Diabetologia (1984) 27: 66–69

Diabetologia

Long-term effects of cyclosporin A on cultured mouse pancreatic islets

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Summary. In the light of recent attempts to treat newly-diagnosed Type 1 (insulin-dependent) diabetic patients with cyclosporin A, and reports suggesting an impaired glucose tolerance following immunosuppresion therapy with cyclosporin A, we investigated the long-term effects of cyclosporin A on islet β-cell morphology and function in vitro. Collagenase-isolated mouse pancreatic islets were cultured free-floating for 7 days in medium RPMI 1640 + 10% calf serum in the presence of cyclosporin A (0.1 or 1.0 mg/l). Islets cultured in the presence of the higher cyclosporin A concentration had impaired islet proinsulin biosynthesis and insulin release when challenged with high glucose concentration. Moreover, the insulin content of the drug-exposed islets was decreased and so was the rate of DNA synthesis. The glucose oxidation and respiratory rates, however, remained unaffected, suggesting that the impaired insulin production was not a result of defective oxidative metabolism. There were no changes in the ultrastructure or phospholipid biosynthesis of the islets after the drug treatment. These data indicate that cyclosporin A affects islets in culture, the clinical implications of which are so far difficult to assess. The inhibitory effect of cyclosporin A on islet cell DNA synthesis must nevertheless be considered in attempts to ameliorate Type 1 (insulin-dependent) diabetes, and when grafting islet cells in numbers primarily insufficient to cure the recipient.

Key words: Islet β cell, cyclosporin A, mouse.

Despite its liver and kidney toxicity cyclosporin A (CsA) has rapidly become the immunosuppressive agent most commonly used in clinical transplantation [1] including pancreatic transplantation [2]. CsA has also become an interesting therapeutical alternative for immune suppression therapy in Type 1 diabetes, since there is evidence that an autoimmune process directed at the insulin-producing β cells may play a role in the development of this disease [3]. Indeed, CsA has been found to prevent the onset of insulin-dependent diabetes in BB Wistar rats [4]. On the other hand, it was recently reported that when switching four diabetic patients with combined cadaver kidney and pancreatic transplants from azathioprine-prednisolone to CsA-prednisolone immunosuppressive therapy there was a deterioration in glucose metabolism [5]. Against this background, we investigated the possible adverse long-term effects of CsA on several aspects of pancreatic islet function by adding the drug, in the range of therapeutic concentrations, to media used for organ culture of pancreatic islets isolated from mice.

Materials and methods

Islet preparation and culture

Male, adult, non-inbred NMRI-mice (Anticimex, Sollentuna, Sweden) were used. Pancreatic islets, prepared by a collagenase method [6], were transferred in groups of approximately 100, not permitting cell attachment, to plastic Petri dishes (Sterilin, Teddington Middlesex, UK) containing 5 ml tissue culture medium RPMI 1640 (glucose 11 mmol/l) supplemented with 10% calf serum and antibiotics [7]. Cyclosporin A (1 mg, generously given by Sandoz, Täby, Sweden) was dissolved in absolute ethanol 0.1 ml, to which 0.02 ml Tween 20 was added. Finally, medium RPMI 1640 was added dropwise to this solution to a final volume of 1 ml. Islets serving as controls were cultured in a medium supplemented with the appropriate solvents only. The medium was changed on days 3 and 5 of culture. Immediately before islet harvest at the end of the 7-day culture period, samples of the culture media were collected for radioimmunoassay of insulin [8].

Islet viability tests

Procedures for the incubation of the cultured islets in vitro have been described previously in detail: insulin release and content [7], proinsulin biosynthesis [7, 9] glucose oxidation and islet perifusion [10], oxygen uptake [11], phospholipid biosynthesis [12] and DNA synthe-

Table 1. Long-term effects of cyclosporin A on insulin production

	Control islets	Islets cultured with CsA (0.1 mg/l)	Islets cultured with CsA (1 mg/l)
Insulin accumulation (ng/dish)	1845 ±180	1475 ±180	1580 ±300
Islet insulin content (ng/5 islets)	304 ± 23	218 ± 15[a]	223 ± 19[a]
Insulin release (ng/60 min per 5 islets)			
Glucose (1.67 mmol/l)	1.2± 0.2	1.3± 0.2	1.0± 0.2
Glucose (16.7 mmol/l)	21.7± 1.7	17.7± 1.8	10.7± 1.2[c]
Proinsulin biosynthesis (dpm × 10^{-3}/120 min per 10 islets)	33.7± 3.8	29.0± 3.8	16.6± 2.0[b]
Total protein biosynthesis (dpm × 10^{-3}/120 min per 10 islets)	140.7± 14.5	122.4± 13.9	90.6± 10.5[a]
Proinsulin biosynthesis as proportion of total protein biosynthesis (%)	24.3± 1.7	23.5± 1.5	18.6± 1.2[a]

The islets were cultured for 1 week in RPMI 1640 supplemented with 10% calf serum and CsA.
CsA was not added to the short-term incubation media. Values are expressed as mean ± SEM for six experiments.
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ versus control islets

Table 2. Long-term effects of cyclosporin A on islet cell replication and glucose metabolism

	Control islets	Islets cultured with CsA 1 mg/l
DNA synthesis (dpm/ng DNA)	5.0 ± 0.4 (12)	3.9 ± 0.3[a] (12)
Autoradiographic islet-cell labelling index (%)	4.24± 0.35 (6)	1.57± 0.34[b] (6)
DNA content (ng/islet)	20.2 ± 1.0 (12)	23.2 ± 1.2 (12)
Glucose oxidation (16.7 mmol/l) (pmol/120 min per 10 islets)	708 ±36 (6)	631 ±33 (6)
Respiration (nl/islet)		
In glucose (1.67 mmol/l)	6.6 ± 0.6 (5)	5.4 ± 0.4 (6)
In glucose (16.7 mmol/l)	11.9 ± 1.3 (5)	8.8 ± 0.8 (6)
Percentage respiratory change	75 ±11 (6)	64 ±12 (6)
D-(U-^{14}C)glucose incorporation (16.7 mmol/l) (pmol glucose/120 min per 25 islets) into:		
Phosphatidylinositol	1.89± 0.15 (13)	1.86± 0.23 (12)
Phosphatidylserine	1.34± 0.24 (13)	1.07± 0.19 (12)
Phosphatidylcholine	9.41± 0.56 (13)	9.94± 1.17 (12)
Phosphatidylethanolamine + phosphatidylglycerol	3.58± 0.23 (13)	3.52± 0.42 (12)

The islets were cultured for one week in RPMI 1640 supplemented with 10% calf serum and CsA. CsA was not added to the short-term incubation media. Values are expressed as mean ± SEM with number of experiments within parentheses. [a] $p < 0.05$ and [b] $p < 0.001$ versus control islets

sis [13]. The procedure for determination of islet-cell labelling index has recently been described [14]. In the present experiments the concentration of ^{3}H-thymidine added to the culture medium was 1 mCi/l and the labelling period was extended to 16 h. Details of the preparation of the electron micrographs were published recently [15].

Results

Insulin production

Culture of islets for 1 week in the presence of CsA/l (0.1 or 1 mg) produced a slight reduction of the islet insulin content, whereas no effects of the drug were observed on the accumulation of insulin in the culture medium (Table 1). When challenged with high glucose concentration in the subsequent short-term incubations, the islets exposed to the higher CsA concentration secreted less insulin than the control islets (Table 1). They were, however, still responding to high glucose with a tenfold increase of insulin secretion. In perifusion experiments islets cultured in the presence of CsA (1 mg/l) showed a marked attenuation of both the first peak and the second, lower phase of insulin release when stimulated with high glucose (16.7 mmol/l) (data not shown).

Glucose-stimulated proinsulin biosynthesis of islets exposed to CsA (1 mg/l) was reduced to < 50% that of the controls (Table 1). A significant but less pronounced impairment of total protein biosynthesis led to a decrease in the percentage of radioactivity incorporated into proinsulin in relation to the totally incorporated radioactivity (Table 1).

Islet cell DNA synthesis

There was a slight reduction in the rate of DNA synthesis in islets exposed for 1 week to CsA (1 mg/l) as shown by the incorporation of ^{3}H-thymidine into islet DNA (Table 2). Likewise, the labelling index of the CsA-exposed islet cells was significantly decreased. This impairment, however, did not affect the islet content of DNA (Table 2).

Glucose metabolism

No long-term metabolic effects of the drug on the oxidative islet metabolism were recorded in the glucose

oxidation and oxygen uptake experiments (Table 2). Thus, exposure of islets for one week to CsA (1 mg/l) did not affect the subsequent production of $^{14}CO_2$ from D-(U-^{14}C)glucose during a 2-h incubation period. Neither did the drug exposure influence the increase in respiratory rate when the islets were challenged with high glucose concentration in Cartesian divers. Finally, no effects were observed on the incorporation of ^{14}C-glucose into the different phospholipid classes of islets exposed to CsA for one week (Table 2).

Islet cell ultrastructure

Ultrastructural studies of the CsA-exposed islet cells showed no signs of nuclear pycnosis. The mitochondria and endoplasmic reticulum were not affected. The β cells were well granulated and, as in the controls, lysosomal structures were frequently observed.

Discussion

Knowledge about the mechanism of action of CsA still remains incomplete, although it has been suggested that this drug interferes with the process of primary lymphocyte activation [16]. Even less is known about the mechanisms behind the toxic effects of CsA on kidney and liver cell function. From a clinical point of view, it has been found that these side effects are reversible and dose-dependent. The impairment of glucose tolerance reported by Gunnarsson et al. [5] following a change in immunosuppressive treatment from azathioprine to CsA in four kidney-plus-pancreas-grafted patients were attributed to an increased insulin resistance, perhaps following an increased circulating glucocorticoid concentration. It should be noted that in another study treatment with CsA did not lead to a deterioration in endocrine function of the grafted pancreas [17]. It was furthermore shown that CsA failed to affect β cell function in acute experiments in vitro [18].

The findings of the present study nevertheless suggest that prolonged exposure to CsA impairs islet β cell function, although the present drug concentration (1 mg/l) somewhat exceeds that measured in the blood of CsA-treated patients. It seems as if this chronic deterioration is not effected via an influence either on the oxidative metabolism of the islets, or on the phospholipid metabolism. The detrimental effect of CsA appears to be located at the level of protein biosynthesis. This could lead to a reduced insulin content of the CsA-exposed islets, which may well explain the impairment of glucose-stimulated insulin release. An influence of CsA on the glucose recognition system of β cells cannot, however, be excluded.

The present finding that CsA reduces the rate of DNA synthesis in cultured islets is perhaps not surprising, since the biologically unique activity of this drug is the immunosuppressive effect mediated through blocking of the precursor T cell in G_0 or early G_1 phase of the cell cycle [16]. It may be speculated that the active mitogen in the present islet culture experiments is glucose (11 mmol/l), the stimulatory effect of which is inhibited by CsA. It is intriguing that corticosteroids, which are often used in combination with CsA in immunosuppressive therapy, have been likewise found to inhibit islet-cell replication in vitro [19]. It should be mentioned in this context that in none of these studies has a preferential effect on any of the different cell types of the islets been documented. There is however ample evidence to suggest that β cells are actively dividing in these preparations [13, 14].

Great caution must be exercised when transferring the present data obtained in vitro to the clinical situation. The present observation of inhibition of DNA synthesis by CsA should nevertheless be considered in the current attempts to ameliorate early Type 1 diabetes [20]. Likewise, this effect of CsA could be deleterious when grafting islet cells into diabetics patients [21], since the long-term success of such treatment may depend not only on immune reactions but also on the replicatory activity in the islet graft.

Acknowledgements. We are grateful to K. Flink, E. Forsbeck, I.-B. Hallgren, A. Nordin and P. Wentzel for excellent technical assistance and to A. Snellman for preparation of the manuscript. Financial support from the Swedish Medical Research Council (12x-109; 12x-6538), the Swedish Diabetes Association, the Kroc Foundation and the Nordic Insulin Fund is gratefully acknowledged.

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