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The effect of 8-cyclopentyl-1,3-dipropylxanthine on the development of cyclosporin-induced acute renal failure

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Abstract—The effect of the selective A_1-adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·1 mg kg^{-1} i.v.) administered twice daily to rats has been assessed on the development of renal dysfunction induced by four daily injections of cyclosporin (60 mg kg^{-1} i.p.). The series of cyclosporin injections resulted in a polyuria accompanied by a 64–70% increase in plasma urea and creatinine concentrations and a 50% reduction in inulin clearance. However, cyclosporin administration resulted in no change in *p*-aminohippurate clearance nor was there any evidence of tubular necrosis or vascular damage. CPX treatment did not improve any index of renal function perturbed by cyclosporin. The findings provide evidence that adenosine does not play a role in the pathophysiology of cyclosporin nephrotoxicity.

Cyclosporin is now established as the immunosuppressant of choice in human organ transplantation with the most serious side effect of treatment being nephrotoxicity. This is characterized by renal vasoconstriction and an associated fall in glomerular filtration rate (Murray et al 1985). The increase in vascular resistance mainly arises from constriction of the afferent glomerular arterioles (Mason 1989). The mediator of this change in resistance is not known, but one candidate is adenosine which constricts the afferent arteriole via activation of the A_1-subtype of adenosine receptor (Murray & Churchill 1985). Indeed, Churchill & Bidani (1982) have proposed that adenosine is an important mediator of haemodynamic changes that occur within the kidney in some forms of acute renal failure (ARF). This hypothesis is supported by the finding that treatment with non-selective adenosine antagonists such as theophylline reduces the severity of various types of ARF (Bidani & Churchill 1983; Bowmer et al 1986; Heidemann et al 1989). However, theophylline treatment has been reported to have no beneficial effects in cyclosporin-induced ARF in the rat (Gerkens & Smith 1985). This lack of effect of theophylline could be due to its non-selective blockade of adenosine receptor subtypes or the dose administered (40 mg kg^{-1} twice daily) being insufficient to block the renal actions of adenosine. We have re-examined the role of adenosine in the pathogenesis of cyclosporin-induced ARF by assessing the effects of treatment with the selective A_1-adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX). Treatment was carried out with a dose of CPX known to antagonize adenosine-induced falls in renal blood flow and which has proved effective in ameliorating glycerol and cisplatin-induced ARF (Kellett et al 1989; Knight et al 1990).

Materials and methods

Materials. [^{3}H(G)]Inulin (180–215 mCi g^{-1}) of stated radioactive purity 98–99% and *p*-[glycyl-1-^{14}C]aminohippuric acid (46·4–57·6 mCi $mmol^{-1}$, radioactive purity 97·5–98%) were both obtained from New England Nuclear Ltd and were used without further purification. CPX was synthesized by Dr R. James of ICI. Kits for the assay of creatinine and urea were obtained from Pierce and Warriner and BDH Ltd, respectively. Cyclosporin (Sandimmun oral solution 100 mg mL^{-1}, Sandoz) was diluted with castor oil to give a stock solution of 60 mg mL^{-1}.

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Proctocol. Male Wistar rats, 250–300 g, received four daily injections of either cyclosporin (60 mg kg^{-1} i.p.) or its vehicle (castor oil, 1 mL kg^{-1} i.p.). Immediately after the first injection of cyclosporin rats were treated with either CPX (0·1 mg kg^{-1} i.v.) or drug vehicle (1 mL kg^{-1} i.v. of 1% v/v dimethyl sulphoxide (DMSO), 0·75% v/v 1 M NaOH in 0·9% NaCl). Treatment with CPX or vehicle was repeated 12 h later and then every 12 h for the next 3 days. Four additional groups of rats were studied which received either no treatment (control), castor oil, castor oil and CPX, or cyclosporin alone.

Three days after the first injection of cyclosporin or castor oil, rats were placed in metabolism cages and urine collected for 24 h. Four days after the commencement of cyclosporin or castor oil injections, rats were anaesthetized and the clearances of [^{3}H]inulin (CL_{IN}) and [^{14}C]*p*-aminohippuric acid (CL_{PAH}) determined to estimate glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. At the end of the experiment a blood sample (0·75 mL) was taken from the carotid artery.

Measurement of plasma urea and creatinine concentrations. Plasma urea and creatinine concentrations were determined using standard spectrophotometric assays: urea by reaction with diacetyl monoxime and creatinine by reaction with picrate in alkaline solution.

Urine analysis. Sodium concentration was measured by flame photometry using a Corning 480 flame photometer and urine osmolality was estimated by measuring freezing point depression using an Advanced Digimatic Osmometer model 3D11.

Determination of [^{3}H]inulin and [^{14}C]p-aminohippurate clearances. Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.). The trachea was cannulated to maintain a clear airway and cannulae were inserted into the left jugular vein and right carotid artery. The animals were heparinized (500 int. units kg^{-1} i.v.) and the single injection method of Hall et al (1977) was then used to simultaneously measure the clearances of [^{3}H] inulin (100 mg kg^{-1}; 20 μCi kg^{-1} i.v.) and [^{14}C]*p*-aminohippurate (40 mg kg^{-1}; 4 μCi kg^{-1} i.v.).

Kidney histology. At the end of the experiment, kidneys were removed, cleared of adherent tissue and their weights recorded. The kidneys were bisected longitudinally, washed and placed in formal saline (BDH). A longitudinal section was cut from one kidney of each rat and stained with haematoxylin and eosin. Sections were examined for evidence of tubular necrosis and vascular damage by a pathologist who was unaware of the treatment the donor animal had received.

Statistical analysis. Results are expressed as mean ± s.e.m. A comparison of data from control rats and rats which received cyclosporin only was made using Student's unpaired *t*-test. One-way analysis of variance (ANOVA) was employed to analyse data from the various groups of cyclosporin-injected rats and

Table 1. Urine output, osmolality and sodium excretion in control rats and rats injected with cyclosporin (60 mg kg^{-1} i.p.) daily for 4 days and treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·1 mg kg^{-1} i.v.) or its vehicle (1·0 mL kg^{-1}).

Group	Urine output (mL/24 h/100 g body wt)	Osmolality (mOsm kg^{-1})	Sodium excretion (mmol/24 h/100 g body wt)
Control	3·1 ± 0·3	2068 ± 163	0·53 ± 0·05
Cyclosporin	14·3 ± 2·8*	1117 ± 155**	0·33 ± 0·05*
Cyclosporin + vehicle	5·6 ± 0·9†	1140 ± 206	0·25 ± 0·05
Cyclosporin + CPX	8·3 ± 2·1	1053 ± 191	0·36 ± 0·04

Results are given as mean ± s.e. m, n = 12. *$P < 0·01$; **$P < 0·001$ relative to control group (Student's t-test); †$P < 0·05$ relative to cyclosporin group (ANOVA).

data from control rats and rats which received either castor oil or CPX and castor oil. Following ANOVA the means were compared where appropriate using Scheffe's test.

Results

The values of all indices of renal function recorded in the groups of rats which received either castor oil (the vehicle for cyclosporin) or CPX and castor oil were not significantly different ($P > 0·05$) from the values noted in control rats. In addition there was no histological evidence of tubular necrosis or vascular damage in either of these groups of rats. These results indicate that neither castor oil nor CPX caused any disturbance of renal function in normal animals.

The plasma urea and creatinine concentrations in rats which received cyclosporin alone were significantly ($P < 0·001$) higher (64–70%) than the levels recorded in the control group (Fig. 1). Repeated cyclosporin injections resulted in a polyuria with urine output increasing approximately five-fold relative to controls (Table 1). Analysis of the urine showed that osmolality and sodium excretion were significantly ($P < 0·01$) reduced when compared with control values. CL_{IN} and CL_{PAH} in cyclosporin-injected rats were, respectively, 53 and 22% lower than the clearance values obtained in control animals with a significant change ($P < 0·001$) occurring only for CL_{IN} (Fig. 2). The plasma levels of urea and creatinine in cyclosporin-injected rats which were treated with CPX or its vehicle were not significantly different ($P > 0·05$) from the untreated cyclosporin-injected group (Fig. 1). Treatment with either CPX or its vehicle reduced the polyuria of cyclosporin-injected rats by 42 and 61%, respectively, although a significant change ($P < 0·05$) was only noted with vehicle treatment (Table 1). However, urine osmolality and sodium excretion in CPX- and vehicle-treated cyclosporin-injected rats were not significantly different ($P > 0·05$) from the untreated cyclosporin group. The result of treatment of cyclosporin-injected rats with CPX or its vehicle was an increase in CL_{IN}, but only vehicle treatment produced a significant ($P < 0·01$) elevation. By contrast CL_{PAH} in these CPX- and vehicle-treated rats showed a small reduction (Fig. 2) with neither change being statistically significant ($P > 0·05$) compared with the clearances recorded in the untreated cyclosporin group.

Mean total kidney weights recorded in the cyclosporin-injected groups were not significantly different from the kidney weight noted in control rats (2·22 ± 0·07 g). Histological examination revealed no evidence of necrotic tubules or vascular damage in any of the rats which received cyclosporin.

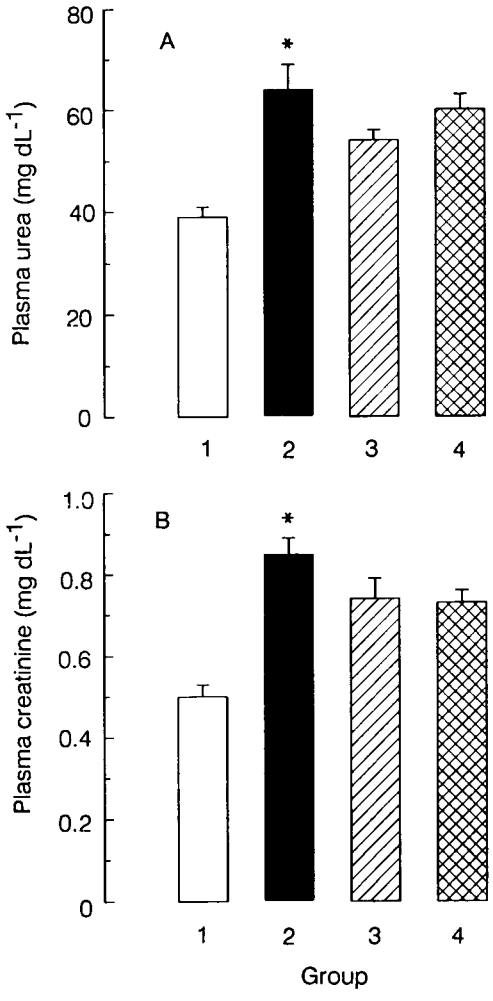

FIG. 1. Plasma urea (A) and creatinine (B) concentrations measured in rats 4 days after daily administration of cyclosporin (60 mg kg^{-1} i.p.). Rats were treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·1 mg kg^{-1}) or its vehicle (1·0 mL kg^{-1}) twice daily i.v. for 4 days. Key to groups: (1) control (no treatment): (2) cyclosporin; (3) cyclosporin + vehicle; (4) cyclosporin + CPX. Columns represent means and vertical bars s.e.m (n = 12). *$P < 0·001$ relative to group 1 (Student's t-test).

Discussion

A series of four daily injections of cyclosporin (60 mg kg^{-1} i.p.) resulted in a moderate impairment in renal function associated with significant increases in plasma urea, creatinine and urine output and a significant decrease in CL_{IN}. Similar increases in urea and creatinine levels were reported by Racusen et al (1986) who employed an identical dosage regime for cyclosporin. In contrast, the marked increase in urine output and associated decrease in osmolality was surprising since in other studies of rats given cyclosporin, urine volume and osmolality have been found to be either unaltered or the volume marginally increased and osmolality slightly decreased (see Mason 1989). Cyclosporin administration produced a greater reduction in GFR compared with ERPF, a finding which has been documented in a previous

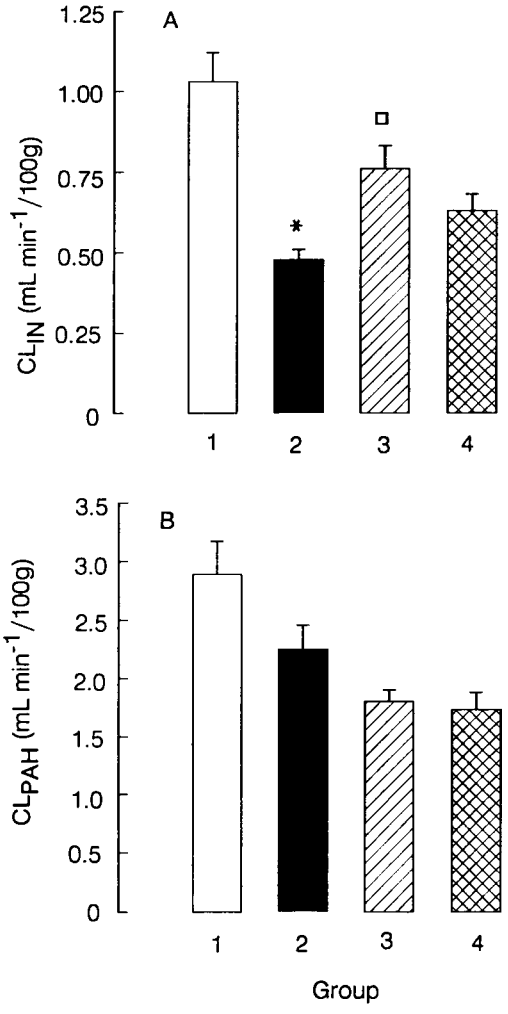

FIG. 2. The clearances of (A) [^{3}H]inulin (CL_{IN}) and (B) [^{14}C]*p*-aminohippurate (CL_{PAH}) determined in rats 4 days after daily administration of cyclosporin (60 mg kg^{-1} i.p.). Rats were treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0·1 mg kg^{-1}) or its vehicle (1·0 mL kg^{-1}) twice daily i.v. for 4 days. Key to groups: (1) control (no treatment); (2) cyclosporin; (3) cyclosporin + vehicle; (4) cyclosporin + CPX. Columns represent means and vertical bars s.e.m (n = 12). *$P < 0·001$ relative to group 1 (Student's *t*-test); □ $P < 0·01$ relative to group 2 (ANOVA).

study of cyclosporin nephrotoxicity in the rat (Barros et al 1987). Those workers suggested that this disproportionate decline in GFR was a result of a concomitant reduction in the ultrafiltration coefficient (K_f) due to a decrease in the glomerular surface area produced by mesangial cell contraction. This suggestion was supported by subsequent studies which demonstrated that local application of cyclosporin produced mesangial cell contraction in addition to potentiating the contractile response of other vasoconstrictor agents active at the mesangium (Meyer-Lehnert & Schrier 1988; Rodriguez-Puyol et al 1989).

In agreement with other investigations (Jackson et al 1987; McNally et al 1990) there was no histological evidence of tubular necrosis despite the fall in GFR. This indicates that cyclosporin nephrotoxicity is not primarily a result of tubular toxicity and, indeed, reviews of the numerous studies on this topic have concluded that renal dysfunction induced by cyclosporin is a consequence of a direct or indirect renal vasoconstrictor action (Racusen & Solez 1988; Mason 1989). The increase in vascular resistance provoked by cyclosporin mainly arises in the afferent glomerular arterioles (Mason 1989). Adenosine can produce afferent arteriole constriction and a subsequent fall in GFR (Murray & Churchill 1985) and its production and release may be involved in the pathophysiology of various forms of ARF (Churchill & Bidani 1982). A reflection of this is that treatment with adenosine antagonists can markedly improve renal function in ARF induced by either cisplatin (Heidemann et al 1989), myohaemoglobinuria (Bidani & Churchill 1983; Bowmer et al 1986), ischaemia (Lin et al 1986) or hypoxaemia (Gouyon & Guignard 1988). However, the present study has shown that treatment with the A_1-selective adenosine antagonist CPX does not significantly reduce the severity of renal dysfunction produced by cyclosporin. It was apparent that any small improvements in renal function produced by CPX treatment could be accounted for by a beneficial effect of its vehicle. This is possibly a result of the DMSO component since this highly polar compound has been shown to have, by an undefined mechanism, a protective effect in ischaemic ARF induced by renal artery clamping (Kedar et al 1981). However, in the study of Kedar et al (1981) the amount of DMSO administered (5 g kg^{-1}) was 60 times the total dose injected in the present study. Furthermore, in our previous investigation of CPX and glycerol-induced ARF we could not detect any beneficial effect of this vehicle (Kellett et al 1989).

The failure of CPX treatment to ameliorate cyclosporin nephrotoxicity is not due to an inadequate dose of CPX since we have shown that 0·1 mg kg^{-1} of CPX markedly inhibits in-vivo the decreases in renal blood flow produced by close arterial injection of adenosine (Kellett et al 1989). The findings indicate that adenosine is not involved in the pathophysiology of cyclosporin nephrotoxicity and support studies which have shown that the non-selective weak adenosine antagonist theophylline did not attenuate either the acute or chronic reductions in renal function produced by cyclosporin (Gerkens & Smith 1985; Churchill et al 1990).

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