
EXPERIMENTAL RESEARCH

An Experimental Saccular Aneurysm Model and Its Pathological Evaluation

Deneysel Bir Sakküler Anevrizma Modeli ve Patolojik Değerlendirmesi

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Abstract: An experimental saccular aneurysm model was made at the bifurcation of the common carotid artery in rats. Thirty albino rats were used. We histologically examined induced aneurysms in common carotid bifurcation of rats in the first, seventh and fourteenth day postoperatively and discussed the results. This aneurysms model has several advantages and it is also possible to use this technique to produce satisfactory experimental saccular aneurysms in other studies.

Key Words: Experimental, rat, saccular aneurysms.

Özet: Bu çalışmada rat karotid kommunis arteri bifürkasyonunda sakküler aneurizma modeli yapıldı. 30 albino rat kullanıldı. Ratların karotid kommunis bifürkasyonunda oluşturduğumuz aneurizmaları postopertaif birinci, yedinci ve ondördüncü günlerde histolojik olarak inceledik ve sonuçları tartıştık. Bu aneurizma modeli birçok avantajlar taşımaktadır ve bu teknik kullanılarak diğer çalışmalar için deneysel sakküler aneurizma oluşturmak olasıdır.

Anahtar Sözcükler: Aneurizma, deneysel, sakküler aneurizma, sıçan

INTRODUCTION

To explain the developmental mechanisms of the cerebral aneurysms, particularly of the saccular type, various hypotheses have been proposed, such as the medial defect theory (8), the internal elastic lamina injury theory (8), degenerative theory (15) and congenital theory (1). Particularly, intracranial aneurysms are the major cause of non-traumatic subarachnoid hemorrhage but their pathogenesis and pathological changes are still obscure. Considerable controversy in pathogenesis has focused on the question of which layer of the vessel wall is the crucial layer in the evolution of aneurysms (1).

Our study describes an experimental model for the generation of saccular aneurysms at the bifurcation of the common carotid artery. Transluminal removal of a part of the bifurcation immediately resulted in a saccular aneurysm.

MATERIALS AND METHODS

Three groups, each consisting of ten albino rats, were formed. In each group, both sexes were equal in number. The age of these rats varied from 12 to 26 weeks and they weighed 210- 330 g. Animals were anaesthetized with ketamine hydrochloride (Ketalar, Parke-Davis, England) 2 mg per 100 g via IM injection. After administering the anesthetic, the right bifurcation of the common carotid artery was exposed. Two small clips (8 mm Keinert-Kutz vascular clips) were used under the operating microscope Opmi-6, (Zeiss, Germany). One blocked the internal and external carotid arteries, the other one clipped the common carotid artery. An 1-1.5 mm arteriotomy near the apex of the bifurcation was made with a no. 11 surgical blade, parallel to the long axis of the vessel. The arterial lumen was irrigated and washed free of blood with physiological saline. In the wall opposite to the arteriotomy, 0.3-0.5 mm²

of the inner vessel wall at the apex of the bifurcation was removed with microsurgical forceps. 4-6 stay sutures of 10-0 silk were placed at the arterial incision to close the vessel. After suturing the arteriotomy wound, removing the clips and restoring the blood flow, a prominent bulging of the vessel wall was seen at the site of the intimal defect resembling a real minute saccular aneurysm (Figure 1).

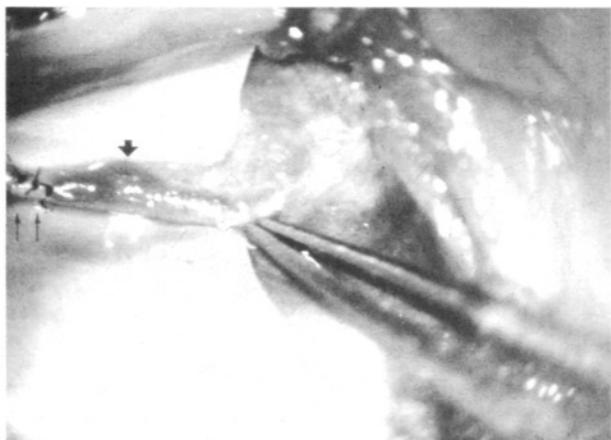


Figure 1: Gross photomicrography showing an aneurysmal sac, appearing in the apex of the bifurcation (large arrow). Two small arrows show suture sites. (X 14)

Thirty rats were used to examine pathological changes in these aneurysms:

Group I: The operation sites and the experimental aneurysms were checked and then, rats were sacrificed by high doses of ketamine at the first day of surgery.

Group II: The operation sites and the experimental aneurysms were checked and then, rats were sacrificed by high doses of ketamin at the seventh day of surgery.

Group III: The operation sites and the experimental aneurysms were checked and then, rats were sacrificed by high doses of ketamin at the fourteenth day of surgery.

The vessels were fixed in 10% formalin and then paraffin embedded. A series of 250-300 transverse 6µm sections were obtained from each specimen with aneurysm and stained with haematoxylin and eosin (H&E). Pathological studies were made in the department of pathology where the height of the aneurysms was measured using an ocular micrometer.

All aneurysm sections were observed histologically and height of the biggest aneurysm in every section was measured microscopically (Table I).

Table I. Mean height (mm) of experimental saccular aneurysms. Mean height and SD (standard deviation) of aneurysms were measured by an ocular micrometer.

Days of operation	Cases	Height of aneurysms
First day of operation	10	0.42±0.074 (0.3-0.6)
Seventh day of operation	10	0.69±0.186 (0.3-1.1)
Fourteenth day of operation	10	0.75±0.217 (0.3-1.2)

RESULTS

All aneurysms were located at the apex of the bifurcation of the common carotid artery. None of them had ruptured. The operated arteries were patent and under the operating microscope no thrombosis was found in the aneurysm sacs.

Macroscopically, small aneurysm can not be seen clearly in the material obtained at the end of the first day. However, histologically the existence of the aneurysm was demonstrated with serial sections and aneurysms measured 0.3-1.0 mm (mean 0.42 ± 0.074 mm) in height (Figure 2). Tunica adventitia was intact. No fibrin was found on the wall of aneurysm.

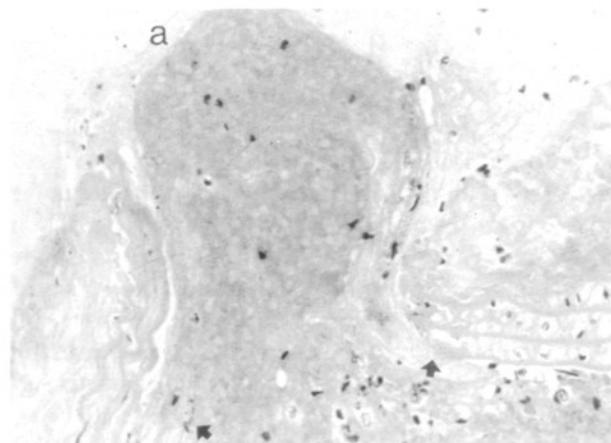


Figure 2: The first day. There is continuity of tunica adventitia. No fibrin is existent on the wall of the aneurysm. a: tunica adventitia, arrows show neck of the aneurysm. (H&E, X400)

At the end of the seventh day, aneurysms measured 0.3-1.0 mm (mean 0.69 ± 0.186 mm) in height. Microscopic examination showed an aneurysm and minimal fibrin deposits on its wall.

Proliferation of smooth muscle and fibroblast also were seen in the same area. No endothelial continuity was found. Inflammatory cell infiltration in the perianeurysmal area accompanied these findings (Figure 3).

Aneurysms measured 0.3-1.2 mm (mean 0.75 ± 0.217 mm) in height at the end of the fourteenth day. Histologically endothelium was intact and there was a minimal increase in collagenization in the wall of aneurysm (Figure 4). Fibrin was not seen in this area.

Table I shows measurement of the aneurysm height at the end of the first, seventh and fourteenth day respectively. The mean height of these aneurysms on the seventh and fourteenth days

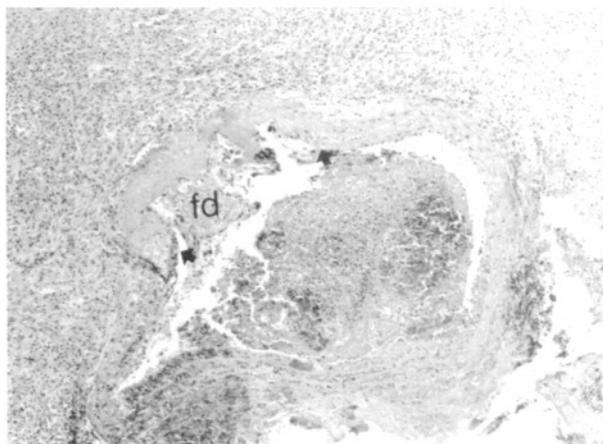


Figure 3: The seventh day. Note regeneration findings in the wall of the aneurysm. There are fibrin deposits in the lumen and on the wall of the aneurysm. fd: fibrin deposit, arrows show neck of the aneurysm. (H&E, X100)

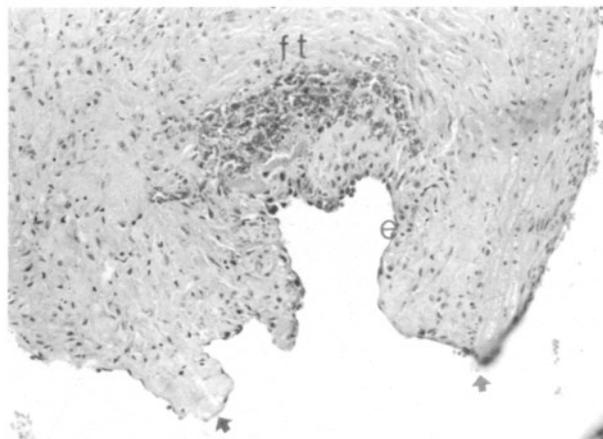


Figure 4: Fourteenth day. Endothelium is intact. Minimal increase of fibrous tissue is noted. ft: fibrous tissue, e: endothelium, arrows show neck of the aneurysm. (H&E, X100)

increased significantly compared with first postoperative day ($p < 0.01$). After 7 days the mean height of the aneurysms had not increased ($p > 0.50$).

DISCUSSION

There are two major pathological theories on saccular aneurysm formation; the medial defect theory and the internal elastic lamina injury theory. Forbus (7) found medial defect in about two thirds of the bifurcations of normal cerebral arteries, in children as well as in adults. He postulated that these defects would provide the groundwork for saccular aneurysm formation. In some reports, it was stated that some remnants of the embryonic vascular system, which were short of tunica media or internal elastic lamina, could form aneurysms(9,11,16). However, some authors reported that medial defects could often be found at the bifurcation of intracranial arteries (12,13).

Many experimental studies also showed that medial defects do not play an important role and it was theorized that internal elastic lamina defects might be a major factor in aneurysm formation (5,6,8,11,15). Some authors thought that both defects must be present for the formation of a cerebral saccular aneurysm (3). We are inclined to believe that the internal elastic lamina is a critical layer in saccular aneurysm formation. Since we saw the formation of a saccular aneurysm after removing a part of this tissue of the artery.

In human saccular aneurysm formation, the tunica media and internal elastic lamina are always involved (15). The removal of the internal elastic membrane together with the tunica media from an extracranial carotid bifurcation and that from the human intracranial arteries at bifurcations after damaging the internal elastic lamina have a similar effect.

Histologically, the wall of the saccular aneurysm seemed very strong. In comparison with natural human saccular aneurysms there were many endothelial cells and fibroblast-like cells (4,14,17) in those of rats. Our pathological result showed that no endothelial continuity was found at the end of the seventh day. However, at the end of the fourteenth day endothelium was intact and there was a minimal increase in collagenization in the wall of aneurysm. Although it seemed that a strong repairing process had taken place, these aneurysms still retained their aneurysmal shape and had even

enlarged because of their abnormal histological structure and the action of hemodynamics at the apex of the arterial bifurcations.

We assume that this reaction took place from the surrounding tissue in the rat's neck and aneurysms did not grow after the seventh day postoperatively. A free position in the subarachnoid space is more likely to contribute to aneurysmal growth and rupture (2,10). If the experimental saccular aneurysms had evolved in a free space, such as the subarachnoid space, the aneurysms would probably have shown fewer regenerative features and thinner and larger sacs than those that we observed in this experiment; they might even have ruptured.

This aneurysm model has several advantages; first it can be developed quickly. Second, the formation rate approached 100%, and third it is possible that this method can be used in cerebral arteries of large animals to produce experimental intracranial saccular aneurysms.

CONCLUSION

The result of this experiment suggests that the internal elastic lamina is a crucial structure in the pathogenesis of aneurysms. We think that this animal model is suitable for such a study. The present study confirms that this experimental saccular aneurysm model is a reliable animal model for investigating the formation of saccular aneurysms.

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