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Effects of a selective adenosine A_1 receptor antagonist on the development of cyclosporin nephrotoxicity

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1 The clinical application of cyclosporin as an immunosuppressive agent is limited by its nephrotoxicity.

2 The effect of FK453, a selective A_1-receptor antagonist, administered twice daily to rats at a dose of 100 mg kg^{-1} was assessed on the development of nephrotoxicity induced by cyclosporin (10 mg kg^{-1} i.p. daily) administered for 14 days. The effects of nifedipine administered twice daily (0.3 mg kg^{-1} s.c.) for 14 days, on cyclosporin nephrotoxicity were also studied.

3 Cyclosporin induced a 46.58% and 35.78% decline in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) respectively and a reduction of 16.69% in filtration fraction (FF). Co-administration of FK453 resulted in falls of 30.5%, 18.59% and 14.7% in GFR, ERPF and FF respectively, the former two significantly less than the falls seen with cyclosporin (CyA) alone ($P<0.05$ vs CyA, ANOVA).

4 Nifedipine appeared to have a more pronounced protective effect resulting in a decline of only 20.91% in GFR, with no significant change in ERPF (increase of 0.93%) when co-administered with CyA.

5 These observations indicate adenosine plays a minor role in the pathophysiology of CyA nephrotoxicity.

Keywords: Adenosine A_1 receptor antagonist; FK453; cyclosporin; nephrotoxicity; glomerular filtration rate; effective renal plasma flow; nifedipine

Introduction

The use of cyclosporin A (CyA) as an immunosuppressive agent has contributed significantly to successful solid organ transplantation with a substantial increase in graft survival. Nephrotoxicity, however, continues to be a significant limiting factor in its clinical application. It appears to be dose-related with functional renal vascular changes at lower doses (Sabatini *et al.*, 1990) and structural damage induced at high doses of the drug (Thomson *et al.*, 1981). Although the precise pathophysiological mechanisms remain unclear, intrarenal vasoconstriction is a characteristic feature of cyclosporin nephrotoxicity (Murray *et al.*, 1985; Curtis *et al.*, 1986) with an increase in total renal vascular resistance (RVR) leading to a concomitant reduction in renal blood flow (RBF) and glomerular filtration rate (GFR) (Murray *et al.*, 1985; Curtis *et al.*, 1986; Conte *et al.*, 1989).

Within the intrarenal circulation, the major target appears to be the afferent arteriole, with experimental studies showing increased afferent arteriolar resistance as measured by micropuncture techniques (Thompson *et al.*, 1989). A variety of mechanisms have been postulated to account for the intrarenal vasoconstriction associated with this agent, including intrinsic vasoconstrictor activity of the drug, increased activity of the renin-angiotensin system, altered prostaglandin metabolism and excessive sympathetic nerve stimulation (McNally & Feehally, 1992).

Adenosine, the endogenous nucleoside, has been proposed as a mediator of cyclosporin nephrotoxicity, because of its characteristic receptor-mediated effects on renal haemodynamics.

Micropuncture studies clearly show that adenosine induces a transient decline in renal blood flow and a more sustained reduction in glomerular filtration rate as a result of cortical afferent arteriolar vasoconstriction (Osswald, 1983). Experimental studies using non-metabolised, selective receptor agonists suggest this pre-glomerular vasoconstriction is induced by activation of adenosine A_1 receptors (Murray & Churchill, 1985). In addition, adenosine appears to play a significant role in the tubuloglomerular feedback response as well as in renin release (Osswald, 1984). Adenosine has also been proposed as a mediator in some forms of acute renal failure (ARF), an hypothesis supported by the protective effect conferred by adenosine receptor antagonists in several animal models of ARF (Bidani & Churchill, 1983; Bowmer *et al.*, 1986; Heidemann *et al.*, 1989). Furthermore, a more recent morphometric study of human acute renal failure suggests the renin-angiotensin system, together with adenosine which is released in kidneys with ischaemic or toxic damage, play a critical role in the pathogenesis of ARF (Bohle *et al.*, 1990). FK453 a pyrazolopyridine derivative, has been shown *in vitro*, to be a highly selective A_1 receptor antagonist (Terai *et al.*, 1990). In studies involving anaesthetized rats FK453 displayed a >300 fold selectivity for the A_1 receptors compared to the A_2 receptors (Kuan *et al.*, 1992). In these animals, FK453 produced a range of pharmacological actions including a selective increase in RBF and GFR, decreased RVR, reduced filtration fraction, a natriuresis, diuresis and increased uric acid excretion (Terai *et al.*, 1990). In addition, FK453 appears to have a protective effect in glycerol, gentamicin and cisplatin animal models of acute renal failure (Andoh *et al.*, 1991; Ishikawa *et al.*, 1991).

The present study was designed to investigate the role of endogenous adenosine and a possible protective effect of FK453 in an animal model of cyclosporin nephrotoxicity. The model was designed to allow repeated measurements of renal haemodynamics in individual animals using methods that cause only minimal stress. Structural and functional changes in the kidney were studied over a period of time and at a dose of CyA, appropriate to the development of clinical nephrotoxicity.

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Methods

Studies were performed on five groups of male Sprague-Dawley rats (A. Tuck and Sons Ltd., Battlebridge, Essex, UK) weighing 264–339 g, 10 rats in each group. Grouping was as follows:-

Group 1 Cremophore 0.5 ml kg^{-1}, i.p., daily (polyoxyethylated castor oil derivative) + 0.5% methylcellulose, oral gavage.

Group 2 Cyclosporin A 10 mg kg^{-1}, i.p. daily.

Group 3 FK453 100 mg kg^{-1} bd twice daily, given orally.

Group 4 Cyclosporin A 10 mg kg^{-1} daily + nifedipine 0.3 mg kg^{-1}, s.c., twice daily (positive control).

Group 5 Cyclosporin A 10 mg kg^{-1}, daily + FK453 100 mg kg^{-1}, twice daily.

The animals underwent an acclimatization period when they were handled, weighed and had their blood pressure measured. They were housed 5 per cage and were kept at a constant temperature of 25°C. They had free access to tap water, fed on a standard diet (Pilsbury's modified rat and mouse breeding diet, Birmingham, UK) and subjected to 12 h cycles of light and dark. The cyclosporin dissolved in cremophore was administered in the morning between 09 h 00 min and 11 h 00 min by intraperitoneal injection at a dose of 10 mg kg^{-1} daily for 14 days (day 1–14) to the rats in groups 2, 4 and 5. FK453 and nifedipine were administered 1 h preceding the dose of cyclosporin in the morning on days 1–14 and between 21 h 00 min and 22 h 00 min at night. FK453 was given at doses of 100 mg kg^{-1} by oral gavage twice daily and nifedipine at a dose of 0.3 mg kg^{-1} s.c., twice daily. The following investigations were carried out:

(1) Daily general observations and blood pressure measurements on days -2, 5 and 12. Systolic blood pressure was measured with a tail blood pressure cuff, a pulse sensor attached to a piezoelectric crystal and an automated electrosphygmomanometer (Narco Biosystems Inc., Houston, Texas, U.S.A.). The value recorded was the mean of three measurements. For this procedure the animals were lightly sedated with Hypnorm (fentanyl 3.15 μg and fluanisone 0.1 mg).

(2) Urine flow rate was estimated on days 0, 7 and 14 from clean urine collected in a metabolic cage during a 12 h overnight starvation period.

(3) Lean body weights were measured on days 0, 7 and 14.

(4) Measurement of GFR and ERPF Each rat underwent clearance studies on days 0 and 14. A single injection and single blood sample isotopic technique using [^{51}Cr]-EDTA and [^{125}I]-hippuran (0.5 MBq) was employed (Provoost *et al.*, 1983; Ferguson *et al.*, 1992; 1993). The animals were lightly anaesthetised in an ether chamber and vascular access was obtained by puncture of the ventral tail vein with a 23 gauge butterfly needle. After confirmation of good flow, the isotope (in 0.4 ml of 0.9% NaCl) was injected and the animals allowed to recover from the anaesthesia. One hour later, using the same technique, 1–2 ml of blood was taken from the tail vein at a site distant from the injection site. The blood was anti-coagulated with lithium heparin and the plasma separated. The dose of radioactivity administered, the plasma samples and the residual radioactivity in the syringe used for the injection were counted on a scaler time ST7 scintillation counter (Nuclear Enterprises, Thorn EMI Ltd, UK). Clearances were calculated according to the formulae $C = V \times (Po/Pt)/t$, where C = clearance, V = volume of distribution (where V = As/Ai, where Ai is the total activity injected and As is the activity per ml plasma), Pt = amount of radioactivity in the plasma after 't' minutes, and Po = I/V, where I is the amount of radioactivity at time '0'.

(5) *Renal histology* The kidneys were prepared for histological studies following the final clearance studies on day 14. One kidney from each animal was fixed by perfusion of the renal artery with buffered formalin at a constant pressure of 90 mmHg. The kidney was then bisected in a frontal plane and immersed in fixative for at least 48 h. A 4 mm slice was embedded in paraffin wax and 4 μm sections were stained with haematoxylin and eosin, periodic acid Schiff and elastin van Giesen. The sections were examined for changes in the glomeruli, blood vessels, tubules and interstitium using a semi-quantitative scoring method. Quantitative assessment of the cortex was carried out by 3 observers independently and without knowledge of the treatment group. Fields of cortex at ×200 magnification on a Reichert Visoplan were selected by a stepped protocol and points on a 25 point screen overlay were recorded as lying over either tubular epithelium, tubular lumen, interstitium, blood vessel or glomerulus. A total of 125 points were counted in each case. Counts from each treatment group were pooled and the resulting contingency table analysed by the chi-squared test.

Statistical analysis

Data on GFR, ERPF and filtration fraction were expressed as percentage changes from baseline (day 0). The paired *t* test was used to assess changes within a group. Changes in parameters from baseline between groups were compared by a 1-factor analysis of variance (ANOVA). If a significant difference among the five groups was detected with ANOVA, Fisher's least significant difference (LSD) test was used to explore which groups were significantly different from each other. The difference in the counted incidence of histological parameters was assessed by the chi-squared test. Significance was defined as $P<0.05$.

Results

Effective renal plasma flow, glomerular filtration and filtration fraction

Results are summarised in Tables 1, 2. Haemodynamic data are missing for 1 rat in group 3 on day 0, and 1 rat in group 4 on day 14 due to technical problems during the clearances studies.

Group 1 (vehicle control)

There was a significant increase in GFR in the control group (14.05%, $P=0.0002$) between day 0 and day 14. There was also an increase in ERPF (9.95%) and filtration fraction (5.55%) but these changes did not reach levels of statistical significance. The increases probably reflect normal growth of these animals.

Group 2 (CyA 10 mg kg^{-1}, daily)

There was a significant fall in GFR between day 0 and day 14 (−46.58%, $P=0.001$). This change was also significantly different when compared to the vehicle control ($P<0.05$, ANOVA). ERPF declined significantly from baseline (−35.78%, $P=0.001$) and also in comparison to the control group ($P<0.05$, ANOVA). The relative difference in the reduction of these parameters from baseline, resulted in a significant fall in filtration fraction (−16.69%, $P=0.005$ and $P<0.05$ vs vehicle, ANOVA).

Group 3 (FK453, 100 mg kg^{-1} twice daily)

There were no significant changes in haemodynamic parameters from baseline or in comparison with the vehicle control in this group. There were small increases in GFR, ERPF and filtration fraction (1.96%, 0.57% and 6.05% respectively).

Group 4 (Cyclosporin A 10 mg kg^{-1}, daily + nifedipine 0.3 mg kg^{-1}, twice daily)

GFR declined significantly in this group (−20.91%, $P=0.0017$, $P<0.05$ vs vehicle, ANOVA). The decline in GFR was, however, significantly less pronounced when compared to the more marked reduction of 46.58% observed in the CyA group ($P<0.05$, ANOVA). There was no significant change in ERPF from baseline (0.93%) or in comparison with vehicle group, although it was significantly different from the decline in ERPF seen in the CyA group ($P<0.05$, ANOVA). Filtration fraction declined significantly from baseline (−20.77%, $P=0.001$) and in comparison with vehicle control ($P<0.05$, ANOVA).

Group 5 (Cyclosporin A 10 mg kg^{-1} daily + FK453 100 mg kg^{-1}, twice daily)

GFR (−30.50%, $P=0.001$, $P<0.05$ vs vehicle) and ERPF (−18.59%, $P=0.009$, $P<0.05$ vs vehicle) were both significantly reduced. However, as with Group 4, the changes in GFR and ERPF were significantly less pronounced in comparison to the CyA group ($P<0.05$, ANOVA). In addition as a result of the disproportionate changes in these parameters there was a significant fall in FF (−14.7%, $P=0.0168$, $P<0.05$ vs vehicle). There were no significant differences between groups 4 and 5 with respect to the changes in GFR and filtration fraction, although there was a significant difference between them with respect to the changes in ERPF ($P<0.05$, ANOVA).

Body weight (Table 3)

The control and FK453 groups gained weight during the study (327.5 g to 340.1 g and 295 g to 300.9 g respectively, $P<0.05$ for both groups). The other groups all lost weight at the end of 14 days ($P<0.05$ vs baseline for these groups).

Table 1 Glomerular filtration rate (ml min^{-1}), effective renal plasma flow (ml min^{-1}) and filtration fraction (%)

Groups		*Day 0*	*Day 14*
Vehicle	GFR	3.04 ± 0.14	3.44 ± 0.11
	ERPF	8.81 ± 0.48	9.38 ± 0.40
	FF	35.31 ± 1.89	36.27 ± 1.57
CyA	GFR	2.49 ± 0.10	1.32 ± 0.10
	ERPF	7.67 ± 0.17	4.91 ± 0.33
	FF	32.4 ± 1.01	26.68 ± 0.93
FK453	GFR	3.28 ± 0.17	3.30 ± 0.14
	ERPF	8.88 ± 0.55	8.57 ± 0.20
	FF	37.63 ± 2.89	38.71 ± 1.91
CyA + Nifed	GFR	3.06 ± 0.12	2.41 ± 0.2
	ERPF	8.04 ± 0.36	8.09 ± 0.44
	FF	38.68 ± 2.28	29.54 ± 1.32
CyA + FK453	GFR	2.89 ± 0.10	1.96 ± 0.13
	ERPF	8.50 ± 0.35	6.75 ± 0.25
	FF	34.23 ± 1.06	28.86 ± 1.13

Data as mean ± s.e.mean.

Table 2 Percentage change in glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and filtration fraction (FF)

Groups		*% change*	P *value* vs baseline*
Vehicle	GFR	14.06 ± 2.37	.0002
	ERPF	9.95 ± 8.55	.2744
	FF	5.55 ± 6.82	.4371
CyA	GFR	−46.58 ± 4.34†	.0001
	ERPF	−35.78 ± 4.41†	.0001
	FF	−16.69 ± 4.45†	.0046
FK453	GFR	1.96 ± 4.96‡	.7031
	ERPF	0.57 ± 8.07‡	.9458
	FF	6.05 ± 7.18‡	.4242
CyA + Nifed	GFR	−20.91 ± 4.5†‡	.0017
	ERPF	0.93 ± 5.83‡	.8768
	FF	−20.77 ± 4.12†	.001
CyA + FK453	GFR	−30.50 ± 6.7†‡	.0014
	ERPF	−18.59 ± 5.6†‡	.009
	FF	−14.71 ± 5.02†	.0168

Data as mean ± s.e.mean.
*Paired *t*-test; †$P<0.05$ *vs* vehicle, ANOVA; ‡$P<0.05$ *vs* CyA, ANOVA.

Systolic blood pressure

There were no significant changes in blood pressure both within and across groups (Table 4).

Renal histological changes

Semi-quantitative assessment of the renal cortex showed no abnormalities of glomeruli or blood vessels and no appreciable interstitial fibrosis was identified. Point counting showed a significant increase (chi^2 test, $P<0.001$) in points falling on the tubular lumina in Groups 3 (FK453 100 mg kg^{-1}, twice daily) and 5 (CyA 10 mg kg^{-1} + FK453 100 mg kg^{-1}) indicating possible tubular dilatation.

Discussion

In the present study, cyclosporin induced a characteristic decline in renal function with a fall in GFR, ERPF and body weight. These changes, occurring in the absence of any appreciable evidence of histological changes are consistent with the results from previous studies demonstrating functional renal impairment at relatively low doses of cyclosporin (Sabatini *et al.*, 1990). Cremaphor (the vehicle for CyA) is potentially vasoactive and has been shown to reduce RBF in the rat (Theil *et al.*, 1986) and in the isolated perfused kidney (Besarah *et al.*, 1987). This is, however, unlikely to have had a significant effect in the present study since the control group (cremaphor + methylcellulose) showed no adverse effect on renal function. The relative change in body weight is also unlikely to have influenced the decline in renal function in the cyclosporin-treated groups as the fall in GFR and ERPF was disproportionately greater than the decrease in body weight. The animals in the control group, in contrast, gained weight and increased their renal function at a normal rate. The changes in renal haemodynamics are probably related to the well documented intrarenal vasoconstriction particularly at the site of the afferent arteriole (Thompson *et al.*, 1989). Cyclosporin administration produced a greater reduction in GFR compared with ERPF (resulting in a marked reduction in filtration fraction), a finding which has been documented in previous studies of cyclosporin nephrotoxicity in the rat (Barros *et al.*, 1987; Ferguson *et al.*, 1993). This observation suggests that additional mechanisms may be involved, influencing GFR independent of any changes in renal plasma flow. Barros *et al.* (1987) have suggested that the disproportionate decline in GFR is related to a decline in ultrafiltration coefficient (Kf) due to a decrease in the glomerular surface area produced by mesangial cell contraction. This hypothesis is supported by studies which have demonstrated that cyclosporin contracts mesangial cells in culture, in addition to potentiating the contractile response of other vasoconstrictor agents active at the mesangium (Meyer-Lehnart & Schrier, 1988; Rodriguez-Puyol *et al.*, 1989).

As in previous studies (McNally *et al.*, 1990a) there was no histological evidence of tubular necrosis despite the decline in renal function. This indicates that acute cyclosporin nephrotoxicity is not related to tubular toxicity and arises primarily as a consequence of renal dysfunction induced by direct or indirect renal vasoconstriction. The increase in vascular resistance provoked by cyclosporin arises mainly in the glomerular afferent arterioles (Thompson *et al.*, 1989). Adenosine can induce afferent arteriolar vasoconstriction by A_1 receptor activation causing a decline in GFR (Murray & Churchill, 1985). In addition *in vitro* studies demonstrate adenosine produces an A_1 receptor-induced contraction of cultured mesangial cells (Olivera *et al.*, 1989). These effects are similar to the pathophysiological changes observed in cyclosporin nephrotoxicity and may indicate a role for adenosine in this form of nephrotoxic injury.

FK453, a potent non-xanthine adenosine A_1 receptor an-

Table 3 Urine flow rate (μl min^{-1}) and body weight (g)

Groups		*Days* 0	7	14
Vehicle	UFR	6.6±0.7	6.2±1.2	6.2±0.9
	Wt	327.5±2.4	331.5±2.3*	340.1±3.0*
CyA	UFR	7.0±0.5	3.6±0.7*	5.9±0.5
	Wt	312.1±2.6	293.0±2.9*†	282.8±3.7*†
FK453	UFR	6.1±0.5	7.2±0.9‡	5.9±1.1
	Wt	295±3.7	292.4±4.4†‡	300.9±3.7*‡
CyA + Nifed	UFR	6.1±0.7	6.0±0.8‡	7.0±1.3
	Wt	289.8±4.4	285.0±4.9*†‡	283.7±4.7*†‡
CyA + FK453	UFR	6.7±1.1	4.9±0.7	7.5±1.1
	W	296.2±3.1	282.6±2.8*†	279.1±3.6*†‡

Data as mean ± s.e.mean.
*$P<0.05$ *vs* Baseline (day 0), Paired *t*-test; †$P<0.05$ *vs* vehicle, ANOVA; ‡$P<0.05$ *vs* CyA, ANOVA.

Table 4 Rat tail systolic blood pressure (mmHg)

Groups	*Days* −2	5	12
Vehicle	108.7±3.1	108.6±3.8	111.3±3.7
CyA	108.9±2.6	105.8±3.2	107.2±2.7
FK453	110.5±3.8	110.4±4.4	115.1±2.8
CyA + Nifed	108.2±2.5	109.6±2.3	106.5±1.9
CyA + FK453	106.1±2.2	101.3±4.3	103.4±2.7

Data as mean ± s.e.mean.

tagonist, appeared to have a limited protective effect on this model of cyclosporin nephrotoxicity, as demonstrated by a less pronounced decline in GFR and ERPF in group 5 when compared to the cyclosporin group. The production and release of adenosine has been implicated in the pathophysiology of various types of experimental acute renal failure. In support of this hypothesis, treatment with adenosine receptor antagonists has been shown to confer protection in ARF induced by ischaemia (Lin *et al.*, 1986), myohaemoglobinuria (Bidani & Churchill, 1983; Bowmer *et al.*, 1986), cisplatin (Heidermann *et al.*, 1989) and hypoxaemia (Gouyon & Guignard, 1988). However, theophylline, a non-selective adenosine receptor antagonist as well as CPX (8-cyclopentyl-1,3-dipropylxanthine), a more selective A_1 receptor antagonist had no beneficial effects in previous studies of acute cyclosporin nephrotoxicity in the rat (Gerkens & Smith, 1985; Panjehshahin *et al.*, 1991). In the present study we used a model of cyclosporin nephrotoxicity employing doses of the drug comparable to those used in man, administered over a period of time sufficient for haemodynamic and structural changes to become evident (Ferguson *et al.*, 1993). However, the inability of the selective A_1 receptor antagonist, FK453 to reverse completely the decline in renal function induced by CyA appears to confirm the multifactorial nature of the pathophysiology of CyA-induced nephropathy and is consistent with previous studies which have failed to demonstrate a protective effect for adenosine antagonists in different rat models of cyclosporin nephrotoxicity.

The histological changes of tubular dilatation observed in groups 3 (FK453 only) and 5 (CyA + FK453) were similar to that observed in a previous study in which significant tubular dilatation was observed in rats treated with CyA at doses of 10 mg kg^{-1}, daily and above (Ferguson *et al.*, 1993). There were, however, no histological abnormalities in any of the other cyclosporin-treated groups in this study (groups 2 and 4) and no functional renal impairment in group 3 (FK453 alone). No tubular dilatation was observed in either 13 or 26 weeks toxicological studies in rats with doses of FK453 ranging from 32–1000 mg kg^{-1}, daily (Internal Report, Fujisawa Pharmaceutical Co., Osaka, Japan). Therefore since there were no other histological tubular abnormalities such as swelling, vacuolisation or necrosis the significance of the histological changes observed in this study in the FK453 treated rats is unclear.

In contrast to FK453, nifedipine, a dihydropyridine calcium-channel antagonist, appeared to be far more protective in this model of cyclosporin nephrotoxicity. This is consistent with previous studies that have demonstrated a protective effect for this class of drugs in both experimental and clinical cyclosporin nephrotoxicity (McNally *et al.*, 1990a, b). They are potent renal vasodilators with a preferential effect on preglomerular vessels (Loutzenhiser & Epstein, 1987). Their inability to reverse completely the decline in GFR induced by cyclosporin (in contrast to the effect on renal plasma flow) may be related to their relative inability to reverse cyclosporin induced decline in K_f. This is supported by the observation that verapamil, a calcium channel antagonist, produces only partial inhibition of cyclosporin-induced mesangial cell contraction (Rodriguez-Puyol *et al.*, 1989).

In conclusion FK453 provided only partial protection against cyclosporin nephrotoxicity. This suggests that adenosine plays a minor role in the vasoconstriction induced by this immunosuppressive agent. However, this study does not exclude the possibility that the administration of cyclosporin may induce a 'hypersensitivity' of the renal microvasculature to adenosine. Perhaps, a study demonstrating a dose-dependent inhibition of the effects of adenosine by a selective A_1 receptor antagonist in chronic cyclosporin therapy may clarify further the role of adenosine in cyclosporin nephrotoxicity.

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