Journal of Neurological Sciences [Turkish] **31:(4)**# 42; 726-735, 2014
http://www.jns.dergisi.org/text.php3?id=823
Research Article

Vascular and Hemodynamic Changes Induced by Experimental Subarachnoid Hemorrhage

Murat ULUTAŞ[1], Ender KORFALI[2]

[1]Konukoglu Hospital of Sanko University, Neurosurgery, Gaziantep, Türkiye [2]University of Uludag School of Medicine, Neurosurgery, Bursa, Türkiye

Summary

Objective: In this experimental subarachnoid hemorrhage (SAH) model, pathophysiological processes that accompany acute vasospasm, morphological changes in the basilar artery, and the effect of altered cerebral blood flow (CBF) on mortality were examined.
Material and Methods: In the hemorrhage group of rats (n=51), experimental subarachnoid hemorrhage was induced and mean arterial pressure (MAP) and cerebral blood flow (CBF) measurements were performed following bleeding. Histopathological assessments were performed in controls (n=7) as well as in rats surviving for 72 hours.
Results: An abrupt reduction of 63.2% ± 10% was observed in CBF, followed by 38.1 ± 13% and 29.1 ± 11% lower values as compared to baseline at 35 and 60 minutes, respectively ($p < 0.001$). The average CBF following hemorrhage was significantly lower in rats that died within the first 24 hours and than those that survived for 3 days ($p < 0.01$). Histopathological examination showed thickening in arterial walls, reduced vessel lumen diameter, and vasculopathy findings.
Conclusion: Our findings suggest that following subarachnoid bleeding the fall in CBF alone does not impact the mortality rates and that the duration of the reduction in CBF seems to represent an important prognostic factor for survival.

Key words: Subarachnoid hemorrhage (SAH), vasospasm, cerebral blood flow, mortality

Deneysel Subaraknoid Kanamanın Yol Açtığı Vasküler ve Hemodinamik Değişiklikler

Özet

Amaç: Çalışmamızda deneysel subaraknoid kanama (SAK) modelinde, akut vazospazma eşlik eden patofizyolojik süreçler, baziler arterdeki morfolojik değişiklikler ve serebral kan akımı (SKA) değişikliğinin mortaliteye etkisi incelenmiştir.
Yöntem ve Gereç: Kanama grubundaki sıçanlarda (n=51) deneysel subaraknoid kanama oluşturuldu ve bunlarda kanama sonrasında ortalama arter basıncı (OAB) ve serebral kan akımı ölçümleri yapıldı. Kontrol grubunda (n=7) ve 72 saatten uzun yaşayan kanama grubundaki sıçanlarda histopatolojik değerlendirmeler yapıldı.
Bulgular: SKA'daki %63.2±10 oranındaki ani düşme, 35. dakikada bazal değerin %38.1±13 ve 60. dakikada %29.1±11 altında seyretti ($p<0.001$). İlk 24 saatte ölen ve 3 gün yaşayan sıçanların kanama sonrası ortalama SKA'ları arasında anlamlı farklılık saptandı ($p<0.01$). Histopatolojik incelemeler ile arter duvar kalınlığında artma, damar iç çapında azalma ve arter duvarında vaskülopati bulguları saptandı.
Sonuç: Çalışmamızın bulguları subaraknoid kanama sonrası SKA'daki düşmenin tek başına mortaliteyi etkilemediğini, düşük kalma süresinin uzunluğunun prognozu etkileyen daha önemli faktör olduğunu düşündürmektedir.

Anahtar Kelimeler: Subaraknoid kanama (SAK), vazospazm, serebral kan akımı, mortalite

INTRODUCTION

Although recurrent bleeding is one of the most significant complications of intracranial aneurysmal bleedings, it can be prevented by early diagnosis and treatment. In contrast with recurrent bleeding, vasospasm, another complication of aneurysmal hemorrhage, remains a serious problem despite studies examining potential prophylactic and therapeutic measures to decrease its occurrence[33]. Following aneurysmal subarachnoid hemorrhage (SAH), vasospasm occurs in 67% of the cases[9] with a biphasic course. Typically, the acute phase has a rapid course of 3 to 4 hours and may spontaneously resolve. The chronic phase starts between days 3 and 5, reaches a peak in 6 to 8 days, and resolves approximately at day 14[38].

In experimental models of SAH, the presence of an abrupt reduction in cerebral blood flow (CBF) and cerebral perfusion pressure (CPP), and an increase in intracranial pressure (ICP) has been clearly established[5,6,15,3]. Immediately after a subarachnoid hemorrhage, occurrence of ischemia has been explained on the basis of the quick fall in CBF and acute vasospasm, although its pathophysiological mechanisms are still not very well defined.

In this study, pathophysiological processes that accompany acute vasospasm, morphological changes in the basilar artery, and the effect of altered cerebral blood flow (CBF) on mortality were examined in an experimental SAH model involving a transclival approach.

MATERIAL AND METHODS

Experimental Model and Induction of SAH

A total of 58 female Sprague-Dawley (SD) rats (210-280 g) were used for the study. Rats were fasted 12 hours in temperature (21 ºC) and light (12 hours on/12 hours off) controlled boxes and were allowed to drink water only in this period. The experimental protocol and procedures were approved by the Uludag University Institutional Committee for Animal Experiments. Rats were categorized into hemorrhage and control groups. While rats in the control group (n=7) were not subjected to any experimental procedures initially and were used for histopathological assessments, experimental subarachnoid hemorrhage was induced in the hemorrhage group (n=51).

After rats were intubated transorally following intraperitoneal injection of thiopental 30 mg/kg, they were ventilated with 1% isoflurane, 70% N2% and 30% O2 mixture (Small Animal Ventilator Model 683; Harvard Apparatus, Inc., Holliston, MA, USA). The left femoral artery and vein were catheterized with a PE-50 polyethylene catheter for blood pressure (BP) and heart rate monitoring at 5-min intervals. From the arterial catheters, pCO2, paO2, pH, hematocrit, and oxygen saturation values were measured by microcapillary sampling before and after SAH (Model 348;Chiron Diagnostics, Halstead, Essex, England). Blood sugar measurements were done with glukostix (Medisense Inc. Woltham MA, USA) before induction of SAH.

In the neck region of each rat fixed within the stereotactic frame in supine position, a 2 cm midline skin incision was made and the cervical muscles were dissected to expose the clivus. With the aid of a surgical microscope (Carl Zeiss, Inc., Göttingen, Germany) a high-speed drill cooled with physiological saline (Microton GC 412; Eesculap Co., Tuttlingen, Germany) was utilized to open a 2-mm burr-hole in the 1/3 of the clivus leaving transparent lamella in order to expose the origin of the anterior inferior cerebellary artery (AICA) from the basilar artery under the dura. Another 1-mm burr-hole was opened in the left lateral side of the midline of the clivus to measure the

parenchymal blood flow; a Laser Doppler Flowmetre probe (0.8 mm diameter, Model P-433; Vasomedics, Inc., St Paul MN) was stabilized on the dura at a 90 degree angle to the parenchyma using a stereotactic holder. Brainstem compression, closure of the subarachnoid space, and opening of the dura were avoided and blood flow measurements were performed always at the same location in the brainstem (Figure 1). After 15 minutes of CBF and mean arterial pressure (MAP) monitoring, the bony lamella at the midline burr-hole was penetrated and a glass-penetrator with a 20 μm tip was used to cause vascular wall and endothelial injury at the origin of AICA at basilar artery for inducing diffuse SAH extending into the cisternae in all rats (Figure 1). Caution was exercised for penetrating the anterior wall of the vessel only and for avoiding local or parenchymal hematoma formation. The burr-hole was sealed with wax to avoid cerebrospinal fluid leak as well as leakage from the hemorrhage. Rectal temperature, brainstem parenchymal blood flow, and MAP were recorded 15 minutes before and for 60 minutes after the hemorrhage with 5-minute intervals. Following measurements, anesthesia was terminated and the rats were observed to wean from anesthesia with self-extubation within approximately 30 minutes.

Rats that could survive beyond three days were intubated using the same anesthesia technique and were placed into ventilator. After cerebral blood flow measurements with 5-minute intervals were performed during the same surgical procedure, the rats were sacrificed with 75 mg/kg intraperitoneal thiopental sodium.

Morphological Study

Rats in the control group and rats in the hemorrhage group that survived beyond 72 hours were included in the morphological study. Following decapitation, brains were removed without damaging the Willis circle and were fixed in 10% paraformaldehyte solution for 72 hours for subsequent histopathological studies. Rats with parenchymal bleeding (n=7) were not included. Coronal cross-sections were obtained 0.5 rostral to the vertebrobasilar junction (cross-section V), 0.5 mm proximal to the junction of AICA-basilar artery (cross section a), and 0.5 mm proximal to the branching point of the posterior cerebral artery (cross-section p) (Figure 2). In each cross-section (v, a and p), four separate measurements were performed. The wall thicknesses were measured at 3, 6, 9, 12 o'clock positions from lumen to the outer border of the media without inclusion of the adventitia. The mean of four measurements was calculated to obtain vessel wall thickness for each cross-section in each rat. Using the same approach, the circumferential length was determined using the luminal surface of the intima for each section and intraluminal diameter was measured. The measurements were performed with National Institutes of Health Image J software.

Multiple sections of 10 μm thickness from the basilar artery and brainstem were fixed in paraffin blocks, and stained with hematoxylene and eosin and masson trichrome. Using the light microscopy at a magnification of x400 the following were assessed: ischemia of the brainstem, and pathological findings in the arterial wall of the basilar artery wall (separation from lamina elastic, misalignment), internal elastic lamina (curling, interrupted continuity), media (edema, pale staining, proliferation), smooth muscle cells (vacuolization, necrosis and fibrozis), and adventitia (inflammation).

Statistical Analyses

Within group comparisons for CBF and MAP was performed using Friedman's test, followed by Wilcoxon test. The wall thickness and inner diameter comparisons were performed using Mann-Whitney U test. A p value less than 0.05 was considered significant.

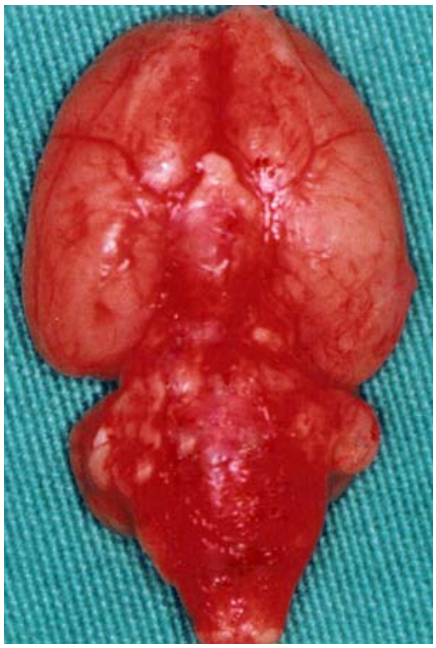

Figure 1: *Diffuse SAH of rat brain extending into the cisternae, which was induced by a glass-penetrator with a 20 µm tip at the origin of AICA.*

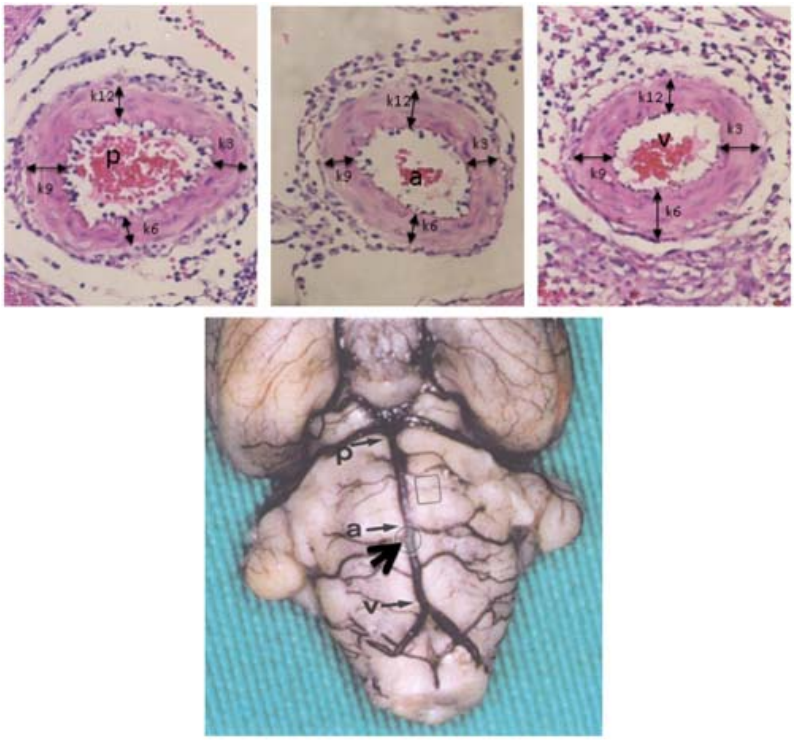

Figure 2: *Upper raw: an example of the calculation of mean vessel thickness in p, a, and v sections using k3, k6, k9, and k12/4 measurements. Lower image: thin arrows indicate the levels for p, a, and v cross-sections; the circle and the square indicates the point where the SAH was induced and parenchymal blood flow measurement was done, respectively.*

RESULTS

Rectal temperature, arterial blood pH, pCO2, pO2, oxygen saturation, hematocrit, and blood glucose values before and after SAH in the hemorrhage group did not differ significantly ($p > 0.05$). In this group, a total of 34 rats survived beyond 72 hours with no neurological deficits except for hypoactivity. Seven rats developing parenchymal focal hematoma were not included in the analyses.

Change in mean arterial pressure in the hemorrhage group

The baseline MAP of 77.9 ± 6.2 mmHg increased to 87.1 ± 10.8 mmHg immediately after the induction of bleeding along with the abrupt fall in CBF ($p < 0.001$). Within the first 15 minutes following bleeding an increase up to 14.5 ± 2.2% was observed which gradually declined and approached baseline values on day 3 ($p > 0.05$) (Figure 3).

Cerebral blood flow alterations in the hemorrhage group

The mortality rate in the first 24 hour and between 24-48 hours were 25.5% (n=13) and 7.8% (n=4), respectively. The mean CBF before bleeding in rats dying within the first 24 hours was 59.2 ± 23.8 mlLD/100g/min, while it went down to 21.6 ± 14.4 mlLD/100g/min after bleeding ($p < 0.001$) and was 36.8 ± 16.05 mlLD/100g/min at 60 minutes ($p < 0.001$). There was an acute decrease in CBF in the order of 63.51 ± 9%, followed by a 50 ± 11% decrease below the baseline at 35 minutes, and 38.7 ± 10% decrease below the baseline at 60 minutes ($p < 0.001$). (Figure 3)

In the hemorrhage group, the pre-hemorrhage mean CBF was 55.5 ± 17.03 mlLD/100g/min in rats surviving beyond 72 hours (n=27), while it decreased to 20.5 ± 10.9 mlLD/100g/min after bleeding, and raised to 39.4 ± 12.3 mlLD/100g/min at 60 minutes ($p < 0.001$). There was an acute fall in CBF of 63.2 ± 10%, followed by values that are 38.1 ± 13% and 29.1 ± 11% below the baseline at 35 and 60 minutes, respectively ($p < 0.001$) (Figure 3). CBF on day 3 was 10.7 ± 8% lower than the baseline, but the difference was not significant ($p > 0.05$). On the other hand, there was a significant difference in blood flow changes between rats that died within the first 24 hours and rats that survived beyond 3 days ($p < 0.01$).

Morphological examination

There were no significant differences in v a and p cross-sections of the vessel wall in control rats ($p > 0.05$). In the hemorrhage group the v, a and p cross-sections were 24.7%, 20.1% and 13.1% higher as compared to controls, respectively ($p<0.001$, $p<0.001$, and $p<0.05$, respectively). Also, within the hemorrhage group, p and a cross-sections (closer to the hemorrhage area) were significantly higher as compared to v cross-sections of the vessel wall ($p < 0.001$ and $p < 0.05$, respectively).

As compared to controls, there was a significantly more marked decrease in v (33.09%), a (38.8%), and p (37.4%) cross-sections of the inner lumen of the basilar artery in the hemorrhage group ($p < 0.001$, $p < 0.001$, and $p < 0.05$, respectively).

Histopathological examination revealed no differences between basilar artery cross-sections (v, a and p). Seventy-two hours after hemorrhage the following diffuse histopathological changes were observed along the whole vessel length in the basilar artery: pale cytoplasm (25/27), separation of endothelium from the internal elastic lamina (15/27), interrupted continuity of the internal elastic lamina (2/27), pale staining of the media (24/27), vacuolization of the smooth muscles (24/27), periadventitial inflammation (27/27), curved endothelium (15/27), curved internal elastic lamina (22/27), and edema of the media (20//10) (Figure 4). None of the rats had fibrozis or necrosis in the media and smooth muscles of the basilar artery wall, and there was no ischemia in the brainstem.

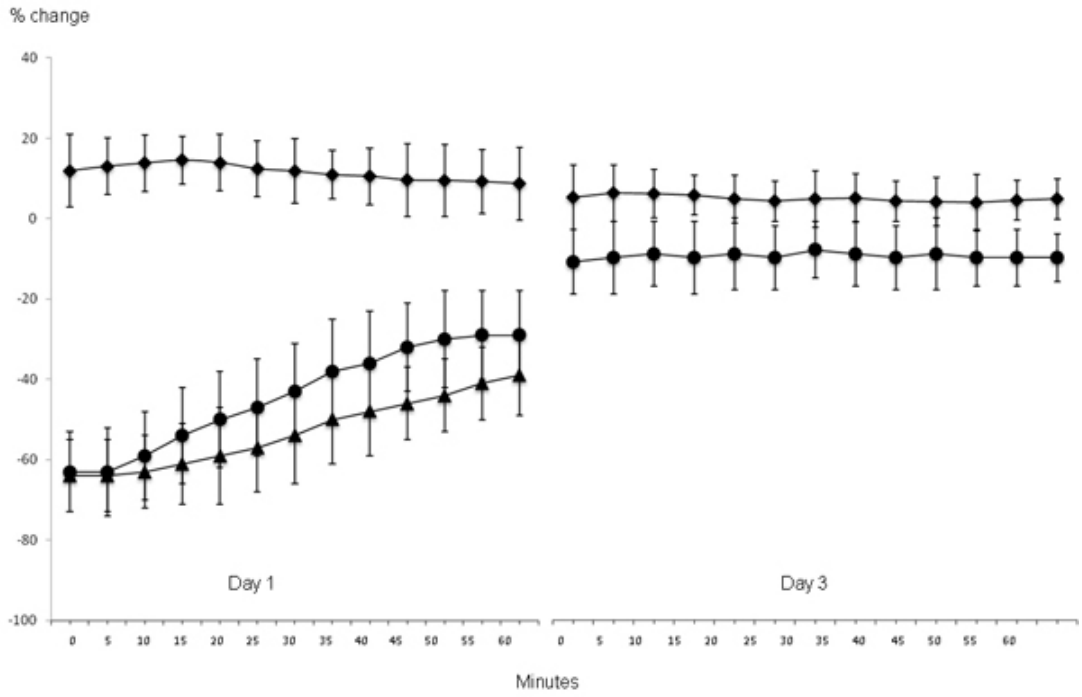

Figure 3: *Graphical view of percent changes in cerebral blood flow (CBF) and mean arterial pressure (MAP) during the experiment. The sudden decrease in CBF and increase in MAP was observed after hemorrhage ($p<0.001$). The parameters was started to improve in the first hour but CBF still remained lower in dead animals compared to survived ($p<0.01$). This has been referred as the most important reason affecting the mortality. CBF on day 3 was 10.7 ± 8% lower than the baseline, but the difference was not significant ($p > 0.05$).*

● *(Circles), changes in CBF*

▲*(triangles), changes in CBF of the subjects that died within 24 hours;*

◆ *(diamonds), changes in MAP*

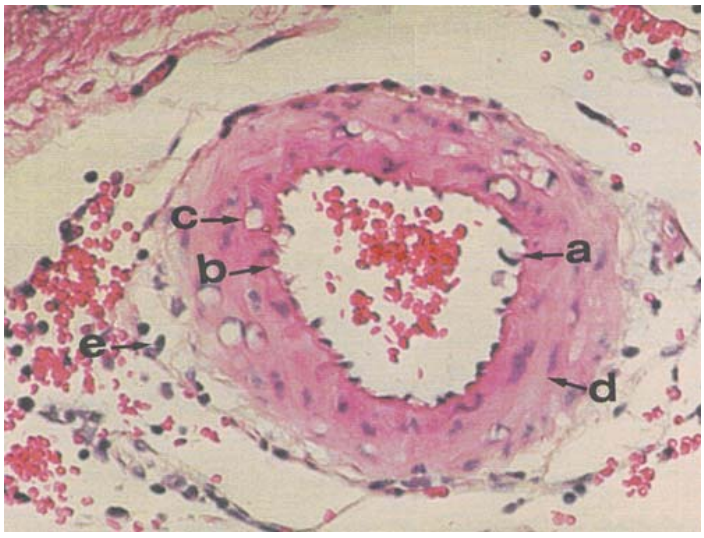

Figure 4: *An example of the pathological changes and basillar artery thickening after SAH (H&E x400). (a) cytoplasmic paleness of the endothelium, separation and curving of endothelium from the internal elastic lamina, (b) curved elastic lamina, (c) vacuolization of the smooth muscle, (d) edema, proliferation and paleness of media, (e) periadventitial inflammation*

DISCUSSION

Our experimental study showed the presence of cerebral hemodynamic changes occurring immediately after the induction of subarachnoid bleeding, while no ischemic cells could be detected in the brainstem. Histologically, there was acute vasospasm along with the pathological vessel wall changes starting in the endothelium and elastic lamina.

CBF was 63.2 ± 10% lower than the baseline after 5 minutes in rats with experimentally-induced SAH, and it remained below the baseline for 60 minutes ($p < 0.001$) despite an increase starting from 10 minutes and reaching a 29.1 ± 11% decline at its highest point during this time period. These results are similar to previous observations[5,15,28]. In another animal model where saline injections were used for controls, CBF was not reported to change significantly despite a 20% decrease in cerebral perfusion, while up to 50% decrease in CBF was found in the SAH group in conjunction with lowered perfusion pressure and impaired cerebral autoregulation[15].

It has been proposed that, compared to fall in CBF, the increase in ICP and lowered cerebral perfusion following SAH have a negligible significance in terms of clinical outcomes[24]. In our clinical practice, the temporary loss of consciousness is generally regarded to arise from this drop in cerebral blood flow, and the increase in intracranial pressure together with lowered CBF may actually represent protective mechanisms against further bleeding.

Our findings suggest the lower CBF over the time course may be of higher importance than the initial fall after SAH. In the rats that died within the first 24 hours, CBF was 64 ± 9% lower than baseline after bleeding, and was still 50 ± 11% below the baseline at 35 minutes ($p < 0.001$) (Figure 3). Previously, the extent of the reduction in CBF was suggested to carry a prognostic value in terms of mortality that is independent of the cerebral perfusion pressure and ICP[2,6]. While the abrupt fall in cerebral perfusion pressure at the time of bleeding and the increase in ICP start to return to normal values within minutes of the incident, decrease in CBF persists, again suggesting a more important role for CBF change in the emergence of early brain injury than alterations in CPP and ICP[5,6,8,15].

In experimental and clinical microdialysis studies, an increased lactate/private ratio and glutamate concentrations, a marker for the presence of ischemia, have been noted within minutes following subarachnoid hemorrhage, and this finding was thought to be suggestive of the development of ischemia[26,29]. In addition, an increase in neuron specific enolase, a marker of neuronal injury, has been found in the first 24-h period following aneurysmatic SAH and this has correlated with the amount of bleeding in the subarachnoid space and poor neurological condition at presentation[18,22]. Also in experimental studies, cell death has been reported to occur within the first 24 hour not only for neurons, but also for astrocytes and oligodendrocytes[20,25] leading to a suggestion that the death of these cells could be related to the acute fall in CBF and its duration[20,25]. In our study, CBF and MAP approached baseline values at 72 hours ($p > 0.05$). In rats that had hypoactivity without neurological deficits, histopathology showed no ischemic cells in the brainstem despite the presence of vasospasm in the basilar artery. Other studies in our laboratory examining chronic vasospasm in acute or recurrent subarachnoid hemorrhage also showed no histopathological signs of ischemia[2,37]. Despite the demonstration of diffuse ischemic injury in human brain immediately after subarachnoid bleeding[16,39] absence of this finding in experimental models might be related to

the differences in vascular wall structure or in the blood flow in collateral vessels.[4,10,21].

In animal models, vasospasm develops in large and small cerebral vessels within minutes following SAH[6,30,31] with accompanying morphological and functional changes[15,35,36]. Also, structural changes in the human brain has been shown to occur in the time course following SAH[12]. Disturbed alignment of the endothelial cells and separation from basal lamina have been shown to occur within 10 minutes following a subarachnoid hemorrhage[11] followed by apoptosis in the endothelial nuclei within 3 hours[12]. Disintegration in the proteins of lamina terminalis continue for 24 hours[27,32] and the effects on the blood-brain barrier results in an increase in vascular permeability and edema[7,13,23]. Following SAH, periadventitial inflammatory cell recruitment reaches a peak in 48 hours, and the transformation from cellular response to humoral immunity response contributes to the development of vasospasm[17].

Light microscopic examination of the basilar artery 72 hours after subarachnoid hemorrhage showed a similar extent of pathological changes in different cross-sections. None of the rats had necrosis or fibrosis of the media, while there was pale cytoplasm of the endothelium, curved elastic lamina, pale staining in the media, and intense edema and proliferation. Cytoplasmic paleness of the endothelium was more marked in areas close to bleeding (87.5% in a cross-sections). Particularly in areas close to the site of hemorrhage (v and a cross-sections) there was a significantly more marked increase in the thickness of the vessel wall and media ($p < 0.001$ and $p < 0.05$, respectively) as compared to the cross-section more distant to bleeding site (p cross-section), together with vasculopathy accompanied by intense periadventitial cellular infiltration. In another study by our team using the same experimental model[2], although there was no other vessel wall pathology apart from curved elastic lamina at 60 minutes, vasospasm with decreased inner lumen diameter of the basilar artery and increased vessel wall thickness was observed. Signs of vasculopathy persisted well up to 72 hours that started with curved elastic lamina 1 hour after bleeding[2].

Although apoptosis[3,12], genetic factors[1], and perivascular, cellular, and humoral factors[17] have been previously shown to be associated with the changes in enzyme activity[19], it is still unclear why elastic lamina and endothelium are affected first. The angiographic vasospasm observed after acute SAH does not result in clinical manifestations associated with vasospasm and ischemia in all patients, suggesting a genetic difference resulting in the absence of a consistent correlation between the volume of blood in the subarachnoid space and development of vasospasm.

CONCLUSION

Low CBF triggered by the presence of oxyhemoglobin in the subarachnoid space and endothelial injury, and the subsequent pathophysiological processes result in the occurrence of vascular and neuronal injury even before hemorrhagic patients are admitted. The results of our study suggest that not only the extent of the fall in CBF but also its duration may be important for the prognosis after SAH.

Correspondence to:
Murat Ulutaş
E-mail: nrsrgn@yahoo.com

Received by: 02 August 2014
Revised by: 03 September 2014
Accepted: 11 September 2014

The Online Journal of Neurological Sciences (Turkish) 1984-2014
This e-journal is run by Ege University Faculty of Medicine,
Dept. of Neurological Surgery, Bornova, Izmir-35100TR
as part of the Ege Neurological Surgery World Wide Web service.
Comments and feedback:
E-mail: editor@jns.dergisi.org
URL: http://www.jns.dergisi.org
Journal of Neurological Sciences (Turkish)
Abbr: J. Neurol. Sci.[Turk]
ISSNe 1302-1664

REFERENCES

1. *Aihara Y, Kasuya H, Onda H, Hori T, Takeda J: Quantitative analysis of gene expressions related to inflammation in canine spastic artery after subarachnoid hemorrhage. Stroke 2001; 32:212-217*
2. *Alkan T, Tureyen K, Ulutas M, Kahveci N, Goren B, Korfali E, Ozluk K: Acute and delayed vasoconstriction after subarachnoid hemorrhage: local cerebral blood flow, histopathology, and morphology in the rat basilar artery. Arch Physiol Biochem 2001; 109:145-153*
3. *Atalay B, Caner H, Cekinmez M, Ozen O, Celasun B, Altinors N: Systemic administration of phosphodiesterase V inhibitor, sildenafil citate, for attenuation of cerebral vasospasm after experimental subarachnoid hemorrhage. Neurosurgery. 2006; 5:1102-7*
4. *Bagley LJ, Hurst RW: Angiographic evaluation of aneurysms affecting the central nervous system. Neuroimaging Clin N Am 1997; 7: 721-737*
5. *Bederson JB, Germano IM, Guarino L: Cortical blood flow and cerebral perfusion pressure in a new noncraniotomy model of subarachnoid hemorrhage in the rat. Stroke 1995; 26:1086-1091*
6. *Bederson JB, Levy AL, Ding WH, Kahn R, DiPerna CA, Jenkins AL, 3rd, Vallabhajosyula P: Acute vasoconstriction after subarachnoid hemorrhage. 1998; Neurosurgery 42:352-360*
7. *Borel CO, McKee A, Parra A, Haglund MM, Solan A, Prabhakar V, Sheng H, Warner DS, Niklason L: Possible role for vascular cell proliferation in cerebral vasospasm after subarachnoid hemorrhage. Stroke 2003; 34:427-433*
8. *Dorsch N, Branston NM, Symon L, Jakubowski J: Intracranial pressure changes following primate subarachnoid haemorrhage. Neurol Res 1989; 11:201-204*
9. *Dorsch NW, King MT: A review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage Part I: Incidence and effects. J Clin Neurosci 1994; 1:19-26*
10. *Frederickson RG, Low FN: Blood vessels and tissue space associated with the brain of the rat. Am J Anat 1969; 125:123-145*
11. *Friedrich V, Flores R, Muller A, Sehba FA: Escape of intraluminal platelets into brain parenchyma after subarachnoid hemorrhage. Neuroscience 2010; 165:968-975*
12. *Friedrich V, Flores R, Sehba FA: Cell death starts early after subarachnoid hemorrhage. Neurosci Lett 2012; 512:6-11*
13. *Hamann GF, Okada Y, Fitridge R, del Zoppo GJ: Microvascular basal lamina antigens disappear during cerebral ischemia and reperfusion. Stroke 1995; 26:2120-2126*
14. *Hughes JT, Schianchi PM: Cerebral artery spasm. A histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. J Neurosurg 1978; 48:515-525*
15. *Jackowski A, Crockard A, Burnstock G, Russell RR, Kristek F: The time course of intracranial pathophysiological changes following experimental subarachnoid haemorrhage in the rat. J Cereb Blood Flow Metab 10:835-849, 1990.*
16. *Kassell NF, Sasaki T, Colohan AR, Nazar G: Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 1985; 16:562-572*
17. *Kubota T, Handa Y, Tsuchida A, Kaneko M, Kobayashi H: The kinetics of lymphocyte subsets and macrophages in subarachnoid space after subarachnoid hemorrhage in rats. Stroke 1993; 24:1993-2000*
18. *Kuroiwa T, Tanabe H, Arai M, Ohta T: [Measurement of serum neuron-specific enolase levels after subarachnoid hemorrhage and intracerebral hemorrhage]. No Shinkei Geka 1994; 22:531-535*
19. *Kusaka G, Ishikawa M, Nanda A, Granger DN, Zhang JH: Signaling pathways for early brain injury after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2004; 24:916-925*
20. *Lee JY, He Y, Sagher O, Keep R, Hua Y, Xi G: Activated autophagy pathway in experimental subarachnoid hemorrhage. Brain Res 2009; 1287:126-135*
21. *Liszczak TM, Varsos VG, Black PM, Kistler JP, Zervas NT: Cerebral arterial constriction after experimental subarachnoid hemorrhage is associated with blood components within the arterial wall. J Neurosurg 1983; 58:18-26*
22. *Mabe H, Suzuki S, Mase M, Umemura A, Nagai H: Serum neuron-specific enolase levels after subarachnoid hemorrhage. Surg Neurol 1991; 36:170-174*
23. *Ohta T, Satoh G, Kuroiwa T: The permeability change of major cerebral arteries in experimental vasospasm. Neurosurgery 1992; 30:331-335*
24. *Prunell GF, Mathiesen T, Diemer NH, Svendgaard NA: Experimental subarachnoid hemorrhage: subarachnoid blood volume, mortality rate, neuronal death, cerebral blood*

flow, and perfusion pressure in three different rat models. Neurosurgery 2003; 52:165-175

25. *Prunell GF, Svendgaard NA, Alkass K, Mathiesen T: Delayed cell death related to acute cerebral blood flow changes following subarachnoid hemorrhage in the rat brain. J Neurosurg 2005; 102:1046-1054*
26. *Sarrafzadeh AS, Haux D, Ludemann L, Amthauer H, Plotkin M, Kuchler I, Unterberg AW: Cerebral ischemia in aneurysmal subarachnoid hemorrhage: a correlative microdialysis-PET study. Stroke 2004; 35:638-643*
27. *Scholler K, Trinkl A, Klopotowski M, Thal SC, Plesnila N, Trabold R, Hamann GF, Schmid-Elsaesser R, Zausinger S: Characterization of microvascular basal lamina damage and blood-brain barrier dysfunction following subarachnoid hemorrhage in rats. Brain Res 2007; 1142:237-246*
28. *Schwartz AY, Sehba FA, Bederson JB: Decreased nitric oxide availability contributes to acute cerebral ischemia after subarachnoid hemorrhage. Neurosurgery 2000; 47:208-214*
29. *Sehba FA, Bederson JB: Mechanisms of acute brain injury after subarachnoid hemorrhage. Neurol Res 2006; 28:381-398*
30. *Sehba FA, Ding WH, Chereshnev I, Bederson JB: Effects of S-nitrosoglutathione on acute vasoconstriction and glutamate release after subarachnoid hemorrhage. Stroke 1999; 30:1955-1961*
31. *Sehba FA, Friedrich V, Jr., Makonnen G, Bederson JB: Acute cerebral vascular injury after subarachnoid hemorrhage and its prevention by administration of a nitric oxide donor. J Neurosurg 2007; 106:321-329*
32. *Sehba FA, Mostafa G, Knopman J, Friedrich V, Jr., Bederson JB: Acute alterations in microvascular basal lamina after subarachnoid hemorrhage. J Neurosurg 2004; 101:633-640*
33. *Siasios I, Kapsalaki EZ, Fountas KN: Cerebral vasospasm pharmacological treatment: an update. Neurol Res Int 2013; 57:13-28*
34. *Solomon RA, Antunes JL, Chen RY, Bland L, Chien S: Decrease in cerebral blood flow in rats after experimental subarachnoid hemorrhage: a new animal model. Stroke 1985; 16:58-64*
35. *Takemae T, Branson PJ, Alksne JF: Intimal proliferation of cerebral arteries after subarachnoid blood injection in pigs. J Neurosurg 1984; 61:494-500*
36. *Tanabe Y, Sakata K, Yamada H, Ito T, Takada M: Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. An experimental study. J Neurosurg 1978; 49:229-238*
37. *Tureyen K, Nazlioglu HO, Alkan T, Kahveci N, Korfali E: Single or multiple small subarachnoid hemorrhages by puncturing a small branch of the rat basilar artery causes chronic cerebral vasospasm. Neurosurgery 2005; 56:382-390*
38. *Weir B, Grace M, Hansen J, Rothberg C: Time course of vasospasm in man. J Neurosurg 1978; 48:173-178*
39. *Weir B, Macdonald RL, Stoodley M: Etiology of cerebral vasospasm. Acta Neurochir Suppl 1999; 72:27-46*