

The Effect Of Defibrotide On Cerebral Vasospasm Following Subarachnoid Haemorrhage: A Light And Electromicroscopic Study In A 3-Way Subarachnoid Haemorrhage In Cats

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Abstract : Cerebral arterial vasospasm is the major complication of subarachnoid haemorrhage. This process of unclear aetiology causes significant increases in the morbidity and mortality of patients with subarachnoid haemorrhage. In this study all physiopathological characteristics of subarachnoid haemorrhage were established in a 3-way model in cats. Using this model, we investigated the efficacy of defibrotide in preventing cerebral

arterial vasospasm following subarachnoid haemorrhage. Although the process of vasospasm could not be prevented by the use of defibrotide, our results suggest that defibrotide can significantly decrease delayed histopathological damage after subarachnoid haemorrhage.

Key words: Subarachnoid haemorrhage, Cerebral vasospasm, Defibrotide, Cerebral ischemia, Transient

INTRODUCTION

Cerebral vasospasm (CV) that follows subarachnoid haemorrhage(SAH) causes a significant increase in morbidity and mortality. Delayed cerebral vasospasm after SAH is a complex pathological process characterized by an abnormal proliferation and necrosis of the cellular constituents of the arterial wall (1,13). Although the mechanism of this disorder is still unclear, it has been considered that multiple endogenous vasoactive substances are involved. Therefore several studies aiming the prevention and treatment of CV were performed, and the subject is still under extensive investigation in the current literature. In 1964, Crompton (8) reported pathological findings in the cerebral arteries of 119 patients suffering from cerebral infarcts following aneurysmal SAH. Today, CV is considered to be an acute proliferative vasculopathy similar to atherosclerosis (28).

Blood surrounding the vessel is held to be the responsible factor for this vasculopathy(11,18,22,26). Also laboratory studies revealed perivascular blood as the cause of CV (4,13,16,30,32).

In this study we investigated the efficacy of defibrotide, an antithrombotic agent (27,34), in preventing cerebral arterial vasospasm following subarachnoid haemorrhage.

MATERIALS AND METHODS

Eighteen hybrid adult cats were used in the study. They were treated first with forane inhalation in a closed box, then catheterized via to femoral vein and anaesthetized with profol(3mg/kg/hour) and alfentanil(0.1mg/kg/hour). The surgical procedure was performed at 21 C room temperature. Cats were fixed on the operating table in the prone position, and the

lumbar region was shaved. The surgical field was cleaned using hibiscrub and betadine. A middle skin incision was followed by dissection of the subcutaneous tissues and paravertebral muscles. Two laminae were exposed and laminectomy was performed. A one millimetre thick sterile shunt tube was inserted in the subarachnoid space through a 2mm dural incision. The length of the tube was adjusted to the distance between the dural incision and skull base. Subsequently, the surgical area was closed using the appropriate surgical technique, and a stopcock was placed at the external end of the tube. After observing the free drainage of 1 ml cerebrospinal fluid through the tube, 1 ml autogenous arterial blood obtained via the femoral artery was intrathecally administered at a slow rate, causing isobaric SAH. Subarachnoid haemorrhage as described above induced on the first, third and seventh days. Ten days after the last bleeding, the cats were sacrificed and the basilar arteries were harvested using a surgical microscope (Kapps, Germany). The dissected arteries were immersed in 10% formaldehyde solution for 1 week, then dehydrated and embedded in wax. Sections of the vessels were stained with haematoxylin and eosin.

GROUPS

A) Group 1 (SAH Group): SAH was established in 6 cats as described above.

B) Group 2 (SAH and treated with defibrotide): Following the first SAH 6 cats in this group received intravenous defibrotide at a dose of 2mg/kg/day for 17 days.

C) Group 3 (Control group): Intrathecal saline solution was administered to the cats.

PATHOLOGICAL EVALUATION

1) Light Microscopy: Three transverse sections 5mm apart were made across each basilar artery. These 8µm thick transverse sections of all basilar arteries, stained with Haematoxylin-Eosin were photographed under x100 magnification. Measurements of distance from the adventitia to the lumen (wall thickness) and the diameter of the lumen were performed by two independent and blind observers (MMO and AS) and mean value was obtained.

2) Transmission Electron Microscopic study(TEM): Basilar arteries were first washed with a special isotonic 1% solution, and then passed through an isotonic acetone series. They were then treated with Vestopal 1, 2, and 3 for one hour each, and placed in capsules with vestopal 4. The capsules were incubated at 60 C for 23 hours to reach a solid state, and then studied under the TEM microscope (JOEL-JEM,100C)

3) Scannig Electron Microscopic study (SEM): The basilar arteries were also prepared for SEM studies (JOEL-SEM).

FINDINGS

Although most of animals showed lethargy caused by SAH during the entire observation period, we observed no appreciable difference in behaviour between the treated and untreated group.

A) Macroscopic findings:

Twelve brains in Groups 1 and 2 exhibited thickening of the basal arachnoid and obviously thinner basilar arteries compared to the control group. Blood or haematoma was not present. Colour change due to haemosiderin was detected in the basal cisterns of 5 cats. No macroscopic pathology was present in the brains of the normal cats.

B) Microscopic findings:

1) Light microscopy: When basilar arteries from the three groups were compared, decreased basilar artery calibres, thickened adventitia, increased collagen content in the fibrous tissue and monoclear cell infiltration were noticed in the SAH and defibrotide group. Haemosiderin either free or in macrophages was observed in a scattered pattern (Fig. 1 and Fig.2). Tunica media hypertrophy and vacuolization were also noticed. In the SAH group which did not receive defibrotide, subendothelial oedema and fields of disrupted continuity were also observed. When basilar arteries in Groups 1 and 2 were compared with arteries from the control group, significant widening, volume increase and narrowing of the lumen were found in all. The regular and concentric character of the narrowing was observed in the group that did not receive defibrotide. The control group showed normal histological architecture (Fig.3).

2) TEM findings: Significant pathological changes were noticed in the TEM examination of the 1st and

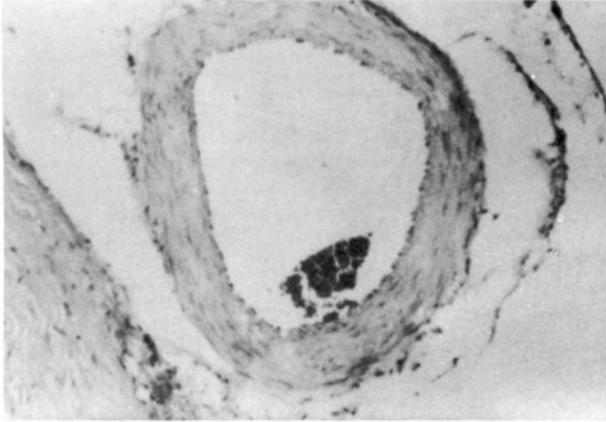


Fig. 1 : Note the mural thickening of the basilar artery with prominent tortuosity of the internal elastic membrane. The Diameter is reduced.

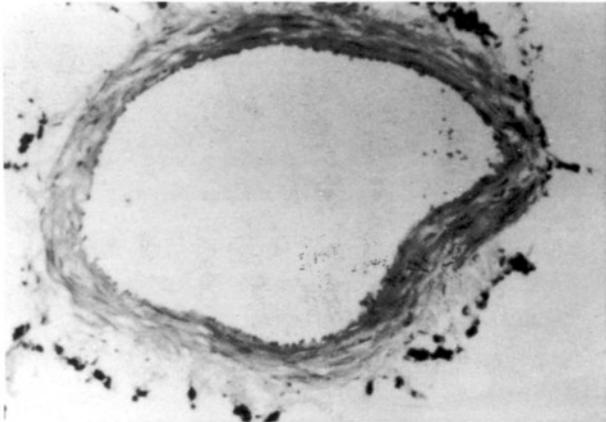


Fig. 2 : Lack of tortuous internal elastic membrane and thinned almost normal blood vessel wall signify a mildly dilated muscular type of blood vessel.

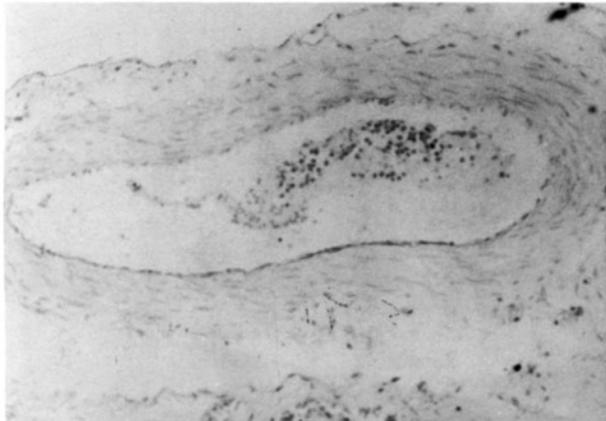


Fig. 3 : Almost normal thickness of the muscular blood vessel and regularity of the internal elastic membrane are characteristic features of a normal basilar artery.

2nd groups, compared with the controls. This pathological destruction was much more in the group that did not receive defibrotide. The first finding was an increase in the folds and undulations of the lamina elastica. Under high magnification, some disruption and loosening were noticed. Disruption and severe vacuolization of the endothelial cells were observed especially in the SAH group. These cells showed a tendency to penetrate into the disrupted internal elastic lamina (Fig.4). Necrosis in the tunica media (myonecrosis) was the most prominent finding. Pathological smooth muscle cells were scattered in the tunica media. Vacuolization was present in all cells. Nuclei were pyknotic and electron dense myofilaments were observed. Some cells exhibited juxtannuclear vacuolization, and the vacuoles contained some granules. Because of intracellular debris, these structures appeared highly electron dense. Meanwhile, extracellular debris was formed by dead smooth cells. As mentioned, endothelial cell vacuolization and desquamation, as well as smooth muscle cell was limited in the defibrotide group (Fig. 5). The control group almost always showed a normal appearance (as described) (Fig 6).



Fig. 4 : Intensive undulation of the internal elastic lamina and vacuolization of the endothelial cells (Group 1).

3) SEM findings: As in light microscopy findings, alteration in vascular calibres were demonstrated in different groups. SEM showed convolution of the endothelium and tunica intima, narrowing of the lumen, and thickening of the vessel wall in the basilar arteries. In both SAH groups, the external calibres were also decreased (Fig. 7 and Fig. 8). Therefore, the subarachnoid space was larger and fibrous bands were noticed especially in the pure SAH group.

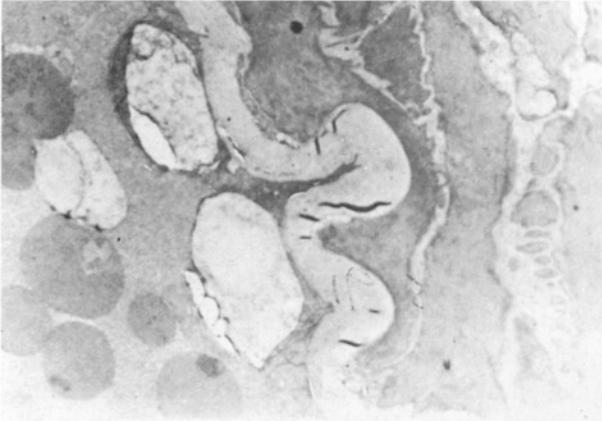


Fig. 5 : Minimal configuration defect in the elastic lamina (Group 2).

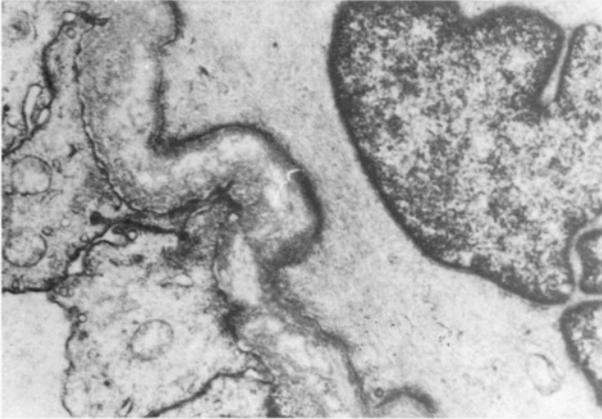


Fig. 6 : Normal basilar artery of the cat.

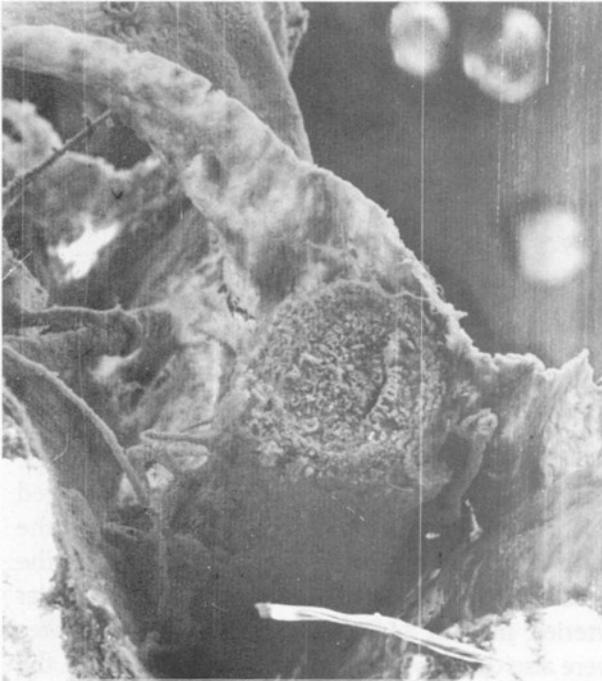


Fig. 7 : Highly narrowed basilar arter after SAH. Note the relative increase of subarachnoid space (Group 1).



Fig. 8 : Limited arterial spasm in the defibrotide group.

The control group did not show any of these irregularities (Fig. 9). C) Quantitative evaluation: Basilar artery calibres were determined in 6 cases each of the three groups. The mean value of the lumen

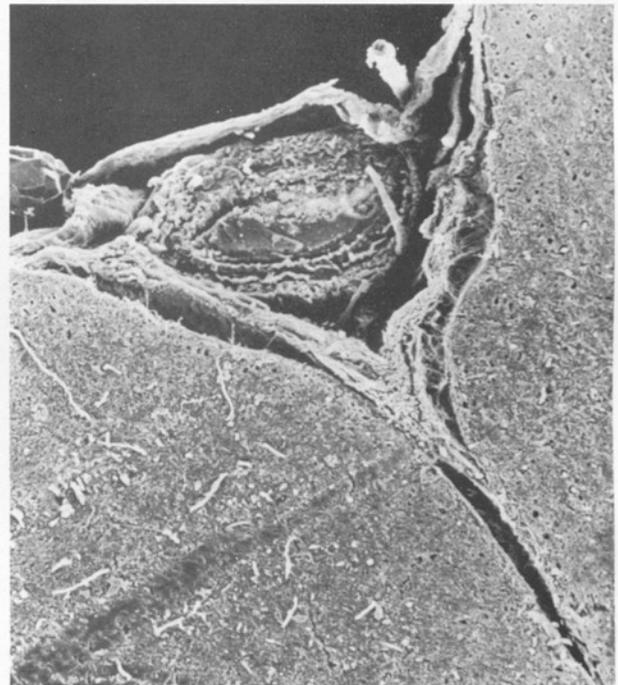


Fig. 9 : Normal SEM appearance of the cat basilar artery.

the three groups. The mean value of the lumen diameter was 5.0 ± 0.2 in the 1st, 7.5 ± 0.3 in the 2nd 9.5 ± 0.1 in the 3rd group. Wall thickness measured 1.2 ± 0.1 , 1.1 ± 0.2 and 1.0 ± 0.1 in the following three groups. Division of both parameters was defined as vasospasm index. It was 0.24 in the SAH group, 0.14 in the defibrotide and 0.10 in the control group (Table. 1). When the normal basilar artery diameter was considered 100%, the average value of narrowing was found to be 53% and 79% in groups 1 and 2, respectively ($P < 0.005$). We used the non-parametric Mann-Whitney U-test for statistical comparison of the vessel calibres between the control and treated groups.

DISCUSSION

Histopathological alterations following subarachnoid haemorrhage have been investigated in numerous studies (17,21,23). Crompton (8) detected necrosis, oedema and subendothelial polynuclear leucocyte infiltration in the vascular wall of 119 patients who died following SAH. Convey and McDonald (7) studied the relation between pathology and clinical status and suggested that the clinical picture is due to narrowed vascular lumen secondary to intimal thickening. However, Eldevik et al (12) failed to show any morphological alteration in the cerebral arteries of patients who had vasospasm in the antemortem period. At this point, the need for reproducible experimental models to clarify the aetiopathogenesis became obvious.

In the models used to study vasculopathy following SAH, blood was administered to the subarachnoid space via the cisterna magna (13,24,36) or placement of a subarachnoid clot around the middle cerebral artery (23,30). In earlier single bleeding models, Weir (35) and Mayberg (24), in monkeys and cats respectively, failed to show any morphological vascular change either by light microscopy or by SEM. Alksne (1), Alksne and Greenwood (2), and Fein (13) were able to show only limited pathological changes in the tunica media of monkey arteries. Using a double-bleeding model in pigs, Takemae et al (33), observed narrowing of vascular lumen, intimal proliferation and necrosis in the tunica media. Although several investigators (3,28) agree on the similarity of these histopathological changes to the arteriosclerotic process, the cause of intimal proliferation is still unclear.

In the literature, the best model to demonstrate histopathological changes was the one described by Takemae et al (33). We suggest that previous models in monkeys and cats failed because of the insufficiency of a single bleeding. In our model, narrowing of the vascular lumen, thickening of the vascular wall, pathological undulation in internal elastic lamina and adventitial mononuclear cell infiltration were clearly demonstrated by light microscopy after the third bleeding. In addition, increased intimal undulation was demonstrated in more detail by TEM. Vacuolization and myonecrosis were also observed in both endothelial, and smooth muscle cells by TEM. Therefore, our study to demonstrate histopathological alterations due to chronic cerebral vasospasm in cats is original. Late cerebral vasospasm is a two-phased process. First vascular spasm, and second vasculopathy. Following these two consecutive phases, the vessel diameter and a smaller volume of blood passes through the lumen within the same time frame. Vasculopathy seems to be secondary to vascular constriction (3,30,35). Recently the capability of endothelial cells to secrete heparin and its inhibitory effect on smooth muscle hypertrophy were shown (34). At the initial phase of cerebral vasospasm, vascular constriction causes endothelial damage.

Therefore, the inhibitory effect of endothelial cells on the tunica media disappears, and this process supports media proliferation. The main factor causing vascular spasm is not clear. Although the effects of autonomous nervous system (2,6,9), platelets (31), red blood cells (19,23), prostaglandins (10,29), angiotensin (5), cyclic nucleotides (14), histamin (20), and neutrophils (15), have been shown to cause vascular spasm in experimental models, the pathogenesis of late cerebral vasospasm remains unclear.

Defibrotide, a deoxypolyribonucleotide, has been found to modulate endothelial cell function causing an increase in t-PA and a decrease in PAI levels and also an increase in PGI₂ production (34). Defibrotide inhibits platelet aggregate formation in vitro experiments as well as end-to-end anastomosis in rats (27). Besides an increase of protein C and S levels, a synergic action of heparin was observed (34). Defibrotide appeared to be a potent drug in partial prevention of experimental delayed cerebral vasospasm. The mechanisms of the antivasospasm effect of defibrotide remain to be clarified. One

likely possibility is that defibrotide may have inhibited the induction of endothelin the activity of which is potent and extremely long-lasting(25). Another possibility is the synergic action of heparin which prevent smooth muscle contractions (34). Although we must be careful when extrapolating these in vitro observations, it is tempting to hypothesize that defibrotide may be helpful in clinical practice

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