Ultrastructural Changes of the Basilar Artery Following Experimental Subarachnoid Haemorrhage

A Morphological Study on the Pathogenesis of Delayed Cerebral Vasospasm

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Summary

Recent experimental studies have shown, that the endothelium of cerebral vessels undergoes significant changes after subarachnoid haemorrhage which may lead to biochemical changes at the endothelial surface with disturbance of the delicate homeostasis of vasodilating and vasoconstricting mechanisms which are thought to be responsible for preservation of the tones of the cerebral vasculature. Ultrastructural studies incorporating different forms of microscopic observations of the endothelium after SAH representing a prerequisite for further investigations on the pathogenesis of cerebral vasospasm are scarce. This experimental study was performed in order to investigate and define more precisely the pathomorphological changes at the endothelial surface of the basilar artery of dogs after experimental SAH.

Two separate injections of autologous blood into the cisterna magna within 72 hours resulted in extensive angiographic narrowing of the diameter of the basilar artery of all animals. Histological studies of the basilar artery including light microscopic, transmission electron microscopic, scanning electron microscopic and freeze cracking microscopic examinations demonstrated severe pathomorphological changes at the endothelial surface. These consisted mainly of infolding and corrugation of the endothelium, disorientation and desquamation of endothelial cells as well as of vacuolation and ingrowth of fibrous tissue between the endothelial and muscular layer. No pathomorphological changes could be observed in the muscular layer.

As the described post-haemorrhagic ultrastructural changes of the endothelium of cerebral vessels in spasm are likely to represent the morphological basis of the delayed form of cerebral vasospasm future research on its pathogenesis should primarily focus on the structural and biochemical changes taking place at the endothelial surface of the cerebral vasculature after SAH.

Keywords: Cerebral vasospasm; experimental subarachnoid haemorrhage; basilar artery; ultrastructural studies.

Introduction

Despite the advent of microsurgical techniques, sophisticated neuroradiological methods as well as improvement in neuro-anaesthesia with subsequent reduction of peri-operative morbidity and mortality the toll of subarachnoid haemorrhage (SAH) from ruptured intracranial aneurysms is still high. Concerning recent clinical studies only slightly more than 30% of the patients suffering from SAH can be expected to achieve complete functional recovery16, 21, 22.

The occurrence of delayed ischaemic deficits due to the development of chronic cerebral vasospasm is considered to be responsible for a significant percentage of the mortality and permanently disabling morbidity of subarachnoid haemorrhage1, 10, 23, 31, 32.

Despite extensive experimental and clinical investigations the aetiology of this phenomenon remains obscure.

However necropsy studies following SAH as well as experimental investigations of cerebral vessels in spasm have demonstrated the development of morphological changes of the arterial wall which are thought to play an important role especially in the pathogenesis of the delayed form of cerebral vasospasm6, 7, 11, 13, 14, 17, 18, 25, 27, 36-38, 43, 44, 45, 47.

Most of these studies have focussed attention on the pathological changes of the muscular layer of the cerebral arteries which have for a long time been considered to be of utmost importance for the development of post-haemorrhagic vasospasm. However recent experimental studies have shown, that the endothelium of cerebral vessels undergoes significant pathological alterations after SAH which may lead to biochemical changes at the endothelial surface with disturbance of the delicate homeostasis of vasodilating and vasoconstricting mechanisms being responsible for the pres-
ervation of the tonus of the cerebral vasculature. Ultrastructural studies incorporating different forms of microscopic examinations of the endothelium after SAH which represent a prerequisite for further investigations on the pathogenesis of cerebral vasospasm are scarce. This experimental study was performed in order to investigate and define more precisely the pathomorphological changes at the endothelial surface of the basilar artery using a canine model of delayed cerebral vasospasm after experimental subarachnoid haemorrhage.

Material and Methods

The experimental procedure was performed according to the "two-haemorrhage model" of chronic cerebral vasospasm. Mongrel dogs of both sexes weighing between 20 and 25 kg were used for the experiments. Anaesthesia was induced with an intravenous injection of pentobarbital sodium (15 mg/kg). Thereafter endotracheal intubation was performed and controlled ventilation was maintained with a combination of nitrous oxide and oxygen. Blood gas parameters were checked routinely during the course of the experiments and were kept within normal limits by adjusting the tidal volume and frequency of the respirator. Following these procedures the dogs were placed into supine position. After either surgical exposure of the femoral artery or percutaneous catheterization of the vessel an angiographic catheter was advanced to the left vertebral artery under fluoroscopic control. A baseline angiogram of the basilar artery was performed using 5 ml of iopromid corresponding to 200 mg iodine/ml.

After the angiographic procedure the dogs were rolled into prone position and the cisterna magna was aseptically punctured. For simulation of the subarachnoid haemorrhage 4 ml of autologous blood were injected manually over a period of 2 minutes. Thereafter the dogs were kept in headdown position of 30 degrees for 30 minutes to allow distribution of the blood within the basal cisterns and especially around the basilar artery.

On day three of the experiment using light anaesthesia a second injection of the same amount of blood into the cisterna magna, was performed. On day 8 the dogs were again anaesthesized and put on artificial ventilation. Angiography of the basilar artery was repeated in order to demonstrate the presence and severity of cerebral vasospasm as demonstrated by the angiographic narrowing of the vessel diameter.

After the last angiographic procedure an anterior thoracotomy was performed. The descending aorta was clamped as well as the thoracic part of the vena cava before entering the right atrium. Following incision of the descending aorta an infusion of 2,000 ml of physiologic saline was started in order to wash out the blood from the cerebral circulation followed by intravital perfusion-fixation of the cerebral vasculature with 2.5% cacodylate-buffered glutaraldehyde.

Thereafter a craniotomy was performed, the brain of the dog was quickly removed, the basilar artery was dissected using the surgical microscope and put into the above described solution until further dissection.

Examination of the changes of the angiographic diameter of the basilar artery from day 1 to day 8 were made using a Zeiss stereo-microscope and a ten-fold magnification. The diameter of the basilar artery was measured at 7 corresponding locations along the course of the vessel.

Fig. 1. Example of an angiogram of the basilar artery of a dog with experimental subarachnoid haemorrhage. Comparison of the angiogram performed on day one (a) versus day 8 (b) demonstrates severe angiographic narrowing of the vessel diameter. Arrows pointing to the locations along the course of the vessel where measurements of the diameter were performed.