Histopathologic evaluation of neurogenic pulmonary edema after subarachnoid hemorrhage in rabbits

Cemal Gundogdu, MD, Mehmet Kizilkaya, MD, Mehmet D. Aydin, MD, Canan Atalay, MD, Zuhtu U. Sevimli, MD.

ABSTRACT

Objective: To investigate the effects of subarachnoid hemorrhage (SAH) on lung tissue.

Methods: We conducted this study on 20 rabbits in the Ataturk University Medical Faculty, Erzurum, Turkey in 2005. Experimental SAH was applied to all animals under general anesthesia. After 20 days, all animals were sacrificed. Their lungs were examined histopathologically.

Results: Foamy hemorrhagic parenchymal lesions, alveolar rupture, and subintimal fluid collection in the pulmonary vasculature were observed in the lungs of the non-surviving animals. However, minimal changes were found in the lungs of the surviving animals ($p<0.01$).

Conclusions: Our results suggest that luminal narrowing of the lung vessels due to subintimal fluid collection plays an important role in the development of pulmonary hypertension and neurogenic pulmonary edema in SAH.

Neurosciences 2006; Vol. 11 (3): 158-161

Extracerebral organ dysfunction after SAH can influence outcome.[1] Neurogenic pulmonary edema (NPE) is a rare and very serious extracerebral organ dysfunction in patients with SAH. Neurogenic pulmonary edema may effect the outcome, and may increase the ratio of death in the SAH patients.[2,3] Neurogenic pulmonary edema may occur in the patients with SAH, being mainly due to cardio circulatory changes related to imbalance of the central neuro-vegetative control.[4] Life-threatening arrhythmias, ventricular wall motion dysfunction, and contraction band necrosis are also seen in patients with SAH.[5] The myocardial injury in SAH may be due to a massive sympathetic stimulation of the myocardium in response to rapidly increasing intracranial pressure.[6] Cardiac dysfunction may lead to hemodynamic instability and contribute to the NPE. This hemodynamic instability results in pulmonary vasoconstriction, increased alveolar permeability, and hemorrhagic pulmonary edema.[6,7] There are very few studies investigating the effects of SAH on lung dysfunction. This may be the result of the lack of a reliable experimental model to examine the association between SAH and lung injury. For this reason, this study was conducted to investigate the effect of the NPE after SAH by analyzing pulmonary histopathologic changes.

Methods. This study was carried out in the Ataturk University Experimental Research Center. This study was performed on 20 anesthetized adult male New Zealand rabbits [(3.7 ± 0.4 (SD) kg. body weight]. They were left hungry 6 hours before surgical intervention. Experiments were carried out in anesthetized spontaneously breathing rabbits, which is known to alter the parenchymal stresses and is,

From the Departments of Pathology (Gundogdu), Anesthesiology and Reanimation (Kizilkaya, Atalay, Sevimli), and Neurosurgery (Aydin), School of Medicine, Ataturk University, Erzurum, *Turkey*.

Received 11th October 2005. Accepted for publication in final form 7th February 2006.

Address correspondence and reprint request to: Dr. Cemal Gundogdu, Ataturk Universitesi Lojmanlari, No. 55/5, 25240 Erzurum, *Turkey*. Tel. +90 (542) 232 3669. Fax. +90 442 3166340. E-mail: mkizilkaya65@hotmail.com

in fact, a possible cause of lung lesions. A balanced injectable anesthesia was used for reducing pain and mortality. After inducing anesthesia with isoflurane by a face mask, 0.2 mL/kg of the anesthetic combination (Ketamine HCL, 150 mg/1.5 mL; Xylazine HCL, 30 mg/1.5 mL; and distilled water, 1 mL) was subcutaneously injected before surgery. During the operation, 0.1 mL/kg anesthetic combination was used when required. A 22-gauge needle connected to a 3-way stopcock was inserted via the atlanto-occipital membrane into the cisterna magna (intrathecal space), 0.5 ml of blood was taken from the auricular vein of the animals and injected into their cisterna magna over 35 seconds. After the injection, the needle was taken out, then anesthesia discontinued and the animals observed until completely normally. The animals were followed up for 20 days. After the 20 days, the animals were sacrificed and the left upper lobe of the lungs was removed for histopathologic procedures. The remaining total wet lung was weighed and then dried to a constant weight at 60^0C over 48 hours in an oven. To assess tissue edema, the wet/dry (W/D) ratio was calculated as indicators of lung edema. In order to observe histopathologic changes in the pulmonary tissues, the lungs, after being kept in 10% formalin solution for 7 days, were taken into paraffin blockage. Sections of 1 μm were obtained and stained with hematoxylin–eosin and analyzed under light microscope in order to observe histopathologic changes in the lungs. Light microscopic analysis was performed by blinded pathologists. Lung injury was scored under light microscopy from 0 (no damage) to 4+ (maximum damage), according to the combined assessment of pulmonary alveolar edema, interstitial or septal edema, alveolar congestion, alveolar hemorrhage, perivascular or septal hemorrhage, alveolar neutrophilic infiltration, septal neutrophilic infiltration, pneumocytes, and alveolar macrophages. The scores obtained for each rabbit were recorded. Analysis was performed using SPSS for Windows. The W/D ratio and histopathologic scores between non-survivors and survivors were compared statistically (by Mann-Whitney U test). A value of $p<0.05$ was considered significant.

Results. Seven animals died within 20 days of the experimental period. The remaining animals were followed up for 20 days. The W/D lung weight ratio increased in the non-survivors (9.2 ± 0.9 g), compared with the survivors (3.4 ± 0.5 g) ($p<0.01$). In the histologic analysis of the lung, significantly more alveolar edema, congestion, perivascular-subintimal fluid collection, or alveolar septal hemorrhage or both, increase in the number of pneumocytes and monocytes were observed in the non-survivors compared with survivors. In addition, pulmonary cortical hemorrhagic focuses were also observed in these 7 animals. Macroscopic appearances of lungs of the survivors and non-survivors are seen in **Figure 1**. The SAH developed brain is seen in **Figure 2**. Normal pulmonary tissue, and massive pulmonary alveolar hemorrhage/alveolar infiltration developed lung in a non-surviving animal are seen in **Figure 3**. The median (range) of the lung histology scores were: survivors = 0 (0-1), non-survivors = 3.5 (3-4). There was a significant difference between scores ($p < 0.01$, Mann-Whitney U-test).

Discussion. Many clinical and experimental studies reported that SAH may cause neurogenic pulmonary edema. Experimental SAH can easily be developed by the injection of fresh autologous blood into the cisterna magna. In our model, SAH was formed similarly to this method. The resulting mortality was approximately 35%. Marked or diffuse NPE occurred in non-survivors, but minimal changes occurred in survivors. Pulmonary vascular subintimal fluid collections were more prominent in the non-surviving animals. Neurogenic pulmonary edema is rare in patients with reversible neurological injury, but is more common in those with severe and fatal neurological diseases.[8,9] Weir et al,[9] studied 78 patients with fatal SAH from ruptured aneurysm and found pathological evidence of NPE in 71%. However, Hijdra et al,[10] reported that SAH lead to NPE in 17%. The NPE is characterized as an acute, protein-rich lung edema occurring shortly after cerebral lesions associated with an acute rise of intracranial pressure. Pathophysiological mechanisms include a rise of the pulmonary vascular hydrostatic pressure, either due to massive sympathetic innervation with pulmonary vasoconstriction, or increased left atrial pressure following systemic arterial hypertension, or an increase in pulmonary capillary permeability.[7] Massive sympathetic discharge during the initial insult to the central nervous system is once thought to cause generalized vasoconstriction with a shift of blood from the high resistance systemic circulation to the low resistance pulmonary circulation. Consequently, this condition resulted in fluid overload to the pulmonary circulation, damaging the pulmonary capillaries and altering their permeability. The leakage of red blood cells and high protein edema fluid through the pulmonary capillary membrane suggests that there might be disruption and damage to the capillary endothelium.[10-13] Whereas, our results suggested that luminal narrowing of the lung vessels due to subintimal fluid collection may have an important

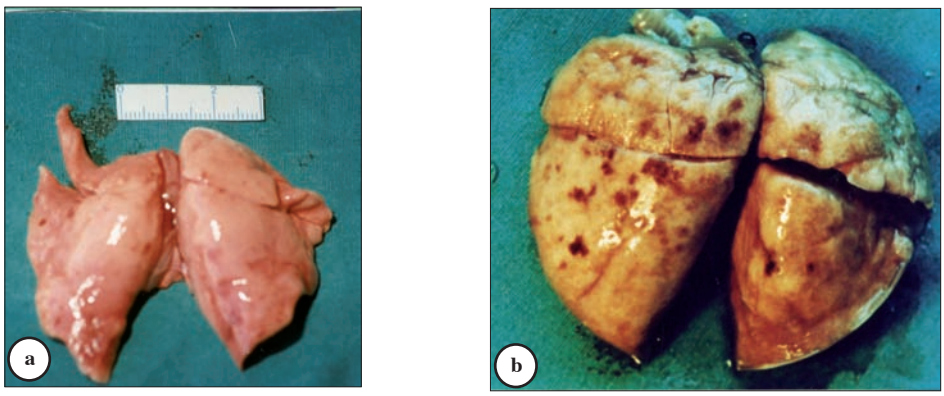

Figure 1 - Macroscopic appearance of the lung. **a)** Normal lung in the survivors. **b)** Severe edematous appearance in the non-survivors.

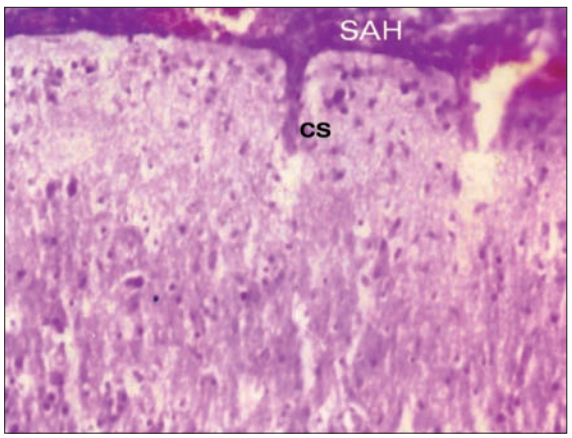

Figure 2 - Microscopic appearance of the brain showing SAH. SAH - subarachnoid hemorrhage, CS - cortex. (Hematoxylin & Eosin x 100).

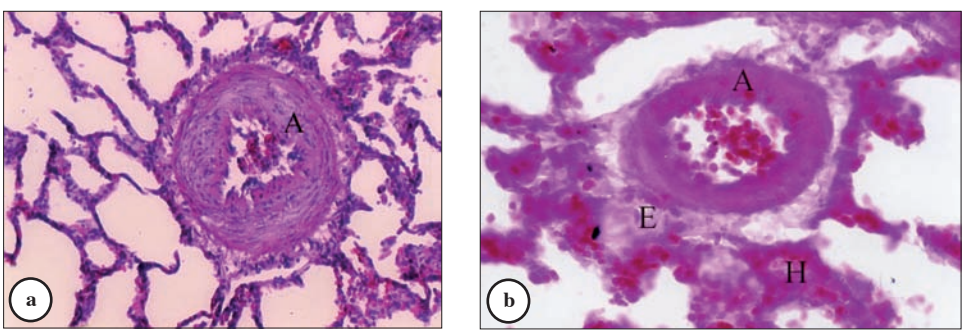

Figure 3 - Microscopic appearances of the lungs (x 100, Hematoxylin & Eosin). **a)** Normal lung tissue. **b)** Edema, infiltration, and hemorrhage are seen in the lung. A - arteriol, H - hemorrhage.

role in the development of pulmonary hypertension and NPE in SAH. Myocardial dysfunction is a risk factor in the development of pulmonary edema in SAH.[6] Impaired left ventricular hemodynamic performance may contribute to cardiovascular instability, pulmonary edema formation, and cerebral ischemia. Pulmonary artery wedge pressures may reach high levels [>16 mm Hg].[14] In the end, cerebro-pulmonary function may be worse and death may be inevitable. Hypoxia, increasing intracranial pressure may appear due to failed lung function. It has been reported that the mean interval between the onset of SAH and the diagnosis of NPE on chest film was 2.5 hours.[15] Left ventricular functions return to normal 2-6 weeks after SAH.[14]

Neurogenic pulmonary edema occurs in approximately 10-71% of patients with SAH.[4,7] In patients with sudden death from SAH, more than 90% present with acute pulmonary edema.[16,17] Walder et al,[17] reported that the development of NPE, characterized by an acutely increased capillary permeability to proteins, was independent of the degree of intracranial pressure increase. Neurogenic pulmonary edema secondary to subarachnoid hemorrhage has not been clearly reported so far.

We can conclude that SAH is an important predisposing factor in NPE, and there may be a positive feedback loop between SAH and NPE. In our study, response of stress reaction in the same animal species with the same stressor was not the same in the survivors and non-survivors. Namely, SAH resolution occurred in a short time in the survivors in our study. These differences may be explained by genetically determined or socially conditioned differences in stress regulation. As a rule, the animal model is not the same in humans in view of the clinic outcome in such conditions. In this study, postmortem anatomic-histopathologic findings were similar to that in humans, as described in the literature.[10-13] However, we think that this topic needs further investigations.

References

1. Gruber A, Reinprecht A, Illievich UM, Fitzgerald R, Dietrich W, Czech T, et al. Extracerebral organ dysfunction and neurologic outcome after aneurysmal subarachnoid hemorrhage. ***Crit Care Med*** 1999; 27: 505–514.
2. Gruber A, Reinprecht A, Gorzer H, Fridrich P, Czech T, Illievich UM, et al. Pulmonary function and radiographic abnormalities related to neurological outcome after aneurysmal subarachnoid hemorrhage. ***J Neurosurg*** 1998; 88: 28–37.
3. Dragosavac D, Falcao AL, Araujo S, Terzi RG. Neurogenic pulmonary edema. Report of two cases. ***Arq Neuropsiquiatr*** 1997; 55: 305-308.
4. Di Giugno, G, Rosa G. Neurogenic pulmonary edema during subarachnoid hemorrhage. ***Minerva Anesthesiol*** 1988; 64: 229-230.
5. Di Pasquale G, Andreoli A, Lusa AM, Urbinati, S, Biancoli S, Cere E, et al. Cardiologic complications of subarachnoid hemorrhage. ***J Neurosurgery Sci*** 1998; 42: 33-36.
6. Rorbakken G, Brunvand H, Gundersen T, Farstad G. Cardiopulmonary complications in acute subarachnoid hemorrhage. ***Tidsskr Nor Laegeforen*** 1998; 118: 3430-3434.
7. Schwarz S, Schwab S, Keller E, Bertram M, Hacke W. Neurogenic disorders of heart and lung function in acute cerebral lesions. ***Nervenarz*** 1997; 68: 956-962.
8. Mayer SA, Fink ME, Homma S, Sherman D, Limandri G, Lennihan L, et al. Cardiac injury associated with neurogenic pulmonary edema following subarachnoid hemorrhage. ***Neurology*** 1994; 44: 815-820.
9. Weir BK. Pulmonary edema following fatal aneurysm rupture. ***J Neurosurg*** 1978; 49: 502-507.
10. Hijdra A, Vermeulen M, Van Gijn J, Van Crevel H. Respiratory arrest in subarachnoid hemorrhage. ***Neurology*** 1984; 34: 1501-1503.
11. Garcia-Uria J, Hoff JT, Miranda S, Nishimura M. Experimental neurogenic pulmonary edema. Part 2: The role of cardiopulmonary pressure change. ***J Neurosurg*** 1981; 54: 632-636.
12. Lagerkranser M, Pehrsson K, Sylve'n C. Neurogenic pulmonary edema. A review of the pathophysiology with clinical and therapeutic implications. ***Acta Med Scand*** 1982; 212: 267-271.
13. Millen JE, Glauser FL. Low levels of concussive brain trauma and pulmonary edema. ***J Appl Physiol Resp Environ*** 1983; 53: 666-670.
14. Simon RP, Bayne LL. Pulmonary lymphatic flow alterations during intracranial hypertension in sheep. ***Ann Neurol*** 1984; 15: 188-194.
15. Watanabe T, Sekiguchi K, Inoue A, Taniguchi Y, Sato S. Clinical evaluation of neurogenic pulmonary edema following acute stage of subarachnoid hemorrhage. ***No Shinkei Geka*** 1992; 20: 417-422.
16. Schievink WI, Wijdicks EF, Parisi JE, Piepgras DG, Whisnant JP. Sudden death from aneurysmal subarachnoid hemorrhage. ***Neurology*** 1995; 45: 871–874.
17. Walder B, Bründler MA, Tötsch M, Elia N, Morel DR. Influence of the type and rate of subarachnoid fluid infusion on lethal neurogenic pulmonary edema in rats. ***J Neurosurg Anesthesiol*** 2002; 14: 194-203.