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Original Article

Coronary fat embolism following subarachnoid hemorrhage: an experimental study

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ABSTRACT

Background: Subarachnoid hemorrhage (SAH) can lead to neurogenic pulmonary edema (NPE), and chylomicron metabolism may be altered unfavorably in acute lung injury. This study aimed to investigate the possible effect of NPE on the development of coronary fat embolism.
Methods: This study was conducted on 27 rabbits, 5 of which were used as the control ($n=5$). Experimental SAH was induced in 15 of the animals by injecting homologous blood into the cisterna magna, and the remaining 7 animals were administered only isotonic saline solution (Sham, $n=7$) in the same manner under general anesthesia. After 21 days, all the animals were euthanized, and their hearts, lungs, and brains underwent histopathological examination.
Results: Six animals died of SAH during the experiment, and foamy hemorrhagic parenchymal lesions and intraalveolar hemorrhage were observed in their lungs. The histopathologic findings revealed minimal changes in the lungs, heart, and brains of the surviving animals; however, an abundant amount of fat globules was found in the coronary arteries of the six nonsurviving animals. There was a meaningful difference between the number of occluded coronary arteries with fatty globules in the surviving and nonsurviving animals ($P<.001$). However, the difference between the survivors and the isotonic-saline-injected group was not meaningful ($P>.05$). Coronary fat embolism was an important mortality factor following SAH ($P<.005$).
Conclusions: In SAH-induced NPE, the leakage of chylomicrons into the systemic circulation may lead to coronary fat embolism, which has not yet been reported in the literature.

1. Introduction

Neurogenic pulmonary edema (NPE) is the most serious complication of subarachnoid hemorrhage (SAH). Because vagal nerves play a vital role in lung functions, vagal ischemia and sympathetic overactivity may have a causative role in the pathogenesis of NPE [1]. SAH causes sympathovagal imbalance-based lung–heart injury via decreased parasympathetic and increased sympathetic overactivity [2]. Consequently, the development of NPE can result in fat embolism (FE) following SAH [3]. FE is a dangerous problem as a major complication of severe trauma. It is known that fat droplets enter the circulation from bone fracture sites, whereas absorbed lipids from the intestines are transported for catabolism into the lungs via the thoracic duct. Therefore, systemic lipid embolism may not occur unless bone fractures lead to pulmonary injury because, in FE, pulmonary contusion is more important than long bone fracture [4]. FE requires mobilization of free fat, which then enters the circulation and lodges as fat globules in the fine venous capillaries [5]. Coronary artery embolism is recognized as an important nonatherosclerotic cause of acute myocardial infarction [6]. Cardiac dysfunction, including echocardiographic wall motion abnormalities, electrocardiographic changes, and positive troponins suggestive of myocardial damage, may also occur after SAH [7]. It is unknown whether there is a causal relationship between early release of the biomarkers of myocardial injury after SAH and neurological sequelae. Although myocardial injury after SAH has been primarily attributed to the release of catecholamine by the sympathetic nerve endings in the myocardium or as a result of an imbalance in the autonomic nervous system, the pathophysiological mechanism of the association between SAH and myocardial injury remains controversial [8–11]. Moreover, it has been reported that left ventricular systolic dysfunction and contraction-band necrosis of the myocardium after experimental SAH can occur in

Conflict of interest: The authors declare that there is no conflict of interest.

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the absence of persistent myocardial hypoperfusion [12]. The present study aimed to evaluate the effect of NPE, if present, on the development of coronary FE as a cause of myocardial injury in SAH.

2. Methods

2.1. Animal selection

Twenty-seven adult male New Zealand rabbits (3.7±0.4 kg) were used in this study. Five of the 27 animals were used as the control (n=5). Experimental SAH was induced in 15 of the animals by injecting homologous blood into the cisterna magna, and the remaining 7 animals were only administered isotonic saline solution (Sham, n=7) in the same manner under general anesthesia. After 21 days, all the animals were euthanized, and their lungs, hearts, and brains underwent histopathological examination.

2.2. Ethical and anesthetic procedures

This study was performed according to the guidelines set by the Ethics Committee of our faculty (B.30.2.ATA.0.23.85-41). All the animal experiments complied with the Animal Research: Reporting of In Vivo Experiments guidelines, and they were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 8023, revised 1978). The animals were fasted for 6 h before surgical intervention. The experiments were carried out in anesthetized spontaneously breathing rabbits. A balanced injectable anesthesia was used to reduce pain and mortality. After inducing anesthesia with isoflurane via 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 of the anesthetic combination was used when required. In addition, 0.5 ml of autologous blood was taken from the auricular arteries and injected into the cisterna magna of 15 of the animals via a 22-gauge needle over the course of approximately 1 min, and 0.5 ml of isotonic saline solution was injected in the same way into the remaining 7 animals. The surviving animals were followed up for 21 days without any medical treatment. Six of the 15 animals with SAH died from SAH complications after 2 weeks.

2.3. Histopathological and statistical analysis

It is necessary to apply special staining procedures to diagnose coronary FE. Because the free fat in the paraffin-embedded tissue sections can be dissolved by ethanol and the slides do not demonstrate free fat, frozen fresh tissue sections were used in this study to observe fat. Moreover, frozen heart and lung sections (1 μm) were taken from the epicardial coronary arteries and pulmonary vessels and then stained with Sudan-III Black. After storing the hearts and lungs in 10% formalin solution for 7 days, heart and lung tissue sections were embedded in paraffin, and then 1 μm of the pulmonary cortical and myocardial sections was removed and stained with hematoxylin and eosin (H&E) to provide more detail of cell and tissue structures.

The occluded branches of the coronary arteries with fat particles were counted, and the Mann–Whitney *U* test was used to conduct a statistical analysis of the differences between the nonsurviving, the surviving, and the isotonic-saline-injected animals.

3. Results

3.1. Clinical outcome

Cardiorespiratory disturbances, motor inability, signs of meningeal irritation, stiff neck, decreased Glasgow Coma Scale (<10), and electrocardiogram abnormalities were detected in the 15 SAH-induced animals. In the isotonic-saline-injected group, one animal died due to iatrogenic SAH within the first week. Six of the 15 SAH-induced animals died of SAH during the experiment; 2 of the 6 animals died in the first week, and 4 of the 6 animals died between 16 and 20 days.

3.2. Gross anatomical findings

Postmortem macroscopic examination of the deceased animals showed massive brain swelling, meningeal inflammation, and cortical injury. Foamy hemorrhagic parenchymal lesions and intra-alveolar hemorrhage were observed in the lungs of the dead animals. Pleural hemorrhagic focuses and a purple-reddish appearance were observed in the deceased animals due to SAH.

3.3. Histopathological findings

An abundant amount of fat globules was found in the coronary arteries of all six of the nonsurviving animals. However, minimal histopathological changes were found in the lungs, heart, and brains of the surviving animals. Coronary FE was only detected in two of the animals that were given isotonic solution and three of the surviving animals. Significant intra-alveolar hemorrhage, congestion, and pulmonary artery (PA) medial hypertrophy were observed in the histologic analysis of the lungs of the nonsurviving animals. In the remaining animals, only minimal edematous and hemorrhagic changes were observed in the lungs, and FE was only detected in two animals. Fig. 1 shows the macroscopic appearance of a heart with an occluded coronary artery (base), a brain with an induced SAH (black arrow/A), a lung that developed neurogenic lung edema (blue arrow/B), and the histological findings of a coronary artery occluded with fat globules (LM, Sudan-III, ×20). Fig. 2 shows the microscopic appearance of a SAH-induced brain and the

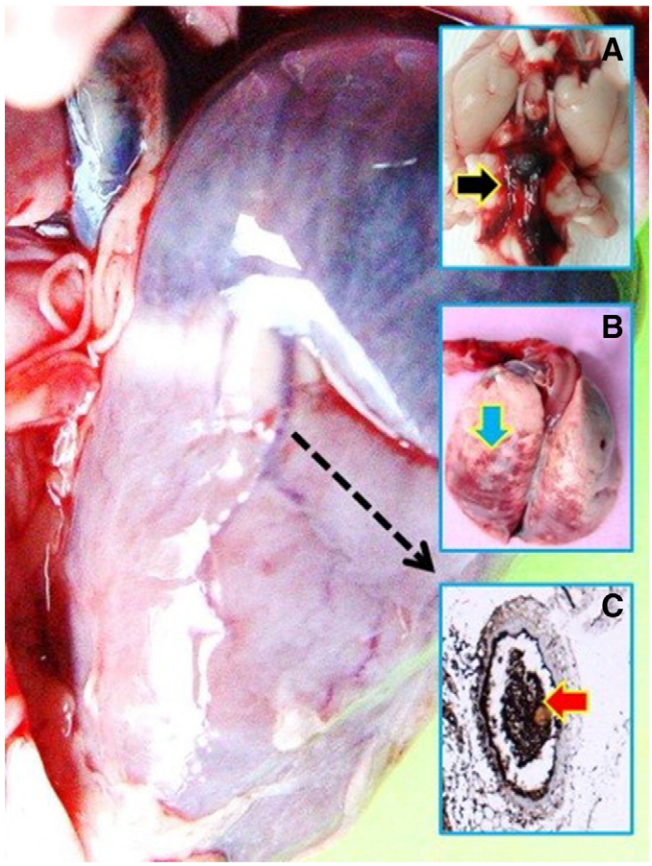

Fig. 1. Macroscopic appearance of a heart with a thrombosed coronary artery (base), a brain with an induced SAH (black arrow/A), a lung that developed neurogenic lung edema (blue arrow/B), and the histological findings of a coronary artery occluded with fat globules (red arrow/C) (frozen section, LM, Sudan-III, ×20).