Effects of Cyclosporine on Glucose Tolerance in the Rat

J. F. YALE, R. D. ROY, M. GROSE, T. A. SEEMAYER, G. F. MURPHY, AND E. B. MARLISS

SUMMARY

In a study of prevention of spontaneous diabetes in BB rats by therapeutic doses of cyclosporine (10 mg/kg/day), the male control non-diabetes-prone rats showed glucose intolerance after a 0.25 g/kg glucose load by gavage, at 90 and 130 days of treatment. Non-BB male Wistar rats treated similarly showed glucose intolerance at 1 wk of treatment, with progressive worsening for 5 wk, then sustained up to 12 wk of treatment. Fasting euglycemia was maintained, but both pre- and postchallenge plasma insulin levels were significantly lower with cyclosporine at several time points. Total pancreatic insulin was decreased to one-third that of control after 5 wk. After withdrawal of cyclosporine, glucose tolerance returned to normal in 2 wk. Sprague-Dawley rats responded similarly and in both strains, an increase in the cyclosporine dose to 15 mg/kg/day augmented the glucose intolerance. These results demonstrate that therapeutic doses of this agent induce reversible glucose intolerance due, in part, to inhibition of insulin secretion and also possibly inhibition of synthesis, though a peripheral effect is not excluded. This hyperglycemic effect of cyclosporine has implications for its potential use in type I diabetes mellitus, transplantation, and other autoimmune disease. DIABETES 1985; 34:1309–13.

The immunosuppressive properties of cyclosporine were discovered by Borel in 1972. Its action is novel, differing from other current immunosuppressive drugs in that it is not an alkylating, antimitotic, or lympholytic agent. Its adverse effects include dose-related nephrotoxicity[1,2] and hepatotoxicity.[1] Other side effects, usually mild, include tremor (15%), hirsutism (12%), and gingival hyperplasia (6%).[3] This article documents another "side effect" of cyclosporine: induction of glucose intolerance.

The spontaneously diabetic BB rat is a widely studied animal model of type I insulin-dependent diabetes mellitus (reviewed in ref. 4). The rat syndrome (as also the human) appears to have an autoimmune basis (reviewed in ref. 5). Cyclosporine has been reported to prevent diabetes occurrence in the BB rat when given before the usual age of onset,[6-9] but is unable to reverse either the hyperglycemia or the beta cell destruction when given at onset.[10] When cyclosporine therapy was initiated shortly after diagnosis in humans, either a ≥50% reduction in insulin dose or cessation of insulin was reported in a substantial proportion.[11,12]

In a previous study where we examined the immunologic changes induced by cyclosporine in relation to diabetes prevention in diabetes-prone BB rats,[13] non-diabetes-prone BB rats were used as controls. After 90 days of cyclosporine treatment, an oral glucose tolerance test was performed that unexpectedly showed glucose intolerance in the male non-diabetes-prone BB rats treated with cyclosporine. This article presents those results, as well as showing the reproducibility of the finding that diabetes does not occur in Wistar rats unrelated to the BB colony, nor in a totally unrelated strain, Sprague-Dawley rats.

MATERIALS AND METHODS

Fourteen non-diabetes-prone male and female BB rats (Animal Resources Division, Health and Welfare Canada, Ottawa, Ontario, Canada) were treated with cyclosporine (10 mg/kg/day; Sandoz Canada Inc., Dorval, Quebec, Canada) by gavage from age 30 to 160 days. Glucose tolerance tests were performed at 120 and 160 days of age. Forty-five male Wistar rats, unrelated to the BB rats, were studied, 21 of which were treated with the same dose of cyclosporine from age 60 days, and 19 others acting as controls. Half of these rats had an oral glucose tolerance test performed after 4 h, 2 days, and 1, 2, and 5 wk of cyclosporine, and were then prepared for study of pancreatic morphology (by light mi-

From the McGill Nutrition and Food Science Centre, Royal Victoria Hospital, Montreal, Quebec, Canada.
Received for publication 24 June 1985.
Address reprint requests to Dr. Errol B. Marliss, McGill Nutrition and Food Science Centre, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, H3A 1A1, Canada.
This work was presented in part at the 45th Annual Meeting of the American Diabetes Association in Baltimore, Maryland, June 16, 1985 [Diabetes (Suppl. 1) 1985; 34:6A].

TABLE 1
Glucose tolerance tests in non-diabetes-prone BB rats during cyclosporine (CsA) treatment*

	Plasma glucose (mg/dl)		Plasma insulin (ng/ml)			
	0 min	60 min	0 min	60 min	Weight (g)	Cyclosporine levels (ng/ml)
Male BB rats, 90 days						
CsA-treated	117 ± 6	273 ± 29†	—	—	437 ± 9	364 ± 58
Controls	104 ± 2	144 ± 3			430 ± 17	
Male BB rats, 130 days						
CsA-treated	107 ± 3	253 ± 26†	0.37 ± 0.26	0.80 ± 0.29	464 ± 18	666 ± 171
Controls	104 ± 0	134 ± 19	0.38 ± 0.16	0.77 ± 0.30	467 ± 41	
Female BB rats, 90 days						
CsA-treated	105 ± 2	165 ± 5	—	—	263 ± 1	253 ± 46
Controls	104 ± 4	144 ± 1			256 ± 13	
Female BB rats, 130 days						
CsA-treated	103 ± 1	157 ± 7	0.31‡	0.59‡	289 ± 5	451 ± 115
Controls	110 ± 4	139 ± 4	0.18‡	0.41‡	290 ± 0	

*Times given represent duration of treatment.
†$P < 0.01$, versus controls.
‡$N = 2$.

croscopy) and pancreatic insulin content measurements. The other rats had glucose tolerance tests after 4, 7, 9, and 12 wk of cyclosporine, then again at 1, 2, and 4 wk after stopping the drug. The last 5 Wistar rats were treated concomitantly with 16 Sprague-Dawley rats, 8 of which received cyclosporine while the other 8 received the olive oil vehicle. These rats were started at age 60 days and had glucose tolerance tests at 1, 2 , and 4 wk of cyclosporine treatment at 10 mg/kg/day, then 2 wk after the increase in dose to 15 mg/kg/day. Glucose, 0.25 g/kg, was given by gavage without anesthesia after a 16-h fast, with measurements of plasma glucose and insulin from tail vein blood obtained before the challenge (0 time) and at 60 min. Wistar and Sprague-Dawley rats were obtained from Charles River Laboratories of Canada, Inc., St. Constant, Quebec, Canada. Plasma glucose was measured on a Beckman II glucose analyzer (Beckman Instruments, Fullerton, California). Plasma and diluted pancreatic acid-ethanol extract immunoreactive insulin levels were measured using antibeef insulin antibody (Dr. Peter Wright), ^{125}I-porcine-insulin and rat insulin standards (24.5 U/mg; Novo Research Laboratory, Copenhagen, Denmark), and with dextran-coated charcoal separation of bound from free insulin. Concentrations ≤1000 ng/ml of cyclosporine had no effect on the insulin assay. Cyclosporine levels were measured using radioimmunoassay kits (Sandoz Canada Inc.) on plasma separated from blood without prior incubation.

RESULTS

The cyclosporine treatment was well tolerated by all rats, and their body weights were not different from those of the control rats that received the same volumes of olive oil vehicle (Tables 1 and 2). After 2 wk of cyclosporine in Wistar rats, their plasma concentrations were: 1018 ± 82 ng/ml, 4 h postdose; 1004 ± 67 ng/ml, at 8 h; 879 ± 86 ng/ml, at 16 h; and 373 ± 42 ng/ml, at the 24-h trough values. In BB rats, at 90 and 130 days of treatment (ages 120 and 160 days), all treated males showed hyperglycemia at 60 min, compared with controls (Table 1). Female non-diabetes-prone BB rats did not show glucose intolerance, but their plasma cyclosporine levels were lower (Table 1).

In the Wistar rats, glucose intolerance occurred after 1 wk of treatment, followed by a progressive increase of the 60 min plasma glucose during the first 5 wk of treatment (Figure 1). Plasma insulin was significantly lower at time 0 and 60 min at both 7 and 12 wk of therapy. Although there was a significant increase after glucose challenge, it was less than that in controls. The fasting plasma glucose was not systematically affected (Table 2). However, 24-h urine collections done after 12 wk of cyclosporine treatment showed glycosuria (data not shown). After 5 wk of the drug, the total pancreatic insulin was significantly lower in the cyclosporine-treated rats (35.9 ± 2.9 versus 116.8 ± 15.0 μg/g pancreas, in controls $P < 0.001$). The pancreatic morphology was normal in all cyclosporine-treated Wistar rats, with an absence of the classic signs of autoimmune involvement of the pancreas seen in the spontaneous BB rat diabetes syndrome (e.g., insulitis, periductular inflammation, or pancreatic lymphocytic infiltration).

When cyclosporine was stopped, 60-min glucose values

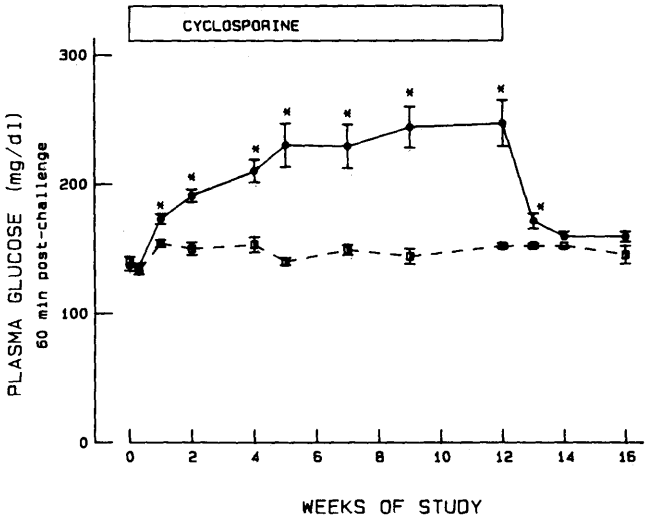

FIGURE 1. Time course of plasma glucose (mg/dl) 60 min after 0.25 g/kg glucose by gavage, without anesthesia, in cyclosporine-treated and control male Wistar rats. Data are presented as mean ± SEM. For both groups, N = 10. *Significantly different ($P < 0.05$) than controls.

TABLE 2
Glucose tolerance tests in Wistar rats during cyclosporine (CsA) treatment

	Plasma glucose (mg/dl)		Plasma insulin (ng/ml)		
	0 min	60 min	0 min	60 min	Weight (g)
4 h					
CsA-treated (N = 11)	95 ± 2	137 ± 4	0.42 ± 0.08	0.81 ± 0.08	239 ± 2
Controls (N = 9)	99 ± 3	140 ± 4	0.46 ± 0.09	0.68 ± 0.05	238 ± 2
2 days					
CsA-treated (N = 11)	109 ± 3	136 ± 3	0.54 ± 0.08†	1.22 ± 0.12	237 ± 3
Controls (N = 9)	113 ± 2	133 ± 3	1.03 ± 0.19	1.18 ± 0.11	237 ± 2
1 wk					
CsA-treated (N = 11)	110 ± 2	173 ± 4*	—	—	263 ± 2
Controls (N = 9)	113 ± 3	154 ± 3			263 ± 3
2 wk					
CsA-treated (N = 11)	92 ± 3*	191 ± 5*	—	—	281 ± 2
Controls (N = 9)	107 ± 3	150 ± 5			281 ± 4
4 wk					
CsA-treated (N = 10)	98 ± 2*	210 ± 9*	—	—	299 ± 3
Controls (N = 10)	116 ± 3	153 ± 6			299 ± 2
5 wk					
CsA-treated (N = 11)	120 ± 3	230 ± 17*	—	—	332 ± 5
Controls (N = 9)	120 ± 2	140 ± 3			336 ± 7
7 wk					
CsA-treated (N = 10)	121 ± 7	229 ± 17*	0.48 ± 0.02†	0.84 ± 0.11†	337 ± 6
Controls (N = 10)	133 ± 3	149 ± 4	1.06 ± 0.09	1.31 ± 0.12	328 ± 3
9 wk					
CsA-treated (N = 10)	127 ± 4*	244 ± 16*	0.85 ± 0.08	1.26 ± 0.15†	358 ± 6
Controls (N = 10)	110 ± 2	144 ± 6	0.99 ± 0.11	2.32 ± 0.21	363 ± 4
12 wk					
CsA-treated (N = 10)	126 ± 2	247 ± 18*	0.47 ± 0.08†	0.76 ± 0.13†	372 ± 28
Controls (N = 10)	120 ± 2	152 ± 2	0.89 ± 0.06	1.92 ± 0.19	370 ± 4
1 wk postwithdrawal					
CsA-treated (N = 10)	95 ± 2	171 ± 6*	—	—	371 ± 9
Controls (N = 10)	120 ± 2	152 ± 2			370 ± 4
2 wk postwithdrawal					
CsA-treated (N = 10)	114 ± 2	159 ± 4	0.29 ± 0.05†	0.78 ± 0.12†	382 ± 9
Controls (N = 10)	120 ± 2	152 ± 2	0.89 ± 0.06	1.92 ± 0.19	370 ± 4
4 wk postwithdrawal					
CsA-treated (N = 10)	109 ± 2	159 ± 4	0.38 ± 0.06	1.29 ± 0.09	399 ± 10
Controls (N = 10)	113 ± 3	145 ± 7			399 ± 7

*Glucose significantly different ($P < 0.05$) in CsA-treated versus controls.
†Insulin significantly different ($P < 0.05$) in CsA-treated versus controls.
Unpaired *t*-tests were used for statistical analysis.

decreased to near-normal levels at 1 wk, and to control levels by 2 wk (Table 2 and Figure 1). Plasma insulin responses remained decreased, but tended to rise at 4 wk. In the 5 Wistar rats that received the higher dose of cyclosporine, resulting in 16-h postdose plasma levels of 1758 ± 70 ng/ml, the glucose intolerance was greater (60-min value, 313 ± 17 mg/dl), whereas the fasting glucose was again unaffected.

In the Sprague-Dawley rats, glucose intolerance also occurred after 1 wk of cyclosporine treatment (60 min glucose,

190 ± 5 versus 173 ± 6 in controls, $P < 0.05$), despite lower plasma cyclosporine levels than those obtained in either Wistar or BB rats (378 ± 94 ng/ml 16 h postdose, $P < 0.05$). An increase of the dose to 15 mg/kg/day was followed by a rise in the 16-h postdose plasma cyclosporine levels to 932 ± 118 ng/ml, with a sharp rise in the glucose 60 min postchallenge (242 ± 16 versus 146 ± 7 for controls, $P < 0.001$). The fasting plasma glucose remained normal.

DISCUSSION

We have demonstrated a glucose intolerance-inducing effect of cyclosporine in male BB rats, Wistar non-BB rats, and Sprague-Dawley rats, using doses of oral cyclosporine that achieve "therapeutic" plasma cyclosporine levels.[1,6] This suggests that this effect is not strain related. The glucose intolerance occurred after 1 wk, but was absent after 4 h and 2 days. Intolerance seems related to circulating plasma cyclosporine levels, as suggested by the aggravation in both Wistar and Sprague-Dawley rats following an increase in the cyclosporine dose. (The data also suggest strain-related differences in drug kinetics, since the Sprague-Dawley rats had lower levels at the same dose.) Peripheral hypoinsulinemia was present during several of the tests, and this combined with markedly diminished total pancreatic insulin content after 5 wk of the drug treatment suggest that the major mechanism of glucose intolerance induction is via decreased insulin release, and possibly, decreased insulin biosynthesis. The absence of insulitis and inflammation in the pancreas of glucose-intolerant non-BB rat strains suggests that the immune process seen in diabetes-prone BB rats is not responsible for the B-cell dysfunction. After withdrawal of cyclosporine, the glucose tolerance improved after 2 wk, although plasma insulin responses remained decreased. This suggests that altered insulin sensitivity or insulin resistance may also have been present during cyclosporine administration, and that this effect was reversed more rapidly than was restoration of insulin secretion after withdrawal of the agent. A peripheral component is also suggested by the normal insulin values found in the non-diabetes-prone BB rats. The fasting hypoinsulinemia with euglycemia is harder to reconcile by such a mechanism.

Such effects of cyclosporine on glucose and insulin have been suggested by recent in vivo and in vitro studies. In man, it was reported that when azathioprine-prednisolone was replaced by cyclosporine-prednisolone in four diabetic men with both cadaver kidneys and pancreatic transplants, there was a deterioration in glucose metabolism despite an increase in fasting and stimulated plasma C-peptide.[14] It has been suggested that this effect could be related to a decrease in prednisolone clearance with cyclosporine,[15] but a direct effect of the drug could not be excluded. The usual use of corticosteroids with cyclosporine in human transplantation may explain why glucose intolerance has not been associated more frequently with cyclosporine therapy, in light of the frequency of its occurrence with steroids alone. No study has yet reported on glucoregulation with cyclosporine alone in man.

In cyclosporine-treated male Wistar rats, high doses of cyclosporine (50 mg/kg/day for 7 days) caused hyperglycemia with hypoinsulinemia associated with severe degranulation and hydropic degeneration of islet B-cells.[16] Mouse pancreatic islets in culture showed impaired islet proinsulin biosynthesis and insulin release in the presence of high cyclosporine concentrations.[17] One study, using a short in vitro incubation with the drug, failed to show effects on insulin release.[18] This, taken with the present results, suggests that time of exposure is an important factor in the effect on insulin secretion. At doses in the therapeutic range, we could not identify morphologic changes in the pancreas at the light microscopic level, including the immunoperoxidase stain for insulin. The marked decrease in pancreatic insulin content would be consistent with impaired insulin biosynthesis even at the lower cyclosporine levels.

The mechanisms of this glucose intolerance effect remain to be defined, although our data suggest that: (1) insulin secretion and possibly synthesis is impaired either in absolute terms or in relation to the glucose levels; (2) altered insulin binding and/or action may also play a role; and (3) the glucose intolerance is reversible upon cyclosporine withdrawal. The implications of the present findings are important. In diabetes prevention studies, it could be difficult to differentiate glucose intolerance due to the appearance of the diabetic diathesis, itself, from the cyclosporine effects. In studies of early cyclosporine treatment at diagnosis of diabetes, the glucose intolerance related to cyclosporine therapy may mask beneficial effects of the immunosuppressive therapy at the level of the insulitis. If the glucose intolerance is also found in humans, it may have the same confounding effect in current studies of the early therapy of diabetes with cyclosporine, and in eventual studies aimed at prevention in susceptible individuals. It is noteworthy that 100 ng/ml of cyclosporine in the medium of human islet cultures in vitro is sufficient to cause decreased insulin response to glucose.[19]

ACKOWLEDGMENTS

This study was supported by a grant from the Medical Research Council of Canada (MC-6540). J.-F. Yale was the recipient of a Postdoctoral Fellowship from the Medical Research Council of Canada. The authors express appreciation to Dr. P. Thibert for the supply of BB rats, to Marie Montambault and Madeleine Faucher for technical assistance, and to Jessie Blais for secretarial assistance.

REFERENCES

[1] Stiller, C. R.: The Canadian multicentre transplant study group: a randomized clinical trial of cyclosporine in cadaveric renal transplantation. N. Engl. J. Med. 1983; 309:809–15.

[2] Myers, B. D., Ross, J., and Newton, L.: Cyclosporine-associated chronic nephropathy. N. Engl. J. Med. 1984; 311:699–705.

[3] Ota, B., and Bradley, M.: Side effects of cyclosporine in 100 renal allograft recipients. Transplant. Proc. 1983; 15 (Suppl. 1):3150–55.

[4] Marliss, E. B., Ed.: The Juvenile Diabetes Foundation Workshop on the spontaneously diabetic BB rat: its potential for insight into human juvenile diabetes. Metabolism 1983; 32 (Suppl. 1):1–116.

[5] Yale, J. -F., and Marliss, E. B.: Altered immunity and diabetes in the BB rat. Clin. Exp. Immunol. 1984; 57:1–11.

[6] Stiller, C. R., Laupacis, A., Keown, P. A., Gardell, C., Dupre, J., Thibert, P., and Wall, W.: Cyclosporine: action, pharmacokinetics, and effect in the BB rat model. Metabolism 1983; 32 (Suppl. 1):69–72.

[7] Like, A. A., Dirodi, V., Thomas, S., Guberski, D. L., and Rossini, A. A.: Prevention of diabetes mellitus in the BB/W rat with cyclosporine A. Am. J. Pathol. 1984; 117:92–97.

[8] Laupacis, A., Gardell, C., Dupre, J., Stiller, C. R., Keown, P., Wallace, A. C., and Thibert, P.: Cyclosporine prevents diabetes in BB Wistar rats. Lancet 1983; I:10–11.

[9] Jaworski, M. A., and Honore, L.: Effect of prophylactic cyclosporine on the development of insulin-dependant diabetes mellitus and lymphocytic migration to target organs in autoimmunity in the BB rat. Clin. Res. 1984; 32:349A.

[10] Yale, J. F., Grose, M., Seemayer, T. A., and Marliss, E. B.: Response to cyclosporine administration at onset of diabetes in BB rats. Clin. Res. 1985; 33 (Suppl. 2):447A.

[11] Stiller, C. R., Laupacis, A., Dupre, J., Jeuner, M. R., Keown, P. A., Rodger, W., and Wolfe, B. M. J.: Cyclosporine for treatment of early type I diabetes: preliminary results. N. Engl. J. Med. 1983; 308:1226–27.

[12] Assan, R., Fenten, G,. Debray-Sachs, M., Quiniou-Debrie, M. C., Laboire, C., Thomas, G., Chatenoud, L., and Back, J. F.: Metabolic and immunological effects of cyclosporine in recently diagnosed type I diabetes mellitus. Lancet 1985; 1:67–71.

[13] Yale, J. -F., Grose, M., Seemayer, T. A., and Marliss, E. B.: Immunological and metabolic concomitants of cyclosporine prevention of diabetes in BB rats. Diabetes Research and Clinical Practice 1985; (Suppl. 1):S610.

[14] Gunnarsson, R., Klintmalm, G., Lundgren, G., Wilczek, H., Ostman, J., and Groth, C. -G.: Deterioration in glucose metabolism in pancreatic transplant recipients given cyclosporine. Lancet 1983; 2:571–72.

[15] Ost, L.: Effects of cyclosporine on prednisolone metabolism. Lancet 1984; 1:451.

[16] Helmchen, U., Schmidt, W. E., Siegel, E. G., and Creutzfeldt, W.: Morphological and functional changes of pancreatic B-cells in cyclosporine-A-treated rats. Diabetologia 1984; 27:416–18.

[17] Andersson, A., Borg, H., Hallberg, A., Hellerstrom, C., Sandler, S., and Schnell, A.: Long-term effects of cyclosporine A on cultured mouse pancreatic islets. Diabetologia 1984; 27:66–69.

[18] Laube, F., and Hahn, H. J.: Effect of cyclosporin-A on insulin secretion in vitro. Horm. Metab. Res. 1985; 17:43–44.

[19] Nielsen, J. H., Mandrup-Poulsen, T., and Nerup, J. *In* Diabetes Research and Clinical Practice 1985; (Suppl. 1):S405.