

# The Role Of Hypertension On The Formation Of Cerebral Saccular Aneurysms: An Experimental Study In Rats

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**Abstract :** The function of experimentally induced hypertension in the formation of saccular aneurysms in rats was the subject of this study. In four group of rats, the left common carotid artery and the posterior branch of both renal arteries was ligated in the first and third group, and only left common carotid artery in the second group. The fourth group was the control group and nothing was done. Rats in the second and third groups were given an injection of SC deoxycorticosterone acetate. There were offered standard drinking water was replaced by 1 % NaCl solution.

Three months later, the rats were sacrificed by 10 % formol perfusion. Vessels with aneurysmatic appearance were examined using an operating microscope. Two aneurysms in the first group and four in the third were traced. The results show that saccular cerebral aneurysms are inducible in experimental animals.

**Key Words :** Aneurysm Cerebral Aetiology Hypertension

## INTRODUCTION

Saccular aneurysms are extremely rare in animals (5,12), this rarity may partly explain why the developmental mechanism of such aneurysms in man is still controversial (16). Several techniques for the production of experimental aneurysms have been reported. These include intramural injection of nitrogen mustard or hypertonic aqueous sodium chloride (6,17,26), closing an arterial wound with methyl-2-cyanoacrylate (24), and insertion of a vein pouch (4,18). However, these aneurysms differ greatly from naturally occurring saccular cerebral aneurysms, and throw no light on their aetiology and development.

In 1978, Hashimoto et al. (7) induced saccular cerebral aneurysms in rats by ligating the unilateral common carotid artery, making the rats hypertensive and feeding them betaaminopropionitrile. Recently, other studies of the same group have followed (9-10,13-15,19,27). These observers induced aneurysms by enhancing haemodynamic stress on

the cerebral arteries such an increasing local blood flow and intramural pressure.

Microscopically, the elastic lamina and the media ended around the beginning of the aneurysm. The thin aneurysmal wall consisted of fibrous connective tissue. All findings were generally in accordance with spontaneous lesions in humans (9). The results prove that this procedure is a suitable method for the formation of experimental saccular aneurysms.

In our study, we attempted to induce cerebral saccular aneurysms experimentally in rats and modify less invasive models.

## MATERIALS AND METHODS

Four groups, each consisting of ten albino rats aged from 12 to 26 weeks and weight 210-330 g, were used. Sexes were equal.

Group I: Ligation of the posterior branches of both renal arteries and the left common carotid artery was performed under ketamine hydrochloride (Ketalar,

Parke-Davis, England) anaesthesia 2 mg per 100 g. One week after ligation, 1 % saline solution was substituted for drinking water.

Group II: Ligation of the left common carotid artery was performed under ketamine hydrochloride anaesthesia. One week after ligation, 1 % saline solution was substituted for drinking water, and deoxycorticosterone acetate (Doca, Organon, Netherland) was injected subcutaneously once a week in a dose of 0.25 mg per 100 g.

Group III : Ligation of the posterior branches of both renal arteries and left common carotid artery was performed under ketamine hydrochloride anaesthesia. One week after ligation 1 % saline solution was substituted for drinking water and deoxycorticosterone acetate was injected subcutaneously once a week in a dose of 0.25 mg per 100 g.

Group IV : This group was the control group and they were merely given standard drinking water.

All the rats were on standard diet and were checked every day. All tolerated the procedures well. Three months later, the right common carotid artery was exposed under ketamine hydrochloride anaesthesia. A polyethylene catheter was cannulated through a small arteriotomy using an operating microscope and the blood pressure was measured using the manometric method. The rats were sacrificed using 10 % formol perfusion. After fixing the heads of the rats in 10 % formol for ten days, vessels with aneurysmatic appearance were subjected to histopathological examination under a light microscope.

All the surgical procedures were done under an operating microscope (Zeiss, OPMI-VI, Germany)

### RESULTS

The observed blood pressures were  $100 \pm 5$  mm Hg in group I,  $66-6$  mm Hg in group II,  $110 \pm 10$  mm Hg in group III, and  $30 \pm 5$  mm Hg in group IV (Control)

Under an operating microscope, 6 of 40 cases showed aneurysmatic dilatation of the arterial wall at the right anterior cerebral arter (ACA), and olfactory artery (OA) bifurcation (Figure 1).

Among ten rats of group I (Ligation) 2 aneurysms in two rats were found; and among ten rats of group III (Ligation+injection) 4 aneurysms in four rats. The

sex ratio was 1/1 in the rats with aneurysms (Table 1). In groups II and IV, no apparent aneurysmal changes were observed in the arterial system.

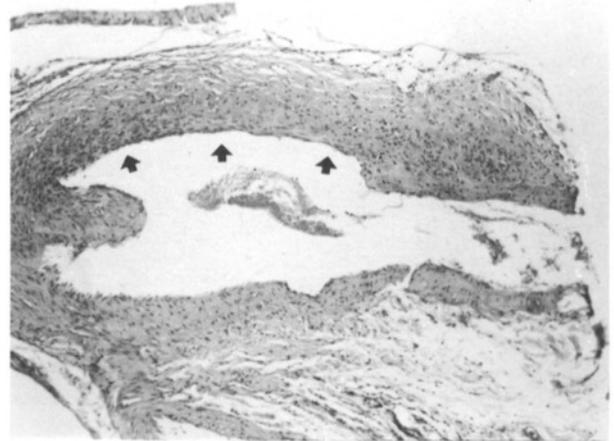


Fig. 1 : Showing aneurysmal dilatation at the anterior cerebral artery and olfactory artery bifurcation (ACA/OA). Left side aneurysmal sac (Arrows) (HEX100)

Table 1 : Induced lesions and blood pressure				
Groups	Number of Rats	Duration of experiments (Months)	Cerebral aneurysm	Blood Pressure
Group I	10	3	2	$100 \pm 5$
Male	5		1	$102 \pm 3$
Female	5		1	$99 \pm 4$
Group II	10	3	0	$66 \pm 6$
Male	5		0	$68 \pm 4$
Female	5		0	$65 \pm 5$
Group III	10	3	4	$110 \pm 10$
Male	5		2	$106 \pm 6$
Female	5		2	$113 \pm 7$
Group IV	10	3	0	$30 \pm 5$
Male	5		0	$32 \pm 3$
Female	5		0	$29 \pm 4$

Microscopically, the intima in two rats was normal. Intimal thickening at the mouth of the aneurysms in four rats was seen. In all rats, the internal elastic lamina first fragmented and then ceased rapidly in the neck of the aneurysm (Figure 2). There was no internal elastic lamina in the wall of the aneurysm. Necrotic areas and cells without nuclei were observed in the media at the mouth of the aneurysms, and the layer of media was not present around the aneurysmal sac (Figure 3). Thickness of the aneurysmal wall in 6 rats with aneurysm varied and was composed of fibrous connective tissue. Adventitial pathology was not observed. Rupture and luminal thrombosis were not found in any rat. Atherosclerotic change was observed at the entrance of the sac in one rat.

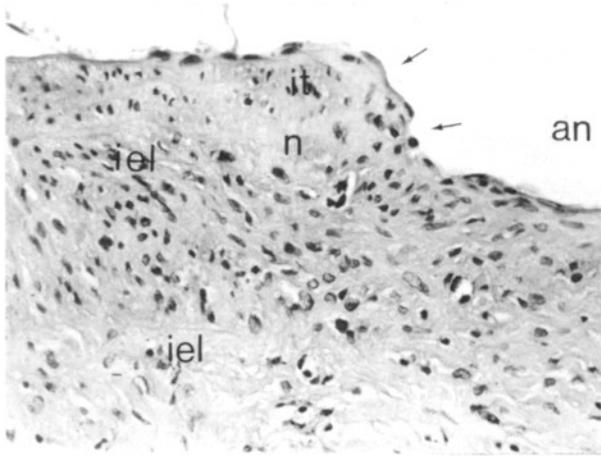


Fig. 2 : Note the fragmentation and disappearance at the internal elastic lamina it: Intimal thickening (Arrows) n: Necrosis iel: Internal elastic lamina an: Aneurysm (HEX400).

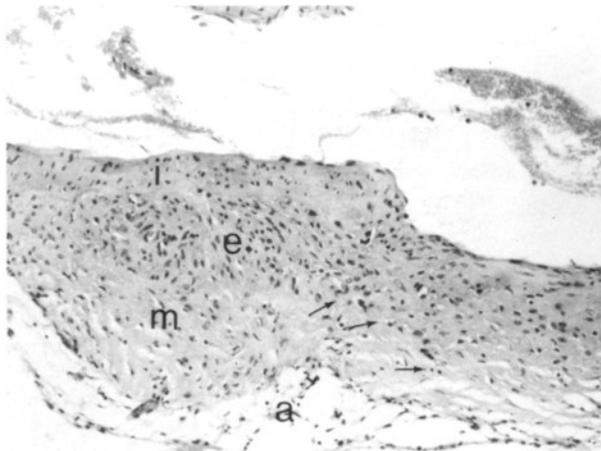


Fig. 3 : Appearance of the aneurysmal mouth and the ending of the media (Arrows) i: Intima e: Endothelial proliferation M: Media a: Adventitia (HEX200)

### DISCUSSION

So far only 4 cases of spontaneous aneurysm in animals have been reported: in a llama, a cow, a chimpanzee and a rat (1,11,16,23). In the case of the llama and the cow, however no reliable macroscopic or microscopic descriptions and no details of the aetiology were given. Eight cerebral aneurysms were found in the chimpanzee, combined with severe atherosclerotic changes in the cerebral arterial system.

Hashimoto et al. (7-10) induced saccular cerebral aneurysms in rats by ligating a unilateral carotid artery, making the rats hypertensive, and feeding them beta-aminopropionitrile. When growing animal

are fed beta-aminopropionitrile, one of the lathyrogens, connective tissue becomes abnormally fragile and a variety of connective tissue disorders result (20, 25). Animals die from haemothorax caused by the dissecting aneurysm of the aorta or become critically ill with dislocation of the vertebrae and other skeletal lesions. These changes are called "Angiolathyrism" and "Osteolathyrism", respectively (3,22). The common pathological changes in the vessels, especially in the aorta, are loss of tensile strength and elasticity, swelling, disruption, and elimination of elastic fibres of the vessel wall (20, 25). These experiments were performed on the hypothesis that if haemodynamic stresses were increased on the fragile, cerebral arterial wall of beta-aminopropionitrile-fed animals, cerebral aneurysm might be produced.

Kim et al. (14,15) succeeded in the induction of saccular aneurysms in monkeys on the same concepts as pertained to the rat model. Recently, Hasimoto et al. (8) induced saccular cerebral aneurysms without beta-aminopropionitrile, however, the incidence was definitely lower than that of groups with beta-aminopropionitrile. This method has been used in other experiments (10,19). In previous studies, unilateral nephrectomy was done to induce renal hypertension (7-9), the main subdivisions of which are renovascular and renal parenchymal hypertension. A simple explanation for renovascular hypertension is that decreased perfusion of renal tissue due to stenosis of a main or branch renal artery activates the renin-angiotensin system. Renin acts on the basic substrate angiotensinogen to form angiotensin I, which is then enzymatically converted by converting enzyme to angiotensin II. Angiotensin II elevates arterial blood pressure by direct vasoconstriction and stimulation of aldosterone secretion with resultant sodium retention (2). Recently, the posterior branches of both renal arteries were ligated (10,13-15,19,27) and deoxycorticosterone acetate injected subcutaneously twice a week in a dose of 2,5 mg pr 100 g of rat (7-9). We injected subcutaneously once a week in a dose of 0,25 mg per 100 g deoxycorticosterone acetate, which also serves as a precursor of aldosterone and increases the renal distal tubular exchange of intratubular sodium for secreted potassium and hydrogen ions, the hypertension is due to increased sodium reabsorption and extracellular volume expansion (21).

In previous studies, blood pressure was measured in the unanaesthetized state by the tailcuff, auto-pick-up method and systolic hypertension greater than 150 mm Hg was recorded in all rats (7-10,13-15,19,27). In our study, the maximal blood pressure was found to be 120 mm Hg. The difference between the blood pressures may be explained by the altered methods of measurement. In Hashimoto et al. (8) and in our study, there were no differences in incidence between males and females. In the present study we found a total of six cerebral saccular aneurysms in groups I and III. In group II no aneurysm could be induced without ligation of the posterior branches of both renal arteries. These results support the idea that sufficient haemodynamic stress can not be achieved without forming renal hypertension (10).

In the literature, almost all the aneurysms induced in the rats, localized at the anterior part of the circle of the Willis, as we also found, but aneurysms were observed at the posterior part of the circle of Willis in a group with bilateral carotid ligation (10). However, Kim et al. (14,15) achieved aneurysm induction in monkeys at different sites such as the internal carotid artery (ICA), middle cerebral artery (MCA), posterior cerebral artery (PCA), and posterior communicating artery (PCoA).

Microscopically, in saccular cerebral aneurysms, the intima of the parent artery at the entrance to the aneurysmal sac is thickened. The internal elastic lamina, which is hypertrophied and intensely stained in the parent arteries, often ended abruptly at the mouth of the aneurysm. The media at the entrance ended abruptly and is replaced by fibrous connective tissue. The aneurysm sacs vary considerably in thickness from one aneurysm to another and even within the same aneurysm. Swollen endothelial cells are often observed along the inner aspect of the sac of the aneurysm. However, in some places, endothelial cells are completely absent. The internal elastic lamina and smooth muscle cells are not apparent in the walls of the sacs and the walls of the aneurysms are composed of fibrous connective tissue sometimes with hyaline degeneration. The adventitia, which is continuous with that of the parent artery, is usually thicker at the neck, then thinned out (9,13).

In our histopathological findings, the internal elastic lamina extended into the neck of the aneurysm for a short distance and then suddenly

disappeared. Medial necrotic areas were present at the mouth of the aneurysmal sac. The medial layer ended abruptly in the neck of the aneurysm. There was no evidence of other inflammatory reaction.

Yamazoe et al. (27) examined the elastic skeleton of experimentally-induced cerebral aneurysm in rats by scanning electron microscopy. The first noted change was the loss of folds protruding from the internal elastic lamina. Morphological changes of the internal elastic lamina, considered to be primarily responsible for aneurysm formation occurred after the loss or disintegration of the elastic skeleton of first the intima, then the media.

Nagata et al. (19) showed that examination by scanning electron microscopy of the luminal surface of cerebral aneurysms revealed variations in the shape of the endothelial cells from fusiform to polygonal. They observed crater-like depressions on the endothelial surface and small holes and enlarged gaps at the junction of the endothelial cells. Besides, many leucocytes with or without fine processes were found adhering to the endothelial surface near or on the crater-like depressions and interendothelial gaps. The inner surface of the domes of the aneurysms frequently showed a thick layer of platelets, particularly at the junctions between the endothelial cells.

## CONCLUSION

This recently-developed animal model proved valid for saccular cerebral aneurysms in man. Haemodynamic stresses on cerebral arteries were increased with ligation of the unilateral common carotid artery and the posterior branches of both renal arteries, salt hypertension and deoxycorticosterone, and saccular cerebral aneurysms were induced. In this study, deoxycorticosterone with its hypertensive effect was administered in addition to the surgical procedures of previous studies including ligation of the posterior branches of the renal arteries. Analyzing this model, it is suggested that hypertension and haemodynamic stress are very important for the development of saccular cerebral aneurysms in man.

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