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Influence of cyclosporin A on the respiration of isolated rat kidney mitochondria

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In vitro exposure of isolated rat kidney mitochondria to cyclosporin A, a new immunosuppressive agent with serious nephrotoxic side-effects, leads to alterations of both succinate- and glutamate plus malate-supported respiration in a dose-related manner. ADP- and 2,4-dinitrophenol-stimulated respiration, respiratory control indices, and ADP/O ratios are decreased. The mitochondrial alterations are discussed as possible pathogenetic reasons of cyclosporin A nephrotoxicity.

Cyclosporin A toxicity *Rat kidney* *Mitochondrion respiration*

1. INTRODUCTION

Cyc A has proved to be a powerful immunosuppressive agent in renal, liver, heart, and pancreatic transplantation (for a recent review, see [1]). However, nephrotoxicity has been described as one of the most serious side-effects of this novel pharmacological product [1–3]. Ultrastructural studies have shown that the proximal tubular cells, but not the glomerulus and other parts of the nephron are affected [4,5]. Hitherto, the molecular processes mediating this nephrotoxic effect of Cyc A were unknown. One possible mechanism may be the injury of energy metabolism in the affected cells.

The present study was designed to evaluate the influence of Cyc A on respiration in rat renal cortical mitochondria. The results provide evidence that Cyc A nephrotoxicity may be caused by an alteration of mitochondrial respiration.

2. MATERIALS AND METHODS

Male Wistar rats (VEB Versuchstierproduktion Schönwalde, GDR) weighing 250–300 g were killed by a blow to the head and both kidneys were rapidly excised and immediately immersed in an ice-cold preparation medium. The medium contained 210 mM mannitol, 70 mM sucrose, and 0.5 mM EDTA buffered at pH 7.4 with traces of Tris. The kidneys were split in half longitudinally and the medulla was removed. The remaining cortical tissue was placed in the preparation medium, minced, rinsed and homogenized in a Potter-Elvehjem glass homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 800 × *g* in a refrigerated centrifuge (Model K 24, VEB Zentrifugenbau Leipzig, GDR). The resulting supernatant was centrifuged for 5 min at 12000 × *g*. The pellet was then resuspended in preparation medium to a concentration of about 10 mg mitochondrial protein per ml and stored on ice. Throughout the isolation procedure the tissue and all solutions were kept at 0–4°C.

Respiratory measurements were carried out with a Clark oxygen electrode (VEB Metra Radebeul, GDR) at 25°C in a closed oxygraph cell. The medium contained 210 mM sucrose, 10 mM KCl, 10 mM KH_2PO_4, 0.5 mM EDTA, 60 mM Tris-HCl (pH 7.4), either 10 mM succinate or a mixture of 10 mM glutamate and 10 mM malate, and an aliquot of mitochondrial suspension (about 1 mg

Abbreviations: Cyc A, cyclosporin A; DNP, 2,4-dinitrophenol; RCI, respiratory control index

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protein) in a final volume between 2.20–2.26 ml. 30 μl of different Cyc A dilutions in 154 mM NaCl were added. The following parameters of mitochondrial respiration were studied: state 3 respiration, the rate of O_2 consumption measured in the presence of 100 μM ADP added 1 min after adding Cyc A; state 4 respiration, the slower rate measured following the depletion of ADP; the RCI, the ratio of state 3 to state 4; the ADP/O ratio, the ratio of added ADP to the oxygen consumed in state 3; and the DNP-stimulated respiration, oxygen consumption measured in the presence of 50 nM DNP. All respiratory rates were calculated as nmol O_2 utilized/min per mg mitochondrial protein. The calculations were related to an O_2 solubility of 240 mM in the medium at 25°C.

Protein determinations were made by a Biuret method [6] with crystallized human serum albumin as standard. ADP was determined with an ADP-test combination (Boehringer, Mannheim; FRG). Statistical analyses were performed with the Student's *t*-test.

ADP and succinate were from Boehringer, glutamate from Reanal (Budapest) and Cyc A from Sandoz, Basle. Other chemicals and biochemicals on analytical grade were obtained from Merck, Darmstadt and VEB Laborchemie Apolda (GDR).

3. RESULTS

Both succinate- and glutamate plus malate-supported respiration of renal cortical mitochon-

Table 1

Glutamate plus malate supported respiration of rat kidney mitochondria depending on cyclosporin A concentration in the incubation medium

Cyc A (μg/ml)	Respiratory rates (nmol O_2/mg protein per min)			RCI	ADP/O
	State 4	State 3	DNP		
0	8.7 ± 0.6	69.0 ± 9.0	72.4 ± 11.3	7.99 ± 1.4	2.61 ± 0.1
1	8.9 ± 0.9	65.8 ± 8.1	62.1 ± 9.4	7.45 ± 1.5	2.64 ± 0.1
5	10.2 ± 1.9	54.8 ± 8.4	56.6 ± 8.5	5.48 ± 1.4	2.49 ± 0.1
10	11.0 ± 1.8[b]	43.3 ± 5.2[b]	47.1 ± 5.5[b]	3.98 ± 0.7	2.37 ± 0.3
50	17.3 ± 2.1[c]	24.1 ± 1.3[c]	29.5 ± 7.9[b]	1.41 ± 0.2	2.06 ± 0.2[b]
75	19.7 ± 1.6[c]	21.8 ± 1.9[c]	23.8 ± 2.1[c]	1.14 ± 0.0[c]	2.42 ± 0.4

Data are given as arithmetic means ± 1 SD of 5 separate preparations. Differences to the controls: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. Further details, see section 2

Table 2

Succinate supported respiration of rat kidney mitochondria depending on cyclosporin A concentration in the incubation medium

Cyc A (μg/ml)	Respiratory rates (nmol O_2/mg protein per min)			RCI	ADP/O
	State 4	State 3	DNP		
0	16.0 ± 0.8	112 ± 13.3	109 ± 15.5	6.96 ± 0.6	1.93 ± 0.2
1	16.2 ± 0.9	113 ± 14.1	110 ± 15.7	6.92 ± 0.8	1.94 ± 0.1
5	16.7 ± 1.2	100 ± 11.4	101 ± 12.9	5.98 ± 0.3[a]	1.81 ± 0.1
10	16.7 ± 0.4	90.6 ± 9.8[a]	97.7 ± 13.9	5.43 ± 0.6[a]	1.70 ± 0.2
50	20.3 ± 1.3[c]	60.2 ± 6.5[c]	70.1 ± 9.8[b]	2.99 ± 0.5[c]	1.69 ± 0.1[a]
75	21.3 ± 1.1[c]	54.1 ± 8.9[c]	62.7 ± 12.1[b]	2.56 ± 0.5[c]	1.68 ± 0.1[a]

For further details, see table 1

dria appear affected by Cyc A in a dose-related fashion (tables 1,2). State 3 and DNP-stimulated respiration are inhibited and state 4 respiration is increased. Therefore, the decline of RCI seems to be the most sensitive parameter for detecting the mitochondrial dysfunction. The ADP/O ratio, however, is only slightly affected. The influence of Cyc A on mitochondria is more evident for the glutamate plus malate- than succinate-supported respiration.

4. DISCUSSION

Our results indicate that Cyc A interferes, like other nephrotoxic drugs, especially aminoglycoside and cephalosporin antibiotics [7], with the mitochondrial energy generation. The in vitro exposure of renal mitochondria to Cyc A has shown a deterioration of mitochondrial respiration in a manner similar to that observed with gentamicin: the ADP- and DNP-stimulated respiration is decreased and the resting state (state 4) respiration is increased. Cyc A alters the mitochondrial properties already at concentrations between 40 and 80 nM. Threshold concentrations of gentamicin for the same effects were at higher concentrations, between 1 and 2 mM [8]. These effects of Cyc A suggest that this drug acts both as inhibitor of mitochondrial electron transport system and uncoupler. Since state 3 of glutamate plus malate-supported respiration is influenced at a lower concentration than succinate-supported respiration, it seems very likely that Cyc A inhibits mitochondrial electron transport system at several sites.

The uncoupling effect is evident at higher concentrations of Cyc A. Since this effect on state 4 respiration includes both the inhibitory effect on the respiration and the uncoupling action, the true value of this effect would be underestimated under such conditions.

Detailed knowledge of the tissue distribution of Cyc A depending on the kind of administration of this drug is lacking. Nooter et al. [9] demonstrated that already a single oral dose of 82 mg/kg in the rats led to a concentration of about 80 μg per g kidney tissue. In general, immune suppression in rats has been achieved with dose ranges of Cyc A between 5 and 50 mg/kg per day [1]. Taking into account the pharmacokinetic behaviour of Cyc A [9] we suggest that Cyc A levels in kidneys occur which could provoke the effects demonstrated here by in vitro experiments. In fact, structural alterations of renal mitochondria have been reported in patients receiving Cyc A [10].

In summary, the study clearly demonstrates that renal mitochondria exposed to Cyc A in vitro exhibit alterations in respiratory parameters. This mitochondrial injury may be an important pathogenetic mechanism of Cyc A nephrotoxicity.

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