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Cyclosporine-induced insulin release in rats is related to an increase in plasma lipid levels

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Abstract. We studied the modifications of plasma lipid levels induced by cyclosporine (CsA), streptozocin (STZ) or both drugs in rats. Male Wistar rats (RT1y) were administered i.p. with CsA or STZ or both at the dosage of 15 mg/kg daily for 8 days and were sacrificed on day 9. Total lipid, triglyceride and total cholesterol plasma levels were measured. The plasma total lipid content was significantly increased in CsA-treated and in CsA + STZ-treated rats with respect to controls (662 ± 29 and 632 ± 32, respectively, vs 472 ± 27 mg/dl). The triglyceride content was significantly higher in CsA-treated and in CsA + STZ-treated animals than in controls (137 ± 8.7 and 188 ± 14.1, respectively, vs 79 ± 7.7 mg/dl). The total cholesterol level was not significantly different in CsA- and STZ-treated rats with respect to controls. CsA-treated and STZ-treated rats concomitantly revealed a significant impairment of glucose tolerance. In fact, 150 min after orogastric administration of 350 mg glucose, glycaemia was significantly more elevated in treated animals than in controls. We conclude that the increase in lipid levels induced by CsA treatment could be related to drug-induced damage to the pancreas islets, as shown by the early insulin release and fatty tissue degeneration.

Key words: Cyclosporin A – Plasma lipids – Diabetic rats

A role for cyclosporine (CsA) in the pathogenesis of a hyperlipidaemic status in patients undergoing renal transplantation was recently proposed [10]. In fact, in stable kidney graft recipients with good transplant function, long-term immunosuppression with CsA and low-dose prednisolone was associated with higher serum lipid levels than therapy with azathioprine and prednisolone. Lipid abnormalities in renal transplant recipients treated with CsA have been observed 2 [8] and even 3 years [11] after transplantation. Since hyperlipidaemia represents an important atherogenic risk, the use of CsA theoretically could be limited in kidney-grafted patients in whom the previous uraemia [9] and treatment with antihypertensive drugs and steroids [1] can also accelerate atherosclerosis. A significant increase in the levels of plasma lipids has been observed in rats treated with CsA [6]. The use of fish oil (a lipid-reducing agent) as the CsA vehicle rather than olive oil induced an increase in the levels of plasma triglycerides but not of cholesterol in treated with CsA animals for 28 days [6].

In a previous study we demonstrated that CsA administration in rats treated with multiple low doses of diabetogenic streptozocin (STZ) precipitated the onset of diabetes [3]. We subsequently showed that CsA treatment increased in vitro insulin release from pancreatic beta cells [4]. Finally, we found that CsA potentiated the in vivo effect of STZ in impairing the insulin content of the rat pancreas [5].

In the present study we evaluated whether the CsA-induced insulin release was related to modifications of the plasma lipid levels and glucose tolerance. The bilirubin content was examined as an indicator of liver function. We also compared the effect of CsA alone with that of STZ alone and of the two drugs given together. STZ was administered in multiple low doses, thereby assuring the onset of an autoimmune diabetes which mimicked type I insulin-dependent diabetes mellitus in man [7].

Materials and methods

Animals and pharmacological treatment. Four groups, each containing 20 male Wistar rats (RT1y) weighing 250–300 g, were studied. Animals of the first group were untreated (controls). In the second group, CsA (a gift from Sandoz, Basel, Switzerland) was injected intraperitoneally (i.p.) at the dosage of 15 mg/kg daily for 8 days; the drug was mixed prior to the injection with Tween 80 and 96% ethanol in physiological saline to achieve the final concentration of 2 mg/ml. The third group of rats was treated with multiple low doses of STZ (Upjohn, Kalamazoo, Mich., USA); the drug was dissolved in sterile 0.05 *M* sodium citrate buffer, pH 4.5, and injected i.p. within 5 min at the dosage of 15 mg/kg daily. The fourth group received

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Table 1. Plasma content (in mg/dl) of total lipids, triglycerides, total cholesterol and bilirubin (total and direct) (mean ± SEM of 10 experiments) in rats after 8 days of treatment with cyclosporine (CsA), streptozocin (STZ) or both drugs (CsA + STZ) and in controls (C)

Treatment	Total lipids	Triglycerides	Total cholesterol	Bilirubin	
				Total	Direct
C	472 ± 27	79 ± 7.7	65 ± 2.6	0.17 ± 0.01	0.23 ± 0.04
CsA	662 ± 29*	137 ± 8.7*	69 ± 5.4	0.60 ± 0.06*	0.60 ± 0.08*
STZ	477 ± 61	77 ± 4.7	66 ± 4.6	0.22 ± 0.02	0.21 ± 0.03
CsA + STZ	632 ± 32*	188 ± 14.1*,+	54 ± 6.1	0.65 ± 0.06*	0.63 ± 0.07*

* $P < 0.01$ versus C, + $P < 0.05$ versus CsA

Table 2. Glycaemia values (in mg/dl) measured before and 150 min after orogastric administration of glucose (350 mg in 1 ml) to rats fasted for 18 h after being treated for 8 days with CsA, STZ or both drugs (CsA + STZ) and controls (C) (mean ± SEM of 10 experiments)

Treatment	Glucose administration	
	Before	After
C	65 ± 6.0	89 ± 3.8
CsA	86 ± 3.0*	128 ± 8.4**
STZ	101 ± 5.1**	134 ± 13.5*
CsA + STZ	128 ± 7.7**,+	177 ± 16.0**,+

* $P < 0.05$ versus C, ** $P < 0.01$ versus C, + $P < 0.05$ versus CsA and versus STZ

both CsA and STZ i.p. at the same dosages daily for 8 days. On day 9 following 18 h of fasting during the night with free access to water, 10 rats per group were exsanguinated at the aorta bifurcation level after brief ether anaesthesia. The blood was used for the biochemical assays. The remaining 10 rats were used for the glucose tolerance test.

Biochemical assays. The plasma triglyceride and cholesterol concentrations were determined by routine enzymatic methods (Boehringer, Mannheim, FRG). Lipids and bilirubin were measured by using colorimetric tests (Boehringer).

Glucose tolerance test. Following 8 days of pharmacological treatment, 10 rats were given orogastrically an aqueous glucose solution (350 mg in 1 ml) while under light ether anaesthesia and following 18 h of fasting. Glycaemia was evaluated before and 150 min after glucose administration. The blood glucose level was determined by using an enzymatic test (hexokinase/G6P dehydrogenase, Boehringer).

Statistical analysis. The results were expressed as mean ± SEM and subjected to variance analysis. Significance was assumed when P was < 0.05.

Results

Table 1 gives the plasma levels (mg/dl) of total lipids, triglycerides, total cholesterol and bilirubin (total and direct) in control rats and in those treated for 8 days with CsA, STZ, or CSA and STZ. The data represent the mean ± SEM of 10 experiments. Plasma levels of total lipids were significantly higher in CsA-treated rats than in controls. Treatment with STZ did not increase the total lipid levels with respect to controls, whereas the concomitant administration of both drugs did not seem to modify the effect due to CsA alone. Plasma levels of triglycerides were significantly more elevated in CsA-treated animals than in controls. Treatment with STZ alone did not alter the plasma triglyceride content with respect to that of the controls, but concomitant treatment with STZ and CsA produced a significant increase compared with CsA treatment alone. CsA, STZ and CsA + STZ treatments did not determine a significant difference in plasma total cholesterol content, which appeared to be similar to that of the controls. The level of total bilirubin (which was all present as direct bilirubin) was significantly higher in CsA-treated and in CsA + STZ-treated rats than in the controls.

Table 2 gives the glycaemia values (mg/dl) of rats subjected to orogastric administration of 350 mg glucose in 1 ml. Blood glucose levels were studied before and 150 min after administration. Before glucose administration, the rats were fasted for 18 h, following 8 days of CsA, STZ or CsA + STZ treatment. The data represent the mean ± SEM of 10 experiments. Glycaemia levels in the CsA-treated rats were significantly more elevated than in the controls before glucose administration; hyperglycaemia was more evident in these rats than in the controls after administration. STZ-treated rats were significantly hyperglycaemic compared with the controls before glucose administration, and they maintained less evident but significantly more elevated glycaemia values than the controls after administration. CsA + STZ-treated rats, before and after glucose administration, presented significantly higher glycaemic levels than those of the controls; these levels were significantly more elevated than those of the CsA-treated and STZ-treated rats.

Figure 1 depicts the results of the glucose tolerance test in the pharmacologically treated rats. Treatment with CsA and with CsA + STZ significantly reduced, with respect to the controls, the glucose tolerance of the animals.

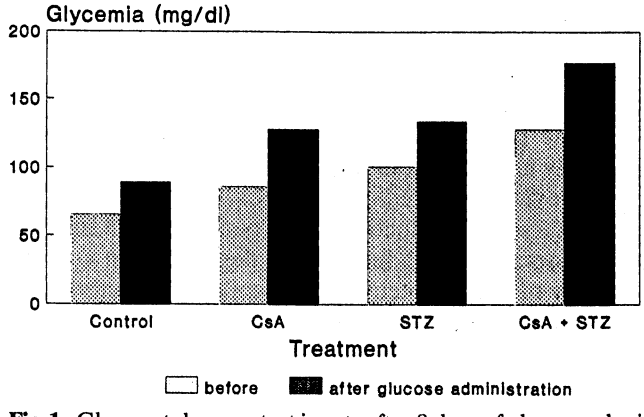

Fig. 1. Glucose tolerance test in rats after 8 days of pharmacological treatment. CsA Cyclosporin; STZ Streptozotocin

Discussion

In a previous study we demonstrated that the treatment of rats with CsA increased the release of insulin from the pancreas [5]. In fact, the total pancreatic insulin content was significantly lower (−65% of controls) in the CsA-treated rats and was related to a reduction of the total pancreatic protein content (−31% of control values). The effect was amplified by the concomitant treatment with CsA and STZ, and we presume that CsA has a direct toxic effect on the beta cells. Owing to the usefulness of CsA as an immunosuppressive drug in grafted patients and the modifications induced in the serum lipid content in stable renal transplant recipients [10], we believed it worthwhile to study the plasma content of total lipids, triglycerides and total cholesterol in the same experimental model we previously used [5].

In the present study we demonstrated that after 8 days of CsA treatment, the plasma total lipid levels significantly increased with respect to those of the controls. We also showed that the plasma triglyceride values were significantly more elevated in the CsA-treated animals than in the controls, whereas the cholesterol content did not vary.

We hypothesize that the early severe drop in the total pancreas insulin content of CsA-treated rats could be related to a mobilization of fatty acids from the adipose tissue. In fact, in STZ-treated rats, in which the total insulin pancreatic content was reduced to a lesser extent (−26% of controls) [5], the plasma triglyceride values were not significantly different from those of the controls. Moreover, in CsA + STZ-treated animals, in which the total pancreatic insulin content was dramatically reduced (−80% of controls) [5], the plasma triglyceride levels were significantly higher than in the controls and CsA-treated animals. During 8 days of treatment with CsA, the plasma cholesterol level did not appear to vary with respect to that of the controls. Since the increase in plasma cholesterol has also been demonstrated in 28-day treated animals [6], the modification in the plasma cholesterol content could be attributable to CsA treatment for more than 8 days. We agree that the increase in the lipid levels could have been due to a substantially altered hepatic synthesis of lipoprotein, as previously suggested [6]. In fact, in the present study we demonstrated an altered hepatic function at 8 days of treatment, as indicated by the roughly threefold increase in total bilirubin with respect to controls. In rats, the presence of only direct circulating bilirubin is indicative of severe hepatic liver alteration. It is also possible that the CsA treatment was responsible for the elevation of the plasma lipid levels through a reduction of the peripheral lipid catabolism, as observed in chronically uraemic rat models [9].

We previously revealed after CsA and CsA + STZ treatment a severe reduction in the pancreas protein content [5] associated with fatty tissue degeneration and hypoatrophy of the parenchyma. We concluded that the increase in lipid levels induced by CsA treatment could have been related to the fatty pancreas degeneration due to drug-induced damage to the islets. We believe that the beneficial effect obtained by CsA administration in diabetic patients with recent onset type I disease [2] deserves to be thoroughly evaluated. In fact, the usefulness of CsA treatment could be related to insulin release from undamaged beta cells in these patients. However, continuous drug administration could lead to significant and irreversible pancreas degeneration. Moreover, the subsequent drug-induced increase in the plasma lipid levels could favour the rise in diabetic metabolic disorders. The same considerations apply to diabetic patients who undergo pancreas transplantation.

With the experimental model of the present study, it was demonstrated that CsA significantly modified the plasma lipid levels more than the diabetogenic STZ. Our demonstration that CsA treatment significantly impaired the rat glucose tolerance capacity is in agreement with other results.

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