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SHORT COMMUNICATIONS

The reversal of experimental cyclosporin A nephrotoxicity by thromboxane synthetase inhibition

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Abstract—The ability of thromboxane synthetase inhibition to reverse acute cyclosporin A (CsA)-induced nephrotoxicity in the rat was investigated. CsA administration (50 mg/kg/day p.o. for 14 days) to male Sprague–Dawley rats caused a significant 50% decline in creatinine clearance rates, an increase in *N*-acetyl-β-D-glucosaminidase (NAG) enzymuria and renal tubulointerstitial damage by day 14. These changes were associated with a 5–6-fold increase in urinary thromboxane B_2 excretion (from pretreatment values of 28.1 ± 7.9 to 122.6 ± 38.9 and 165.8 ± 39.0 ηg/24 hr body weight on days 7 and 14, respectively). Excretion rates of 6-keto-prostaglandin $F_1\alpha$ and prostaglandin E_2 were, however, unaffected by CsA administration. Co-treatment with a thromboxane synthetase inhibitor (CGS 12970; 8-[3-methyl-2-(3-pyridyl)-1-indolyl]-octanoic acid) from day 7 (10 mg/kg/day) normalized thromboxane B_2 excretion, resulted in creatine clearance rates which were similar to pretreatment values on days 10 and 14, reduced NAG enzymuria on day 10 and prevented acute proximal tubular vacuolation. However, the severity of chronic CsA nephrotoxicity, namely chronic tubular damage and microcalcification at the corticomedullary junction, was not diminished by the thromboxane synthetase inhibition. These results demonstrate that (i) elevated thromboxane synthesis plays an important role in the development of acute CsA nephrotoxicity and (ii) that different and/or additional mechanisms are involved in the pathogenesis of chronic nephrotoxicity.

Acute cyclosporin A (CsA*) nephrotoxicity is characterized functionally as a decrease in glomerular filtration rate (GFR) resulting from altered renal haemodynamics, in particular reduced renal blood flow and increased renal vascular resistance [1–3]. Although the renin–angiotensin–aldosterone system (RAAS), sympathetic nervous system and altered eicosanoid metabolism have all been implicated in the pathogenesis of CsA-induced nephrotoxicity the extent, nature and interaction of these mechanisms in this process remains controversial. However, there is increasing evidence that CsA-induced renal vasoconstriction is associated with increased excretion of the vasoconstrictor eicosanoid thromboxane A_2 (TxA_2) although effects on vasodilatory eicosanoid species are less clear [4–7].

Previous studies from this and other laboratories have demonstrated that thromboxane synthetase inhibitors (TSI) can ameliorate CsA nephrotoxicity in experimental animals [8, 9] and also that thromboxane receptor antagonists have a similar effect in experimental but not clinical nephrotoxicity [10, 11]. Acute CsA nephrotoxicity is commonly observed in clinical practice and its successful treatment has obvious clinical advantages. This study therefore investigates the additional potential for TSI to reverse the functional and structural consequences of CsA administration in the rat.

Materials and Methods

Animals. Adult male Sprague–Dawley rats (initial weight 245–310 g), obtained from Charles River Ltd (Margate, U.K.) were allowed free access to food (CRM diet, Biosure Ltd, Cambridgeshire, U.K.) and water throughout the experimental period.

Drugs. A stock solution of CsA (Sandimmun®, Sandoz Ltd, Basle, Switzerland) was diluted with olive oil (Boots Company Ltd, Nottingham, U.K.) to a concentration of 50 mg/mL. The TSI (CGS 12970; 8-[3-methyl-2-(-3-pyridyl)-1-indolyl]-octanoic acid; Ciba Geigy, Horsham, U.K.) was suspended in 0.9% (w/v) NaCl (pH 9.5 with 0.1 M NaOH) to give a concentration of 2 mg/mL. Drugs or their vehicles were administered to the conscious animal by gavage using a 4FG cannula.

Protocol. Animals were randomized into three groups of six animals. Rats received CsA (50 mg/kg) once daily for 14 days either alone or in combination with either the TSI CGS 12970 (10 mg/kg) or 0.9% (w/v) NaCl, daily from day 7. In addition, untreated animals or those receiving CsA vehicle (olive oil) with or without the addition of either 0.9% (w/v) NaCl or CGS 12970 from day 7 were also studied. Creatinine clearance rates (CCR) and urine *N*-acetyl-β-D-glucosaminidase (NAG) activity [measured pretreatment (day 0) and on days 4, 7, 10 and 14] and trough whole blood CsA concentrations (measured on day 14) were estimated as described previously [8]. Urine thromboxane B_2 (TxB_2), the stable metabolite of TxA_2, prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_1\alpha$ (6-$KPGF_1\alpha$) concentrations were also measured both pretreatment (day 0) and on days 7 and 14 using specific monoclonal radioimmunoassay kits based on magnetic separation (Amersham International, Amersham, U.K.) The cross reactivity of the TxB_2 antibodies with PGE_2 and 6-KPGF1α was 0.3% and 0.15%, respectively.

At the end of the experimental period animals were killed by cervical dislocation under ether anaesthesia and kidneys removed and prepared for histological examination as described previously [8]. Sections (5 μm thick) were randomized and examined blind under light microscopy for the presence of drug-induced changes, particularly proximal tubular vacuolation (PTV), chronic tubulo-interstitial damage (CTD) and calcification (CA). Each feature was assessed on a scale from 0 to 4, where 0 was no abnormality and 1, 2, 3 and 4 represented mild, moderate, moderatively severe and severe abnormalities, respectively.

Statistics. For multiple comparisons results were

* Abbreviations: CsA, cyclosporin A; GFR, glomerular filtration rate; CGS 12970, 8-[3-methyl-2-(-3-pyridyl)-1-indolyl]-octanoic acid; PTV, proximal tubular vacuolation; CTD, chronic tubulointerstitial damage; CA, calcification; CCR, creatinine clearance rates; NAG, *N*-acetyl-β-D-glucosaminidase; TxB_2, thromboxane B_2; PGE_2, prostaglandin E_2; 6-$KPGF_1\alpha$, 6-keto-prostaglandin $F_1\alpha$; TSI, thromboxane synthetase inhibitors; RAAS, renin–angiotensin–aldosterone system.

Table 1. The effect of CGS 12970 on experimental CsA nephrotoxocity

Group	Days				
	0	4	7	10	14
Creatinine clearance rate (mL/hr/kg body weight)					
(1) CsA alone	362 ± 50	272 ± 68	247 ± 104	208 ± 25†	153 ± 55†
(2) CsA + saline from day 7	376 ± 125	284 ± 84	266 ± 82	178 ± 58†	160 ± 50†
(3) CsA + TSI from day 7	317 ± 64	304 ± 73	255 ± 40	283 ± 54	285 ± 54*
NAG enzymuria (U/mmol urinary creatinine)					
(1) CsA alone	57 ± 14	124 ± 41	195 ± 60†	228 ± 25†	180 ± 59†
(2) CsA + saline from day 7	71 ± 23	154 ± 52	175 ± 55†	231 ± 88†	165 ± 34†
(3) CsA + TSI from day 7	78 ± 18	148 ± 41†	224 ± 61†	131 ± 18*†	124 ± 43†

Results are expressed as the mean ± SD from six determinations.

Significant differences ($P < 0.05$), assigned using Neuman–Keul's test, are denoted as * for (3) vs (1) or (2) at each time point and as † for comparisons to pretreatment values.

CsA was administered for 14 days at 50 mg/kg body weight either alone or with the addition of either saline or TSI from day 7.

compared by one-way analysis of variance with significances assigned using Neuman–Keul's test. For single comparisons, results were compared using unpaired Student's t-test.

Results

The administration of CsA (50 mg/kg) for 14 days resulted in weight loss (1–2 g/day) which was similar in all three treatment groups. Normal weight gain (2–4 g/day) was observed in untreated animals and those receiving either drug vehicles or CGS 12970. Consequently, body weight was increased by 10–15% in untreated and vehicle-treated animal groups and decreased by 10–15% in all three CsA treatment groups over the experimental period. In addition, no significant changes in renal function, NAG enzymuria or urinary prostaglandin excretion were observed over the experimental period in the untreated and vehicle treatment groups (results not shown).

CsA administration resulted in a progressive decline in CCR over the experimental period (Table 1). By day 14, CCR had decreased by around 40% in animals treated with CsA either alone or in combination with saline from day 7. A progressive increase in NAG enzymuria, maximal on day 10 was also noted in these treatment groups. Treatment with CGS 12970 from day 7 prevented any further decrease in CCR resulting in clearances similar to pretreatment values on days 10 and 14. A similar, but more striking, effect of TSI on NAG enzymuria was also noted. By day 10, urine NAG activity was significantly lower than the values obtained previously on day 7 ($P < 0.05$) and also compared with the values demonstrated by the other CsA treatment groups (both $P < 0.05$).

Although CsA treatment was without significant effect on the urinary excretion of either 6-KPGF$_1\alpha$ or PGE$_2$, a 5–6-fold increase in TxB$_2$ excretion was observed by day 14 in animals receiving CsA either alone or in combination with saline from day 7 (Table 2). Co-treatment with CGS 12970 from day 7, without effect on either 6-KPGF$_1\alpha$ or PGE$_2$ excretion, reduced TxB$_2$ excretion from 112.6 ± 56.3 to 37.8 ± 13.4 ηg/24 hr by day 14 ($P < 0.05$).

Trough whole blood CsA concentrations were similar in all CsA treatment groups on day 14 with values ranging from 4.5 ± 0.5 to 5.5 ± 0.6 μg/mL.

The kidneys of animals treated with CsA either alone for 14 days or in combination with saline from day 7 onwards demonstrated a similar severity of CA, CTD and PTV (Table 3). Varying degrees of CTD and CA affecting the proximal convoluted tubule (S1 and S2) and the corticomedullary junction, respectively, were noted in all CsA-treated groups. CA occurred initially as minute calcific deposits which gradually formed larger masses. CTD was most often evident at the surface of the kidney as small wedge-shaped foci of tubular atrophy and dilatation with secondary interstitial fibrosis associated with a focal lymphoid infiltrate. In the most severely damaged kidneys these foci of CTD extended deeply into the cortex causing radial scars. Whereas virtually all animals showed some CTD, CA was a more variable feature of CsA treatment. PTV, observed in around 50% of deep cortical (S3) tubules, consisted of multiple vacuoles within the epithelium but in the absence of lethal cell damage. Although a similar severity of both CTD and CA were observed in animals co-treated with CGS 12970, no PTV was observed.

Discussion

The results of this study which clearly demonstrate that the development of acute CsA nephrotoxicity, characterized functionally by reduced CCR and increased NAG enzymuria and structurally by a tubulopathy, is associated with increased urinary excretion of TxB$_2$ confirms previous results from this and other laboratories [4–7]. The observation though that TxB$_2$ excretion reached a plateau before reductions in GFR were observed also suggests that additional factors are involved in the development of CsA nephrotoxicity [1]. However, the results do demonstrate that TSI can prevent further deterioration in renal function and reverse the PTV observed following CsA administration in the presence of consistent CsA concentrations. Other structural correlates of CsA nephrotoxicity, namely those of CTD including microcalcification, basophilia and tubular dilatation were not, however, reduced by CGS 12970 administration from day 7 confirming other reports [4, 12] that the chronic nephrotoxicity is not reversible. These results also demonstrate that while elevated thromboxane synthesis plays an important role in the development of acute CsA nephrotoxicity, different and/or additional mechanisms are involved in the pathogenesis of chronic nephrotoxicity.

Of particular interest is the observation that inhibition of TxA$_2$ synthesis from day 7 onwards essentially normalized TxB$_2$ excretion by day 14 and was as effective with respect to both functional and structural parameters as the effect of co-administration of the inhibitor for the entire experimental period as observed in a previous study [8]. The ability of TSI to reverse the functional toxicity associated with chronic CsA treatment has also been demonstrated [4]. In this study rats were treated with CsA at 40 mg/kg 48 hr for 7 weeks; UK-38,485 was administered during the final week resulting in a significant increase in GFR associated with reduced TxB$_2$ excretion.

The effect of CsA on the excretion of vasodilator prostaglandin species, however, remains unclear. While this study failed to demonstrate any significant effect of

Table 2. The effect of CsA alone or in combination with CGS 12970 on urine prostaglandin excretion

Group	Days 0	7	14
Urine TxB_2 excretion (ηg/24 hr)			
(1) CsA alone	28.1 ± 7.9	122.6 ± 38.9†	165.8 ± 39.0†
(2) CsA + saline from day 7	33.1 ± 15.1	150.1 ± 31.5†	184.9 ± 46.7†
(3) CsA + TSI from day 7	22.3 ± 8.6	112.6 ± 56.3†	37.8 ± 13.4*
Urine 6-KPGF$_{1\alpha}$ excretion (ηg/24 hr)			
(1) CsA alone	25.2 ± 12.2	19.4 ± 12.7	18.6 ± 14.1
(2) CsA + saline from day 7	29.5 ± 11.5	31.5 ± 9.4	21.8 ± 15.4
(3) CsA + TSI from day 7	31.2 ± 13.7	36.2 ± 19.4	26.5 ± 17.3
Urine PGE_2 excretion (ηg/24 hr)			
(1) CsA alone	105.8 ± 49.7	81.2 ± 32.8	90.6 ± 33.3
(2) CsA + saline from day 7	131.1 ± 35.3	106.5 ± 34.2	111.4 ± 45.4
(3) CsA + TSI from day 7	100.5 ± 59.0	101.8 ± 32.8	74.9 ± 24.9

Results are expressed as the mean ± SD from six determinations.

Significant differences ($P < 0.05$), assigned using Neuman–Keul's test, are denoted as * for (3) vs (1) or (2) on day 14 and as † for all comparisons to pretreatment values.

CsA was administered for 14 days at 50 mg/kg body weight either alone or with the addition of either saline or TSI from day 7.

Prostaglandin excretion was measured over a 16–18 hr period.

Table 3. Morphological indices of CsA nephrotoxicity

Treatment Group	PTV	CTD	CA
CsA alone	2.4 ± 1.5	2.0 ± 0.8	1.5 ± 1.2
CsA + saline from day 7	1.8 ± 1.7	2.2 ± 0.8	1.2 ± 1.0
CsA + TSI from day 7	0 ± 0	1.9 ± 1.3	0.9 ± 0.8

Results are expressed as the mean ± SD from six animals.

CsA was administered for 14 days at 50 mg/kg body weight either alone or with the addition of either saline or TSI from day 7.

Each feature was assessed on a scale from 0 to 4, where 0 was no abnormality and 1, 2, 3 and 4 represented mild, moderate, moderatively severe and severe abnormalities, respectively.

CsA on the excretion of either PGE_2 or 6-KPGF$_{1}\alpha$, similar findings to those obtained by Perico *et al.* [4], increased excretion rates of the latter have also been noted [5–7] and both reduced [5] and increased excretion [7] of PGE_2 have been observed.

In the present study prevention of further CsA-induced renal dysfunction by TSI was associated with normalization of TxB_2 excretion. While this suggests that TSI improves GFR by preventing TxA_2 constriction at the afferent arteriole [13], the accumulation of prostaglandin endoperoxides potentially capable of metabolism to vasodilatory products [14] cannot be excluded.

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