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Metabolic and ultrastructural effects of cyclosporin A on pancreatic islets

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Abstract We investigated the effect of different doses of cyclosporin A (CyA) on glucose and insulin levels, as well as its residual effects on pancreatic islets ultrastructure after discontinuation of the drug. We studied four groups of Wistar rats. One control- ($n = 5$) and three experimental groups, $n = 10$ each, were treated with different doses of CyA IM for 14 days: group I, 5 mg/Kg; group II, 15 mg/Kg; and group III, 25 mg/Kg. Five animals of each group were sacrificed after 14 days, and the remaining five after 21 days to assess residual CyA effects. On the day of sacrifice, the rats underwent maltose absorption test, and glucose and insulin levels were measured. Pancreatic biopsies were obtained on day 21 to evaluate islets ultrastructure by electron microscopy. As a result, statistically significant, dose dependent ($P < 0.05$) increases in glucose and insulin levels were observed in CyA-treated groups. Groups II and III showed insulin levels significantly higher after fasting ($P < 0.05$) on day 14 comparing to the controls, while in groups I and II values returned to normal after CyA discontinuation. Group III showed persistently increased insulin levels on day 21. Pancreatic ultrastructural changes were observed only in group III. We can conclude that CyA effects on glucose and insulin levels were temporary and reversible at low doses. Ultrastuctural changes in the pancreatic islets may occur with high doses of CyA.

Key words Cyclosporin A · Langerhans · Insulin

Introduction

The immunosuppressive effect of Cyclosporin A (CyA) is well known in the field of organ transplantation, and has improved the success rate in organ transplantation since its introduction. However many toxic effects of Cyclosporin A on kidney, pancreas, and liver, which sometimes require drug discontinuation, have been described [12, 13].

In a previous study we had examined the effects of CyA on glucose metabolism. Here CyA was shown to have a hyperglycemic effect in rats after small bowel transplantation as was confirmed by maltose absorption test (Mab-test) after surgery [2]. The hyperglycemia has

not been attributed to increased absorption from the graft but mainly to a CyA side effect on glucose metabolism.

To confirm these observations, we evaluated in the present study the influence of different doses of CyA on serum glucose and insulin levels, CyA residual effects, and drug induced pancreatic ultrastructural changes after discontinuation of the medication.

Materials and methods

Adult Wistar rats weighing between 250- and 350 g were divided in four groups: one control group of untreated rats ($n = 5$) and three experimental groups ($n = 10$ each) treated with varying doses of CyA for 14 days (group I, 5 mg/kg; group II, 15 mg/ kg; and group III, 25 mg/kg, administered once a day IM). Five animals of each group were sacrificed on day 14 (end of CyA administration); the remaining five animals of each group were sacrificed on day 21 to evaluate the residual CyA effect on pancreatic islets ultrastructure by histology.

On the day of sacrifice, animals were submitted to the Mab test. Blood samples were collected for measurements of plasma glucose after fasting, as well as after 60, 120, 180, and 240 min following maltose administration (0.5 g/kg) by gavage. For measurement of serum insulin levels by immunofluorometric assay, blood samples were obtained after fasting and 120 min after maltose intake. Insulin monoclonal antibodies were obtained from ascitic fluid of BALB/C mice challenged with tumoral cells.

Tissue Processing and Microscopy

At the time of sacrifice on day 21, the pancreas was excised. The pancreatic tissue was fixed for 60 min at room temperature with 2.5% glutaraldehyde and 2% formaldehyde (modified Karnowsky) in 0.1 M sodium cacodylate buffer at pH 7.2. After a buffer rinse (overnight) the material was post-fixed for 60 min at room temperature with buffered 1% osmium tetroxide and dehydrate in ethanol and propylene oxide. The material was embedded in araldite; semithin sections were stained with toluidine blue for light microscopy, and ultrathin sections were stained with 2% uranyl acetate in ethanol 50% and lead citrate for transmission electron microscopy (Jeol JEM-1200 EXII). The statistical significance was determined by using the non-parametric Mann Whitney and Kruskal-Wallis test ($P < 0.05$ was considered to be statistically significant).

Results

Statistically significant increases of glucose levels were observed in CyA-treated groups, and were dose dependent. Group II (15 mg/kg) and group III (25 mg/kg) showed statistically significant increases of glucose levels on day 14 ($P < 0.05$) as compared to those of the control group (Fig. 1 a). In addition, persistent increases of glucose levels could be seen on day 21 in group III ($P < 0.05$) even after 7 days of CyA interruption (Fig. 1 b). Serum insulin measurements also were CyA-dose-dependent. In groups II (15 mg/kg) and III (25 mg/kg) the fasting serum insulin values were significantly higher ($P < 0.05$) on day 14, as compared to those of group I and those of the control group (Fig. 1 c).

However, insulin levels returned to normal values in groups I and II after CyA interruption. Only animals of group III (25 mg/kg) showed persistently higher insulin levels on day 21 (Fig. 1 d). In group III, a decrease in serum insulin values was observed during the Mab test after 120 min.

Electron microscopy showed vacuolar degeneration of the pancreatic islet cells, with cytoplasmic disorder and nuclear pyknosis in group III (25 mg/kg) (Fig. 2 D) on day 21; the histological appearance in the remaining groups was normal (Fig. 2 A–C).

Discussion

Various studies on the side effects of CyA on humans and animals have shown controversial results, thus the mechanisms by which these effects are produced are mostly unknown. Much cytotoxic action has been reported in a variety of organs, and the pancreas is considered to be a possible target of immunosuppressive agents [5, 11]. Not only CyA but also FK 506, recently introduced among immunosuppressive medications, has shown to produce, though to a lower degree, some pancreatic changes.

The possible functional and ultrastructural alterations induced by immunosuppressive agents should be recognized, since, when a pancreatic transplant is performed with administration of CyA, graft viability may be altered [4]. Experimentally, high doses of CyA induce alterations on glucose metabolism that have been attributed to a toxic effect of the drug upon pancreatic beta cells [6]. Toxicity of the drug upon exocrine pancreas has already been shown [7]. Hirano et al. [7] demonstrated an alteration of carbacol induced amylase and lipase exocrine secretion under administration of CyA (50 mg/Kg), without affecting basal secretion of the enzymes. According to Suzuki et al. [14] and Anderson et al. [1], reduction in the synthesis of DNA, RNA, and protein is involved in the inhibitory effects of CyA on exocrine and endocrine pancreatic functions.

In our study, by using less toxic doses of CyA, we demonstrated an increase of fasting levels of glucose and insulin that are dose dependent. Hyperglycemia, through high levels of insulin, indicates a possible drug-induced peripheral resistance to insulin action. Nakaga-

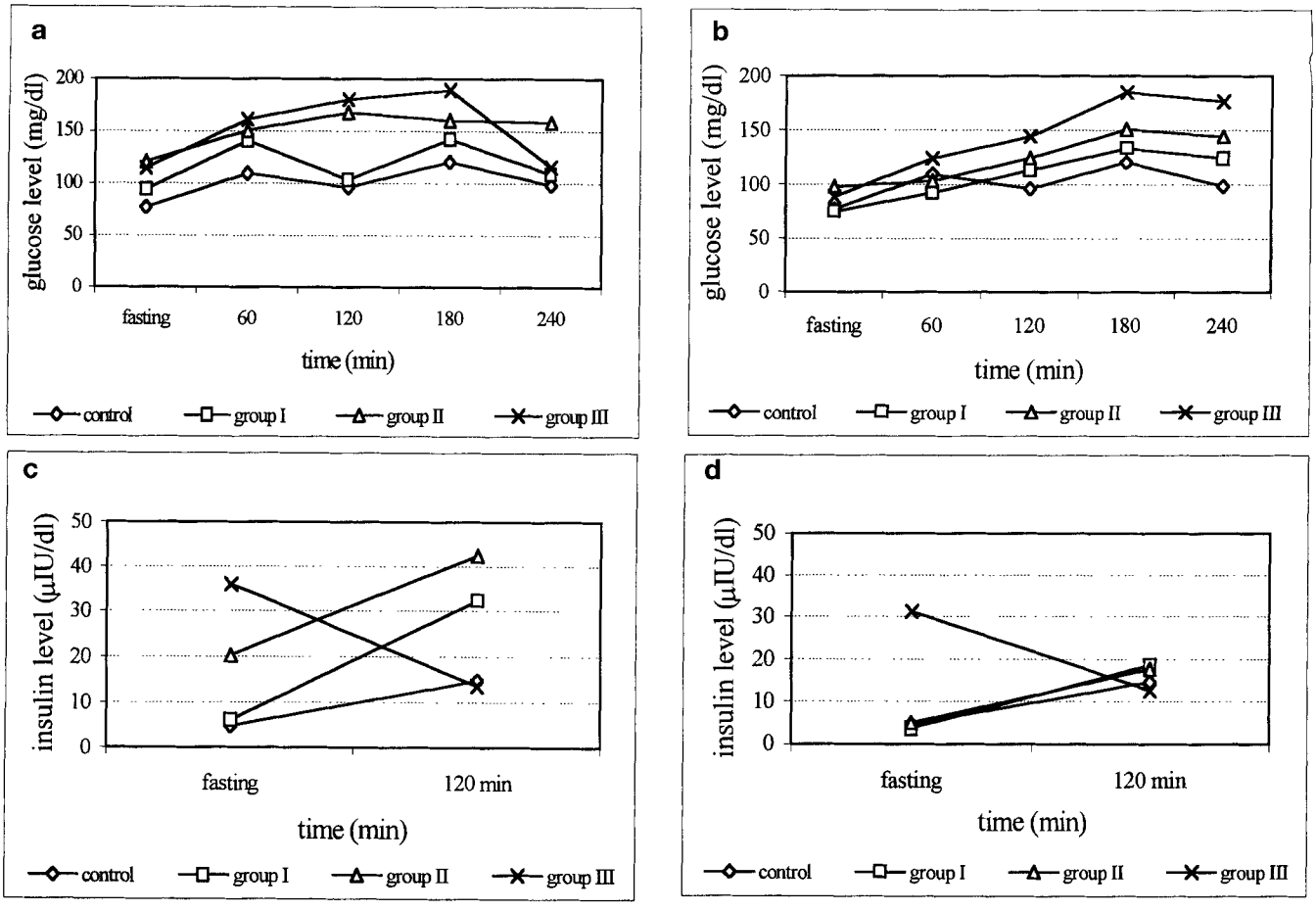

Fig. 1 Comparison of average plasma glucose levels at fasting and 60, 120, 180, 240 min after maltose administration on day 14 (**a**) and 21 (**b**). **c** and **d** illustrate the average serum insulin levels at days 14 and 21, respectively

wa et al. [10] reported raised blood glucose levels and significant abnormalities in a glucose tolerance test in parallel with CyA administration. The insulin-specific binding rates of erythrocytes in this study indicates changes of affinities of insulin receptors, but not changes in the value themselves. The rates obtained by this method were significantly lower in CyA-treated groups than those of controls, suggesting that the affinity of insulin receptors can be influenced by CyA. High levels of glucose with simultaneous high levels of insulin, which we observed in group II and group III after maltose administration, strengthen the hypothesis of an increased drug induced peripheral resistance to insulin action. The mechanisms involved on glucose intolerance, and the relationship between CyA and changes in insulin receptors, remain to be answered.

Our data show that, under therapeutic doses of CyA, insulin synthesis and secretion by pancreatic islets is not affected, as demonstrated by increase in the plasma insulin levels after maltose administration. This is in contrast to reports by Ishizuka et al. [8], Fehmann et al. [3] and Hahn et al. [5], who administered low doses of CyA and observed a decreased synthesis and secretion of insulin. Probably, a reduction in the synthesis is related to a reduction of insulin production in the pancreatic islets [5], but this aspect was not determined in our study. On the other hand, group III showed a reduction on insulin levels after maltose administration.

It remains uncertain whether the synthesis of insulin, the conversion of proinsulin to insulin and/or the secretory pathway itself is a consequence of a functional CyA effect on insulin metabolism or of the ultrastructural damage in the islets as was observed in group III. In contrast to the group III, the effects were temporary and reversible after discontinuation of the drug in groups I and II, with normalization of the plasmatic insulin at a basal levels . As already demonstrated by Nakagawa et al. [10] and Wahltrom et al. [15], glucose intolerance observed during CyA administration is caused by decreased insulin secretion and peripheral insulin resistance, dependent on the dose of CyA as well as on the duration of administration. Withdrawal of short-term

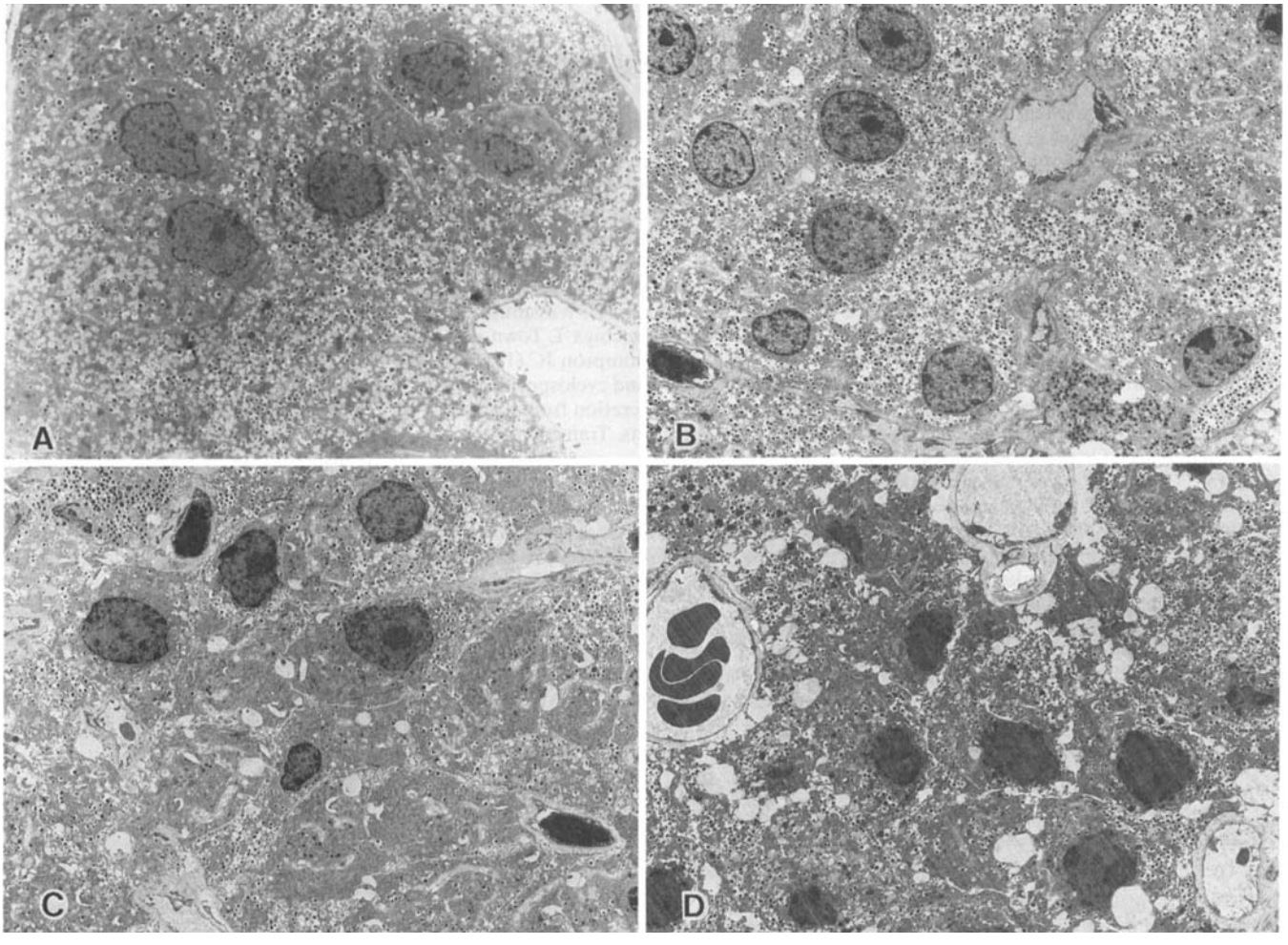

Fig. 2 Electron micrographs of pancreatic islets obtained at day 21. A normal histological appearance is seen in control (**A**) and animals treated with a CyA dose of: 5 mg/kg (**B**), 15 mg/kg (**C**). Vacuolar degeneration (*black arrow*), cytoplasmic disorder and nuclear pyknosis (*white arrow*) is observed in animals treated with 25 mg/kg of CyA (**D**), (× 2,250)

CyA treatment allows rapid normalization of the glucose intolerance, with normalization and overcompensation of both insulin secretion and sensitivity.

Electron microscopy of pancreatic biopsies in group III, obtained after sacrifice of the animals, showed apparent islet vacuolar degeneration, with cytoplasmic disorder and nuclear pyknosis. It is unclear if these ultrastructural changes explain the decline in serum insulin levels after 120 min of maltose administration in group III. The evaluation of insulin content in the pancreatic islet cells in this group could provide an answer to this question. According to Metrakos et al. [9], CyA exerts a blocking effect on proliferation and differentiation of islet cells, probably, by inhibition of trophic activity on ornithine-decarboxylase. In groups I and II we did not observe any important histological alteration on day 21, in accordance with Hahn et al. [5] who reported that functional alterations occur earlier than ultrastructural changes. However, as no histological examination was performed on day 14, we may have overlooked any minimal ultrastructural changes in the islets during the first two weeks.

In conclusion, the effect of administration of low doses of CyA on glucose and insulin metabolism is temporary and reversible. Ultrastructural changes in the pancreatic islets may occur only after administration of high doses of CyA. Whether morphological alterations induced by high doses of CyA are reversible or not, has yet to be demonstrated in a study with a longer period of observation.

References

1. Andersson A, Borg H, Hallberg A, Hellerstrom C, Sandler S, Schnell A (1984) Long-term effects of cyclosporin A on cultured mouse pancreatic islets. Diabetologia 27: 66–69
2. Bakonyi Neto A, Koh I, Goldenberg S (1994) Validity of the maltose absorption test in the heterotopic small bowel transplant model. Transplant Proc 26(3): 1670–1672
3. Fehmann HC, Haverich R, Stockmann F, Creutzfeldt W (1987) Cyclosporine A in low dose induces functional and morphologic changes in rat pancreatic beta cells. Transplant Proc 19: 4015
4. Gunnardson R, Klintmaln R, Lundgren G, Wilczek H, Ostman J, Groth CG (1983) Deterioration of glucose metabolism in pancreatic transplantation recipients given cyclosporin A. Lancet II: 571
5. Hahn HI, Laube R, Lucke S, Besch W (1994) Alteration of pancreatic beta cells in Wistar rats treated with non-diabetogenic doses of cyclosporin A. Pharmacol Toxicol 70: 188–191
6. Helmchen U, Schmidt WE, Siegel KG, Creutzfeldt W (1984) Morphological and functional changes of pancreatic beta cells in cyclosporin A–treated rats. Diabetologia 27: 416–418
7. Hirano Y, Hisatomi A, Ohara K, Noguchi H (1992) The effects of FK506 and cyclosporin A on the exocrine function of the rat pancreas. Transplantation 54: 883–887
8. Ishizuka J, Gugliuzza K, Wassmuth Z, Hsieh J, Sato K, Tsuchiya T, Townsend CM Jr, Fish JC, Thompson JC (1993) Effects of FK506 and cyclosporine on dynamic insulin secretion from isolated dog pancreatic islets. Transplantation 56: 1486–1490
9. Metrakos P, Hornby L, Rosenberg L (1993) Cyclosporine and islet mass – Implications for islet transplantation. J Surg Res 54: 375–380
10. Nakagawa H, Yamashita T, Nagai Y, Ishikawa T, Ohira M, Park I, Yada K, Yamamoto Y Umeyama K, Sowa M (1992) Influence of cyclosporine on glucose tolerance in rats. Transplant Proc 24: 1635–1637
11. Nielsen JH, Mandrup-Poulsen T, Nerup J (1986) Direct effects of cyclosporin A on human pancreatic beta cells. Diabetes 35: 1049–1052
12. Ota B, Braadley M (1983) Side effects of cyclosporin in 100 renal allograft recipients Transplant Proc 15 (Suppl. 1): 3150–3156
13. Ryffel B, Donatsch P, Madorim M, Matter BE, Ruttimann G, Schon H Stoll R, Wilson I (1983) Toxicological evaluation of cyclosporin A. Arch Toxicol 53: 107–141
14. Suzuki S, Oka T (1989) Inhibitory effects of cyclosporin on exocrine and endocrine functions of the rat pancreas. Transplant Proc 21: 913
15. Wahlstrom HE, Akimoto R, Endres D, Kolterman O, Moossa AR (1992) Recovery and hypersecretion of insulin and reversal of insulin resistance after withdrawal of short-term cyclosporine treatment. Transplantation 53: 1190–1195