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Effects of Cyclosporin A on rat pancreatic β and D cells and glucose levels (An immunocytochemical study)

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Abstract: The effects of cyclosporin A (CsA) on rat pancreatic islet β and D cells and plasma glucose levels at doses of 50 mg/kg body weight applied for 20 days were investigated. Fifteen Swiss Albino rats were used as the study group and 5 were the control group. Rat pancreatic islet β and D cells were stained immunocytochemically by anti-insulin and anti-somatostation antibodies. β and D cells from 15 different islets were evaluated qualitatively and quantitatively. Plasma glucose changes were also compared between the study and the control group. The pancreas morphology was found to be normal in both groups by light microscopy. The difference between the numbers of β and D cells of pancreatic islets among the groups was not statistically significant. The staining intensity of islet β cell cytoplasms was lighter in the CsA administered rats. Plasma glucose levels of CsA-exposed rats increased significantly compared with the control group ($p<0.05$).

In concluson, pancreatic islets were not found to be affected morphologically by CsA at the light microscopic level. The present findings show that CsA impairs the glucose metabolism possibly due to β cell dysfunction.

Key Words: Cyclosporin A, pancreatic islet, β cell, D cell, glucose.

Introduction

Cyclosporin A (CsA), discovered in 1972 by Borel, has rapidly become the most commonly used immunosuppressive agent in clinical transplantation (1-8). It has also become a therapeutical alternative for immune suppressive therapy in Type I diabetes, since there is evidence that an autoimmune process directed toward the insulin producing β cells may play a role in the development of the disease (1, 4, 6, 7). The immunosuppressive effect of CsA was mediated through blocking of the precursor T cell in G_0 or early G_1 plase of the cell cycle (1). CsA can exert hepatotoxic and nephrotoxic effects related to dose and duration. It has also been demonstrated that CsA accumulates in the pancreas resulting in high localized tissue concentrations (2, 3). Other side effects, usually mild, include tremors (15%), hirsutism (12%), and gingival hyperplasia and insulin metabolism are conflicting. Several in vitro studies have shown that CsA has an adverse effect on islet β cell function even with therapeutically recommended doses and it impairs insulin synthesis and secretion. This has resulted in hypoinsulinemia, glucose intolerance, and hyperglycemia accompanied by marked decrease in pancreatic islet insulin content or release (1-3, 6-8). In contrast, some studies have suggested that CsA did not alter directly the insulin producing cells in vitro (4, 5). There is also some information about the effect of CsA on somatostatin producing D cells.

We performed an immunocytochemical investigation to determine the effects of CsA on insulin producing β cells and somatostatin producing D cells in rat pancreas islets. Moreover, we aimed to detect the changes in plasma glucose concentrations due to CsA.

Materials and Methods

This study was performed by the Internal Medicine and Pathology Departments at the Experimental and Clinical Investigations Center of Erciyes University, Medical School. The principles of laboratory animal care were followed. A group of 15 Swiss Albino rats were used in the study. Another group of 5 rats were used as controls. At the beginning of the study, after overnight fasting, 1-2 ml heparinized blood samples were obtained from the left jugular vein in aseptic conditions from both gorups. The first group consisted of 15 rats receiving daily subcutaneous injections of 50 mg/kg body weight of CsA for 20 days. Following the last injection (day 20), the rats were sacrified by dense ether anesthesia and average 1-2 ml blood samples were collected from the same site.

The abdomen of each rat was opened and after dissecting the muscles and intestines, pancreas was removed. Pancreatic tissue was then fixed in 10% formalin. After 24 hours of fixation, the pancreas tissue was embedded in paraffin wax. Sections 5 μm thick were cut and stained with hematoxylin and eosin. Light microscopic examination was performed.

For immunochemistry, same thickness, formaline-fixed, and paraffin embedded sections were stained by polyclonal antisera as anti-insulin (Insulin, polyclonal, cat no: AG029-5P, BioGenex Lab, San Ramon, CA, 94583, USA) and anti-somatostatin (Somatostatin, polyclonal, cat no: AR042-5R, BioGenex Lab, San Ramon, CA94583, USA). Insulin-immunoreactive β and somatostatin-immunoreactive D cells from 15 different areas of islets were counted under a light microscope at 40x magnification for the study and the control group. The intensity of cytoplasmic immunostaining was determined as "normal" or "light". The number of cells stained normal or lightly was also counted in both groups. Fasting plasma glucose levels were measured by routine biochemical methods at university hospital laboratories.

Statistical analysis. All results were expressed as mean±SEM. The differences between CsA administered and control rats were compared with the Mann-Whitney U test and paired t test. A p value <0.05 was considered statistically significant.

Results

Morphological assay. The rat pancreatic islets morphology of CsA administered rats and the control rats had similar features to each other and were found to be normal by light microscopic examination.

Immunocytochemical assays. The changes in the number of insulin-immunoreactive cells in pancreatic islets of CsA administered rats were not significantly different from those in the control group (Table 1). Similarly, numbers of the somatostatin-immunoreactive cells in CsA group rats were not significantly different from these in the control rats (Table 2).

Table 1. Number of insulin-immunoreactive cells in CsA administered and control group in pancreatic islets.

Groups	n	Minimal-Maximal	Median
CsA	15	445-635	515
Control	5	489-574	497

u: 30.0, p>0.05.

Table 2. Numbers of somatostatin-immunoreactive cells in CsA administered and control group in pancreatic islets.

Groups	n	Minimal-Maximal	Median
CsA	15	180-264	230
Control	5	199-223	211

u: 22.0, p>0.05.

In immunostained sections, the insulin-immunoreactive cells showed generally light immunostaining in the CsA administered group (Figure 1) but the cytoplasms in the control rats generally showed normal staining (Figure 2). The number of islets with lightly stained insulin-immunoreactive cells were more than the islet numbers with normally stained cells in CsA administered rats. This difference was not statistically significant (Table 3).

Biochemical assay. On day 20, the mean fasting plasma glucose levels of CsA administered rats were significantly higher than plasma glucose concentrations before CsA administration (198.87±16.8 mg/dl versus 107.40±10.3 mg/dl, p<0.05) (Table 4). No significant change was found in the control group (106.72±10.8 versus 107.38±10.1).

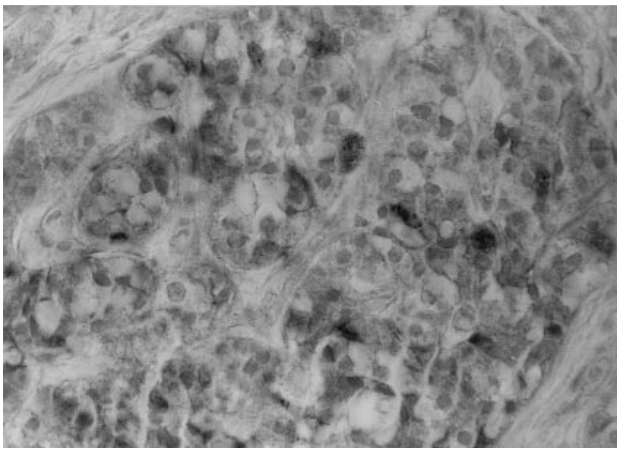

Figure 1. The insulin-immunoreactive cells showed mostly light immunostaining in the CsA administered group (immunochemistry, 40x).

Discussion

We found no morphological changes in rat pancreas islets compared with control rats under light microscopic examination even at doses of CsA as high as 50 mg/kg for 20 days. At therapeutic doses (15 mg/kg), no

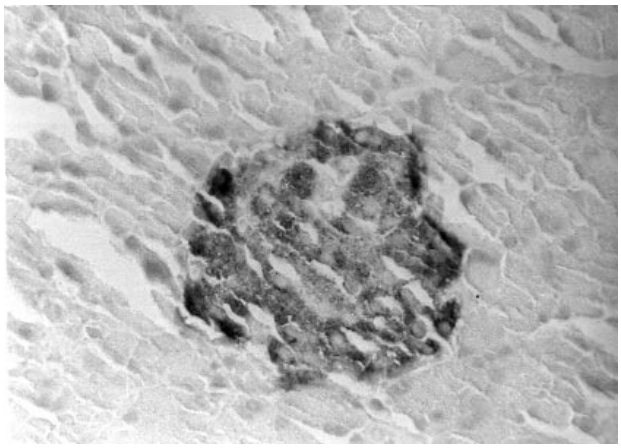

Figure 2. Mostly normally stained β cells in the control rats (immunochemistry, 40x).

Table 3. Intensity of immunostaining of insulin-immunoreactive cells in CsA administered and control group in pancreatic islets.

	Normal		Light	
Islet number (n=15)	Median	Min-Max	Median	Min-Max
CsA	6	3-7	9	8-12
Control	10	9-12	5	3-6

u: 75.0, p>0.05u: 50.0, p>0.05

Table 4. Plasma glucose concentrations in CsA administered rats before and after study.

Time	n	mean±SEM (mg/dl)
Day0	15	107.40±10.3
Day20	15	198.87±16.8

t: 16.23, p<0.05.

morphological changes could be identified in the pancreas at the light microscopic level (8). Ultrastructural studies of the CsA exposed islet cells showed no signs of nuclear pycnosis (1). Andersson et al. (1) reported that the mitochondria and endoplasmic reticulum had not been affected and the β cells were well granulated. These findings were confirmed by Laube et al. (4) since in their research electron micrographs of CsA-treated islets did not differ from cultured control islets. In contrast, Bani-Sacchi et al. (2) observed different degrees of dilatation of rough endoplasmic reticulum cistarnae and enlargement of the Golgi apparatus in β cells, together with a pronounced reduction in the number of secretory granules. They noted that there were no apparent morphological changes in the other cytoplasmic organelles. They suggested that CsA induced a substantial defect in granulogenesis.

We also could not find and significant difference between the number of insulin-immunoreactive cells of the pancreatic islets of CsA administered group and the control group. No direct effect on β cells occurs numerically. Therefore the mechanism of action has to be due to drug-induced dysfunction. Indeed, studies have revealed no β cell degeneration or cell death (2, 6-8). CsA effects on insulin content and the secretion function were determined in isolated pancreas incubations. Most of these studies suggested that insulin content or secretion function decreased due to CsA administration (1-3, 6-8). It was found that CsA impairs islet β cell function and it was suggested that the detrimental effect was located at the level of protein biosynthesis and added that the influence of CsA on the glucose recognition system could not be excluded (1). The decrease in immunoreactive insulin and glucose intolerance together with a reduction in the number of secretory granules were related to impairment in the intracellular transport of the hormone from the sites of synthesis to the secretory granules induced by CsA (2). The impaired carbonhydrate tolerance was thought to be due to the marked reduction of the pancreatic insülin reserve (3). Nielsen et al. (6) reported that CsA has a direct inhibitory effect on insülin release from human islets with a concomitant increase in the residual insulin content. The same study also showed that CsG inhibited insulin release, while CsD had no consistent effects and the authors suggested that there is a difference in immunosuppressive potency between these analogues. Similar results were reported by Robertson (7) showing that CsA inhibits glucose-induced insulin secretion in a dose-response manner at concentrations commonly found in blood. Total pancreatic insulin was decreased to one third of the control after 5 weeks and after withdrawal of cyclosporin, impaired glucose tolerance returned to normal within 2 weeks in one study (8). These results were related to the inhibition of insulin secretion and also possibly the inhibition of synthesis since the plasma insulin levels were significantly low.

In contrast, the results of some studies showed no effects of CsA on the endocrin pancreas in vitro (4, 5). Laube et al. (4) observed minimal changes even with very high levels of CsA in short term effects. Exposure to long term CsA resulted in a minimal decrease in insulin content which was not accompanied by drastic changes in insulin secretion. They assumed that CsA lead to minimal functional changes in the endocrin pancreas even in toxic concentrations. Another study demonstrated no reduction

in the glucose-induced insulin secretion of rat islets (5). Since they observed a slight increase in hormone release, the authors concluded that CsA did not directly and immediately alter the insulin-producing cells.

In immunostained sections, the insulin-immunoreactive cells generally showed a light immunostaining in the CsA administered group but normal staining of the cytoplasms of the control rats. This staining difference was possibly due to a decrease in cytoplasmic granules. We also demonstrated the glucose intolerance-inducing effect of CsA in rats. When both findings were interpreted together, we thought that CsA may have caused a defect in granulogenesis. This result is compatible with the suggestion of Bani-Sacchi et al. (2) who assumed that the impairment in the secretory processes of β cells was due to CsA.

We found no difference between the numbers of the somatostatin-immunoreactive cells in the CsA group and in the control rats. CsA did not seem to effect the somatostatin producing cells, morphologically. In one study, mean areas of somatostatin immunoeactivity showed a significant increase (2). Glucagon content, which we did not investigate in the present study, was determined in several studies. Either glucagon content or mean areas of glucagon immunoreactivity did not change in some of these studies (2, 6). It was reported that the glucagon content of islets was found to be decreased significantly after 3 weeks administration of CsA (3).

In conclusion, the present data reveal that CsA impairs glucose metabolism due to β cell dysfunction. We also observed that CsA did not affect pancreatic islets morphologically.

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