BENEFICIAL EFFECTS OF ACARBOSE ON DAILY PLASMA GLUCOSE PROFILE AND CATARACT DEVELOPMENT IN SAND RATS

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SUMMARY

Sand rats were used as a model for nutritionally induced type II (non-insulin-dependent) diabetes in an effort to evaluate the effect of acarbose on carbohydrate digestion. Daily plasma glucose profiles, insulin levels and weekly cararact development were determined following long-term feeding with a diet containing acarbose (20 or 40 mg/100 g diet). Acarbose not only dramatically decreased daily plasma glucose and insulin levels ($p<0.05$) but also delayed, and possibly prevented, cataract formation in sand rats. The effect of acarbose persisted for 150 days. The control of daily plasma glucose levels and reduction of insulin levels obtained with acarbose may lead to the delay of cataract formation in sand rats. These results could have potential applications to diabetic patients as an adjunct treatment.

Recent studies have shown that the addition of acarbose (BAY G-5421) to a starch-containing diet leads to a significant reduction in post-prandial glucose increments in both non-diabetic and diabetic rats.[1–3] Ample information exists describing the potential of acarbose to reduce glycosuria and plasma glucose levels in both rat and human diabetic subjects.[3,4] However, because of the lack of studies reporting the effects of acarbose on late-stage diabetic complications, such as cataracts, we elected to extend our studies to include observations on cataract formation using sand rats as the model of type II (non-insulin-dependent) diabetes.[5]

The sand rat (*Psammomys obesus*) feeds exclusively on low-calorie, high-salt succulents in its natural environment and demonstrates normal blood glucose levels.[5,6] Long-term feeding of a synthetic chow diet results in obesity and a diabetic syndrome including hyperinsulinaemia, hyperglycaemia, markedly decreased glucose tolerance and insulin resistance.[5,7,8] Hyperglycaemia in experimental animals has been shown to cause lenticular changes, including increased sugar alcohol, eventually leading to cataract formation.[9–12] Our efforts were directed towards evaluating the effect of acarbose on cataract formation in sand rats, particularly on its progression. Additionally, the long-term effect of this drug on daily plasma glucose and insulin levels was assessed.

MATERIALS AND METHODS

Weanling sand rats (70±9 g body weight) were divided into two groups and fed diets composed of (%): soybean protein (20), cornstarch (65), soybean oil (5), cellulose (4) and vitamins (2). Diets were administered with or without acarbose, in pellet form. Rats were housed in individual cages maintained at 22±1°C with a 12 h light/dark phase (0600–1800 hours); water and food were offered *ad libitum*.

Three different experiments were conducted. In experiment 1, 12 rats were used as the control group. Twelve and 11 rats were used for 20 mg and 10 rats for 40 mg acarbose supplementation, respectively. Rats were fed the respective diets for 15 and 35 days, and daily plasma glucose levels were measured. The second experiment was carried out for 150 days in sand rats fed a diet with 40 mg of acarbose, and cataract development was evaluated. Plasma insulin levels were also measured. An additional experiment was carried out to monitor the stages of cataract development. Sand rats (9 per group) were fed a diet with or without acarbose (40 mg/100 g diet) for 60 days.

Blood samples were drawn from the tail tip into tubes which had been prewashed with heparin (400 U/ml) and 0.01 mM sodium flouride at 0900, 1230, 1630 and 2100 hours, to determine daily plasma glucose profiles. Glucose was measured using a Beckman glucose analyser. Insulin levels were also determined by standard radioimmuno-

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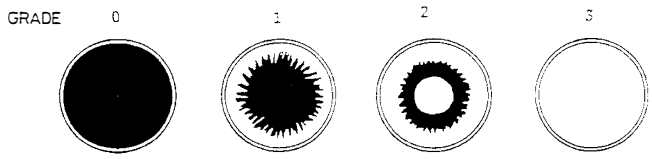

Fig. 1. *Cataract classification. Grade 0, lens completely clear; grade 1, opaque areas at periphery; grade 2, widespread opacities within the lens; grade 3, total lens opacity.*

assay using a kit obtained from CIS Bioindustries International (Oris, France).[13] Because of the low plasma volume, insulin levels were measured in only a small number of sand rats (as indicated in Table IV) at 33, 66 and 94 days into the experimental period. The animals were handled according to guidelines set forth by the Animal Care Centre of the Hebrew University of Jerusalem, Israel.

Classification of Cataracts

The lenses were examined without anaesthesia using a hand-held ophthalmoscope. Both eyes of each animal were evaluated. Prior to examination the pupils were dilated by a single topical application of tropicamide (0.5%) and phenylephrine (10%). The cataracts were assessed in a masked fashion, i.e. the observer was unable to identify the animal's current or previous treatment group. Cataracts were classified using a modification of the method described elsewhere:[14,15] grade 0, lens completely clear; grade 1, isolated or contiguous areas of opacity at the periphery of the lens, vascular features of the retina still visible; grade 2, extension of opacity towards the centre the lens, vascular features of the retina obscured; grade 3, lens totally opaque, cataract visible to the naked eye as a dense white mass (Fig. 1).

The significance of the difference between groups was calculated by Student's *t*-test.

RESULTS

There was no significant effect of acarbose on body weight during the experimental period. The body weight changes of sand rats fed a diet with acarbose for 150 days was 266±35 g compared with 277 g without acarbose. At the start of the experiment the fasting plasma glucose (85±12 mg/dl) and insulin (53±18 μU/ml) were comparable.

The effect of feeding a diet including acarbose (20 or 40 mg) for 15 or 35 days on daily plasma glucose levels in sand rats is shown in Tables I and II (experiment 1). Both concentrations reduced plasma glucose levels during all test periods, although statistically significant differences were obtained only with 40 mg of acarbose. Acarbose dramatically reduced the plasma glucose levels throughout the day. The longer experiment (35 days) was carried out to determine whether the low concentrations (20 mg) of acarbose could also lead to a significant reduction in daily plasma glucose levels (Table II). Both drug concentrations significantly ($p<0.05$) decreased plasma glucose levels. Results from rats fed higher acarbose concentrations were significantly different ($p<0.025$) from those of the control group.

Table III describes the long-term effect of acarbose on morning glucose levels (experiment 2) and cataract appearance. Acarbose significantly decreased plasma glucose levels during all experimental periods. After 33 days on the diets, only 7% of the rats fed acarbose had developed cataracts, as compared with 60% of the control rats. By the end of the experiment (150 days) 42% and 92%, respectively, of the sand rats developed cataracts when fed diets with or without acarbose, indicating the time-dependence of cataract formation (Table III).

The long-term effect of acarbose on insulin levels is detailed in Table IV. Although sand rats developed hyperinsulinaemia after 33 days of feeding, incorporation of acarbose into the diet significantly ($p<0.05$) reduced insulin levels (measured in the morning and in the fed state). The increase in insulin level with time was much more notable in rats fed without acarbose (170±36 and

Table I. Daily plasma glucose profile (mg/dl) of sand rats fed a diet with or without acarbose (20 or 40 mg/100 g diet) for 15 days

		Hours			
Group	*n*	0900	1230	1630	2100
Control	12	262 ± 52	238 ± 52	214 ± 51	209 ± 45
+20 mg acarbose	12	209 ± 41	195 ± 45	218 ± 56	198 ± 40
+40 mg acarbose	10	143 ± 36*	129 ± 37*	126 ± 38*	130 ± 38*

Values are the mean ± SEM.
n, number of animals.
*$p<0.025$ significantly different from control.

Table II. Effect of acarbose (40 or 20 mg/100 g diet) on daily plasma glucose profile (mg/dl) in sand rats fed the diet for 35 days

		Hours			
Experiment	*n*	0900	1230	1630	2100
Control	12	249 ± 21	223 ± 15	204 ± 38	205 ± 25
+20 mg acarbose	12	182 ± 32*	153 ± 14*	133 ± 29*	158 ± 28*
+40 mg acarbose	10	129 ± 23*	113 ± 19*	104 ± 21*	126 ± 21*

Values are the mean ± SEM.
n, number of animals.
*$p<0.05$ versus control.

Table III. Long-term effect of acarbose on morning plasma glucose and cataract formation in sand rats fed a diet with or without acarbose (40 mg/100 g diet)[a]

Day of measurement	Glucose levels (mg/dl) in rats fed a diet: With acarbose	*n*	Without acarbose	*n*	Rats developing cataract[b] fed a diet: With acarbose	Without acarbose
33	141 ± 24	(14)*	217 ± 29	(15)	1/14	9/15
66	156 ± 37	(14)**	296 ± 44	(15)	5/14	9/15
94	142 ± 23	(14)*	298 ± 49	(15)	6/14	12/15
119	135 ± 25	(14)*	272 ± 59	(15)	6/14	12/15
150	125 ± 19	(14)*	290 ± 49	(15)	6/14	13/15

Values are the mean ± SEM.
n, number of rats per group.
*$p<0.05$ versus without drug; **$p<0.06$ versus without drug.
[a]Rats were maintained on their respective diets for 150 days. Glucose levels were measured in the morning between 0800 and 0900 hours.
[b]Cataract appearance evaluated visually.

Table IV. Long-term effect of acarbose (40 mg/100 g diet) on insulin levels (μU/ml) in sand rats

Day of measurement	Without acarbose	(*n*)	With acarbose	(*n*)
33	170 ± 36*	(7)	114 ± 46	(7)
66	213 ± 11*	(6)	106 ± 10	(7)
94	316 ± 56*	(6)	158 ± 50	(7)

Values are the mean ± SEM.
n, number of animals.
*$p<0.05$ versus with acarbose.

316±56 μU/dl at 33 and 84 days, fed without acarbose (170±36 and 316±56 μU/dl at 33 and 94 days, respectively) than in those fed with acarbose (114±46 and 158±5 μU/ml at 33 and 94 days, respectively).

Table V summarises cataract developmental stages in sand rats. The cataract's progression was graded (Fig. 1) by examining the lens directly with an ophthalmoscope. The majority of cataracts in both eyes appeared at week 4 in the group fed acarbose-free diets. Six of 8 sand rats fed acarbose-free diets had grade 3 cataracts after 5 weeks on the diet. Only 1 rat (number 9, Table V) fed acarbose developed cataracts. The other 7 rats on the acarbose diet did not develop any signs of cataracts and were not included in Table V.

DISCUSSION

The glucose-lowering effect of acarbose was seen throughout the day, indicating the benefits of acarbose in reducing both post-prandial glucose levels and controlling daily glucose profiles. This dual effectiveness is presumably due to acarbose's potential to delay starch digestion during eating, and to the free access provided to food and water (Tables I, II). Under such conditions plasma glucose levels are expected to be moderate, with improved renal threshold.

Data from this study support the suggestion that the concentration of acarbose required to control plasma glucose levels can be reduced (from 40 to 20 mg/100 g diet) when acarbose is administered in the long term. This could be useful in averting the possible negative side effects of higher doses of acarbose. Inclusion of acarbose in the sand rat diet also reduced the high insulin levels observed in those fed without acarbose[7,16] (Table IV). We postulate that, as a result of the glucose reduction caused by acarbose, the amount of insulin required for glucose control is decreased. We believe that acarbose may have

Table V. The stage of cataract development (grades 0–3) in the right (R) and left (L) eyes of sand rats

	Week of experiment															
	0		1		2		3		4		5		6		7	
Rat	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
1	0	0	0	0	0	0	2	1	3	2	3	3	3	3	3	3
2	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3
3	0	0	0	0	0	0	2	2	3	3	3	3	3	3	3	3
4	0	0	0	0	0	0	0	0	0	2	3	3	3	3	3	3
5	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	1
6	0	0	0	0	0	0	1	0	3	2	3	3	3	3	3	3
7	0	0	0	0	0	0	1	0	3	2	3	3	3	3	3	3
8	0	0	1	1	2	2	2	3	3	3	3	3	3	3	3	3
9	0	0	0	0	0	0	0	0	1	1	3	2	3	3	3	3

Rats 1–8 were fed a diet without acarbose; rat 9 was fed a diet with acarbose (40 mg/100 g diet).

an additional beneficial effect on carbohydrate metabolism as a protective factor against hyperinsulinaemia.

The close association between diabetes and cataract formation in sand rats has been noted previously.[9] The most striking finding of the present study was the ability of acarbose to delay, and even prevent, cataract formation in sand rats (Table V). The 'high-calorie' carbohydrate-enriched diet produced hyperglycaemia and correlated well with cataract formation. By reducing the plasma glucose levels, acarbose presumably delayed cataract appearance and progression. The mechanism by which acarbose exerts its effect is unknown and requires clarification. We hypothesise that accumulation of sorbitol derived from the metabolism of glucose via the polyol pathway is the major contributor to cataract formation.[11,17] However, there is increasing evidence that non-enzymatic glycation of crytallins may contribute to the formation of cataract.[18] We believe that the preventive effect on cataract development is a consequence of the better control of glucose obtained using acarbose. Aldose reductase and sorbitol concentrations in the lenses of the sand rats fed with or without acarbose are currently being investigated in our laboratory.

Collectively, the data presented here imply that late complications occurring in the diabetic state can be attenuated by acarbose. The reduction in insulin level and the control of the daily plasma glucose levels obtained using acarbose may prevent or delay cataract formation, as well as other diabetes-related complications in sand rats.

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Key words: Acarbose, Cataract, Sand rat.

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