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Original Article

Mechanism of cerebral fat embolism in subarachnoid hemorrhage: An experimental study

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Subarachnoid hemorrhage (SAH) may cause neurogenic pulmonary edema (NPE), and chylomicron metabolism may be destroyed in injured lungs. We aimed to investigate the effect of neurogenic pulmonary edema (NPE), if present, on the development of cerebral fat embolism. This study has been conducted on 20 rabbits. Experimental SAH has been applied to half of the animals by injecting homologous blood into the cisterna magna, and the remaining half was applied only isotonic saline solution in the same manner under general anesthesia. After 20 days, all animals were killed. Their lungs and brains were examined histopathologically. Six animals died of SAH between 16 and 20 days, and foamy hemorrhagic parenchymal lesions and intra-alveolar hemorrhage were observed in their lungs. Fat globules were abundantly found in cerebral arteries of six of all the non-surviving animals. But, minimal histopathological changes were found in the lungs and brains of the surviving animals. Cerebral fat embolism was detected in only one animal that was given isotonic solution. SAH may cause NPE and result in lung tissue destruction. Chylomicron metabolism may be disordered in the destructed lungs and leakage of chylomicrons into systemic circulation may be facilitated via destroyed lung barrier. These pathologic processes may lead to cerebral fat embolism.

Key words: fat embolism, pulmonary edema, subarachnoid hemorrhage.

INTRODUCTION

Neurogenic pulmonary edema (NPE) is a rare and serious complication of subarachnoid hemorrhage (SAH).[1] Cerebral vasoconstriction and increased intracranial pressure (ICP) can aggravate cerebral ischemia and necrosis in SAH.[2] Vasospasm and microvascular aggregation of red blood cells can lead to acute ischemic damage. Swelling of perivascular astrocytes, neurons, and endothelium-induced ischemic insult lead to intra-arterial red blood cells aggregation, increased cerebrovascular resistance, and worsened ischemia.[3,4] ICP can lead to imbalanced neuro-vegetative functions and can trigger cardio-respiratory disturbances.[5] Life-threatening arrythmias, ventricular dysfunction, contraction band necrosis and respiratory disturbances can develop in patients with SAH.[6] Cardiac dysfunctions leading to hemodynamic instability may result in pulmonary vasoconstruction, increased alveolar permeability, alveolar destruction and hemorrhagic pulmonary edema.[7,8]

Di Pasquale *et al.*[6] showed that alveolar wall destruction secondary to alveolar injury could facilitate cerebral fat embolism. It has been reported that free fatty acids orginating from chylomicrons also cause serious cerebral necrosis.[9,10] SAH-induced vasospasm and fat acumulation in the cerebral arteries can lead to high mortality in massive SAH. The mortality rate of SAH is about 25% within 24 h.[11] There is no study investigating the development of cerebral fat embolism in SAH secondary to NPE, so we aimed to investigate this topic.

METHODS

This study was performed on 20 anesthetized adult male New Zealand rabbits (3.7 ± 0.4 kg). Experiments were carried out according to the guidelines set by the ethical committee of our faculty. They were fasted 6 h before surgical intervention. Experiments were carried out in anesthetized spontaneously breathing rabbits. 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

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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. 0.5 mL of autologous blood was taken from the auricular vein and injected into cisterna magna via 22-Gauge needle in about one minute to half of these animals and 0.5 mL of isotonic saline solution was injected in the same way to the remaining animals. The surviving animals were followed up for 20 days without any medical treatment. The six animals with SAH were killed. At the end of experiments, all animals' brains and lungs were removed for histologic examination. After keeping the lungs in 10% formaline solution for 7 days, 1 μm of pulmonary cortical sections were taken and stained with H&E. Also, 1 μm of brain specimens was taken from the bilateral proximal middle cerebral artery territory by frozen-section and stained with Sudan III-Black. Occluded branches of the middle cerebral arteries with fat particles were counted and statistical analysis was carried out between the non-survivors and survivors, and isotonic saline-injected animals by using Mann–Whitney *U*-test.

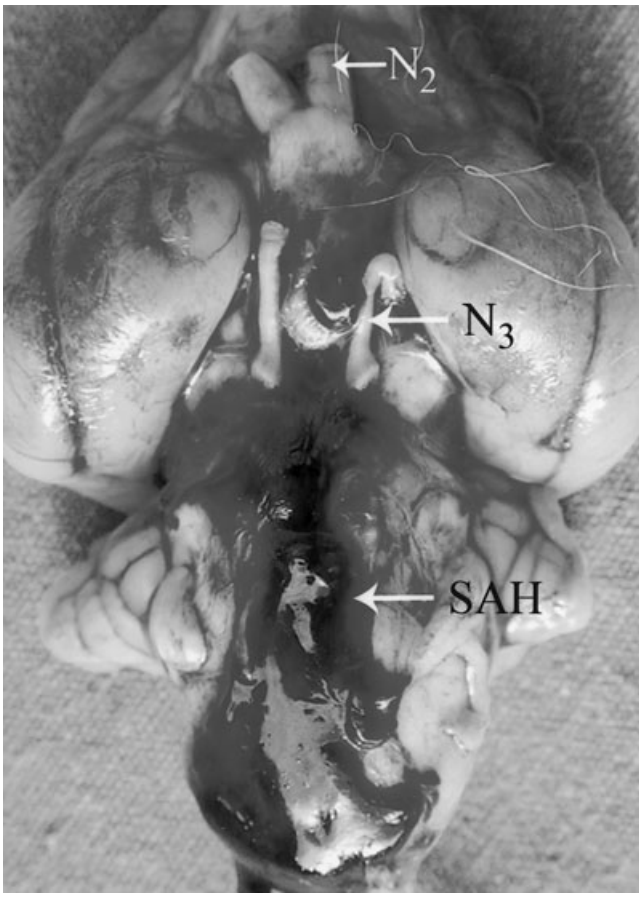

Fig. 1 Macroscopic appearance of a brain with subarachnoid hemorrhage (SAH) in the basal cisterns (N_2, optic nerve, N_3, occulomotor nerve).

RESULTS

In the isotonic saline-injected group, two animals died due to iatrogenic SAH within the first week and two animals were added in the experiment. In the remaining animals, only minimal histopathologic changes were observed in their lungs, and fat embolism was detected in only one animal. In the SAH group, six animals died between 16 and 20 days. Postmortem macroscopic examination in the deceased animals showed massive brain swelling, meningeal inflammation and cortical injury. Macroscopic appearance of the SAH-developed brain is shown in Figure 1 and the microscopic appearance of hemorrhagic subarachnoid compartment is shown in Figure 2. Pleural hemorrhagic focuses and purple-reddish appearances were observed in the deceased animals due to SAH (Fig. 3). Significant intra-alveolar hemorrhage and congestion were observed in the histologic analysis of the lungs of non-survivors (Fig. 4). In the examination of the brains, only one artery was occluded with lipid particles in the brain of a deceased animal in the isotonic-injected group. However, some branches of middle cerebral artery were observed as occluded in three of the non-surviving animals and one of the surviving animals in the SAH group (Fig. 5). The mean number of arteries occluded with fat particles were 4 ± 1 in both hemispheres of the brains in the SAH-developed animals. There was a meaningful difference between the numbers of the occluded arteries of survivor and non-survivor animals ($P < 0.05$). But the difference between the survivors and isotonic saline-injected group was not meaningful ($P > 0.05$) (Table 1).

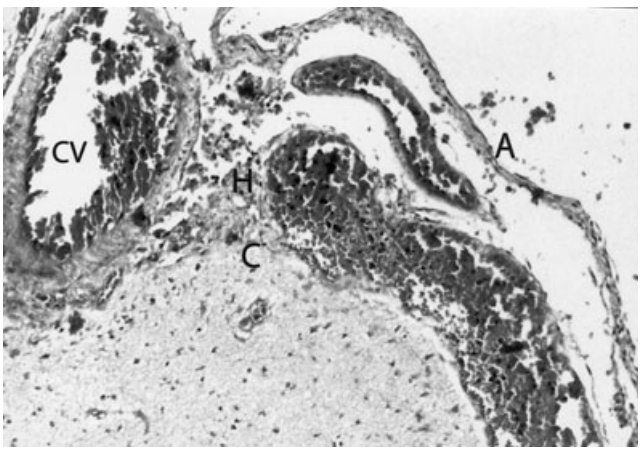

Fig. 2 Massive subarachnoid hemorrhage (H) in subarachnoid space, arachnoidal thickening (A), inflammatory changes, vascular congestion (CV), pia-arachnoid adhesions and subpial cortical ischemic alterations (C) are observed in the brain of a deceased animal (LM, HE, ×400).

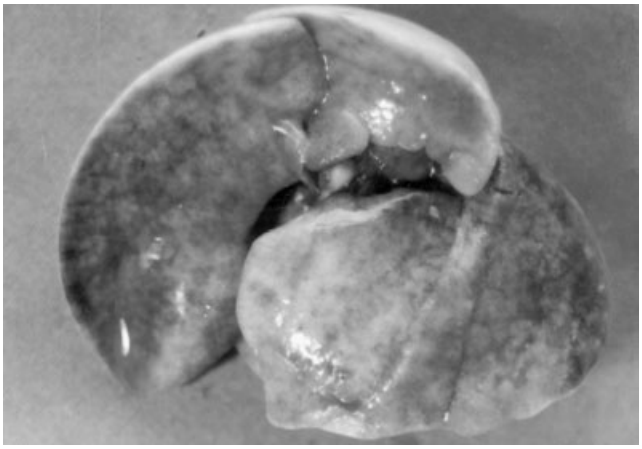

Fig. 3 Macroscopic appearance of lungs with pleural hemorrhagic focuses and purple-reddish lesions were observed in a deceased animal.

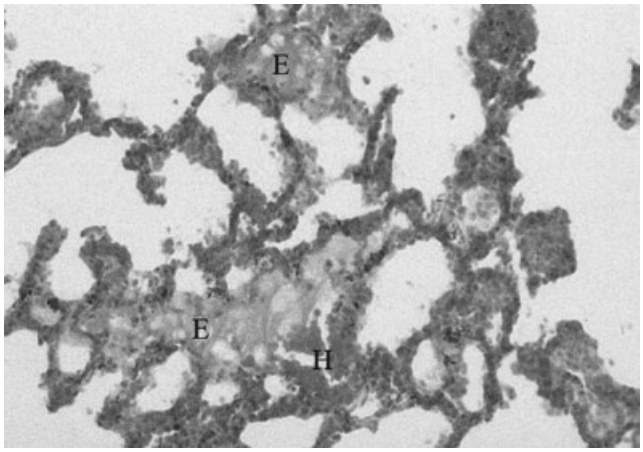

Fig. 4 Intraalveolar fluid collection (E) and hemorrhage (H), alveolar wall hemorrhage are seen in a cerebral fat embolism developed animal (LM, HE, ×400).

Table 1 Mean numbers of the occluded branches of each middle cerebral artery

Rabbit No.	1	2	3	4	5	6	7	8	9	10
SAH group ($n = 10$)	**3**	**0**	**0**	**1**	**2**	**0**	1	0	0	0
Isotonic group ($n = 10$)	**1**	0	0	0	**0**	0	0	0	0	0

The results of deceased animals are shown in bold. The difference was significantly meaningful between the subarachnoid hemorrhage (SAH) and isotonic saline-injected group ($P < 0.05$). The difference was meaningful between the deceased and survivor animals in the SAH group ($P < 0.05$). However, the difference was not significant between survival animals ($P > 0.05$). The deceased animals are shown in bold.

DISCUSSION

Subarachnoid hemorrhage is termed as blood leakage in the subarachnoid space due to vascular rupture in various etiologic factors. Experimental SAH can be formed by autologous blood injection into the cisterna manga and early cerebral vasoconstriction and diminished cerebral blood flow (CBF) occur in all models.[12,13] CBF decreases acutely and persistently after SAH, independently of changes in ICP and cerebral perfusion pressure.[14] Decreased cerebral perfusion pressure may cause increased ICP and perfusion arrest.[15] Severe SAH is associated with loss of autoregulation.[16,17] Microvascular aggregation of red blood cells has also triggered acute ischemic damage of the brain.[4] In the ischemic phase, swelling of perivascular astrocytes, neurons, and endothelium-induced ischemic insult leads to aggregation of red blood cells, increased cerebrovascular resistance, and worsened ischemia.[2] Extensive global ischemic brain damage can result in death shortly after severe SAH.[18] The mortality rate of SAH is about 25% within 24 h, and is 45% within one month.[14]

Neurogenic pulmonary edema is rare in patients with reversible neurological injury but is more common in those with severe and fatal neurological diseases.[19,20] Weir *et al.*[19] studied 78 patients with fatal SAH from ruptured aneurysms and found pathological evidence of NPE in 71%. But Hijdra *et al.* [21] have reported that SAH leads to NPE in

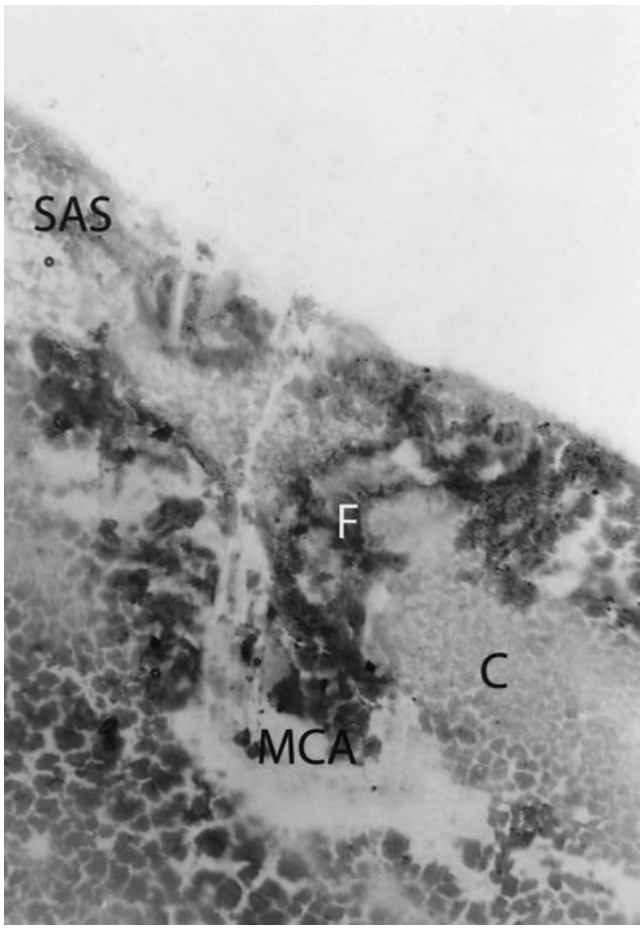

Fig. 5 Intraarteriolar fat accumulation (F) is seen in the branches of the middle cerebral artery (MCA) at the sylvian area (C) (LM, Sudan Black, ×400).

17% of cases. NPE is characterized as an acute, protein-rich lung edema occuring shortly after cerebral lesions associated with an acute rise of ICP. Pathophysiological mechanisms include a rise of the pulmonary vasular 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.[8] Massive sympathetic discharge during the initial insult to the CNS is once thought to cause generalized vasoconstruction with a shift of blood from the high resistance systemic circulation to the low resistance pulmonary circulation. Consequently, this condition results in fluid overload to the pulmonary circulation, damaging the pulmonary capillaries and altering their permeability. The leak of red blood cells and high protein edema fluid through the pulmonary capillary membrane suggest that there might be disruption and damage to the capillary endothelium.[22–25] Myocardial dysfunction is a risk factor in the development of pulmonary edema in SAH.[7] Impaired left ventricular hemodynamic performance may contribute to cardiovascular instability, pulmonary edema formation, and cerebral ischemia. Pulmonary artery wedge pressure may reach high levels [>16 mmHg].[26] In the end, cerebro-pulmonary functions may be worsened and death may be inevitable. Hypoxia, increasing ICP and cerebral fat embolism may appear due to destructed lung function. Fat embolism developing secondary to NPE can worsen the brain insult. It has been reported that the mean interval between the onset of SAH and the diagnosis of NPE on chest film was about 2.5 h,[27] and cardiorespiratory functions may return to normal within 2 to 6 weeks after SAH in non-fatal cases.[19]

Chylomicrons are absorbed from the gastrointestinal tract, transported into lungs via thoracic duct and catabolized in the pulmonary endothelial cells. For this reason, pulmonary pathologies may destroy fat metabolism. Lung disorders such as alveolar wall destruction may destroy chylomicron metabolism and fat particles may leak into systemic circulation through the destructed alveolar wall.[10,26,28]

After 3 weeks of fat embolism, brain tissue can be obtained and evaluated for LM examination using HE and specific fat staining such as Sudan-III.[29] For examination, the specimens are obtained at the Sylvian area. The most lesions may revert to a normal appearance on MRI and correlated with LM findings after 3 weeks. However, small focal lesions may remain in the gray and/or white matter on MR images, and this correlates with the cystic changes on LM findings. Sporadic intracapillary fat vacuoles and disruption of the endothelial cells may be observed.[30] The usual presenting features of fat embolism are altered mental status, focal and generalized seizures, weakness, paresia, plegia, autonomic dysfunctions, deep coma, decerebrate response to noxious stimuli, petechiae, high-grade fever, oliguria and respiratory failure.[31]

Fat embolism syndrome can also be complicated by anemia, coagulopathy due to enhanced coagulation of platelets and thrombo-embolic processes. Postmortem studies show small hemorrhages in the white matter, basal ganglia, brain stem, and cerebellum. Inflammation caused by fat globules in the alveoli can lead to enhanced capillary permeability.[28,32] Patchy necrosis, demyelination, microinfarct, perivascular hemorrhage and white matter atrophy are seen in the brain-stem, cerebrum and cerebellum.[23,26,33] It has been reported that the mean interval between the onset of SAH and the diagnosis of NPE on chest film was about 2.5 h.[27] The first dye used in the determination of fat globules was Sudan III and is still the most common stain today.[29] Our experiment was also planned according to the knowledge that a 2–3-week follow-up period of animals was thought as sufficient.

Neurogenic pulmonary edema occurs in about 10–71% of patients with SAH.[5,8,21] In patients with sudden death from SAH, more than 90% present with acute pulmonary edema.[33,34] Walder *et al.* [34] reported that the development of NPE that was characterized by an acutely increased capillary permeability to proteins was independent of the degree of ICP.

Cerebral fat embolism occurrence in the presence of neurogenic pulmonary edema secondary to SAH has not been reported so far.

In our study, pulmonary tissue destruction was observed and fat globules were found in cerebral arteries of the deceased animals. It is believed that SAH is an important predisposing factor in NPE, and it is speculated that NPE may be the most important factor in cerebral fat embolism. As a rule, we may not think that the animal model is the same in humans in view of the clinical outcome in such conditions. Hovewer, our study showed that our lung findings were similar in humans which have been described in the literature.[22,23,25,27,35–37] In a literature review, the major cause of death in SAH appears to be cerebral insult due to NPE and its complications.[7,19–21,26] Additionally, here we surprisingly observed that cerebral fat embolism is a dangerous complication of NPE; this has not been mentioned in the literature.

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