconstriction occurred at 5 to 10 days, and returned to almost normal size at 20 days in a rat model.

In accordance with Okada's model, we had expected the vessel diameters to return to normal size after 20 days, so we performed the second hemorrhage at the first month. However, the first month's lumen measurements revealed that vessel diameters had significantly decreased, compared to those of the control groups ($p<0.01$) (Figure 3A). The vessel wall measurements also differed significantly between the V1W and control groups ($p<0.05$) (Figure 3B). Even the V1W groups and V1M group differed significantly, indicating that vessel constriction had continued after the first week. However, vessel wall thickness did not differ between the V1W and V1M groups ($p>0.05$). In that sense, our results were not the same as Okada's and other, related, previous studies. Okada et al. also reported that they did not observe endothelial disruption and vacuolization on the muscle layer on day 20 of hemorrhage, following histopathological evaluations. Vessel lumen diameters and vessel wall thickness did not significantly differ between the V1M and V1M+B groups ($p>0.05$) (Figure 2A). Moreover, there was no significant difference between the V1M+B and V1M+S groups ($p>0.05$) (Figure 2A).

We performed the second hemorrhage in the same way as the first, as stated in previous studies. We analyzed the second hemorrhage a week later and, again, injected whole blood into previous silastic cuff after we had removed granulated tissues as much possible. However, as the morphological measurements indicated, there was no significant response to the second hemorrhage. We hypothesize that the reason for this was an extensive fibrous layer inside the silastic cuff and around the artery, which we observed when we reopened the cuff after the first month. This gave rise to the thought that the arteries could not expand because the

extensive fibrous layer or granulation around the artery restricted vasoconstrictive molecules with regard to interaction with the artery. When we evaluated the perivascular tissue inflammatory cell infiltration, first week arteries had mid-level infiltration, whereas this was not observed in first month arteries; instead we found extensive fibrosis (Figure 2. D,E). Conversely, the second hemorrhage also caused infiltration of inflammatory cells, but not as much as we observed in the V1W group. Moreover, the secondary V1M+S and V1M+B groups also showed extensive granulation around the arteries (Figure. 2.D,E).

CONCLUSION

Using the rat femoral artery vasospasm model developed by Okada et al., we indicated that there was no significant difference between first week and first month vasospasm groups in terms of vessel lumen diameter and wall thickness measurements of arteries. Furthermore, first month vasospasm groups showed greater vasoconstriction than first week groups. The secondary hemorrhage had no effect on vessel lumen diameter and vessel wall thickness. This may have been the result of an extensive fibrous structure and granulation around the arteries, preventing interaction between vasospastic molecules and the artery, and possibly preventing vasodilatation. We could not use this model to evaluate the response of the femoral artery to the secondary hemorrhage by means of autoimmune mechanisms, because of the extensive fibrosis tissue and granulation around the artery, so we can conclude that Okada's vasospasm model is not suitable for double hemorrhage studies.

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REFERENCES

1. *Aladag M, Turkoz Y, Sahna E, Parlakpinar H, Gul M. The attenuation of vasospasm by using a sod mimetic after experimental subarachnoidal haemorrhage in rats. Acta neurochirurgica. 2003;145(8):673-677.*
2. *Athar M, Levine J. Treatment Options for Cerebral Vasospasm in Aneurysmal Subarachnoid Hemorrhage. Neurotherapeutics. 2012;9(1):37-43.*
3. *Batjer HH. Cerebrovascular Disease. Lippincott-Raven; 1997.*
4. *Brismar J, Sundbarg G. Subarachnoid hemorrhage of unknown origin: prognosis and prognostic factors. J Neurosurg. 1985;63(3):349-354.*
5. *Clowes A, Reidy M, Clowes M. Mechanisms of stenosis after arterial injury. Laboratory investigation; a journal of technical methods and pathology. 1983;49(2):208.*
6. *Dorsch NWC, King MT. A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage Part I: Incidence and effects. Journal of Clinical Neuroscience. 1994;1(1):19-26.*
7. *Drake C. Formal discussion on Symon L: Vasospasm in aneurysm. In: Moossy J JR, ed. Cerebrovascular Disease, seventh conference. New York1971:241-244.*
8. *Findlay JM, Macdonald RL, Weir BK, Grace MG. Surgical manipulation of primate cerebral arteries in established vasospasm. J Neurosurg. 1991;75(3):425-432.*

9. *Huang CY, Chan FL, Yu YL, Woo E, Chin D. Cerebrovascular disease in Hong Kong Chinese. Stroke. 1990;21(2):230-235.*
10. *Johnston SC, Selvin S, Gress DR. The burden, trends, and demographics of mortality from subarachnoid hemorrhage. Neurology. 1998;50(5):1413-1418.*
11. *Kalström E GT, Honson J et al. Changes in cerebral blood flow after subarachnoid hemorrhage. Paper presented at: Third International Congress of Neurological Surgery1966; Amsterdam.*
12. *Kassell N, Sasaki T, Colohan A, Nazar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke. 1985;16(4):562-572.*
13. *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(1):18-36.*
14. *Kolias AG, Sen J, Belli A. Pathogenesis of cerebral vasospasm following aneurysmal subarachnoid hemorrhage: putative mechanisms and novel approaches. Journal of neuroscience research. 2009;87(1):1-11.*
15. *Lee JY, Huang DL, Keep R, Sagher O. Characterization of an improved double hemorrhage rat model for the study of delayed cerebral vasospasm. Journal of neuroscience methods. 2008;168(2):358-366.*
16. *Lin CL, Calisaneller T, Ukita N, Dumont AS, Kassell NF, Lee KS. A murine model of subarachnoid hemorrhage-induced cerebral vasospasm. Journal of neuroscience methods. 2003;123(1):89-97.*
17. *Maspes PE, Marini G. Intracranial arterial spasm related to supraclinoid ruptured aneurysms. Acta Neurochir (Wien). 1962;10:630-638.*
18. *Mayberg MR, Okada T, Bark DH. The significance of morphological changes in cerebral arteries after subarachnoid hemorrhage. J Neurosurg. 1990;72(4):626-633.*
19. *Mayberg MR, Wayne Houser O, Sundt Jr TM. Ultrastructural changes in feline arterial endothelium following subarachnoid hemorrhage. Journal of neurosurgery. 1978;48(1):49-57.*
20. *Meguro T, Clower BR, Carpenter R, Parent AD, Zhang JH. Improved rat model for cerebral vasospasm studies. Neurological research. 2001;23(7):761-766.*
21. *Nakagomi T, Kassell NF, Sasaki T, et al. Effect of subarachnoid hemorrhage on endothelium-dependent vasodilation. J Neurosurg. 1987;66(6):915-923.*
22. *Okada T, Harada T, Bark DH, Mayberg MR. A rat femoral artery model for vasospasm. Neurosurgery. 1990;27(3):349-356.*
23. *Ono S, Komuro T, Macdonald RL. Heme oxygenase-1 gene therapy for prevention of vasospasm in rats. J Neurosurg. 2002;96(6):1094-1102.*
24. *Rhodin J. Architecture of the vessel wall. In: Bohr DF SA, Sporles HV, ed. Handbook of physiology section. Vol 2. Philadelphia: WB Sounders; 1980:1-31.*
25. *Ross R. George Lyman Duff Memorial Lecture. Atherosclerosis: a problem of the biology of arterial wall cells and their interactions with blood components. Arteriosclerosis, thrombosis, and vascular biology. 1981;1(5):293-311.*
26. *Satoh M, Parent AD, Zhang JH. Inhibitory effect with antisense mitogen-activated protein kinase oligodeoxynucleotide against cerebral vasospasm in rats. Stroke. 2002;33(3):775-781.*
27. *Sudlow CL, Warlow CP. Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. International Stroke Incidence Collaboration. Stroke. 1997;28(3):491-499.*
28. *Vatter H, Weidauer S, Konczalla J, et al. Time course in the development of cerebral vasospasm after experimental subarachnoid hemorrhage: clinical and neuroradiological assessment of the rat double hemorrhage model. Neurosurgery. 2006;58(6):1190-1197; discussion 1190-1197.*