Experimental Medicine

Cerebral Vascular Changes in Experimentally Induced Hypertensive Rats

Jehoram Tei Anim[1], Chitra Sarkar[1], Ganesh Gopalakrishnan[2]
Departments of [1]Pathology and [2]Surgery, Faculty of Medicine, Kuwait University, Kuwait

Kuwait Medical Journal 2001, 33 (3): 235-239

ABSTRACT

Objective: To study the pathogenesis of cerebral vascular changes in hypertension, especially those predisposing to hemorrhage, we set out to produce a hypertensive rat model by the Goldblatt (2 kidney-1 clip) method.

Materials and Methods: Fifty rats were rendered hypertensive by the Goldblatt method in which the left renal artery was ligated surgically. The animals, together with 18 control rats, were divided into three groups as follows: a) on normal diet and water, b) on normal diet and 1% salt water and c) on normal diet and drinking water containing 0.12% of the lathyrogen beta aminoproprionitrile (BAPN). Slices of brain were examined both macroscopically and microscopically for morphological changes in the cerebral vessels.

Results: Mean arterial blood pressures achieved were only moderate (170 mmHg) and corresponding cerebral vascular changes were mild to moderate. None of the severe lesions (hemorrhages, fibrinoid necrosis, microaneurysms, etc.) known to be associated with severe or long-standing hypertension were demonstrated in this study. A few vessels in six animals showed fibrin deposition in the wall, although these could not be directly related to the severity of hypertension or duration of survival of the animal. The six, as well as three additional animals, also showed immunoglobulin G (IgG) deposition in the vessel walls and perivascular tissues within the brain.

Conclusion: We have successfully established a rat model for the study of hypertension-related cerebral vascular changes. The deposits of fibrin and IgG in the vessel wall and surrounding tissues represent early changes, which appear to occur even in the absence of severe hypertensive vascular disease. Further studies are required to determine the relevance of these changes to other known morphological changes of the cerebral vasculature in hypertension.

KEYWORDS: cerebral vessels, experimental rats, fibrin, hypertension

INTRODUCTION

The exact mechanisms underlying spontaneous intracerebral hemorrhage are poorly understood. Rosenblum[1], in lamenting the lack of current studies into the pathogenesis of such hemorrhages, has emphasized that fibrinoid change is the pre-eminent underlying lesion in the cerebral vessels, similar to vascular changes in other tissues in hypertensives. He stressed, however, that the cerebral vessels are more susceptible to the change, which may be responsible for both the hemorrhage and the presence of microaneurysms. Special stains must be used to demonstrate fibrin in the vessel walls in all cases. Fisher[2] introduced the term 'lipohyalinosis' to include changes such as hyaline sclerosis and fibrinoid change and on that basis classified microaneurysms into four types. Fujii et al.,[3] maintained that sclerosis and thickening in the vessel resulting from hypertension produce obstruction such that the proximal parts of the vessel become subjected to excessive luminal pressures, which further accelerate the degenerative changes that may lead to rupture.

We have carried out a detailed pathological study of the cerebral vessels in experimentally-induced hypertensive rats in an attempt to shed some light into the mechanisms underlying the degenerative changes in the vessels that may predispose them to rupture in spontaneous intracerebral hemorrhage.

MATERIALS AND METHODS

Wistar-Kyoto rats, approximately six months old, were used in this study. A total of 50 rats were rendered hypertensive by the Goldblatt (2 kidney-1 clip) method. This was achieved by placing a ligature around the left renal artery. The animals were then divided into three groups. Group 1 was maintained on water and normal diet (n = 16).

Address correspondence to:
Dr. JT Anim, Department of Pathology, Faculty of Medicine, Kuwait University, P.O. Box: 24923, 13110 - Safat, Kuwait. Tel (965) 531-947; fax: (965) 533-8905; e-mail: anim@hsc.kuniv.edu.kw

Group 2 was maintained on 1% NaCl in drinking water and normal diet (n = 18). Group 3 was maintained on 0.12% beta-aminopropionitrile (BAPN) in water and normal diet (n = 14). One month following renal artery ligation, the right carotid artery of alternate animals was cannulated and blood pressure measured using an external transducer connected to a standard Siemens Sirecust 404-1 monitor, followed by ligation of the artery.

The animals were sacrificed at 2, 3, 4, 6, 9, 12 months after renal artery ligation using ether, followed immediately by perfusion of the arterial system with 10% formalin injected through the heart. The decapitated head was fixed in formalin for a further two weeks, and the brain removed, sliced and cleared in methyl salicylate. There were 5 to 6 slices per brain, each approximately 0.2 cm. thick. The slices were examined under the dissecting microscope for aneurysms of larger vessels, hemorrhage, infarcts and any other vascular changes. All slices of brain were processed into paraffin and sections examined using routine Hematoxylin and eosin (H&E), as well as Martius-Scarlet blue (MSB) to demonstrate fibrin within or outside the vessel walls.

Eighteen control animals that had not been subjected to renal artery ligation were divided into three groups (n = 6 each) and maintained under conditions similar to the experimental animals. Control animals were sacrificed using the same schedule and method as the experimental animals.

Hypertensive changes in the cerebral vessels were graded as follows: grade 0 - no hypertensive changes; grade 1 - mild thickening of the media; grade 2 - moderate thickening of the media with or without sclerosis; and grade 3 - severe medial thickening with or without fibrinoid change/necrosis. A similar grading system was also applied to vascular changes in the kidneys, especially the right (unligated), in order to confirm that significant hypertension had been achieved.

Immunohistochemistry

Immunoglobulins and complement, being important plasma constituents, can be utilized to detect seepage of blood constituents into or outside the blood vessel wall. Immunohistochemical detection of IgG in these sites was carried out on brain sections of all experimental animals, using the labeled streptavidin-biotin (LSAB) method. Primary antibody (rabbit anti-human IgG 1:700 DAKO A-423 diluted in antibody diluent with background reducing agent DAKO S-3022) was used. Color development was by diaminobenzidine (DAB)

Table 1
Blood pressure measurements in 24 experimental rats

Group	Number	Range of blood pressure	Mean blood pressure (SD)
1	9	140 - 180	162 (15.7)
2	10	144 - 210	171 (18.5)
3	5	155 - 224	185 (21.4)
Total	24	140 - 224	170 (18.7)

Table 2
Blood pressure measurements according to duration of survival of 24 experimental rats

Duration (months)	Number	Range of blood pressure	Mean blood pressure (SD)
2	5	155 - 210	168 (25.2)
3	6	140 - 224	171 (25.1)
4	4	142 - 180	164 (16.0)
6	5	170-183	175 (5.6)
9	2	155	155 (0)
12	2	180-195	188 (10.6)
Total	24	140 - 224	170 (18.7)

Table 3
Hypertensive cerebral vascular changes in the three groups of experimental rats (n = 40)

Groups	Number	Cerebral vascular changes (grades)				MSB positive
		0	1	2	3	
1	14	3	7	4	0	3
2	16	1	13	2	0	2
3	10	3	6	1	0	1
Total	40	7	26	7	0	6

Table 4
Relationship between hypertensive cerebral vascular changes and duration of survival in 40 experimental rats

Months	Number	Cerebral vascular changes (grades)				MSB positive
		0	1	2	3	
2	8	2	6	0	0	2
3	9	1	7	1	0	1
4	7	2	4	1	0	1
6	7	2	4	1	0	1
9	5	0	4	1	0	0
12	4	0	1	3	0	1
Total	40	7	26	7	0	6

Statistical methods

Simple statistical methods, e.g., chi-square, were used to determine the significance of various associations. A p-value of ≤ 0.5 was considered as significant.

RESULTS

Systolic blood pressure measurements of control rats ranged between 115 and 130 mmHg, with a mean of 120 mmHg. Renal artery ligation was successful in 45 experimental animals, as evidenced by the demonstration of a shrunken left kidney at autopsy. Blood pressure measurements in 24 of these animals ranged between 140 and 224 mmHg. Mean blood pressures for the three groups of experimental animals are shown in Table 1, and according to duration of survival following renal artery ligation in Table 2. The animals were kept in the three groups under the different experimental conditions, to determine if different levels of hypertension could be achieved. There were, however, no significant differences in the mean blood pressures achieved in the three groups of experimental animals; the levels realized being generally moderate.

Only two experimental animals showed grade 3 vascular changes in the unligated (right) kidneys. The rest showed mild to moderate changes. These vascular changes were, however, significantly more than in the cerebral vessels ($p < 0.001$) in which no grade 3 vascular changes could be demonstrated (Table 3). Animals sacrificed at 12 months showed a slight increase in grade 2 cerebral vascular changes compared to those sacrificed earlier (Table 4). There was also no correlation between the degree of cerebral vascular changes and carotid ligation.

Six experimental animals showed deposition of fibrin in the wall of a few vessels (Fig. 1). Blood pressure measurement in three of these six were 155, 160 and 170 mmHg, respectively, and showed that there was no relationship between level of blood pressure and the presence of fibrin deposits in the vessel wall. There was also no correlation with the group of animals as three were in group 1, two in group 2 and one in group 3. The presence of fibrin deposit was associated with grade 2 vascular changes in two animals and grade 1 changes in the remaining four.

Immunohistochemistry demonstrated the presence of IgG in the walls of cerebral vessels of nine experimental animals. These included the six showing fibrin deposition in addition to three other; two of which demonstrated extensive IgG staining in both the vessel wall and perivascular tissues (Fig. 2). In these two animals, the blood pressures were 180 and 165 mmHg, respectively. No true fibrinoid necrosis of cerebral vessels was demonstrated in any of the experimental animals and there were no hemorrhages or microaneurysms. The larger vessels of the Circle of Willis and its branches also showed no aneurysms. Cerebral vessels were normal in all control animals.

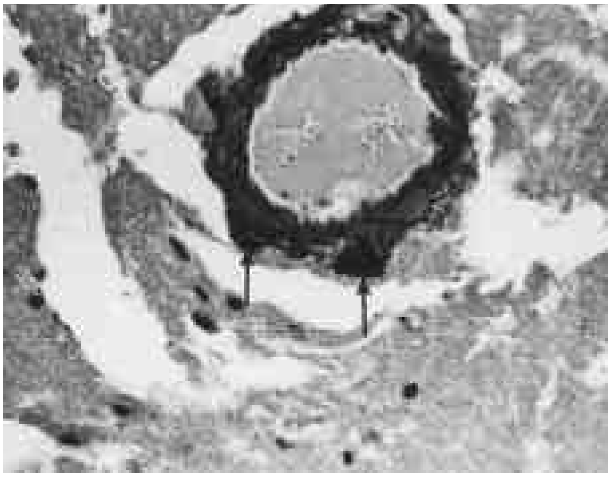

Fig. 1: Photomicrograph of cerebral vessel showing fibrin (arrows) within the wall (MSB x 250)

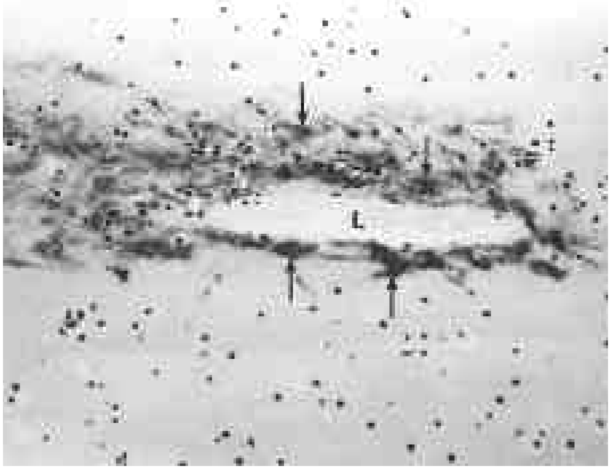

Fig. 2: Photomicrograph of cerebral vessel showing IgG deposits (arrows) in the wall and the surrounding brain. (L) lumen of vessel. (Immunoperoxidase x 100)

DISCUSSION

Hypertension was successfully induced by the Goldblatt method in our study and confirmed by the elevated blood pressure in the experimental rats. The level of hypertension was generally moderate. The corresponding vascular changes in the right (unligated) kidney and brain of the experimental animals were also mild to moderate. Thus, there were only two grade 3 changes in the right kidneys but no grade 3 changes in the cerebral vessels of any of the animals. Although animals sacrificed at 12 months showed a slight increase in grade 2 vascular changes in the brain over those sacrificed earlier, the numbers are too small for any meaningful conclusions to be drawn from this observation. Hyaline changes and microaneurysms, which were notably absent in our study, are known to be dependent on long-standing, sustained hypertension.

Some workers[4,5] have succeeded in maintaining very high blood pressures (230 and 260 mmHg) over a prolonged period of time by intravenous injection of amphetamine or bicuculline. Others have achieved similar results by maintaining the animals on 2% salt solution[6], or by injecting deoxycorticosterone acetate (DOCA), subcutaneously, together with 1% salt in the diet[7]. These higher pressures and prolonged periods of hypertension have enabled the authors to study features such as cerebral edema and permeability to proteins. Fibrinoid necrosis in the vessel wall is commonly associated with malignant hypertension and its absence in all our study animals is a reflection of the moderate hypertension achieved in this study. It is, however, interesting to note the presence of focal fibrin and immunoglobulin deposition in and around the intracerebral blood vessels in some of our experimental animals, even in the absence of very high arterial pressures. Fredricksson et al.,[8] studied deposition of fibrin in the cerebral vessel walls of both renal and spontaneously hypertensive rats and regarded such deposits as early changes in the pathogenesis of cerebrovascular disease in hypertension.

They also concluded that renal hypertensive rats were more prone to show the changes than those who were spontaneously hypertensive. Similar findings have also been reported by Johansson and Linder[4]. The demonstration of fibrin in six of our animals with a further three showing deposition of immunoglobulin outside the vessels is further confirmation of this finding. Like Fredricksson et al.,[8] we conclude that the deposition of fibrin and other proteins in the vessel wall and surrounding brain tissue is an early feature in the pathogenesis of cerebral vascular disease, especially in view of the fact that these changes occurred in rats with only moderate elevation of arterial pressure.

Group 3 animals were given BAPN, a lathyrogen in order to induce weaknesses in the vessel walls and predispose them to aneurysm formation, as demonstrated by Hashimoto et al[7]. No aneurysms were produced in our study, presumably because our animals were older and not the very young animals or weanlings used in the study by Hashimoto et al[7]. Moreover, the absence of microaneurysms in our study may suggest that BAPN has no direct effect on smaller vessels, the usual sites of microaneurysms. According to Hashimoto et al[7] unilateral or bilateral ligation of the carotid artery was necessary to create hemodynamic alterations for the production of aneurysms in larger cerebral vessels. Unilateral carotid artery ligation was carried out in 21 of our experimental animals. It was also expected that this might predispose the smaller vessels to microaneurysm formation. However, no large aneurysms or microaneurysms were observed in our study and there was no correlation between carotid ligation and grade of hypertensive vascular changes in our animals.

This may be due, in part, to the relatively short duration of the study. Our animals were kept for a maximum period of 12 months at which time the oldest animal would be 18 months old. The general view expressed about humans[9] is that degenerative changes in vessel walls are dependent on both the duration and level of hypertension. Microaneurysm formation is also known to be accelerated by the synergistic effect of hypertension and age[10,11]. The duration of our study, coupled with only moderate elevation of arterial pressure were, perhaps, not enough for degenerative changes that predispose the cerebral vessels to hyalinization and microaneurysm formation to manifest in the vessel wall.

In conclusion, we have successfully established the rat hypertensive model in our laboratory. We have also demonstrated that seepage of plasma proteins into the vessel wall and the surrounding brain tissue can occur even in the absence of very high mean arterial pressures in these animals. Further studies are required to determine the relevance of these early changes to other morphological cerebrovascular changes that have commonly been associated with hypertension.

ACKNOWLEDGEMENTS

This study was supported by Kuwait University Research Grant MS-025 for which the authors are grateful. We also wish to thank Mr. K.I. Matthew of the Department of Surgery, Mr. M. Ejaz, Ms. May Al-Agha and Ms. Bency John of the Department of Pathology for their excellent technical assistance.

REFERENCES

1. Rosenblum WI. The importance of fibrinoid necrosis as the cause of cerebral haemorrhage in hypertension. Commentary. J Neuropathol Exp Neurol 1993; 52:11-13.
2. Fisher CM. Cerebral miliary aneurysms in hypertension. Am J Pathol 1972; 66:313-330.
3. Fujii Y, Tanaka R, Takeuchi S, Koike T, Minakawa T, Sasaki O. Hematoma enlargement in spontaneous intracerebral haemorrhage. J Neurosurg 1994; 80: 51-57.
4. Johansson BB, Linder LE. Hypertension and brain edema: an experimental study on acute and chronic hypertension in the rat. J Neurol Neurosurg Psychiat 1981; 44:402-406.
5. Dohi Y, Criscione L, Luscher TF. Renovascular hypertension impairs formation of endothelium-derived relaxing factors and sensitivity to endothelin-1 in resistance arteries. Br J Pharmacol 1991; 104:349-354.
6. Ibarra-Rubio ME, Cruz C, Tapia E, Pena JC, Pedrazza-Chaverri J. Serum angiotensin converting enzyme activity and plasma renin activity in experimental models of rats. Clin Exp Pharmacol Physiol 1990; 17:391-399.
7. Hashimoto N, Nanda H, Nagata I, Hazama F. Animal model of cerebral aneurysms: Pathology and pathogenesis

of induced cerebral aneurysms in rats. Neurol Res 1984; 6:33-40.

8. Fredricksson K, Auer RN, Kalimo H, Nordborg C, Olsson Y, Johansson BB. Cerebrovascular lesions in stroke-prone spontaneously hypertensive rats. Acta Neuropathol 1985; 68:284-294.
9. Anim JT, Kofi AD. Hypertension, cerebral vascular changes and stroke in Ghana: Microaneurysm formation and stroke. J Pathol 1984; 143:171-176.
10. Cole FM, Yates PO. Comparative incidence of cerebrovascular lesions in normotensive and hypertensive patients. Neurology 1968; 18:255-259.
11. Cole FM, Yates PO. The occurrence and significance of intracerebral microaneurysms. J Pathol Bact 1967; 93:393-401.