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INHIBITION OF RENAL GLUCONEOGENESIS BY CYCLOSPORIN A IN RATS

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Summary

Glucose production was investigated in isolated renal tubular fragments from normal Wistar rats treated by Cyclosporin A (CyA), 10–80mg/kg/day for one week. When compared with tubules from untreated animals renal gluconeogenesis from seven substrates of the Krebs cycle was reduced in a dose dependent manner. Renal function studies revealed polyuria and hyperaminoaciduria even with the lowest dose used. With higher doses tubular phosphate reabsorption was decreased and ultrastructural changes were observed in the kidney. This suggests that alterations in tubular function and morphology by CyA in the rat kidney are associated with disturbed renal energy metabolism.

Introduction

Studies of Cyclosporin A (CyA) nephrotoxicity have focused on glomerular and vascular changes, but in addition tubular lesions have been described in man and laboratory animals [1,2]. In the rat it is known that structural alterations occur in the proximal tubular epithelium after doses of CyA which have no adverse effects on glomerular filtration rate (GFR) [3]. The mechanism of this tubular damage and its functional counterpart have so far not been studied in detail. Alterations of tubular function after administration of CyA in rats may be the result of reduced ultrafiltration pressure [4]. A direct metabolic action of CyA on the tubular cells has rarely been discussed.

In the following study we have measured the capacity of isolated tubular fragments to produce glucose from precursors, as a sensitive parameter of renal energy metabolism. This technique which has been used for the study of experimental nephropathies [5] was applied in rats pretreated by CyA. In addition, glomerular and tubular functions were tested and kidney tissue was examined morphologically.

Materials and methods

Normal male Wistar rats weighing between 150–230gm were given CyA 10, 20, 40, 60 or 80mg/kg once a day in olive oil by gavage for a period of seven days. The food contained 18 per cent protein (Altromin, Lage). One day after the last administration of CyA the rats were sacrificed and blood collected for determination of CyA and serum creatinine, urea, electrolytes, transaminases and amino acids. In the 24 hours prior to sacrifice the rats were fasted but a free intake of water was permitted. During this period urine was collected with thymol in a third of all animals tested for renal gluconeogenesis.

For each metabolic experiment four rats were used and for each dose of CyA given six experiments were performed except for the animals receiving 80mg/kg/day (two experiments). Control rats (10 experiments) underwent the same procedures but did not receive any CyA.

Kidney tubules were isolated by collagenase according to the method of Guder et al with some modifications [5]. For determination of renal glucose production the tubular fragments were incubated for 60 minutes at 37°C in Krebs-Henseleit solution with seven gluconeogenic substrates (10mM). Results are given in μmoles glucose produced/g tubular protein/hr. Renal glucose production by tubules of experimental animals is indicated for each precursor in percentage of renal glucose production by tubules of control animals (Table I). The experimental procedure is described elsewhere in more detail [6].

TABLE I. Glucose production of isolated kidney tubules from rats injected with Cyclosporin A. Number of experiments in brackets

Substrates (10mM)	Controls Renal glucose production μmol/g protein/hr Mean ± SEM	Cyclosporin A Renal glucose production in % of controls (mean values) 10mg/kg (6) (1)	20mg/kg (6)	40mg/kg (6) (2)	60mg/kg (6)	80mg/kg (2)
None	56±1	89****	89	76***	74	67
l-lactate	417±6	83**	81	56**	52	52
pyruvate	587±9	80**	77	62***	58	56
α-ketoglutarate	299±5	89***	91	61**	58	50
succinate	396±5	87*	85	61*	58	56
fumarate	290±6	87**	85	71*	64	63
l-malate	328±8	88*	85	70*	66	55
oxaloacetate	263±6	71***	61	54*	52	52
Total (7 substrates)		83	81	62	58	55

p values refer to (1) between 10mg/kg and controls and (2) between 40mg/kg and 20mg/kg

Analytical methods Glucose was determined enzymatically, protein by the biuret method, creatinine by autoanalyzer and aminoacids by column chromatography. CyA was measured in whole blood by radioimmunoassay.

Kidneys of rats who had received 10, 20 and 40mg/kg and control animals were perfusion fixed with 3% glutaraldehyde in 0.2mol phosphate buffer. After dehydration and embedding in epon-araldite, semithin sections (1μm) were stained with methylene blue and basic fuchsin, ultrathin sections with uranylacetate and lead citrate.

Results

The experimental animals appeared drowsy except for those with the lowest dose applied, and showed cramps at a dose of 40mg CyA/kg and more. During the eight day observation period the mean body weight of the animals changed by +4.2, +2.3, -4.5, -6.8 and -15.5 per cent for the rats receiving 10, 20, 40, 60 and 80mg CyA/kg/day compared to +15 per cent in controls.

Renal gluconeogenesis Renal glucose production by isolated tubules was reduced with increasing dosage of CyA. A significant inhibition was found even with the lowest dose used (10mg/kg/day). It concerned all substrates used, but was greatest in the presence of l-lactate, pyruvate and oxaloacetate.

TABLE II. Serum biochemistry, urinary findings and renal function after administration of Cyclosporin A in rats (means of eight animals in each group)

		Cyclosporin A (mg/kg/day)				
	Controls	10	20	40	60	80
Serum						
creatinine (mg/dl)	0.47	0.50	0.51	0.55	0.60	0.66
urea (mg/dl)	52	64	75	77*	80*	99***
K (mmol/L)	6.3	6.0	5.7	5.9	5.5	5.5
P_i (mmol/L)	4.0	3.2	3.3	3.0*	3.8	3.3
creatinine clearance μl/min/g	4.63	4.69	4.12	4.18	3.95	3.16*
Urine						
volume (ml/24hr)	9.9	16.8	12.0	35.3*	37.7*	32.5
osmolality (mOsm/L)	1062	536	676	198***	206***	205***
pH	6.45	6.43	6.33	6.80	6.81	6.79
FE_{Na} (%)	0.4	0.4	0.3	0.5	0.3	0.3
FE_K (%)	12.4	13.6	17.5	17.2	22.0*	19.2
Tp_i (%)	93.1	88.6	84.1	84.6	77.4**	78.5**
total aminoacids/ 24 hr (% of controls)	100	231.8	197.9	250.7	239.1	335.8

Significant differences against controls: *$p<0.05$; **$p<0.02$; ***$p<0.01$ (Student's 't' test)

Serum biochemistry and renal function (Table II) Mean serum creatinine was only slightly higher in rats treated by higher doses of CyA compared to controls. Creatinine clearance did not show any significant reduction up to a dose of 60mg/kg/day. Serum phosphate was decreased with a dose of 40mg/kg/day while percentage tubular reabsorption of phosphate significantly dropped only with 60mg/kg/day. Urine volume increased more than threefold while urine osmolality showed a marked fall. Urine pH and fractional excretion of potassium increased but most changes were not significant.

Even with the lowest dosage of CyA applied *generalized hyperaminoaciduria* was noted, without notable preference for certain acids. Plasma amino acids changed only slightly. Detailed analysis of renal amino acid handling is given elsewhere [6]. Glucose and proteins could never be detected in urine when tested by stix.

Trough levels of CyA determined in whole blood gave the following results (4 rats): 1706±20ng/ml with a CyA dose of 10mg/kg, 1964±24ng/ml with 20mg/kg and 2402±31ng/ml with 40mg/kg (mean ± SEM).

Electronmicroscopy revealed a slight increase of cytolysosomes and occasional enlarged mitochondria in proximal tubular cells after administration of 20 and 40mg/kg/day whereas the appearance of tubules in the 10mg/kg group did not differ from controls; tubular cell necrosis, vascular or interstitial lesions were observed in neither group.

Discussion

The capacity of the kidney to produce glucose from metabolic precursors in the proximal tubular cells is regarded as an important parameter of renal energy metabolism [7]. Earlier studies have demonstrated that isolated kidney tubules are an excellent model to study renal gluconeogenesis in rats [5]. Inhibition of energy metabolism in tubular cells was found to be closely related with changes in tubular transport and morphology in several experimental nephropathies [5,8].

In the present study we observed a dose dependent inhibition of renal glucose production from gluconeogenic precursors eight days after administration of CyA even with a relatively low dose of 10mg/kg/day. The reduced gluconeogenesis might be related to the inhibition of mitochondrial respiration of kidney cortex recently described in the presence of CyA [9]. The alteration of tubular cell metabolism in vitro was accompanied by increased excretion of aminoacids, polyuria and, at higher doses, decreased reabsorption of phosphate. In contrast, GFR did not change significantly except for a fall at the highest dose applied, which is in agreement with other studies in rats [3]. The coincidence of these findings strongly suggests that CyA has a metabolic affect on the tubular cells, which may be responsible for the derangement of some tubular transport functions.

The disturbance of cell metabolism could also be related to the morphological changes of the tubular epithelium described after administration of CyA in man and rat [1–3,10]. In contrast to findings reported by other authors the

morphological alterations observed in our experiments were relatively discrete, consisting only of enlarged mitochondria and an increased number of cytolysosomes in proximal tubular cells. With the lowest dose used (10mg/kg/day) we failed to observe any ultrastructural lesion. The changes were probably not more significant because the total dose of CyA applied was relatively low and kidneys were not damaged at the time of CyA administration.

The relevance of our findings for the human situation is difficult to evaluate, but it appears possible that a derangement tubular cell metabolism is the primary event leading to nephrotoxicity by CyA. Recent histological findings in transplanted patients treated by CyA are in agreement with an initial tubulotoxic action of the drug. Since the defect in renal energy metabolism described was observed in vitro it is assumed that the tubulotoxic effect is not dependent on the vascular actions of CyA. Our results point rather to a defect in energy production in the proximal tubular epithelium by affecting the metabolism of Krebs cycle acids within the mitochondria.

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References

1 Mihatsch MJ, Thiel G, Spichtin HP et al. *Transplant Proc 1983; 15:* 2821
2 Thomson AW, Whiting PH, Simpson IG. In White DJG, ed. *Cyclosporin A.* Amsterdam: Elsevier. 1982: 177–190
3 Ryffel B. *Experimental Toxicological Studies with Cyclosporin A.* Basle: Sandoz. 1982: 1–34
4 Dieperink H, Starklint H, Leyssac PP et al. *Proc EDTA–ERA 1984; 21:* 853
5 Fischer W, Manz F, Schärer K. In Guder W, Schmidt V, eds. *Biochemical Nephrology.* Bern: Huber. 1978: 343–351
6 Beck W. *Doctoral Thesis, Medical Faculty, University of Heidelberg.* Heidelberg 1985
7 Klahr S, Hammerman M. In Seldin DW, Giebisch G, eds. *The Kidney, Physiology and Pathophysiology Vol 1.* New York: Raven Press. 1985: 699–718
8 Schärer K, Yoshida T, Voyer L et al. *Res Exp Med 1972; 157:* 136
9 O'Connor RP, Weinberg IM, Humes HD. *Kidney Int 1984; 25:* 235 (abstract)
10 Waldherr R, Horsch R, Dreikorn K. *Int J Pediatr Nephrol 1982; 3:* 40 (abstract)