

MORPHOMETRY OF THE SMALL INTRAMYOCARDIAL ARTERIES
IN LONG TERM DIABETIC RATS

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ABSTRACT

The small intramyocardial arteries were investigated in 25 streptozotocin-induced diabetic rats at 11 to 40 weeks of diabetic state. The morphometric analysis was made using an eye-piece micrometer on a definite microscopic area. The mean arterial diameter and the mean arterial wall thickness \pm standard deviation were determined in diabetic rats at 11, 19, 26, 33 and 40 weeks and compared with nondiabetic rats of the same age. The difference was statistically significant for the arterial wall thickness in animals with diabetes longer than 19 weeks: $8.84 \pm 1.34 \mu\text{m}$ in diabetic rats at 19 weeks after the streptozotocin injection, versus $7.09 \pm 0.69 \mu\text{m}$ in control group, $10.87 \pm 0.89 \mu\text{m}$ in diabetic rats at 26 weeks versus $8.53 \pm 0.66 \mu\text{m}$ in controls, $12.55 \pm 1.03 \mu\text{m}$ in diabetic rats at 33 weeks versus $8.66 \pm 0.22 \mu\text{m}$ in controls and $12.75 \pm 0.66 \mu\text{m}$ in diabetic rats at 40 weeks versus $8.71 \pm 0.52 \mu\text{m}$ in controls. In nondiabetic animals the arterial wall thickness increased with the normal ageing, but in a lower proportion. Our results are an experimental argument to the fact that the thickening of the small arteries could play a role in the diabetic cardiac damage.

INTRODUCTION

The pathogenesis of the diabetic cardiomyopathy is still under debate. Atherosclerosis of the extramural coronaries has been associated with the increased cardiovascular risk in diabetics (Bell, 1952; Crall and Roberts, 1978; Boucher et al., 1979). But a correlation between arterial atherosclerosis and the severity of diabetes mellitus has not been found by Vigorita et al. (1980). Their study on 185 cases suggest that the diabetic cardiomyopathy is rather caused by a form of microangiopathy. This point of view is also supported by the following findings in the small arteries of diabetic myocardium: endothelial proliferation (Blumenthal et al., 1960; Zoneraich et al., 1980; Gherasim et al., 1985), P.A.S. positive material deposition in the media (Ledet, 1968), capillary and arteriolar microaneurisma (Factor, 1980), and bridges accompanied by subendothelial thickening (Zoneraich and Silverman, 1978). Ultrastructural studies have shown the thickening of the capillary basement membrane in the diabetic human myocardium (Fischer, 1979). In a more recent study, Factor (1981) did not noticed changes of the vascular wall thickness at 8 weeks streptozotocin induced diabetic rats.

In order to investigate the pathogenesis of the heart disease in experimentally induced diabetes, we applied morphometric analysis on the intramyocardial arteries.

MATERIAL AND METHODS

Female Wistar rats 10 weeks old (mean body weight 180 g) fasted for 24 hours, received a single i.v. injection of 30 mg/kg streptozotocin in citrat buffer, pH 4.4. Controls of the same age were injected only with buffer. Blood samples were collected after 24 hours of fasting for measurement of plasma glucose concentration by a glucose oxidase method. In every group the mean plasma glucose concentration was the average of at least four determinations. Fifteen diabetic

animals died spontaneously during the experiment period. All the animals (diabetic and controls) were fed with a low carbohydrate diet.

Five diabetic rats and five controls were sacrificed under aether anesthesia 11, 19, 26, 33, and 40 weeks after the streptozotocin injection.

Heart specimens were taken from each sacrificed animal. Tissue fragments were fixed in formaldehyde and processed routinely. Paraffin sections were stained with hematoxylin-eosin and picrofuchsin van Gieson's. In morphometrical analysis the direct measurement on transverse sections was made using an eye-piece micrometer. For each case a number of 200 microscopic fields, i.e. a surface of 19 mm^2 at a 400 x magnification was scanned. The mean arterial diameter, the mean arterial wall thickness and the mean arterial wall thickness/mean arterial diameter ratio were determined. Student's t-test was used for the statistical analysis.

RESULTS

The mean plasma glucose concentration was $157 \pm 8.66 \text{ mg/100 ml}$ in diabetic animals and $83.5 \pm 5.65 \text{ mg/100 ml}$ in controls.

The results of the morphometric analysis are summarized in Table 1. We found the mean arterial wall thickness significantly increased at 19 weeks after the streptozotocin injection. The difference between diabetic and control animals becomes more and more significant with the experiment duration.

In order to establish the importance of the arterial wall thickness in decreasing the vascular lumen and to exclude a possible error introduced by measuring larger vessels, we computed the mean arterial wall thickness/mean arterial diameter ratio. This parameter is increased after 26 weeks.

On the other hand, the thickening of the small arteries wall is quite constant in the normal process of ageing.

Table 1. Morphometry of the small intramural coronary arteries in diabetic and control rats.

EXPERIMENT DURATION (w)	11	19	26	33	40
Mean arterial wall thickness ($\mu\text{m} \pm \text{SD}$)	D 6.20 \pm 0.93	8.84 \pm 1.34	10.87 \pm 0.89	12.55 \pm 1.03	12.75 \pm 0.66
	n.s.	**	***	***	***
Mean arterial diameter ($\mu\text{m} \pm \text{SD}$)	C 6.86 \pm 0.76	7.09 \pm 0.69	8.53 \pm 0.66	8.66 \pm 0.22	8.71 \pm 0.52
	D 21.90 \pm 7.32	28.72 \pm 3.24	17.97 \pm 5.18	20.01 \pm 3.88	16.86 \pm 2.32
Mean arterial diameter ($\mu\text{m} \pm \text{SD}$)	n.s.	***	n.s.	n.s.	***
	C 22.46 \pm 1.41	23.55 \pm 2.11	21.48 \pm 0.99	20.71 \pm 6.34	22.01 \pm 4.75
Mean arterial wall thickness	D 0.28	0.30	0.60	0.62	0.75
Mean arterial diameter	C 0.30	0.30	0.39	0.41	0.39

w = weeks

D = diabetic rats (five for each group)

C = control nondiabetic rats of the same age (also five for each group)

n.s. = statistically not significant

** = $p < 0.010$; *** = $p < 0.001$; the comparison was made between the same parameter at diabetic and control rats of the same age

SD = standard deviation

DISCUSSION

The main problem in producing an experimental diabetic coronary microangiopathy is to develop a diabetic state which lasts long enough to cause cardiac damage. In our experiment the moderate mean glucose plasma concentration (157 \pm 8.6 mg/100 ml) was due to the small streptozotocin dose and the low carbohydrate diet received by the animals. In any case, the difference between the diabetic groups and the controls injected with buffer alone is statistically significant ($p < 0.001$).

The thickening of the arterial wall in diabetic rats became significant compared with controls of the same age at 19 weeks of diabetic state. In this group an increase of the mean arterial diameter (28.72 \pm 3.24 μm versus 23.55 \pm 2.11 μm in non diabetic rats) was also noticed so that the mean arterial wall thickness/mean arterial diameter ratio was the same as in the

control groups (0.30). The dilatation of the small intramural arteries could represent a compensatory adaptation to the vascular wall damage (McMillan, 1975).

After 26 weeks the wall thickening was more important, so that the adaptative capacity of the intramyocardial arteries was exceeded and the mean arterial wall thickness/mean arterial diameter ratio increased in diabetic rats (0.60 versus 0.30 at 26 weeks, 0.62 versus 0.41 at 33 weeks and 0.75 versus 0.39 at 40 weeks).

Although the thickness of the arterial wall is almost the same at 33 and 40 weeks in diabetic groups (12.55 μm and 12.75 μm) the important difference between the wall thickness/diameter ratio (0.62 versus 0.75) is probably an expression of a significant vascular narrowing secondary to the arterial wall thickening.

We suppose that the difference between Factor's data (1981) concerning the wall thickness/diameter ratio (0.124 in 8 weeks diabetic rats) and our data (0.30 after 11 weeks) is due to the determination in the present study of the internal (luminal) and not of the external diameter.

According to Fein et al. (1981) and Factor et al. (1981) the intramyocardial arterial damage in experimentally induced diabetes can not be due to malnutrition. Although in our experiment both diabetic and nondiabetic animals received the same diet, the diabetic rats showed a significant arterial thickening compared with nondiabetics. In their study, Fein et al. (1981) proved that streptozotocin is not directly cardiotoxic; by giving the streptozotocin inhibitor 3-O-methyl glucose to a series of rats receiving the drug they showed that mechanical abnormalities in papillary muscle function were not observed in the nondiabetic animals given streptozotocin plus inhibitor.

The vascular wall thickening in the normal process of ageing and in diabetes mellitus too, but at a higher level argues the theory of Vracko (1979, 1982) that diabetes accelerates the rate of cell death and cell renewal.

Moreover, in electron microscopy we found the

thickening of the capillary basal lamina in a concentric manner before the beginning of the arterial wall thickening (unpublished data). This finding agrees with the observations of Vracko (1982) in skeletal muscle of aged and diabetic patients. Probably, the capillary bed damage precedes the alterations in small arteries. However it is difficult to establish a causal relationship.

Whatever the cause, we suppose that the results presented here demonstrate the intramyocardial arterial involvement in experimental diabetic heart disease, and are an useful model for explaining the cause of the human diabetic cardiomyopathy.

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