

# Nitric Oxide Level and Adenosine Deaminase Activity in Cerebrospinal Fluid of Patients with Subarachnoid Hemorrhage

## Subaraknoid Kanamalı Hastaların Beyin Omirilik Sıvısında Nitrik Oksit ve Adenozin Deaminaz Aktivitesi Düzeyi

### ABSTRACT

**OBJECTIVE:** Adenosine and nitric oxide (NO) are known as vasodilators. We investigated adenosine deaminase (ADA) activity and NO concentration in the cerebrospinal fluid (CSF) of patients with subarachnoid hemorrhage (SAH).

**METHODS:** Forty patients with SAH and 10 controls were included in the study. Nitrate level and ADA activity were measured in CSF. SAH patients were grouped according to the presence of angiographic vasospasm, Hunt and Hess grading, Glasgow Coma Scale (GCS) and Fisher Grade (FG).

**RESULTS:** The level of NO markers in SAH patients decreased when compared to that in the control group ( $p<0.05$ ). However, NO markers in patients with vasospasm was higher than in that of patients without vasospasm ( $p<0.05$ ). ADA activity increased in patients with SAH ( $p<0.01$ ) and also patients with angiographic vasospasm ( $p<0.05$ ). ADA activity in the poor-grade SAH group was higher than that in the good-grade SAH group. The group with the lower GCS showed increased ADA activity compared to those with a higher GCS score ( $p<0.01$ ). Furthermore, patients with FG 4 had a higher level of ADA activity compared to FG 1 and 2 and FG 3 ( $p<0.001$  and  $p<0.01$ , respectively).

**CONCLUSION:** Decreased NO level may participate in the early development of vasospasm. However, the increased level of ADA activity in the SAH patients with the poor clinical and consciousness level may have resulted from the ischemic cerebral insult.

**KEY WORDS:** Adenosine deaminase activity, Cerebrospinal fluid, Nitric oxide, Subarachnoid hemorrhage, Vasospasm

### ÖZ

**AMAÇ:** Adenozin ve nitrik oksit (NO) vasodilatör maddeler olarak bilinir. Bu nedenle subaraknoid kanamalı (SAK) hastaların beyin omirilik sıvısında (BOS) adenozin deaminaz (ADA) aktivitesi ve NO konsantrasyonunu araştırdık.

**YÖNTEM ve GEREÇ:** Bu çalışmaya 40 SAK'lı ve 10 kontrol hastası dahil edildi. BOS örneklerinde nitrat düzeyi ve ADA aktivitesi çalışıldı. Hastalar, Hunt ve Hess (HH), Glasgow Koma Derecesi (GKD), Fisher derecesi (FD) ve vazospazm varlığına göre gruplara ayrıldı.

**BULGULAR:** NO marker düzeyi, SAK'lı hastalarda kontrol hastalarına göre azalırken ( $p<0.05$ ), vazospazm olan hastalarda olmayanlara göre arttı ( $p<0.05$ ). SAK'lı ve vazospazm gelişen hastalarda ADA aktivitesinde artma saptandı ( $p<0.05$ ). Hunt ve Hess derecesine (HH) göre, ADA aktivitesi HH derecesi IV ve V olan hastalarda, HH derecesi I-III olanlar ve kontrol hastalarına göre daha yüksekti ( $p<0.05$ ). ADA aktivitesi GKD'si  $\leq 8$  olan hastalarda, GKD'si 14 olan hastalar ( $p<0.01$ ) ve kontrol hastalarına göre anlamlı artış gösterdi. Fisher derecesine göre, FD'si 4 olan hastalar, kontrol ( $p<0.001$ ), FD 1 ve 2 ( $p<0.001$ ), ve FD 3 ( $p<0.01$ ) olan hastalardan daha yüksek ADA aktivitesine sahipti.

**SONUÇ:** NO düzeyinin düşük seviyede bulunması, erken vazospazm gelişmesinde NO'nin rolünü önerebilir. Ancak, nörolojik durumu ve şuuru ağır olan hastalarda ADA aktivitesinin yüksek bulunması, SAK'ya eşlik eden imne bulgularından kaynaklanıyor olabilir. Bu nedenle tedavide ADA inhibitörleri yararlı olabilir kanısındayız.

**ANAHTAR SÖZCÜKLER:** Adenosin deaminaz aktivitesi, Beyin omirilik sıvısı, Nitrik Oksit, Subaraknoid kanama, Vazospazm

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**INTRODUCTION**

Nitric oxide (NO) is a major factor in the modulation of cerebral vascular tone. It has been shown that endothelium-dependent relaxation of cerebral vessels is reduced after subarachnoid hemorrhage (SAH). This finding is attributed to necrosis or apoptosis in the endothelial cells of vessels, as well as the presence of hemoglobin and oxy-hemoglobin that bind NO (15,28,45,46). In addition, patients with SAH also suffer from cerebral stroke, which is another source of NO. Evidence shows that an increase in NO concentration within ischemic cerebral tissue is due to up-regulation of constitutive nitric oxide synthase (cNOS) activity in the acute phase of ischemic insult (21,34), while NO produced by inducible nitric oxide synthase (iNOS) increases in the late phase of cerebral ischemia (17,44). Furthermore, a reactive increase of iNOS expression has been shown in endothelial and vascular smooth-muscle cells, as well as in activated microglia, glial networks, and neurons in rats subjected to experimental SAH (41).

Adenosine, a metabolite of adenosine triphosphate (ATP), is thought to possess a housekeeping role in the nervous system. During cerebral ischemic and traumatic insult (2,42), adenosine is released in large quantities and exerts a neuroprotective influence largely via the A1 receptor, which inhibits glutamate release and neuronal activity (9,30). Adenosine deaminase (ADA) is the catalyzing enzyme that converts adenosine to inosine (13). The changes in concentrations of adenosine and its metabolites, including inosine and hypoxanthine, during ischemia depend on ADA activity in the brain (23). The ADA activity in cerebrospinal fluid (CSF) is known to increase in tuberculous meningitis and its use has been suggested to help differentiate this from other forms of meningitis (6). To the best of our knowledge, this study is the first to examine ADA activity in CSF of patients suffering from SAH.

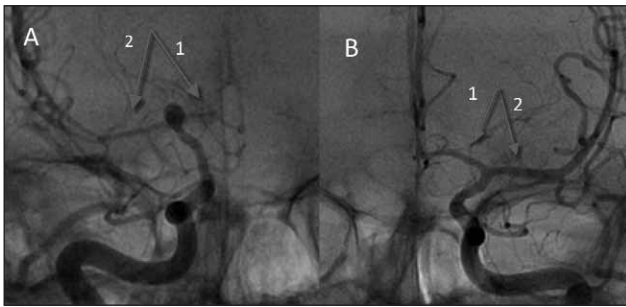
Patients with SAH suffer from the presence of blood in the subarachnoid space and ischemic insult in the acute stage of SAH. Therefore, we investigated ADA activity and the level of NO markers in CSF of patients with SAH according to their neurological conditions, consciousness levels and blood volume in the subarachnoid space on computed tomography (CT) and the presence of vasospasm.

**MATERIALS and METHODS**

Patients included in the present study were admitted to the Neurosurgical Department of Eskişehir Osmangazi University with a diagnosis of SAH between January 2006 and July 2007. This prospective study was approved by the Research Council of Eskişehir Osmangazi University Medical Faculty. A total of 40 patients with SAH were included in the study (Table I). Cerebral aneurysm was diagnosed and located by four-vessel cerebral angiography (31 patients) and CT angiography (4 patients). Accordingly, 35 patients had cerebral aneurysm and five had unknown etiology. Eighteen (58%) of 31 patients undergoing four-vessel angiography procedure had an evidence of vasospasm (Figure 1). The vasospasm was defined as the diameter of basal cerebral arteries narrowed at least 11 % in comparison to the opposite site on

**Table I:** Characteristics of patients with Subarachnoid hemorrhage (SAH.)

Male: female	21:19
Mean Age	57.48
<b>Etiology of SAH</b>	
Aneurysm	35
Unknown	5
Angiographic vasospasm (31 patients were evaluated)	
Vasospasm	18
Without vasospasm	13
<b>Glasgow Coma Scale</b>	
≥14	18
9-13	12
≤8	10
<b>Hunt &amp; Hess Grading</b>	
Grade I	9
Grade II	9
Grade III	8
Grade IV	8
Grade V	6
<b>Fisher Grading</b>	
Grade 1 and 2	17
Grade 3	12
Grade 4	11



**Figure 1:** Figure. 1.A 52-year-old male patient with right internal carotid artery bifurcation saccular aneurysm. Note that the right A1 (1) and M1 (2) arteries had a narrowed diameter when compared to the left side arteriogram. A denotes the right side while B denotes the left side arteriogram.

antero-posterior and lateral projection of radiography using an image analysis system (Osiris, 4.071). The Hunt and Hess grading scale was used for clinical assessment of SAH severity (16). Patients were also classified according to the Glasgow Coma Scale (GCS) on admission (38). The amount of blood observed on initial CT was classified according to the Fisher Scale (11). A total of 10 patients served as controls, including three with lumbar disc diseases, three with trigeminal neuralgia and four with normal pressure hydrocephaly. On the third day of admission, CSF was removed via lumbar puncture or at surgery from the SAH patients and was stored at  $-80^{\circ}\text{C}$  until needed.

#### Determination of NO

The final and stable end products of NO in vivo are nitrate and nitrite, the sum of which reflects the best index of total NO production. Nitrate in CSF was assayed by a modification of the cadmium-reduction method as defined previously by Cortas et al. (8). The nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylethylene diamine. After samples were deproteinized with Somogyi reagent, nitrate was reduced by Cu-coated Cd in glycine buffer at pH 9.7 (2.5–3 g of Cd granules for a 4-ml reaction mixture). The reduction followed pseudo-first-order reaction kinetics, with a convenient time interval for assay being 90 min. Maximum reduction occurred at  $\sim 2$  h.

#### Determination of Adenosine Deaminase Activity

The total adenosine deaminase (ADA: ADA1 plus ADA2) activity was measured in CSF by the modification of Kaplan method (22). Optical density was measured spectrophotometrically at 265 nm in an

assay mixture (final volume 2 ml) containing 0.025 mM adenosine, 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 1.25% glycerol and 0.1 ml CSF. One unit of activity represents one micromole of adenosine per min at  $37^{\circ}\text{C}$  temperature. The ADA activity was expressed as unit (U)/L.

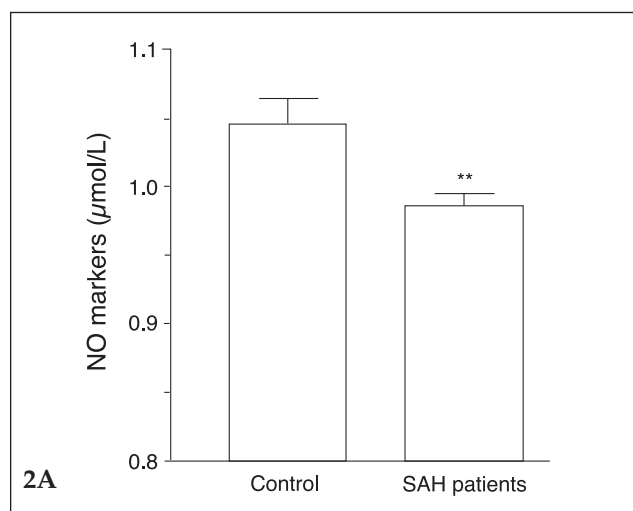
#### Statistical analyses

The data were presented as the mean $\pm$ SEM and analyzed using SPSS for windows 15.0 and Sigmastat 3.1. For assessing multiple groups, Tukey's test following ANOVA was performed if the data had a normal distribution, while Dunn's test followed by the Kruskal–Wallis test were used for data that did not show a normal distribution. Independent sample t test was used for assessing the difference between two groups. Significance was accepted at  $p < 0.05$ .

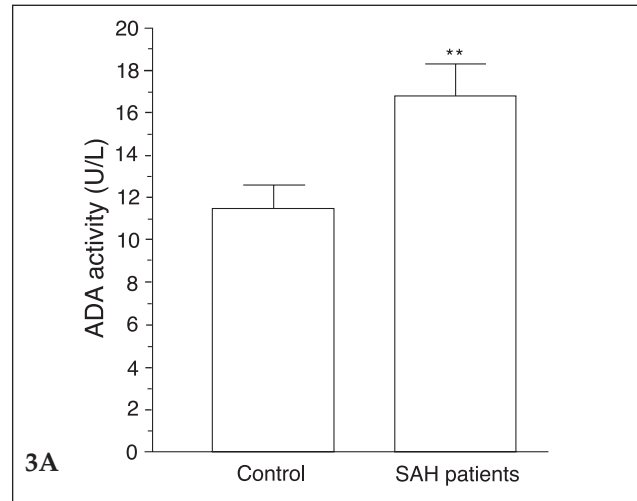
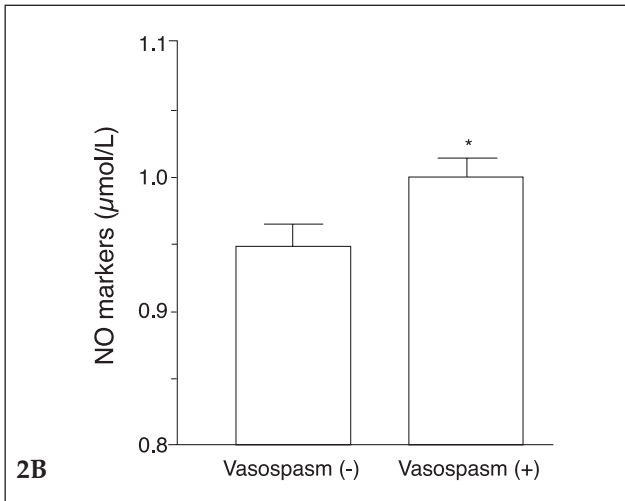
#### RESULTS

Surgery was performed in 26 patients and their aneurysms were clipped successfully. Nine patients could not be operated on because of a high risk of clinical condition for mortality. In the present study, 17 patients including 11 patients with vasospasm died. Mortality was higher in patients with poor grade and with vasospasm.

The level of NO markers in SAH patients ( $0.99 \pm 0.01 \mu\text{mol/L}$ ) decreased when compared to that in the controls ( $1.05 \pm 0.01 \mu\text{mol/L}$ ,  $p < 0.01$ , Figure 2A). On the other hand, no significant differences

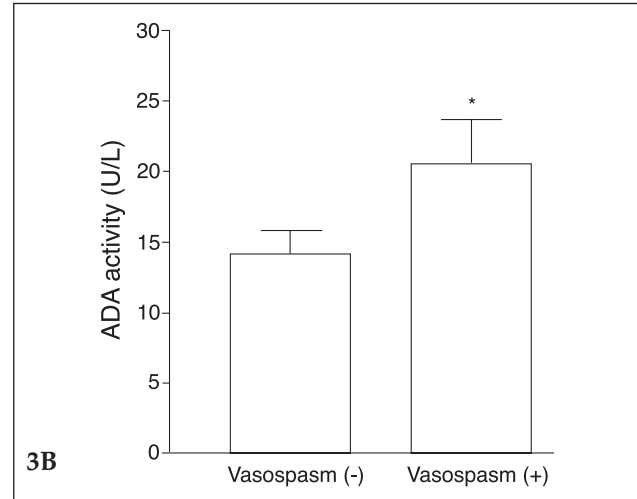


**Figure 2:** Graph showing the level of nitric oxide (NO) markers in CSF; A) in control and SAH patients, B) in patients who had developed vasospasm or not. Values are given as the mean $\pm$ SEM. (independent sample t test). \*\*Significant at  $p < 0.01$ , \*Significant at  $p < 0.05$ .



were found among the groups in relation to Hunt & Hess grade, GCS score and Fisher grade (data not shown). Interestingly, patients with angiographic vasospasm had a higher NO product level (0,100±0,02, Figure 2B) than patients without vasospasm (0,94±0,02) and the difference was significant ( $p<0.05$ ).

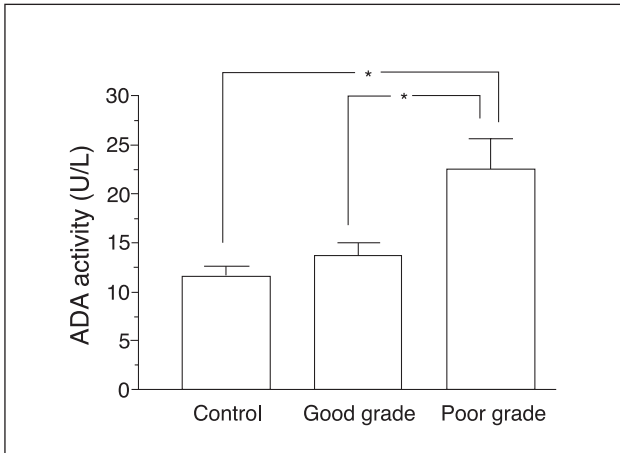
Adenosine deaminase activity in the CSF of patients with SAH (16.78±1.54 U/L) increased significantly ( $p<0.01$ ) in comparison to the controls (11.48±1.10 U/L, Figure 3A). Furthermore, the patients who had angiographic vasospasm had higher ADA activity (20.52±3.19 U/L,  $p<0.05$ ) than patients without vasospasm (14.16 ±1.68 U/L, Figure 3B). ADA activity in the poor-grade SAH group was higher (22.49±3.18 U/L) (grade IV and V) than the good-grade SAH group (grade I-III) (13.70±1.3 U/L, Figure 4) ( $p<0.05$ ) and control patients ( $p<0.05$ ). However, no significant difference was observed between the good-grade SAH and control patients ( $p>0.05$ ).



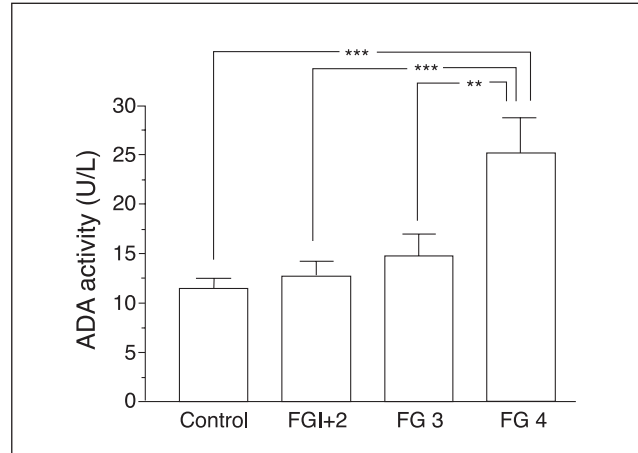
**Figure 3:** Figure. 3. Graph showing adenosine deaminase (ADA) activity in the CSF. **A**) in control and SAH patients, **B**) in patients who had developed vasospasm or not. Values were given as the mean±SEM. A significant elevation in ADA activity was observed in SAH patients when compared to that in the controls and in patients with vasospasm when compared to the patients without vasospasm (independent sample t test). \*Significant at  $p<0.05$ .

Concerning GCS, the increase in ADA activity in patients who had a score of  $\geq 14$  points (12.58±1.32 U/L) and those with a score of 9–13 points (17.76±2.67 U/L) did not differ significantly from that in the control patients ( $p>0.05$ , Figure 5). However, ADA activity in patients who had a score of  $\leq 8$  points (23.14±4.05 U/L) increased significantly compared to that in patients with a score of  $\geq 14$  points ( $p<0.05$ ) and control patients ( $p<0.01$ ). In addition, ADA activity in patients who had a score of 9–13 points did not differ significantly from the patients who had a score of  $\geq 14$  points ( $p>0.05$ ) and control patients ( $p>0.05$ ).

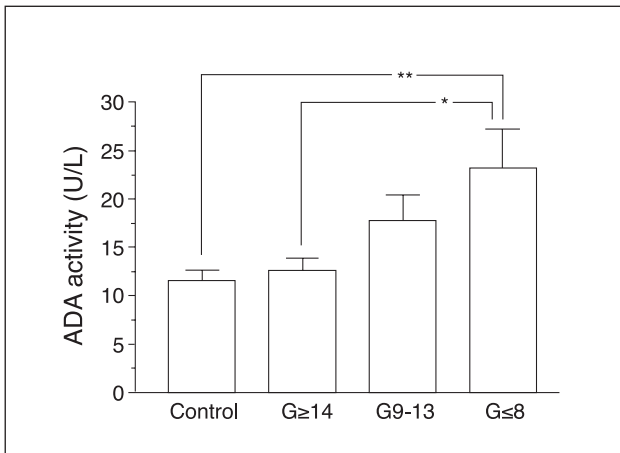
Similarly, ADA activity was also assessed by the amount of blood on CT by using the Fisher SAH grade (Figure 6). ADA activity in patients with Fisher grade 4 (25.19±3.47 U/L) was significantly increased compared to that in Fisher grade 1 and 2 patients (12.75±1.43 U/L,  $p<0.001$ ) and Fisher grade 3 (14.78±2.30 U/L,  $p<0.01$ ) and control patients ( $p<0.001$ ). On the other hand, there was no significant difference between patients with Fisher grade 1 and 2 and the controls ( $p>0.05$ ), Fisher grade 3 and the controls ( $p>0.05$ ), and Fisher grade 1 and 2 and Fisher grade 3 ( $p>0.05$ ).



**Figure 4:** According to the Hunt and Hess grading scale, ADA activity in good-grade (grade I–III) and poor-grade (grade IV and V) SAH and control patients. Values were given as the mean±SEM. There was no significant difference between control and good-grade SAH patients. In contrast, ADA activity in poor-grade SAH patients was significantly increased in comparison with control and good-grade SAH patients (Dunn’s multiple comparisons). \*Significant at  $p<0.05$ .



**Figure 6:** Graph showing adenosine deaminase (ADA) activity related to the Fisher grading system, which is based on the amount of blood seen with CT. Values were given as the mean±SEM. ADA activity in SAH patients with Fisher grade 4 was increased significantly when compared to controls, SAH patients with Fisher grade 1 and 2, and Fisher grade 3 (Tukey test). \*\*Significant at  $p<0.01$ , \*\*\*Significant at  $p<0.001$ . FG: Fisher grade.



**Figure 5:** Adenosine deaminase (ADA) activity in control and SAH patients, according to GCS scores. Values were given as the mean±SEM. ADA activity in SAH patients who had a GCS score of  $\leq 8$  was higher than that in the controls and patients with a GCS score  $\geq 14$  (Tukey’s test). There was no significant difference between the controls and patients with a GCS score  $\geq 14$ , or between patients who with a GCS score  $\geq 14$  and 9–13. G: GCS. \*Significant at  $p<0.05$ , \*\*Significant at  $p<0.01$ .

### DISCUSSION

Under physiological conditions, endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) synthesise NO only when the intracellular calcium concentration is elevated allowing calmodulin binding to the enzyme (27). It is

believed that vasospasm results from the decreased availability of NO and reduced endothelial and neuronal NOS-mediated relaxation of large conductive cerebral arteries (19,20,31). Nitric oxide produced by eNOS is responsible for maintaining vascular tone in addition to its protective effect on the cerebral ischemia by producing an increase of the blood flow in the penumbra area only during the very early stages of cerebral ischemia (27). On the other hand, inflammation of vessel after SAH causes an increased NO level due to activation of iNOS in the cells of the vascular wall and the inflammatory response (41). There have been controversial reports showing that NO is decreased or increased after SAH and SAH-dependent vasospasm (5,20,24,25,32,37,43). Although the level of NO markers was decreased in SAH patients in the present study, the patients with vasospasm had a higher level of NO markers in comparison to those without vasospasm. Similar results have been obtained in patients with SAH-dependent vasospasm (43). Since NO and its metabolite are hazardous in biological systems in high concentrations (18), it is thought that a high level of NO may contribute to the mechanism of vasospasm (43).

In the acute stage of SAH, decreased levels of NO can be explained by the presence of blood and blood products, such as oxyhemoglobin that has been

postulated to bind NO, in the subarachnoid space (15). In addition, reversal of vasospasm after intravascular application of exogenous NO has been demonstrated in experimental SAH (1). NO level in the CSF after SAH has been found to be increased, and the degree of elevation is higher in patients with poor-grade SAH, as well as in those with excess blood in the subarachnoid space, as evaluated by Fisher grading on CT (29,37). A transient decrease in the NO level within 2–3 days is found during investigation of nitrate/nitrite in the CSF of patients with SAH (37), which supports our results. On the other hand, a recent study NO level has been found to be lower in the CSF of SAH patients (25).

The other explanation for the decreasing level of NO in the present study is that the presence of blood around the major cerebral arteries caused morphological changes in the vessels, including the endothelial cells (46). In addition, Zubkov et al. have reported endothelial cell apoptosis in the cerebral arteries of a patient who died from vasospasm after SAH (45). Therefore, endothelial dysfunction due to necrosis or apoptosis is considered to be responsible for the pathogenesis of cerebral vasospasm after SAH. This endothelial injury impairs the equilibrium between NO and prostacyclin whose balance is critical for the maintenance of vascular tone (7,19). Decreased constitutive NOS expression or activity has been shown in experimental vasospasm: endothelial NOS mRNA has been demonstrated to decrease after experimental SAH in monkeys (14), while immunoreactivity for neuronal NOS is virtually absent in nervi vasorum of MCA in cerebral vasospasm (31).

In the present study, ADA activity increased in parallel with the poor neurological conditions (Hunt and Hess classification) (16), a severe decrease in the level of consciousness (GCS) (38), and the presence of excess bleeding (Fisher classification) (11) in patients with SAH. This suggests that adenosine was accumulated in the extracellular space, as observed after hypoxia, ischemia and trauma (3,26,39), since the extracellular space, including CSF, may contain biochemical molecules from blood vessels and the brain. Cerebral ischemia leads to consumption of energy stores by reducing the glucose and oxygen supply (36). Consequently, ATP is metabolized to adenosine and further to inosine, which both accumulate in the extracellular space during ischemia (12). Adenosine exerts its protective effects,

including reduction of glutamate release, via inhibition of presynaptic Ca<sup>2+</sup> channels, direct interference with the vesicle release machinery, and potentially, activation of presynaptic K<sup>+</sup> channels, and also direct inhibition of the N-methyl-D-aspartate receptor (4,9,30). Inosine, a product of ADA, which also rises dramatically during hypoxia/ischemia, may promote increased glutamate release and neuronal activity by possible desensitization of adenosine A<sub>1</sub> receptors (9). Hence, ADA inhibitor causes an increase in adenosine and a decrease in inosine formation in cerebral ischemia in animal studies (23).

During SAH, patients suffer from global or regional cerebral ischemia, shown by cerebral microdialysis monitoring and positron emission tomography (10,40). Degradation products of purine nucleotides (hypoxanthine, xanthine, uric acid and lactate) and the markers of neuronal injury (glutamate and glycerol) in patients with SAH have been shown to be increased in the brain and CSF (10,35,40). In the present study, the increased level of ADA activity may reflect adenosine concentration in CSF. Further increases in ADA activity, in association with poor clinical condition, seem to be related to the ischemic cerebral insult after SAH.

In conclusion, the decreased level of NO in the CSF may be a sign of a decreased vasodilator response in the cerebral arteries in the acute stage of SAH. On the other hand, an increase in the level of NO markers may be associated with cerebral vasospasm. Furthermore, an increase in ADA activity might indicate a use for ADA inhibitors in the treatment of patients suffering from SAH.

## REFERENCES

1. Afshar JK, Pluta RM, Boock RJ, Thompson BG, Oldfield EH: Effect of intracarotid nitric oxide on primate cerebral vasospasm after subarachnoid hemorrhage. *J Neurosurg* 83:118-122, 1995
2. Bell MJ, Kochanek PM, Carcillo JA, Mi Z, Schiding JK, Wisniewski SR, Clark RS, Dixon CE, Marion DW, Jackson E: Interstitial adenosine, inosine, and hypoxanthine are increased after experimental traumatic brain injury in the rat. *J Neurotrauma* 15:163-170, 1998
3. Bell MJ, Robertson CS, Kochanek PM, Goodman JC, Gopinath SP, Carcillo JA, Clark RS, Marion DW, Mi Z, Jackson EK: Interstitial brain adenosine and xanthine increase during jugular venous oxygen desaturations in humans after traumatic brain injury. *Crit Care Med* 29:399-404, 2001
4. Brundage JM, Dunwiddie TV: Role of adenosine as a modulator of synaptic activity in the central nervous system. *Adv Pharmacol* 39:353-391, 1997

5. Chiang MF, Chiu WT, Lin FJ, Thajeb P, Huang CJ, Tsai SH: Multiparametric analysis of cerebral substrates and nitric oxide delivery in cerebrospinal fluid in patients with intracerebral haemorrhage: correlation with hemodynamics and outcome. *Acta Neurochir.* 148:615-621, 2006
6. Choi SH, Kim YS, Bae IG, Chung JW, Lee MS, Kang JM, Ryu J, Woo JH: The possible role of cerebrospinal fluid adenosine deaminase activity in the diagnosis of tuberculous meningitis in adults. *Clin Neurol Neurosurg* 104:10-15, 2002
7. Clower BR, Yamamoto Y, Cain L, Haines DE, Smith RR: Endothelial injury following experimental subarachnoid hemorrhage in rats: effects on brain blood flow. *Anat Rec* 240:104-114, 1994
8. Cortas NK, Wakid NW: Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem* 6:1440-1443, 1990
9. De Mendonca A, Sebastiao AM, Ribeiro JA: Inhibition of NMDA receptor-mediated currents in isolated rat hippocampal neurones by adenosine A1 receptor activation. *Neuroreport* 6:1097-1100, 1995
10. Enblad P, Valtysson J, Andersson J, Lilja A, Valind S, Antoni G, Långström B, Hillered L, Persson L: Simultaneous intracerebral microdialysis and positron emission tomography in the detection of ischemia in patients with subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 16:637-644, 1996
11. Fisher CM, Kistler JP, Davis JM: Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. *Neurosurgery* 6:1-9, 1980
12. Hagberg H, Andersson P, Lacarewicz J, Jacobson I, Butcher S, Sandberg M: Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. *J Neurochem* 49:227-231, 1987
13. Hankiewicz J, Lesniak M. Adenosine deaminase in cerebrospinal fluid. *Enzymologia* 43:385-395, 1972
14. Hino A, Tokuyama Y, Weir B, Takeda J, Yano H, Bell GI, Macdonald RL: Changes in endothelial nitric oxide synthase mRNA during vasospasm after subarachnoid hemorrhage in monkeys. *Neurosurgery* 39:562-568, 1996
15. Hongo K, Kassell NF, Nakagomi T, Sasaki T, Tsukahara T, Ogawa H, Vollmer DG, Lehman RM: Subarachnoid hemorrhage inhibition of endothelium-derived relaxing factor in rabbit basilar artery. *J Neurosurg* ;69:247-253, 1988
16. Hunt WE, Hess RM: Surgical risk as related to time of intervention in the repair of intracranial aneurysms. *J Neurosurg* 28:14-20, 1968
17. Iadecola C, Zhang F, Xu X: Inhibition of inducible nitric oxide synthase ameliorates cerebral ischemic damage. *Am J Physiol* 268:R286-292, 1995
18. Iadecola C: Bright and dark sides of nitric oxide in ischemic brain injury. *Trends Neurosci.* 20:132-139, 1997
19. Iuliano BA, Pluta RM, Jung C, Oldfield EH: Endothelial dysfunction in a primate model of cerebral vasospasm. *J Neurosurg* 100:287-294, 2004
20. Jung CS, Oldfield EH, Harvey-White J, Espey MG, Zimmermann M, Seifert V, Pluta RM: Association of an endogenous inhibitor of nitric oxide synthase with cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg.* 107: 945, 2007
21. Kader A, Frazzini VI, Solomon RA, Trifiletti RR: Nitric oxide production during focal cerebral ischemia in rats. *Stroke* 24:1709-1716, 1993
22. Kaplan NO: Specific adenosine deaminase from intestine. *Methods Enzymol* 2:473-475, 1955
23. Kobayashi T, Yamada T, Okada Y: The levels of adenosine and its metabolites in the guinea pig and rat brain during complete ischemia-in vivo study. *Brain Res* 787:211-219, 1998
24. Lin CL, Hsu YT, Lin TK, Morrow JD, Hsu JC, Hsu YH, Hsieh TC, Tsay PK, Yen HC: Increased levels of F2-isoprostanes following aneurysmal subarachnoid hemorrhage in humans. *Free Radic Biol Med.* 40:1466-1473, 2006
25. Martens-Lobenhoffer J, Sulyok E, Czeiter E, Büki A, Kohl J, Firsching R, Tröger U, Bode-Böger SM: Determination of cerebrospinal fluid concentrations of arginine and dimethylarginines in patients with subarachnoid haemorrhage. *J Neurosci Methods* 164:155-160, 2007
26. Morimoto K, Shimizu H, Hayakawa T, Shimada N, Nii Y, Masana Y, Kato A, Mogami H: Purine catabolites in cerebral interstitial fluid during progression of and recovery from ischemia. *Neurol Med Chir (Tokyo)* 31:129-134, 1991
27. Moro MA, Cárdenas A, Hurtado O, Leza JC, Lizasoain I: Role of nitric oxide after brain ischaemia. *Cell Calcium.* 36:265-275, 2004Review
28. Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Torner JC: Impairment of endothelium-dependent vasodilation induced by acetylcholine and adenosine triphosphate following experimental subarachnoid hemorrhage. *Stroke* 18:482-489, 1987
29. Ng WH, Moochhala S, Yeo TT, Ong PL, Ng PY: Nitric oxide and subarachnoid hemorrhage: elevated level in cerebrospinal fluid and their implications. *Neurosurgery* 49:622-626, 2001
30. Pearson T, Currie AJ, Etherington LA, Gadalla AE, Damian K, Llaudet E, Dale N, Frenguelli BG: Plasticity of purine release during cerebral ischemia: clinical implications? *J Cell Mol Med* 7:362-375, 2003
31. Pluta RM, Thompson BG, Dawson TM, Snyder SH, Boock RJ, Oldfield EH: Loss of nitric oxide synthase immunoreactivity in cerebral vasospasm. *J Neurosurg* 84:648-654, 1996
32. Pluta RM, Jung CS, Harvey-White J, Whitehead A, Shilad S, Espey MG, Oldfield EH: In vitro and in vivo effects of probucol on hydrolysis of asymmetric dimethyl L-arginine and vasospasm in primates. *J Neurosurg.* 103:731-738, 2005
33. 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* 35:638-643, 2004
34. Sasaki T, Hamada J, Shibata M, Araki N, Fukuuchi Y: Inhibition of nitric oxide production during global ischemia ameliorates ischemic damage of pyramidal neurons in the hippocampus. *Keio J Med* 50:182-187, 2001
35. Schulz MK, Wang LP, Tange M, Bjerre P: Cerebral microdialysis monitoring: determination of normal and ischemic cerebral metabolisms in patients with aneurysmal subarachnoid hemorrhage. *J Neurosurg* 93:808-814, 2000
36. Siesjo BK: Pathophysiology and treatment of focal cerebral ischemia. Part I: Pathophysiology. *J Neurosurg* 77:169-184, 1992
37. Suzuki Y, Osuka K, Noda A, Tanazawa T, Takayasu M, Shibuya M, Yoshida J: Nitric oxide metabolites in the cisternal cerebral spinal fluid of patients with subarachnoid hemorrhage. *Neurosurgery* 41:807-811, 1997

38. Teasdale G, Jennett B: Assessment of coma and impaired consciousness. A practical scale. *Lancet* 2:81-84, 1974
39. Van Wylen DG, Park TS, Rubio R, Berne RM: Increases in cerebral interstitial fluid adenosine concentration during hypoxia, local potassium infusion, and ischemia. *J Cereb Blood Flow Metab* 6:522-528, 1986
40. von Holst H, Sollevi A: Increased concentration of hypoxanthine in human central cerebrospinal fluid after subarachnoid hemorrhage. *Acta Neurochir (Wien)* 77:52-59, 1985
41. Widenka DC, Medele RJ, Stummer W, Bise K, Steiger HJ: Inducible nitric oxide synthase: a possible key factor in the pathogenesis of chronic vasospasm after experimental subarachnoid hemorrhage. *J Neurosurg* 90:1098-1104, 1999
42. Winn HR, Rubio R, Berne RM: Brain adenosine production in the rat during 60 seconds of ischemia. *Circ Res* 45:486-492, 1979
43. Woszczyk A, Deinsberger W, Böker DK: Nitric oxide metabolites in cisternal CSF correlate with cerebral vasospasm in patients with a subarachnoid haemorrhage. *Acta Neurochir (Wien)*. 145:257-263, 2003
44. Zhu DY, Deng Q, Yao HH, Wang DC, Deng Y, Liu GQ: Inducible nitric oxide synthase expression in the ischemic core and penumbra after transient focal cerebral ischemia in mice. *Life Sci* 71:1985-1996, 2002
45. Zubkov AY, Ogihara K, Bernanke DH, Parent AD, Zhang J: Apoptosis of endothelial cells in vessels affected by cerebral vasospasm *Surg Neurol* 53:260-266, 2000
46. Zubkov AY, Tibbs RE, Clower B, Ogihara K, Aoki K, Zhang JH: Morphological changes of cerebral arteries in a canine double hemorrhage model. *Neurosci Lett* 326:137-141, 2002