Original articles

TRANSPLANT International

Transplant Int (1989) 2: 8-12

Effect of cyclosporin A on carbohydrate metabolism in the rat*

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Abstract. Liver and kidney carbohydrate metabolism was investigated in rats treated with daily doses of 15 mg/kg body weight cyclosporin A (CyA) for 2 and 8 weeks or of 50 mg/kg body weight CyA for 2 weeks. The higher dosage caused significantly reduced liver glycogen and liver glycogen synthetase activity (of both active I-form and total enzyme activity), whereas the activity of the glycogen-degrading enzyme phosphorylase (active a-form and total activity) remained unchanged. Plasma glucose and glucagon levels, as well as blood ketone bodies of these animals, increased significantly and plasma insulin decreased. In contrast, kidney glycogen and glucose content were higher in rats treated with 50 mg CyA, probably due to enhanced ketone body utilization. Reduced liver glycogen synthetase activity was also found in rats treated with 15 mg CyA. Our data suggest that hypoinsulinemia, induced by CyA, might be a contributing factor to the hyperglycemia, which is mainly due to inhibition of liver glycogen synthesis.

Key words: Cyclosporin A and carbohydrate metabolism in the rat - Carbohydrate metabolism and cyclosporin A in the rat.

Recent studies by Yoshimura et al. [26] have indicated that the incidence of diabetes mellitus requiring insulin therapy is higher in cyclosporin A (CyA)-treated transplant recipients (17.1%) than in azathioprine-treated patients (12.8%), despite significantly lower methylprednisolone dosages at the onset of diabetes mellitus in the CyA group. The onset of diabetes mellitus was related to high CyA trough plasma levels and, in six of the eight patients concerned, insulin therapy could be stopped within 3 months of conversion from CyA to azathioprine treatment [26].

Studies by Hahn et al. [9] suggest that pancreatic β-cells may be sensitive to the toxic effects of CyA. Impaired glucose tolerance and a decrease in islet cell insulin content were observed in rats treated daily with 15 mg/kg body weight CyA. Rats treated with 50 mg/kg body weight developed hyperglycemia and hypoinsulinemia [9]. In CyA-treated rats, high dosages of CyA (50 mg/kg body wt. per day for 7 days) caused hyperglycemia and hypoinsulinemia associated with severe degranulation and hydropic degeneration of islet β-cells [10]. Yale et al. [25] demonstrated that therapeutic dosages of CyA (10 mg/kg body wt. per day) induce reversible glucose intolerance and that an increase in the CyA dosage augments the glucose intolerance, due to inhibition of insulin secretion and/or synthesis. Similar data were obtained by Garvin et al. [7] in the canine model. Dose-dependent impairment of both pancreatic endocrine and exocrine function caused by CyA was observed in rats. Glucose-dependent insulin secretion was significantly impaired with all doses of cyclosporin administered, whereas exocrine pancreatic function displayed lower sensitivity to the noxious action of CyA [15].

To further delineate the mechanism(s) by which CyA affects carbohydrate metabolism, we measured glycogen, glucose, and lactate content, as well as the activities of glycogen synthetase (I-form and total activity) and the glycogen-degrading enzyme phosphorylase (a-form and total activity), in the liver and kidneys of rats treated with 15 mg/kg body weight for 2 and 8 weeks and with 50 mg/kg body weight for 2 weeks. In general, carbohydrate metabolism is regulated by the action of several hormones. The

* This article is dedicated to Professor Dr. August Heidland, Chief of the Division of Nephrology, University of Würzburg, FRG, on the occasion of his 60th birthday

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major effect of adrenaline is activation of glycogenolysis in skeletal muscle, whereas glucagon enhances degradation of glycogen in the liver. Insulin stimulates glycogen synthesis brought about by activation of glycogen synthetase. This enzyme is inactive in its phosphorylated form (D-form) and becomes activated by dephosphorylation (I-form), which is catalyzed by a specific phosphatase. Phosphorylation of synthetase is mediated by cyclic adenosine monophosphate (cAMP)-dependent protein kinase. On the other hand, glycogen breakdown is accomplished by the enzyme glycogen phosphorylase. This enzyme is present in two forms, an activated form (phosphorylase a) and an inactive form (phosphorylase b; for review see Tickett-Gies and Walsh [23]).

Materials and methods

Experimental protocol

Wistar rats with an initial body weight of 200-250 g were used for the experiment. The animals were housed in metabolic cages with free access to tap water. After an acclimation period of 8 days, the rats were randomly assigned to different groups. CyA-treated rats were weight-matched with control animals throughout the study. Body weight of CyA-treated and control rats was determined daily. Body weights before and after the study periods were recorded. The effects of food intake reduction on body weight were comparable in all animals investigated. Controls received the same amount of food that CyA-treated animals had received the day before. One animal from each pair of CyA-treated rats and one from each control pair were used for blood sampling, while the liver and kidneys from the others were removed and examined.

Animals treated for 2 weeks

Group 1. Twenty control rats were fed the vehicle (olive oil) twice daily by oral gavage. Body weight was determined daily. Food intake was reduced when body weight increased faster than in CyA-fed rats. Two weeks after starting the experiments, the animals were sacrified. Blood was drawn from half of the rats ($n = 10$) while liver and kidneys from the other half ($n = 10$) were removed and quick-frozen in liquid nitrogen. The tissue samples were then crushed in order to prevent postmortem alterations of metabolite concentrations. Group 1 acted as a control for groups 2 and 3. Glycogen, glucose, and lactate were analyzed. In five of these ten rats, glycogen synthetase and phosphorylase were measured as described later in this paper. All animals were anesthetized with thiobutabarbital (Inactin, 100 mg/kg; Byk-Gulden, Konstanz, FRG).

Group 2. Twenty rats were given CyA (15 mg/kg body weight daily in two doses) suspended in olive oil by oral gavage. Two weeks after starting the experiments, blood was drawn from the abdominal aorta in half of the rats ($n = 10$) in order to determine CyA trough level as well as blood and serum parameters outlined below. Organs were removed as described above.

Group 3. Sixty rats were given CyA in a daily dose of 50 mg/kg body weight. Two weeks later, half of the rats ($n = 30$) were anesthetized; their blood was drawn and organs were removed as previously described. The other 30 rats treated with CyA in a daily dose of 50 mg/kg body weight were planned to be treated for 8 weeks. However, individual rats began to become symptomatic on day 14, and we therefore decided to sacrifice all the animals immediately and to assign them to group 3. Plasma CyA, creatinine, and urea levels were determined from 20 rats, serum glucose, serum insulin, and plasma glucagon from 10 rats, and blood metabolic intermediates from 10 rats. Liver and kidney parameters were measured from 40 rats.

Animals treated for 8 weeks

Group 4. Twenty control rats were fed the vehicle (olive oil) by oral gavage for 8 weeks. The rats were then anesthetized and treated like those in group 1. This group acted as a control for group 5.

Group 5. Twenty-five rats were given CyA in a daily dose of 15 mg/kg body weight for 8 weeks and then treated like those in group 2.

Analytical methods and measurements

CyA trough levels in blood were determined at the time of sacrifice by a commercially available radioimmunoassay (Sandoz, Nürnberg, FRG) measuring CyA and, to some extent, unknown metabolites using a polyclonal antibody as described by Donatsch et al. [6]. The last CyA dose was administered 12 h before the animals were sacrificed.

Blood lactate, pyruvate, citrate, oxoglutarate, acetoacetate, and β-hydroxybutyrate levels were measured enzymatically using modified microassays in 500 µl samples of deproteinized (5% perchloric acid) whole blood [11]. Serum glucose, urea, and creatinine were determined by routine methods (Hitachi 737, Behring, FRG). Plasma insulin levels were measured using a highly sensitive enzyme immunoassay (Boehringer, Mannheim, FRG). Glucagon was assayed in an aprotinine-ethylenediaminetetraacetate (EDTA)-plasma by a radioimmunoassay (Serono, Freiburg, FRG).

The tissue samples were homogenized with 2.5 vol 4 mmol EDTA/20 m*M* Tris-HCl, pH 7.0 (4° C) containing 25 m*M* NaF. The suspension was centrifuged at 10,000 *g* for 10 min at 4° C and the supernatant was decanted through glass wool. The specific activity of glycogen synthetase was assayed as described by Thomas et al. [22], and phosphorylase according to the method described by Stalmans et al. [18].

Glycogen was determined according to the Bergmeyer [3] method with the following modifications: acid supernatant was precipitated by 67% (v/v) ethanol. The glycogen pellet was dissolved in 0.5 ml of 50 m*M* acetate (sodium) buffer (pH 4.8) which contained 1 mg/ml amyloglucosidase (50 U/mg, Merck, Darmstadt, FRG). After 30 min of incubation at 56° C, glucose was assayed enzymatically using glucose dehydrogenase (Merckotest Gluc-DH, Merck, Darmstadt, FRG). Tissue glucose concentration was assayed enzymatically using the glucose-oxidase method (Merckotest GOD-PAP, Merck, Darmstadt, FRG). Lactate was measured enzymatically by the method described by Bergmeyer [4].

Statistics

The results are expressed as the mean ± SEM. For the analysis of significance, Student's *t*-test was used, with calculation of degrees of freedom according to Fisher Behrensen [16] to correct for the

unequal number of observations in the groups that were compared. Statistical tests were performed for each parameter. Groups 2 and 3 were compared with group 1, group 5 with group 4, and group 3 with group 2. Only P values less than 0.05 were considered significant. In the case of a significant difference in a parameter between group 3 and group 2, indication of significance between group 3 and group 1 was omitted.

Results

Table 1 shows body weight and blood parameters of rats treated with either 15 mg/kg or 50 mg/kg body weight CyA for 2 weeks. These data reveal no differences between rats treated with the 15 mg/kg dose and weight-matched controls. In contrast, an increase in the daily CyA dose to 50 mg/kg body weight for 2 weeks caused higher plasma glucose and glucagon as well as lower serum insulin. There was also a significant rise in plasma creatinine and urea in rats treated with 50 mg/kg compared to rats treated with 15 mg/kg body weight. The CyA level was four times higher when the CyA dose increased but body weight remained comparable. Metabolic intermediates, measured in deproteinized whole blood samples, was unchanged in CyA (15 mg/kg body wt.)-treated and control rats (Table 2). CyA, in a dose of 50 mg/kg body weight, caused enhanced ketone body formation. Blood pyruvate was statistically lower, and blood citrate level higher, in these rats.

Liver and kidney glycogen, glucose, and lactate content of CyA (15 mg/kg body wt.)-treated and weight-matched control rats were also comparable. The glycogen and glucose values of both CyA-treated and control rats were significantly lower after 8 weeks than after 2 weeks of treatment (Table 3), possibly due to lower food intake. Controls received the same amount of food that CyA-treated rats had received the day before. Body weight increased comparably in both groups.

Table 1. Body weight and blood parameters of control rats and rats treated with cyclosporin A (CyA) for 2 weeks. (Data are indicated as mean values ± SEM) $*P<0.05$; $**P<0.01$; $***P<0.001$ versus rats treated with 15 mg/kg body wt. CyA (group 2)

	Control	Cyclosporin A (15 mg/kg body wt.)	Cyclosporin A (50 mg/kg body wt.)
Group	1	2	3
Body weight (g)	260 ± 7 ($n=20$)	250 ± 8 ($n=20$)	266 ± 4 ($n=40$)
Cyclosporin level (ng/ml)	-	1284 ± 82 ($n=10$)	4664 ± 276*** ($n=20$)
Creatinine (mg/dl)	0.56 ± 0.06 ($n=10$)	0.48 ± 0.02 ($n=10$)	0.72 ± 0.05*** ($n=20$)
Urea (mg/dl)	52.0 ± 1.1 ($n=10$)	56.6 ± 1.3 ($n=10$)	71.1 ± 2.2*** ($n=20$)
Glucose (mg/dl)	152 ± 4 ($n=10$)	153 ± 7 ($n=10$)	216 ± 9** ($n=10$)
Insulin (μU/ml)	8.2 ± 0.3 ($n=10$)	8.1 ± 0.9 ($n=10$)	6.4 ± 0.2* ($n=10$)
Glucagon (pg/ml)	126 ± 12 ($n=10$)	95 ± 8 ($n=10$)	253 ± 43** ($n=10$)

Table 2. Metabolic intermediates (μmol/l) in deproteinized whole blood samples of control rats and rats treated with cyclosporin A (CyA) for 2 weeks. (Data are indicated as mean values ± SEM) $*P<0.05$; $**P<0.001$ versus rats treated with 15 mg/kg body wt. CyA (group 2)

	Control	Cyclosporin A 15 mg/kg body wt.	Cyclosporin A 50 mg/kg body wt.
Group	1 ($n=10$)	2 ($n=10$)	3 ($n=10$)
Lactate	1175 ± 198	1137 ± 223	862 ± 106
Pyruvate	90 ± 7	85 ± 9	51 ± 4*
β-Hydroxybuytrate	198 ± 26	181 ± 17	394 ± 103
Acetoacetate	44 ± 8	46 ± 8	235 ± 73**
Citrate	95 ± 11	90 ± 9	137 ± 8*
Oxoglutarate	17 ± 6	18 ± 2	19 ± 2

Table 3. Liver and kidney carbohydrate content of control rats and rats treated with cyclosporin A (CyA) for 2 and 8 weeks. (Data are indicated as mean values ± SEM) $*P<0.05$; $**P<0.01$ versus rats treated with 15 mg/kg body wt. CyA for 2 weeks (group 2); $***P<0.05$ versus rats treated with 15 mg/kg body wt. CyA for 8 weeks (group 4)

	2 Weeks			8 Weeks	
	Control	Cyclosporin A		Control	Cyclosporin A
		15 mg/kg body wt.	50 mg/kg body wt.		15 mg/kg body wt.
Group	1 ($n=10$)	2 ($n=10$)	3 ($n=40$)	4 ($n=10$)	5 ($n=15$)
Liver glycogen	98.7 ± 9.6	94.6 ± 11.8	81.6 ± 5.7*	64.2 ± 4.0	53.1 ± 2.0***
Liver glucose	20.5 ± 1.8	17.9 ± 0.7	15.8 ± 0.6*	11.0 ± 0.6	11.2 ± 0.4
Liver lactate	3.04 ± 0.21	2.83 ± 0.10	1.30 ± 0.07*	1.98 ± 0.26	1.78 ± 0.08
Kidney glycogen	0.28 ± 0.06	0.33 ± 0.03	1.87 ± 0.11**	0.33 ± 0.02	0.28 ± 0.03
Kidney glucose	9.0 ± 0.5	10.9 ± 1.1	17.2 ± 0.6**	8.9 ± 0.4	9.6 ± 0.4
Kidney lactate	1.70 ± 0.11	1.42 ± 0.20	1.73 ± 0.61	1.62 ± 0.44	1.28 ± 0.10

Table 4. *Effect of 2-week cyclosporin A (CyA) therapy on glycogen synthetase and phosphorylase activity (mU/mg protein) in rat liver and kidneys. (Data are indicated as mean values ± SEM) * $P < 0.05$ versus rats treated with 15 mg/kg body wt. CyA for 2 weeks (group 2)*

	Control	Cyclosporin A 15 mg/kg body wt.	Cyclosporin A 50 mg/kg body wt.
Group	1 ($n=5$)	2 ($n=5$)	3 ($n=5$)
Liver	Glycogen synthetase		
Active I-form	2.84 ± 0.39	2.59 ± 0.14	1.32 ± 0.31*
Total activity	6.84 ± 0.8	5.99 ± 0.6	3.59 ± 1.22*
Liver	Phosphorylase		
Active a-form	75.5 ± 5.1	64.4 ± 5.1	71.2 ± 18.2
Total activity	98.4 ± 7.4	80.1 ± 4.5	81.1 ± 1.7
Kidney	Glycogen synthetase		
Active I-form	0.36 ± 0.03	0.34 ± 0.07	0.61 ± 0.04*
Total activity	2.58 ± 0.40	2.14 ± 0.55	1.24 ± 0.16*
Kidney	Phosphorylase		
Active a-form	25.4 ± 1.7	36.4 ± 4.2	35.1 ± 1.7
Total activity	46.3 ± 2.0	48.0 ± 2.4	46.0 ± 1.7

Hepatic glycogen, glucose, and lactate content, however, decreased significantly after 2 weeks of treatment with the high CyA dose (Table 3). On the other hand, kidney glycogen and glucose increased significantly compared to the rats treated for 2 weeks with 15 mg/kg body weight CyA. In rats treated with a daily dose of 50 mg/kg body weight CyA, also liver glycogen and lactate decreased, whereas kidney glycogen and glucose increased (Table 3).

Table 4 shows the specific activities of both liver and kidney glycogen synthetase and phosphorylase. The high dosages of CyA caused significant inhibition of the active I-form and total activity of liver glycogen synthetase compared to activities of both control rats and those treated with 15 mg/kg body weight CyA. In contrast, the active a-form and total activity of the glycogen-degrading enzyme phosphorylase remained unchanged in the liver of all groups of animals. Nor were phosphorylase activities in the kidney influenced by either the high or low dosage of CyA. Rats treated with the latter displayed unchanged kidney glycogen synthetase activity compared with control rats, whereas the active a-form of the enzyme was even higher in the group treated with 50 mg/kg body weight CyA.

Discussion

This manuscript describes the effect of CyA on carbohydrate metabolism in the rat. The effect of CyA, given daily for either 2 weeks at 15 or 50 mg/kg body weight or 8 weeks at 15 mg/kg body weight, on liver and kidney carbohydrate metabolism was assessed by the measurement of metabolite profiles and the activity of both glycogen synthetase and phosphorylase. Hypoinsulinemia and hyperglucagonemia, induced by CyA, may have produced hyperglycemia as a result of the inhibition of hepatic glycogen synthesis. The present study did not include muscle analysis for substrate and enzyme concentrations. The importance of muscle glucose utilization in glucose homeostasis is well established. Therefore, inhibition of liver glycogen synthesis might only have been a contributing factor to the hyperglycemia observed. Since insulin inhibits lipolysis, enhanced ketone body formation (Table 2) may also have been the result of hypoinsulinemia.

The use of CyA in organ transplantation is limited because of the nephrotoxicity and liver toxicity the drug induces [2, 14]. CyA also alters the endocrine pancreas, leading to a decrease in β-cells and the insulin content of individual islets [9]. A deterioration in glucose metabolism in pancreatic transplant recipients given CyA was first described by Gunnarson and coworkers [8], whereas Traeger et al. [24] reported an improved metabolic function of the graft under CyA treatment. When CyA therapy was initiated shortly after newly diagnosed insulin-dependent diabetes mellitus in humans, either a marked reduction in the insulin dosage or complete cessation of insulin was reported in a substantial number of cases [1, 19, 20].

Several studies have demonstrated that an increase in the CyA dose augments glucose intolerance. Hypoinsulinemia, spontaneous hyperglycemia, and impaired glucose regulation have all been reported following an oral or intravenous glucose tolerance test [7, 9, 17, 25]. In the present study, random blood glucose and hormone levels were measured, and it may be argued that oral glucose tolerance tests, including fasting blood glucose levels, give a more accurate estimate of glucose homeostasis. Nevertheless, our data and the results of other studies indicate impaired carbohydrate metabolism as a result of the administration of CyA. Pancreatic glucagon content was normal, even in rats treated with 50 mg/kg body weight CyA [9], but plasma glucagon levels were elevated under these conditions (Table 1).

All of the parameters measured remained unchanged in rats treated with 15 mg/kg body weight for 2 weeks. The liver glycogen, glucose, and lactate levels fell after 8 weeks. Although similar findings were observed in control animals that were partially starved, there was already a tendency for glycogen synthetase activity to decrease in rats treated for 2 weeks with the low-dose CyA. Thus, the reduction

in the concentration of liver substrates in these rats was not due to reduced food intake alone.

In vitro exposure of renal mitochondria to CyA suggests inhibition of the mitochondrial electron transport system [12]. When 50 mg/kg body weight CyA was administered to rats, the state 3 respiration and uncoupled respiration of succinate and glutamate/malate respiration were decreased [13], indicating that structural observations under treatment with CyA may have been a consequence of the mitochondrial dysfunction induced by the drug. Moreover, inhibited Na^+-K^+-ATPase and subsequent damage to tubular basement membrane is assumed to be one of the important biochemical mechanisms underlying the nephrotoxicity of CyA [21]. The present study demonstrated elevated kidney glycogen and glucose content of rats treated with daily doses of 50 mg/kg body weight CyA, probably due to enhanced ketone body utilization. Total glycogen synthetase activity decreased in these animals. However, the active I-form of this enzyme increased from 13.9% in control rats and 15.8% in rats treated with low-dose CyA to 49.2% in rats receiving the higher dosage (Table 4). Renal distal tubular accumulation of glycogen in CyA-treated animals has also recently been demonstrated by Bertani et al. [5].

In sum, our results confirm and extend earlier observations of altered carbohydrate metabolism in both human and animal studies in which CyA was used as the immunosuppressive agent.

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