Oration

MACRO AND MICROANGIOPATHIC LESIONS IN EXPERIMENTAL DIABETES IN RHESUS MONKEYS

Prof. R.N. Chakravarti*

Summary

In an attempt to produce macro and rnicroangiopathic lesions simulting those seen in human diabetes mellitus, experimental diabetes was induced in rhesus monkeys by alloxan injection and observations made for the period of one year. Additionally, immunological injury by sperm antigen-antibody immune complexes was also caused by subjecting half of the animals to bilateral vasectomy. It was observed that vasectomy as such did not cause elevation of serum lipids but antisperm antibodies were detected in the blood of these animals. Induction of diabetes caused marked elevation of blood glucose, TG and FEA while there was marginal increase of cholesterol. Combination of diabetic state with vasectomy depressed the antibody response of the vasectomised animals. Gross grading of aortic sudanophilia showed significant increase in vasectomised non-diabetic animals but in other groups no such effect was detectable. Histological examination of aorta, coronary, carotid, cerebral and renal arteries did show evidence of macro and microangiopathic lesions in variable quantity in different groups, the changes in the diabetic animals being most prominent.

Introduction

In experimental diabetes research one of the greatest drawbacks in the lack of correlation between severity of diabetes and the morphological changes in the arteries arterioles and capillaries, typical of the human disease. In the past many laboratory species have been used from time to time to produce typical diabetic macro and microangiopathic lesions but so far the attempts have been unrewarding by and large. The most successful model of this complication was created in dogs and rhesus monkeys by Engerman *et a1*[1] and Blood worth *et a1*[2], after prolonged duration of the diabetic state for seven to eight years. The diabetes was produced experimentally either by alloxan or by growth hormone injections[1,2]. In these two species these authors observed capillary microaneurysms and dilatation lesions in the retina and typical nodular and diffuse type of glomerulosclerosis in the kidneys. They did not however, study other organs particularly the heart and brain. In the light of these observations it was thought that attempts should be made to produce these diabetic complications in a laboratory species in much shorter time for study of pathogenesis and therapy. As subhuman primates are phylogenetically, metabolically and psychologically akin to human beings the present work was undertaken on rhesus monkeys and the metabolic aberration produced by alloxan was further aggravated by an irnmunological injury following vasectomy.

Material and Methods

Adult healthy male tuberculin negative rhesus monkeys with body weight around 4 Kg were acclimatised in our animal house for a period of one month before starting the experiment. They were given a diet containing soaked bengal gram, wheat chappatis and

*Head of the Department of Experimental Medicine, Post-Graduate Institute of Medical Education and Research, Chandigarh.

fruits providing adequate protein, calories and essential micronutrients. Four groups each containing six animals were made, Group I, sham vasectomised non-diabetic control, Group II vasectomised non-diabetic, Group III diabetic non-vasectomised and Group IV, vasectomised diabetic animals.

Diabetes was induced by a single intravenous injection of freshly prepared alloxan monohydrate, 80 mg/kg body weight in fasting animals. Urine sugar was examined daily for a period of two weeks and blood sugar level monitored every third day upto this period. A small dose of insulin had to be given to protect the animals from developing hyperglycemic coma. After the diabetic state was stabilised insulin was withdrawn.

Surgical vasectomy on both sides was carried out under aseptic conditions and the portion of the resected vas examined histologically. The ligated ends of the vas were examined for any leakage and the skin wound was sutured. After healing of the wound the animals were subjected to electroejaculation and absence of spermatozoa indicated that the operation was properly done. Thus twelve animals were vasectomised and in the other twelve animals only sham vasectomy was performed. The animals were observed for a period of at least one year.

Apart from observing the general condition of the animals, the body weights were recorded every third month, blood pressure monitored periodically and ECG was taken once before starting the experiment and again preterminally. Ocular fundus was examined after mydriasis and the fundus findings recorded by an ophthalmologist.

Blood was sampled from the femoral vein and serum analysed for total cholesterol, phospholipids, TG and FFA by standard methods[3]. Blood glucose was estimated[4]. Circulating antisperm antibodies, agglutinin and immobilizins, were monitored at different intervals by the methods of Kibrick *et al*[5] and Isojima *et al*[6]. Frozen sections from the kidneys, aorta and testes were incubated with FITC labelled human IgG and C 3 at 37°C and then examined under UV light in an Olympus Fluorescent microscope for immuno-fluorescence. The aorta was stained with freshly prepared sudan IV solution to detect the extent of sudanophilia which was mapped and expressed as percentage[7]. Multiple blocks from the aorta, carotid, cerebral and renal arteries were taken and processed to get parafiin sections. The heart was divided transversely into thin slices and step serial sections were cut. These sections were stained with H and E, EVG, PTAH and Alcian blue (pH 2.8) counter stained with PAS. An arbitrary method of scoring for aortic plaques, coronary atherosclerotic index, renal and cerebral atherosclerosis and microangiopathic lesions in all these organs was adopted[8].

RESULTS

The animals of groups I and II remained healthy throughout the experiment and gained weight. However in those monkeys which were made diabetic there were several mortalities. The dead animals were replaced by new animals and, therefore, at the end of experimental period there were six surviving animals in each group. A number of animals from groups II, III and IV showed borderline hypertension at the end of twelve months. The ECG did not reveal any abnormality. Ocular fundus showed bilateral cataract in about one third of the diabetic animals, but fundus abnormality in the form of exudate in one monkey of group III and another with a colloid body of group IV was detected.

TABLE-I

Biochemical parameters in different groups (Mean ± S.E.)

Group	Total cholesterol mg/100ml		Phospholipids mg/100ml		TG mg/100ml		FFA umoles/litre	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
I	161.93± 14.55	150.27± 7.95	197.3 ± 7.69	184.02± 8.8	38.66± 5.54	46.91± 2.9	470.35± 61.89	531.3 ± 41.3
II	150.5 ± 11.27	154.87± 6.68	190.58± 14.03	200.74± 16.43	50.07± 4.27	69.67± 9.17	612.19± 136.0	430.05± 45.04
III	162.84± 12.38	205.65± 27.39	203.14± 14.9	266.6 ± 42.09	46.82± 5.60	122.4±* 29.96	567.16± 86.67	1031.3 ±** 104.34
IV	137.78± 16.62	181.46± 19.24	179.92± 13.5	222.35± 19.05	48.55± 6.73	127.74±** 24.02	558.26± 50.77	904.38± 68.96

*P∠0.05 **P∠0.01

Statistical comparisons made between basal and final readings.

Table - I shows the biochemical alterations in the serum of experimental animals. It is seen that monkeys of group I and II did not reveal any abnormality. However, diabetic monkeys with and without vasectomy showed marked hyperglycemia, hypertriglyceridemia, increased levels of cholesterol, phospholipids and FFA. There was no additional effect of vasectomy on the serum lipids.

From table II it is seen that vasectomised monkeys developed circulating antisperm agglutinins and immobilizins. In group II monkeys the first appearance of these antibodies occured after two or three months and the peak was attained by fifth to sixth month. Thereafter, there was a slow decline upto the end of the experiment. The immobilizins appeared after agglutinins. In diabetic vasectomised monkeys (Gp. IV) there was a much lower titre of circulating antibodies. In the non-vasectomised controls, there was no evidence of circulating antibodies.

Table-2

Sperm agglutinins in serum

Group	Monkey No.	Basal	Peak	Final
	4	-ve	1 : 1024	1:4
	7	-ve	1 : 128	1 : 16
	8	-ve	1:512	1:512
II	18	-ve	1 : 512	1 : 16
	30	-ve	1:16	1:16
	30	-ve	1 : 512	1 : 16
	13	-ve	1:16	-ve
	20	-ve	1 : 4	1 : 4
	23	-ve	-ve	-ve
IV	32	-ve	-ve	-ve
	33	-ve	-ve	-ve
	44	-ve	-ve	-ve

Gross grading of aortic sudanophilia showed significantly increased value in group II monkeys, I vs II 9. 77±2.36 vs 21.1 ± 4.31 ($P<0.01$). In the other two groups the mean values were, group III-15.17±5.58 and group<IV 10.83±1.83 respectively. The frequency incidence of aortic plaques did not show significant variation between the control and the experimental groups, nor was there any significant variation between these groups so far as plaque height was concerned. So far as coronary arteries are concerned there was significant increase of extramural coronary atherosclerosis in vasectomised and diabetic with vasectomy ($P<0.05$), while intramural coronary atherosclerosis was significantly higher in monkeys of group IV ($P<0.001$). Carotid artery atherosclerosis did not show any significant variation among the groups and so was the case with intracranial atherosclerosis (Fig. I). Renal atherosclerosis was more in monkeys of groups II and IV as compared to the other groups.

As regards microangiopathic lesions there was significant thickening of glomerular basement membrane in diabetic monkeys of groups III and IV and also of group II monkeys (Fig. 2). Apart from this the glomerular tufts in these groups of animals showed

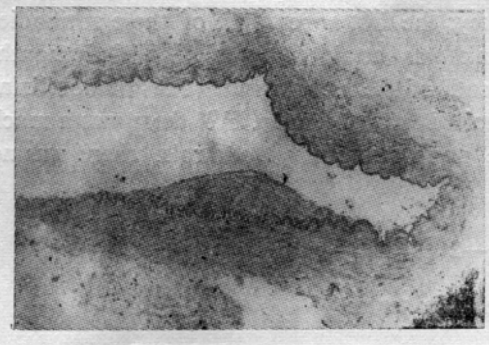

Fig. 1 : Section of the intracranial portion of internal carotid artery showing eccentric fibrous plaque. Hand E × 33

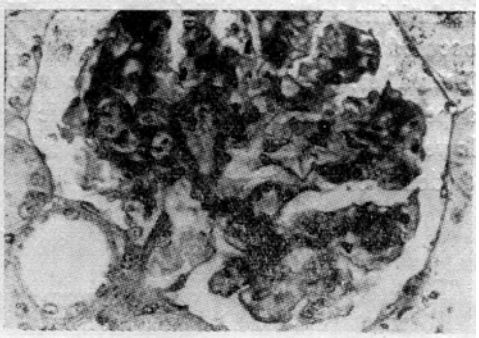

Fig. 2 : Section of the kidney of the diabetic monkey showing diffuse thickening of basemen membrane of glomerular capillaries and mesangial widening. RAS × 132

mesangial cell proliferation and PAS positive material deposition as well as occasionally exudate under the Bowman's capsule. In a few animals hypertensive changs were observed. In the heart small vessel disease was characterised by proliferation of smooth muscle cell of media, intimal hyperplasia, pericollagens is and occasionally intravascular fibrin deposition (Fig 3 and 4). Such small vessel disease was significantly higher in monkeys of groups II, III and IV as compared to group I ($P<0.05$-0.001). Occasionally basement membrane thickening of the vessels of choroid plexus of brain was noted which was seen both in group II and III monkeys.

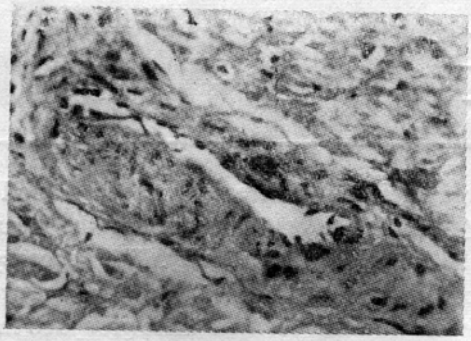

Fig. 3 : Section of the heart of diabetic monkey showing medial smooth muscle cell proliferation and narrowing of the lumen in a small artery. Hand E × 132

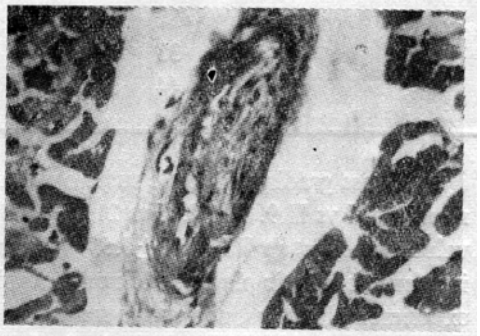

Fig. 4 : Section of the heart of diabetic monkey showing intimal hyperplasia causing extreme narrowing of the lumen of the vessel. Hand E × 132

Immunofluoroscent studies indicated positive IgG and C 3 deposit on the glomerular tufts, seminiferous tubulea of testes and occasionally on the aortic intima of the experimental groups of monkeys. Renal IgG immunoflaorescence was seen in one animal of group I, five of group II, three of group III and six of group IV. C 3 immunofluorescence was absent in group I and was present in 4, 2 and 6 of groups II, III and IV. As regards testes IgG was detected in l, 3, 1 and 2 monk~ys of groups I, II, III and IV respectivcly. C 3 was detected in much less number of animals. Aortic sections showed positive immunofluorescence for IgG and C 3 only in one animal of each groups II, III and 1V.

Discussion

This experimental study has proved that rhesus monkey is a suitable animal model for study of diabetes mellitus. The degree of hyperglycemia produced in these animal was quite high and which indicated that severe type of diabetic state was produced and maintained for a period of one year. With this type of diabetes it could be expected that the various macro and microvascular complications would be enhanced. Further the serum lipids also showed a marked elevation particularly serum TG and FFA which also appeared to play a deleterious role in the pathogenesis of these lesions. In the literature we did not find evidence of such severe diabetic state to have been maintained for such a long time in monkeys with vasectomy, however, the non diabetic monkeys did not show a rise in serum lipids. The later observation is in agreement with those of Clarkson and Alexander[9], who also did not observe increase of these parameters in vasectomised rhesus monkeys. However, Majumdar et al[10] observed a significantly increased level of serum TG and cholesterol in stock diet fed vasectomised rabbits. This indicates that there is quite a bit of species variation.

It was noted that vasectomy in non-diabetic animals caused increase of aortic sudanophilia which could indicate that the immunological injury caused by vasectomy promotes superficial vascular lesions. However, there was no evidence to suggest that this procedure aggravated and produced advanced lesions. In diabetes, macrovascular lesions were increased affecting both the extramural and intramural coronary arteries and renal arteries. Brain vessels both extracranial and intracranial did not show any significant enhancement as compared to other groups. So far as microangiopathic lesions are concerned there was definite evidence of their development in diabetic monkeys with and without vasectomy. The vessels in heart and kidneys were affected. There was further evidence of basement membrane thickening of glomerular tufts as well as deposition of IgG and C3 in the mesangial tissue.

References

1. Engerman, R., Bloodworth, J.M.B., and Susan Nelson, B.S. : Relationship of microvascular disease in diabetes to metabolic control : Diabetes, 1977, 26 : 760.

2. Bloodworth, J.M.B. Jr., Engerman, R.L. and Anderson, P.J. : Microangiopathy in the experimentally diabetic animals. Adv. in metabolic disorder Suppl. 2, 1973, 245.

3. Bansal, N., Majumdar, S., Dahiya, R., Sarkar, A.K. and Chakravarti, R.N : Long term effects of vasectomy in rhesus monkeys with special reference to serum lipids, aorta and heart. Bull P.G.I. 1981, 15 : 151.

4. Sharma, N.C. and Sur, B.K. : Improved method for estimating blood sugar. J. Clin. Path. 1966, 19 : 630.

5. Kibrick, S., Belding, D.L. and Merrill, B. : Methods for the detection of antibody against mammalian spermatozoa. II. A gelatin agglutination test. Fertil, Steril, 1952-3 : 430.

6. Isojima, S., Li, T.S. and Ashitaka, Y. : Immunologic analysis of sperm immobilizing factor found in sera of women with unexplained sterility. Am. J. Obstet. Gynaecol, 1968, 101 : 677.

7. Chakravarti, R.N., Mohan, A.P.and Komal, H.S. Atherosclerosis in macaca mulatta-Histopathological morphometric and histochemical studies in aorta and coronary arteries of spontaneous and induced atherosclerosis, Extpl. Mol. Path, 1976, 25 : 390.

8. Kukreja, R.S., Datta, B.N. and Chakravarti, R.N. : Catecholamine induced aggravation of aortic and coronary atherosclerosis in monkeys. Atherosclerosis, 1981, 40 : 291.

9. Clarkson, T.B. and Alexander, N.J. : Long term vasectomy effects on the occurrence and extent of atherosclerosis in rhesus monkeys. J. Clin. Invest, 1980, 65 : 15.

10. Majumdar, S., Bhatia, N.D. and Chakravarti, R.N. : Immune response to spermatozoal antigens in vasectomised normal and atherogenic diet fed rabbits. Ind. J. Med. Res. 1981, 73, 910.

Bibliography

1. Miller, G.J. & Miller, N.E. The Lancet, (1975) I, 16
2. Gordon, T. Castelli, W.P. Hjoritland M.C., Kennel, W.B. & Dawler, T.R., Am. J. Med. (1977) 62, 707.
3. Misra, K.P. Indian Heart J (1980) 32, I.
4. Turner, R.W.D. Post Graduate Med. J (1980) 56, 38.
5. Wissler, R.W., Vesselinovieten, D. Hughes, R., Turner, D & Frazer, L.E., Circulation (1971) 44 (Suppl-2), 57.
6. Wissler, R.W. & Vesselinveiten, D. Advances in Vet. Sciences and comparative Medicine (1977 a) 21, 351.
7. Wissler, R.W. & Vesselinveiten, D. Animal models of regression atherosclerosis IV. Proceedings 4th International symposium (Ed. Schettlen) (1977b), P. 377, Spreinger-Verlong, Berlin.
8. Tripathy, B.B. (1978) : Epidemiology of diabetes and its vascular complications Eds..Keen. H, Pickup, J.C. and Talwalkar, C.V., IDF, P-65.
9. Visweswar Rao, K.; J. Nutr. and Diabetes (1976) 4, 70.
10. Zak, B. Amer J. Clin Path (1957) 27, 583.
11. Fletcher, H.J. Clin Chem Acta (1968) 22, 393.