Acarbose Treatment and Diabetic Nephropathy in the Cohen Diabetic Rat

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Summary

Groups of Cohen diabetic rats, aged 30 days were treated with Acarbose (40 mg per 100 g sucrose diet) for 3, 5 and 7 months. Siblings of the same sex were used as controls and fed the sucrose diet alone. The dose of Acarbose was adjusted in a preliminary study and found to be tolerated by the animals. Acarbose treatment resulted in a significant reduction of the 2 hr postprandial blood glucose. No elevation of the plasma insulin was noted. A significant decreased incidence and severity of glomerulosclerosis was noted in the 3 months Acarbose treated groups ($P < 0.05$) and in the 5 and 7 months ($P < 0.01$) in comparison with the controls. In the 7 months Acarbose treated group the longevity was significantly longer than in the control ($P < 0.05$). In the 3 and 5 months groups, increased longevity was not apparent as the animals were sacrificed before having the opportunity to manifest the difference.

Key words

Glomerulosclerosis – Diabetes Mellitus – Acarbose – Hyperglycemia – Cohen Diabetic Rat – Sucrose

Introduction

Two main pathological mechanisms have been postulated in the development of diabetic nephropathy. The first implicates "the renal hemodynamic alterations associated with less than optimal metabolic control of diabetes" as reviewed by *Hostetter, Renuke* and *Brenner* (1982), the second implicates hyperglycemia per se, as outlined by *Friedman* (1982). Both views implicate the uncontrolled diabetic state as a cause for the development of diabetic nephropathy. Indeed, control of blood glucose in alloxan and streptozotocin diabetic rats by insulin treatment (*Hagg* 1974; *Rasch* 1979) or by transplantation of the pancreas (*Bloor, Lee, Sayers* and *Orloff* 1977; *Mauer, Steffes, Sutherland, Najarian, Michael* and *Brown* 1975; *Federlin* and *Bretzel* 1984) reduces the incidence of renal lesions. Similar observations were made in KK mice (*Bloor* et al. 1977) following sulfanylurea treatment. In the Cohen diabetic rat the incidence and severity of diffuse glomerulosclerosis and diabetic retinopathy decreased following treatment with glibornuride (*Cohen, Yanko* and *Rosenmann* 1983) and islet transplantation (*Hammes, Klinzing, Wiegand, Bretzel, Cohen* and *Federlin* 1989).

The alpha glucosidase inhibitor, Acarbose was found to inhibit the intestinal sucrase (*Caspary* and *Graf* 1979) and thereby decrease the postprandial hyperglycemia (*Hillebrand, Boehme, Frank, Fink* and *Berchtold* 1979a, b).

Materials and Methods

Dosage of Acarbose

A preliminary study was made in the Cohen diabetic rat to determine the optimum amount of Acarbose* that affected the blood glucose and did not cause loose stools during 3 weeks of feeding. It was found that 40 mg of Acarbose per 100 g food was adequate.

Animals

Six groups of Cohen diabetic rats – 15 animals each – at the age of 28 days were fed the copper-poor sucrose diet (consisting of: sucrose 72%, casein 18%, butter 5%, corn oil 0.5%. USP salt mixture No. II 5%, water and fat soluble vitamins, copper content 1.2 ppm). Groups I, III and V were fed the sucrose diet to which 40 mg Acarbose was added to 100 g of the diet. In Groups II, IV and VI, sex and age matched control siblings were fed the sucrose diet alone.

Groups I and II were fed for 3 months. Groups III and IV were fed for 5 months. Groups V and VI were fed for 7 months.

Postprandial blood glucose and plasma insulin

After 2 months of feeding and at the end of the respective 3, 5 and 7 months terms, the 2 hr postprandial blood glucose and plasma insulin were examined. Food was withdrawn at 14.00 hrs and the animals were refed at 8.00 hrs the next morning. At 10.00 hrs i. e. 2 hrs after feeding, blood from the tip of the tail was examined for glucose and insulin. Blood glucose was determined in a Beckman glucose analyzer (USA) and the plasma insulin by the double immunoassay using insulin kit (Pharmatrope, France).

Urinary protein

At the age of 5 months and onward, the urine was examined monthly for proteinuria. Animals with persistent proteinuria that exceeded 29 mg/24 hrs were sacrificed to enable tissue examination. This procedure was adopted since animals with heavy protein

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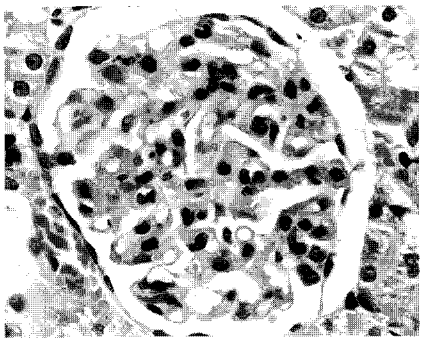

Fig. 1 Grade 2 glomerulosclerosis. Note thickening of mesangial matrix and capillary walls. H&E, x 450.

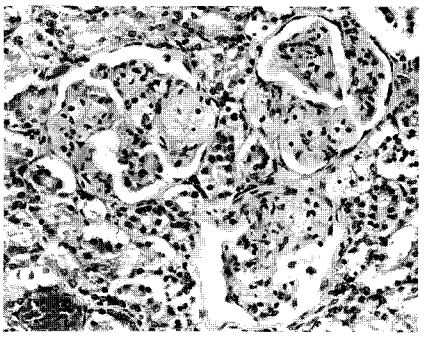

Fig. 2 Grade 4 glomerulosclerosis. Note sclerosis and xanthomatous lesions of the glomerular tufts. H&E, x 110.

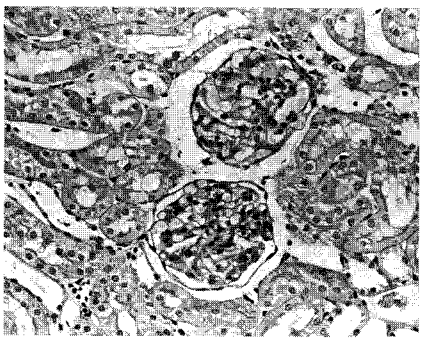

Fig. 3 Histological section of unaffected kidney. Normal glomeruli, PAS, x 110.

loss die in the cage. The other animals in the groups were killed at the end of the appropriate periods i. e. at 3, 5 and 7 months on the respective diets. The kidney samples were fixed in formalin and paraffin-embedded sections were stained with hematoxylin and periodic-acid Schiff (PAS).

Renal changes

Whole transversal sections of both kidneys were examined by the pathologist (ER), and approximately 300 glomeruli were screened in each case. The degree of nephropathy was graded according to the number of glomeruli involved (focal or generalized) and the extent of glomerular involvement (segmental or diffuse). The degree of histopathological changes was rated from 0 to 4+: 0 = no change; Grade 1 = focal, 15–30% of glomeruli segmentally and/or diffusely involved; Grade 2 = focal, 30 to 50% of glomeruli segmentally and/or diffusely involved (Fig. 1); Grade 3 = generalized, 50 to 100% of glomeruli segmentally and/or diffusely involved; Grade 4 = generalized, with secondary tubular atrophy and/or dilatation (Fig. 2) and xanthomatous glomerular lesions. There were, in addition to the glomerulosclerotic changes, segmental lesions in the form of lipohyaline lesions. In sections of the unaffected kidneys, an occasional glomerulus was sclerotic, the rest being normal (Fig. 3).

Statistical evaluation

Student's t-test for nonpaired series and the X^2 method were used to evaluate the results.

Results

Body weight

The body weight of the Acarbose groups was not significantly lower than that of the controls both in males and in females (Table 1).

Blood glucose and plasma insulin levels

The 200 mg/dl was taken as a cutoff point for the mean 2 hrs postprandial blood glucose. In the 3 months Acarbose treated group 25%, in the 5 months group 35% and in the 7 months group 73% had a postprandial blood glucose value smaller than 200 mg/dl compared with none in the respective control groups (Table 1). Moreover in the Acarbose treated groups the mean postprandial blood glucose values that were lower than 200 mg/dl were significantly lower ($P < 0.05$) than in the control groups. In the 3 months treated, 275 ± 9 versus 331 ± 2.5 mg/dl in the controls; in the 5 months Acarbose treated 282 ± 13.5 versus 329 ± 15.8 and in the 7 months 215 ± 4.6 versus 315 ± 31 mg/dl. The plasma insulin levels in the 3 and 5 months Acarbose treated animals were not significantly different from their respective controls. In the 7 months Acarbose treated group the plasma insulin was not different than in the 3 and 5 months Acarbose treated but was significantly higher than in the 7 months controls, 19 ± 2 versus 11.9 ± 1.2 uu/ml. Similarly there was no significant difference between the plasma insulin levels of animals with blood glucose < 200 and those with > 200 mg/dl in the 3 and 5 months Acarbose treated groups, but there was a significant difference in the plasma insulin of the 7 months Acarbose treated animals with blood glucose < 200 mg/dl and those with > 200 mg/dl, 21.2 ± 2.3 versus 13.3 ± 1.8 uu/ml respectively.

Renal changes

In the 3 months Acarbose treated group, 2 out of 15 animals had renal lesions while in the control group 10 out of 15 animals were affected, X^2 (df = 4) = 10.76, $P < 0.05$ (Table 2). In the longer treated 5 and 7 months groups, although renal lesions were observed in all autopsied animals, the severity of nephropathy was significantly milder in the Acarbose treated. In the 5 months group 1 out of 12 and in the 7 months none of the 14 Acarbose treated animal had grade 4 renal changes compared to 6 out of 13 and 5 out of 13 in the respective control groups. X^2 (df = 3) = 12.3, $P < 0.01$. On comparing the severity of grades of renal pathology in all groups, there was a significantly more severe pathology in the controls than in the Acarbose treated rats, X^2 (df = 4) = 15.618, $P < 0.05$.

Longevity

Table 3 details the number of animals and their age at the time of autopsy. Up to 5 months there was no difference in the longevity between the Acarbose treated and control groups. In the 7 months groups, the Acarbose treated longevity was significantly longer; 11 out of 15 animals were alive at term, compared to 3 out of 15 in the controls. X^2 (df = 4) = 10.2714, $P < 0.05$.

Table 1 Metabolic state of animals treated with Acarbose selected according to their postprandial blood glucose levels at term of 3, 5 and 7 months (mean ± SE).

	Spontaneous blood glucose (mg/dl)							
	Acarbose				Control			
	died[a]	< 200	> 200	total	died[a]	< 200	> 200	total
				3 Months on Treatment				
No of animals	0	3 (25)[b]	12	15	0	0	15	15
Blood glucose (mg/dl)	–	163 ± 30	27 ± 9 [c]	251 ± 15.3[c]	–	–	331 ± 2.5	331 ± 2.5
Spontaneous insulin (uu/ml)	–	20 ± 3.1	30 ± 2.8	27.8 ± 2.8	–	–	25.2 ± 2.2	25.2 ± 2.2
Body weight –males				220 ± 5.0				221 ± 11.3
(g) –females				179 ± 6.0				184 ± 8.7
				5 Months on Treatment				
No of animals	3	4 (36)[b]	8	12	6	0	9	9
Blood glucose (mg/dl)	282 ± 17.2	177 ± 13	282 ± 13.5 [c]	233 ± 10.6 [c]	256 ± 20 [c]	–	329 ± 17	329 ± 12
Spontaneous insulin (uu/ml)	13.5 ± 0.9	21 ± 3.7	22 ± 2.8	21.9 ± 3.3	12.9 ± 2	–	16 ± 1.3	16 ± 1.3
Body weight –males					154 ± 9			142 ± 6.9
(g) –females					145 ± 18			–
				7 Months on Treatment				
No of animals	4	8 (73)[b]	3	11	12	0	3	3
Blood glucose (mg/dl)	234 ± 24	181 ± 8.3	215 ± 4.6	188 ± 8	296 ± 23	–	315 ± 31	315 ± 31
Spontaneous insulin (uu/ml)	11 ± 0.5	21.2 ± 2.3 [c]	13.3 ± 1.8	19 ± 2 [c]	11.3 ± 1.4	–	11.9 ± 1.2	11.9 ± 1.2
Body weight –males				160 ± 11				–
(g) –females				151 ± 6.8				162 ± 0.7

[a] during experimental period
[b] % of cases with blood glucose values < 200 mg%
[c] versus control $P < 0.05$

Discussion

Awareness of the importance of control of blood glucose concentrations in reducing long term complications of diabetes stimulated research into new ways of stabilizing postprandial glycemia. The evaluation of the effect of treatment on the development of diabetic vascular complications in man, requires a life time of serial studies. The life time of man is itself an impediment; the use of animal models circumvents these problems, although extrapolation from man to animals is hazardous.

The Cohen diabetic rat has many common characteristics with the human NIDDM (Table 4). In this model diabetes results from the interaction of genetic and environmental factors; it is age related, it responds to oral hypoglycemic agents; with time the enzymatic pattern turns into a diabetic one; it develops diabetic renal and retinal microangiopathy (*Cohen* 1986). About 80% of the animals develop glomerulosclerosis within 6–7 months, a matter that facilitates the study of the effect of treatment on the development and course of diabetic nephropathy.

Table 2 Number of animals and their grade of glomerulosclerosis.

	Acarbose						Control					
Group	normal	1	2	3	4	Total	normal	1	2	3	4	Total
3 mo	13 (86.7)[a]	–	2 (13.3)	–	–	15[b]	5 (33.3)	2 (13.3)	3 (20)	4 (26.7)	1 (6.7)	15
5 mo	–	1 (6.3)	1 (8.3)	9 (7.5)	1 (8.3)	12	–	1 (7.7)	1 (7.7)	5 (38.5)	6 (46.2)	13
7 mo	–	–	–	14 (100)	–	14[c]	–	–	1 (7.7)	7 (53.8)	5 (38.5)	13
Total	13 (31.7)	1 (2.4)	3 (7.3)	23 (56.1)	1 (2.4)	41[d]	5 (12.2)	3 (7.3)	5 (12.2)	16 (39)	12 (29.3)	41

[a] figures in parenthesis (percent)
[b] severity of renal pathology, 3 mo acarbose treated versus control, X^2 (df = 4) = 10.76, $P < 0.05$
[c] severity of renal pathology, 5 and 7 mo acarbose treated versus control, X^2 (df = 2) = 12.133, $P < 0.01$
[d] severity of renal pathology in all groups acarbose treated versus control, X^2 (df = 4) = 15.618, $P < 0.01$

Table 3 Longevity. No of animals at age of death or sacrifice.

Group	Month 3	4	5	6	7	Total
5 months						
Control	2	7 (2)[a]	6	–	–	15
Acarbose	1 (1)	6 (1)	8	–	–	15[b]
7 months						
Control	1	3	7 (1)	1	3	15
Acarbose	0	2	1 (1)	1	11	15[c]

[a] figures in parentheses = number of animals that died in cage and were not examined histologically.
[b] 5 months acarbose-treated groups versus control, X^2 (df = 2) X^2 = 1.5127, P = ns.
[c] 7 months acarbose-treated groups versus control, X^2 (df = 4) X^2 = 10.2714, $P < 0.05$.

Table 4 Common characteristics of the Cohen diabetic rat with the human NIDDM.

1. Diabetes results from interaction between genetic and environmental factors.
2. Age related.
3. Greater prevalence in males than in females.
4. In the beginning there is hyperinsulinemia which turns into normoinsulinemia followed by hypoinsulinemia.
5. In time the enzymatic pattern of carbohydrate and lipid metabolism turns into a diabetic one.
6. Reduced number of insulin receptors.
7. A large percentage develop glomerulosclerosis.
8. 25–35% of diabetic animals develop the early stages of diabetic retinopathy including pericyte cell loss, strand formation and saccular microaneurysms.
9. Osteopathy.
10. Periodontal disease.
11. Embryopathy.

The postprandial rise in blood glucose in diabetes is regulated by hepatic carbohydrate metabolism, insulin release and peripheral glucose uptake. Also the amount of available carbohydrate (*Jenkins, Wolever, Taylor, Barker, Fielden, Boldwin, Bowling, Newman, Jenkins* and *Goff* 1981), its physical form (*O'Dea, Nestel* and *Antonoff* 1980) and complexity (*Crapo, Reaven* and *Olefsky* 1976) are important dietary determinants of the rate of absorption.

The alpha glucosidase inhibitor – Acarbose – has been proven to be effective against intestinal maltase and sucrase (*Caspary* and *Graf* 1979). Because of its inhibiting actions on several key enzymes for carbohydrate digestion, Acarbose decreases postprandial hyperglycemia (*Hillebrand* et al. 1979a, b). Stable partial inhibition of sucrase activity can be attained with this inhibition in which the rate of onset and the steady state are dose related (*Taylor, Backer* and *Caufield* 1983). In the present experiment preliminary study showed that 40 mg per 100 g food is well tolerated by the diabetic animals after 5 days intake.

The Acarbose treated and control groups were siblings of the same sex, thus ruling out the possible role of genetics and sex as factors affecting our results.

In the Acarbose treated groups postprandial blood glucose values were significantly lower than those of the control groups. This difference in blood glucose levels was reflected in a significantly decreased incidence and delayed development of the renal pathological changes in all groups and longer longevity in the 7 months treated groups. The fact that no difference in longevity was noted in the 3 and 5 months

groups derives probably from the fact that they were sacrificed at term and were not given the opportunity to live longer in order to manifest the difference in the 7 months group.

The plasma insulin levels in the 3 and 5 months Acarbose treated groups were not different from the controls. This is compatible with the fact that B cell secretory dynamics are not altered in the perfused pancreas of the rat treated with Acarbose (*Makoto, Sukamoto, Ohki, Okabayashi, Yvu, Maed* and *Baba* 1983). Also, the plasma insulin levels in the human NIDDM were not changed after a short-term treatment with Acarbose (*Baron, Eckel, Schmeiser* and *Kolterman* 1987). The fact that in the 7 months treated group the plasma insulin was higher than that of the controls may be due to a reduction in the level of insulin in the controls and not to the elevation of the plasma insulin in the Acarbose treated group, as the plasma insulin in the 7 months treated group was not different from that of 3 and 5 months Acarbose treated or control groups.

Acarbose treatment in the human NIDDM has improved the glycemic increment following the ingestion of meals (*Baron* et al. 1987; *Jenkins, Taylor, Goff, Fielden, Misiewicz, Sarson, Bloom* and *Alberti* 1981; *Walton, Sherif, Noy* and *Alberti* 1979) and is consistent with the proposed mechanism of action i.e. delaying the rate of digestion of carbohydrate at the level of the gastrointestinal tract.

In conclusion, the results of the present study demonstrate that in the Cohen diabetic rat, which is NIDDM, Acarbose treatment lowered the postprandial blood glucose increments, decreased the incidence and delayed the development of diabetic glomerulosclerosis and its severity. It also prolonged longevity of the treated diabetic animals.

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