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Morphological and functional changes of pancreatic B cells in cyclosporin A-treated rats

U. Helmchen[1], W. E. Schmidt, E. G. Siegel and W. Creutzfeldt

Division of Gastroenterology and Metabolism, Department of Medicine, and [1]Department of Pathology, University of Göttingen, Göttingen, FRG

Summary. Cyclosporin A (50 mg/kg orally for 7 days) produced severe degranulation and hydropic degeneration of islet B cells in rats. These changes were accompanied by hyperglycaemia and hypoinsulinaemia, while the pancreatic insulin content decreased by 75%.

Key words: Cyclosporin A, B cell changes, pancreatic insulin content, insulin levels, hyperglycaemia.

Cyclosporin A (CsA) has proved to be a powerful immunosuppressive agent in the prevention of graft rejection after transplantation of solid organs or bone marrow [1]. Renal and hepatic toxicity are known to be the main untoward effects of CsA in patients and in experimental animals [2, 3]. We wish to draw attention to lesions of pancreatic islet B cells and alterations of insulin secretion in rats treated with high doses of CsA.

Methods

Male Wistar rats were tube fed once daily for 7 days, after 12 h fasting, with either CsA (50 mg/kg) in olive oil or olive oil alone. On day 8, blood was collected during pentobarbital anaesthesia (90 mg/kg Inactin) from the abdominal aorta and the animals were sacrified by bleeding. Creatinine, glucose, insulin and CsA concentrations were measured in serum or blood, respectively.

For light microscopy, paraffin sections of Bouin-fixed pancreas were stained with (1) haematoxylin/periodic acid Schiff, (2) aldehyde-thionin, (3) peroxidase-anti-peroxidase (PAP) technique applying specific antisera raised against insulin, glucagon, somatostatin and pancreatic polypeptide. For electron microscopy, tissue was fixed in 1.5% glutaraldehyde, embedded in araldite and ultra-thin sections were stained with uranyl acetate and lead citrate.

Insulin and CsA were extracted from deep-frozen pancreas using cold 0.7 N HCl/ethanol (1:3). For CsA determination the pellet was re-extracted in 0.05 mol/l Tris-buffer (pH 8.5) with 0.3% Tween 20/ethanol (1:1) after centrifugation (2,000 *g* for 10 min). Insulin and CsA content were estimated by radioimmunoassay.

The data are given as mean ± SEM. $p < 0.05$ was considered significant by Student's t-test.

Results

The CsA-treated rats lost weight during the 8-day period (CsA group 314 ± 3.7 versus 274 ± 5.6 g; oil group 315 ± 4.6 versus 319 ± 4.9 g). At the end of the experiment, the plasma creatinine and glucose concentrations were significantly increased in the CsA group, while the serum insulin levels decreased. The pancreas insulin content decreased significantly while the CsA concentration in blood and pancreas reached very high levels (Table 1).

Table 1. Effect of treatment of rats with cyclosporin A (50 mg/kg orally for 7 days)

	Control rats ($n=9$)	CsA-treated rats ($n=9$)
Creatinine (μmol/l)	48.6 ± 7.1	128.2 ± 8.8[a]
Serum glucose (mmol/l)	7.2 ± 0.4	10.9 ± 0.8[a]
Blood CsA (ng/ml)	Undetectable	4990 ± 454[a]
Serum immunoreactive insulin (U/l)	64 ± 11	< 6.25[a]
Pancreatic CsA (μg/mg wet weight)	Undetectable	2.46 ± 0.26[a]
Pancreatic immunoreactive insulin (U/g wet weight)	2.09 ± 0.22	0.52 ± 0.03[a]

Results expressed as mean ± SEM

[a] $p < 0.001$

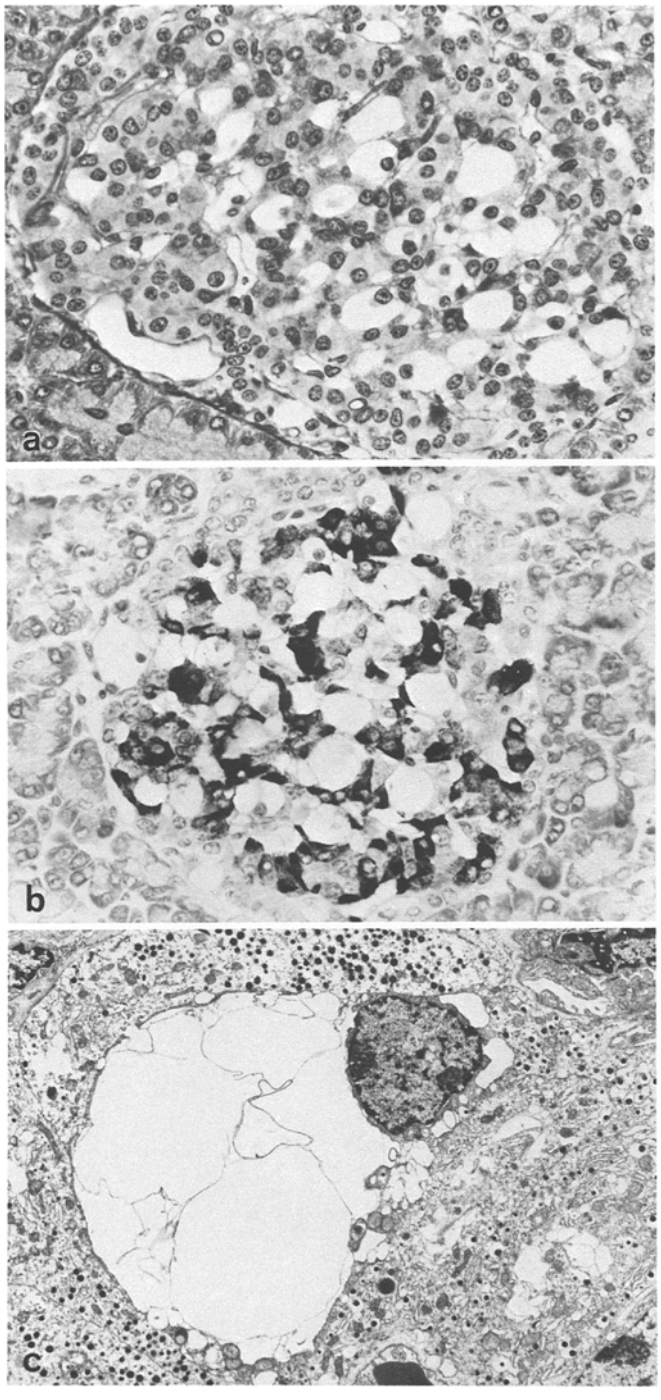

Fig. 1 a–c. Rat pancreatic islets after oral administration of 50 mg/kg CsA for 7 days. **a** Almost complete degranulation and partial hydropic degeneration of B cells (aldehyde-thionin, × 327); **b** pancreatic islet with hydropic B cells, still reacting with anti-insulin serum (PAP technique, × 327); **c** hydropic B cell containing residual membranes and early nuclear pycnosis. Degranulation and focal membraneous dilation within adjacent B cells (glutaraldehyde fixation, × 3960)

Morphology

Most pancreatic islets of CsA-treated rats studied on PAS-stained sections contained several, mainly centrally situated B cells of hydropic appearance due to cytoplasmic vacuolization. The vacuoles were PAS-negative. Aldehyde-thionin staining revealed an almost complete degranulation not only of the vacuolized, but also of the remaining B cells (Fig. 1 a). Neither the pancreatic islets nor the surrounding acinar cells were infiltrated by leucocytes or macrophages. Immunohistochemistry showed that both the vacuolized and the intact B cells still reacted with insulin antibodies (Fig. 1 b). No major alterations were observed in glucagon, somatostatin, or pancreatic polypeptide cells labelled by specific antisera.

Ultrastructurally, the cytoplasmic vacuolization corresponded to a dilated endoplasmic reticulum, ranging from focal changes to complete ballooning of the B cells (Fig. 1 c). In severely hydropic B cells the nuclei displayed signs of early pycnosis.

Discussion

This study clearly shows that in rats high doses of CsA (exceeding the immunosuppressive dose of 5 mg/kg [4] and leading to CsA blood levels of 4 μg/ml) produce severe morphological and functional alterations of the pancreatic B cells. The degranulation and hydropic degeneration of the B cells correspond to a decrease of the pancreatic insulin content and insulin plasma levels and to hyperglycaemia of the animals. They are unlike any known morphological changes produced by β cytotoxins (alloxan, streptozotocin, cyproheptadine) but resemble the hydropic A cells as seen after $CoCl_2$ and synthalin treatment [5].

It is very tempting to relate these changes to the well-known toxic and protein synthesis – inhibitory effects of high dosages of CsA. However, this would not explain the selective damage of the islet B cells. The highest concentrations of CsA have been found in the liver and the pancreas [6]. An accumulation of CsA in the pancreas has been confirmed by this study. It is not known whether CsA specifically accumulated in the B cells, thus interfering with cellular function.

Similar observations have been reported recently in rats [7]. The significance of the finding for other species and for the immunosuppressive therapy with CsA in man is unknown. Preliminary studies in our laboratory have resulted in B cell changes (degranulation and dilatation of the endoplasmic reticulum in a significant number of B cells) in rats treated for 3 weeks with only 6.25 mg/kg CsA daily. After replacement of azathioprine by CsA without simultaneous change of the glucocorticoid dose in kidney-transplanted subjects, deterioration of glucose tolerance has been described [8].

This hitherto unknown side effect of CsA should be investigated further, especially since CsA is being used experimentally in the treatment of acute-onset Type 1 (insulin-dependent) diabetes [9] and various immunological disorders [10].

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Professor Werner Creutzfeldt
Medizinische Universitätsklinik
Robert-Koch-Straße 40
D-3400 Göttingen
FRG