Acta Neurochirurgica 60, 45—54 (1982)

ACTA
NEUROCHIRURGICA
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Blood-Brain Barrier Changes After Experimental Subarachnoid Haemorrhage

By

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With 6 Figures

Summary

Cerebral vascular permeability and blood-brain barrier in the acute stage of subarachnoid haemorrhage were studied in experiments on cats.

The bleeding was induced by a special method, imitating the natural course of subarachnoid haemorrhage.

Fluorescence of the Evans blue injected intravitally was used as an indicator. It was found that one hour after the subarachnoid haemorrhage there are discrete diffuse spots of translucency related to the individual vessels, appearing bilaterally in the cerebral cortex and to a lesser extent in the white substance. Four hours after the bleeding, the extravasations and tissue staining become more widespread and confluent. There are more areas with disturbed blood-brain barrier, and they are better differentiated from the normal tissue. They are distributed bilaterally throughout the brain with predominance on the side of the bleeding vessel. The possible mechanisms of the blood-brain barrier disturbance after subarachnoid haemorrhage are discussed.

It is well established that brain damage after subarachnoid haemorrhage (SAH) is mainly of an ischaemic nature [2, 10]. The reduction of brain perfusion occuring during SAH may also damage the cerebral vessels and the blood-brain barrier (BBB) [18]. This may in turn result in secondary brain oedema with subsequent rise of intracranial pressure (ICP) observed experimentally and clinically [3, 10].

Since the occurrence and distribution of BBB damage and its time course in the acute stage of SAH is not clear, animal experiments employing Evans blue (EB) intravital staining were performed.

0001-6268/82/0060/0045/$ 02.00

Material and Methods

Thirty cats of both sexes, weighing from 2.5 to 4.2 kg, anesthetized with 0.32 mg per kg of body weight intraperitoneal pentobarbitone (Narcoren) were used for the experiments. The animals were placed on heated pads with heads held in a stereotactic frame. They breathed spontaneously through tracheostomies. Arterial and venous pressures, epidural pressure over both hemispheres, heart and respiration rates, EEG, end-expiratory carbon dioxide contents, and subcutaneous temperature were monitored and registered throughout the procedures. Blood gases were analysed at intervals, and were found to remain within the normal limits.

Subarachnoid Bleeding

Close imitation of natural SAH conditions in cat experiments was attempted. Bleeding from an injured artery in the subarachnoid at the base of a closed skull was regarded as the goal of the experiment. Therefore an original method was developed: through the open mouth the soft palate was incised, the nasal mucosa was dissected, and the presphenoid air cell was opened. Removal of its upper wall gave access to the perichiasmatic cistern and of its side to the internal carotid artery and its main branches. Immediately after puncture, producing SAH, the bone opening was closed with a wax plug, partially filling the presphenoid cell. The tightness of such closure was tested up to 180 mm Hg in separate experiments. This experimental layout reproduces important pathogenic factors of SAH: mechanical injury of the vessel, natural pressure of extravasation, and intracranial pressure responses to the event.

Blood-Brain Barrier Examination

The animals were injected intravenously with 1 ml per kg body weight of 3% solution of Evans blue (EB) immediately after the end of the plateau of the increased intracranial pressure, which was usually about four minutes after the onset of haemorrhage. The cats were sacrificed with an i.v. overdose of potassium chloride 10 minutes, one hour, and 4 hours after the haemorrhage. The skull was frozen in liquid nitrogen, embedded in medium for frozen tissue specimens (Tissue-Tek II, Lab-Tek Products, Naperville, Illinois, U.S.A.), and stored at — 25 °C for 12 hours. The head was sectioned horizontally with a cryostat microtome (LKB Sweden). Sections of 10, 20, and 50 microns on Tesa film D (Beiersdorf AG, Hamburg, FRD), were dried at — 25 °C, and fixed on glass for microscopy. Slides from 10 different levels of the brain from each animal were inspected under the operating microscope for blue staining and with a Zeiss Photomicroscope III, (D-7082 Oberkochen, FRG). Epilumination with mercury high pressure lamp HBO 50, exciting filter 546, and suppression filter 590, was used. Fluorescence micrographs of selected areas were taken on Ektachrome 160 (Kodak U.S.A).

Results

In the sham operation cats after 10 minutes, one, and 4 hours no blue staining of the brain could be detected. EB red fluorescence was restricted only to the blood vessels. The brain parenchyma and perivascular spaces remained free from fluorescence (Fig. 1).

Ten minutes after the SAH no change in the appearence of the brain slices in comparison to the control animals could be seen.

One hour later direct and operation microscopic viewing revealed no blue staining, but fluorescence microscopy showed discrete diffuse spots of translucency clearly related to the individual vessels, particularly in the grey matter, and locally in the subcortical white matter

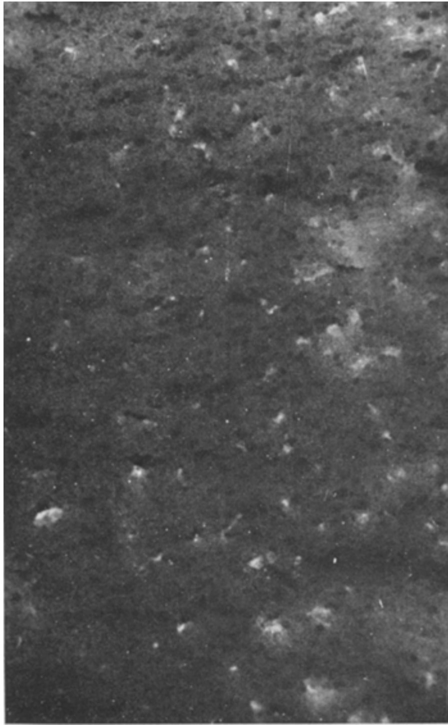

Fig. 1. Cerebral hemisphere of a cat four hours after sham operation and i.v. injection of Evans blue. Distinct intravascular fluorescence. Magnification: ×63

(Fig. 2). The translucencies in the white substance were more evenly distributed throughout both hemispheres, whereas there were more injured foci in the cortex on the side of the bleeding vessel.

Four hours after the bleeding, the extravasations of the dye around the vessels were more distinct and widespread, with staining of the surrounding brain tissue. The cells were not stained selectively; only diffuse translucency was found. The spotty, now partially confluent, areas in the cerebral cortex could be noted bilaterally, particularly on the side of bleeding. The vessels in the white substance

were strongly permeable to the EB in both hemispheres. Increased permeability concerned mainly the precapillaries, capillaries, and venules, but was also distinct in the bigger vessels, including the conducting arteries, with tissue staining in their close vicinity (Figs. 3 and 4). In two cats of the four hours series a very distinct blue coloration was observed macroscopically. Confluent blue foci in the

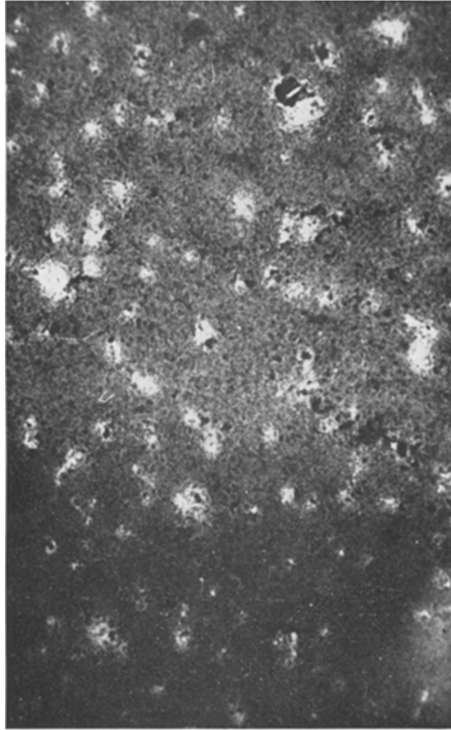

Fig. 2. Perivascular diffusion of Evans blue in the white matter and particularly in the cortex of cats brain one hour after subarachnoid haemorrhage. Magnification: ×63

cerebral cortex were present bilaterally in the temporal lobes, with clear predominance on the side of the injured vessel. The white substance was spared (Fig. 5). In these cases fluorescence microscopy showed massive staining of the cortex and widespread leakage of fluorescent material from the vessels in the white substance (Fig. 6). Both cats differed others in the group by high arterial pressure of mean 160 mm Hg maintained after the SAH, and elevated intracranial pressure to about 40 mm Hg throughout the posthaemorrhagic period.

Discussion

Evans blue dye was extensively used for demonstration of the impaired BBB. Administered intravascularly it forms a bond with blood plasma albumin, to which the BBB is impermeable. It was empirically established that in the first 24 hours after injury, the vital

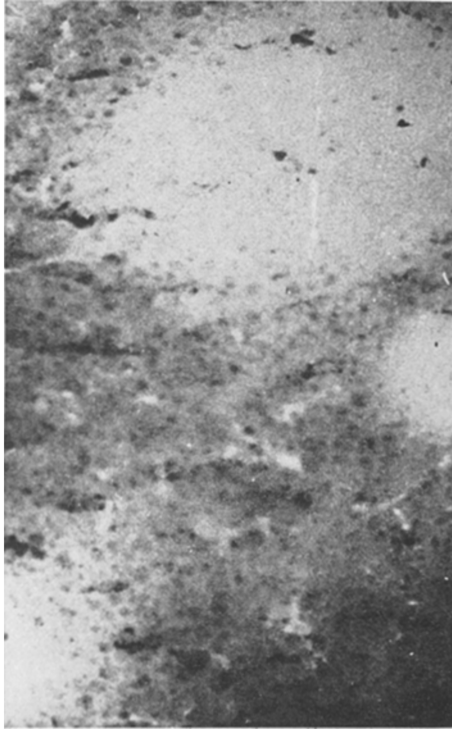

Fig. 3. Discrete diffuse spotty fluorescence of Evans blue in the brain cortex of a cat four hours after subarachnoid haemorrhage. Magnification: ×100

staining corresponded exactly with those areas which were histologically oedematous, provided that the dye was given prior to, or at the time of production of the lesion [1]. Intra vitam extravasation of EB and tissue staining occurs only when the BBB is disturbed. Postmortem dye diffusion is also possible [11], but immediate freezing of the tissue prevents such an event. Fluorescence microscopy with EB has an advantage over light microscopy in its ability to demonstrate even the smallest amount of the dye [14].

Increased fluorescence around the vessels, and tissue staining, may lead to the assumption that after SAH cerebral vessel walls and the

BBB become permeable to the plasma albumin. It appears between 10 and 60 minutes after SAH, and increasingly spreads during the following 4 hours. The changes are universally distributed throughout the brain, with predominance in the grey matter on the side of the bleeding vessel.

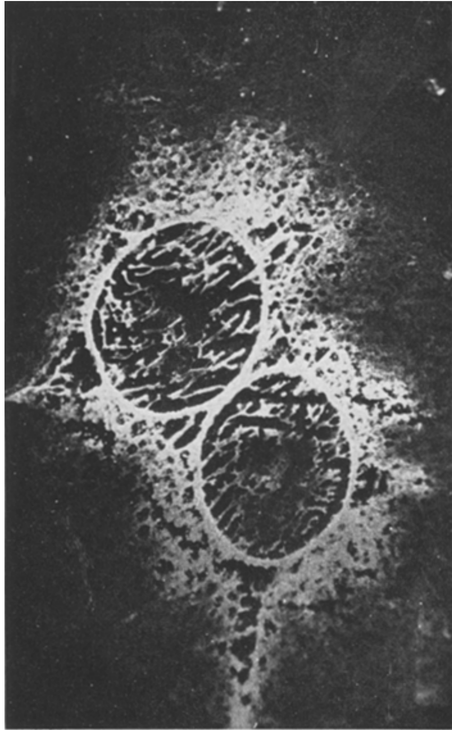

Fig. 4. Evans blue extravasations with tissue staining round conducting arteries of cats' brain four hours after subarachnoid haemorrhage. Magnification: ×63

There are numerous factors in the SAH that may be responsible for the damage of the cerebral vascular permeability and the BBB.

It was suggested that increased capillary permeability after SAH results from the kinins generated in the blood extravasated into the subarachnoid [13]. Vasoparalysis or impaired autoregulation in the ischaemic zones which acutely develop after SAH may provide another explanation of or contribute to the phenomenon. It is also well established that the development of increased permeability of cell membranes closely follows creation of an area of acute ischaemia [17]. It has been shown experimentally that brain swelling suffi-

cient to raise the intracerebral pressure focally may occur within 20 minutes of vascular occlusion [3]. In SAH the capillary circulation is under increased stress, since the acute ischaemic zones are accompanied by vasodilatation due to exhausted autoregulation. Stress disruption of the tight junctions in capillaries may result in the transudation of fluids [12]. More direct transmission of arterial pul-

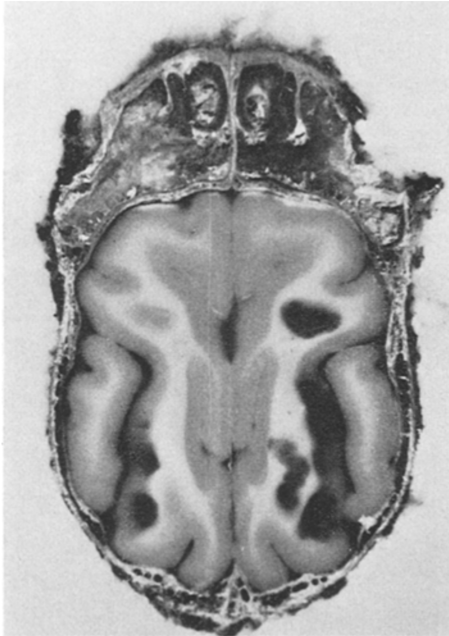

Fig. 5. Horizontal head section of a cat four hours after subarachnoid haemorrhage. Macroscopic staining of the cerebral cortex bilaterally, with predominance of the hemisphere ipsilateral to the bleeding vessel

sations to the capillaries in vasodilatation may also add to that stress [15]. Vascular dilatation itself can lead to fluorescein staining of the periadventitial zones [4]. It has been suggested that postischaemic vasodilatation of arterioles, capillaries, and venules, damaging the BBB, may persist even after onset of vasoconstiction, thus accounting for the increased cerebral blood volume in patients with vasospasm and reduced cerebral blood flow [7]. Increased arterial pressure would have in such circumstances a devastating effect on the BBB. It was suggested by Symon and Pasztor that monitoring of the intracranial pressure could sort out patients in whom induced arterial hypertension improved flow, without the risk of secondary brain swelling.

The patients with marked vasoparalysis after SAH do not benefit from induced therapeutic arterial hypertension, and instead develop secondary brain swelling [16]. This opinion can be supported by the two cases from our experiments with massive macroscopically visible BBB disruption in the cortex. Both had increased arterial pressure and an ICP course corresponding to the ischaemic-edematous type

Fig. 6. Massive Evans blue spots of fluorescence in the cerebral cortex of a cat four hours after the subarachnoid haemorrhage in the area of macroscopically visible blue staining. Magnification: ×63

described by Nornes and Magnaes [10]. They found the oedema formation after SAH to be the main cause of the secondary rise of the ICP.

It has been proved that acute arterial hypertension itself is capable of damaging the BBB as soon as seven minutes after onset of hypertension [8].

Two hours after SAH ultrastructural changes in the vascular intima and myonecrosis were demonstrated [18].

Computerized tomography (CAT) provides further data on the increased cerebral vascular permeability after SAH. CAT is a valuable tool in detecting the permeability of cerebral vessels [9]. It is also established, that contrast enhancement is partly due to the BBB abnormalities [6]. It was demonstrated that after SAH intravascular contrast material migrates to the vicinity of the parent vessel and aneurysm. It could not be detected during the phase of vasospasm [5].

Damage of the BBB and increased vascular permeability after the SAH may in consequence lead to the leakage of vasoactive substances from the blood and promotion of secondary vascular spasm and brain oedema.

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