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Anne Prévot · Jean-Michel Liet · Denis S. Semama
Eve Justrabo · Jean-Pierre Guignard
Jean-Bernard Gouyon

Disparate effects of chronic and acute theophylline on cyclosporine A nephrotoxicity

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Abstract We previously developed a model of acute cyclosporine A (CsA)-induced vasomotor nephrotoxicity in rabbits. As exogenous adenosine infusion mimics the haemodynamic changes that characterize acute renal failure (ARF), we wanted to know whether adenosine was a mediator in this model and whether an adenosine receptor blocker could prevent the CsA-induced ARF. Group 1 were untreated controls. Group 2 received CsA (25 mg/kg per day) for 5 days. Renal function parameters were measured, showing ARF in all animals compared to controls. Theophylline (1 mg/kg i.v. bolus) was then administered and renal function was reassessed. Theophylline significantly reduced renal vascular resistance (–8%) and increased renal blood flow (RBF) (+20%), glomerular filtration rate (GFR) (+50%), filtration fraction (+24%) and diuresis (+73%), suggesting that adenosine was involved in the CsA-induced ARF. In group 3, theophylline (30 mg/kg per day) was given concomitantly with CsA for 5 days. GFR was normalized, but theophylline did not hinder the drop in RBF seen with CsA alone in group 2. Microscopy observation of the kidneys showed that chronic theophylline administration aggravated the morphological changes induced by CsA alone. We conclude that CsA administration for 5 days induced a vasomotor nephropathy with an adenosine-mediated afferent arteriolar constriction which cannot be prevented by concomitant theophylline administration.

Keywords Acute renal failure · Adenosine · Cyclosporine A · Kidney · Nephrotoxicity · Rabbit · Theophylline

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A. Prévot (✉) · J.-P. Guignard
Laboratoire de Néphrologie Pédiatrique, BH11-971,
Centre Hospitalier Universitaire Vaudois,
1011 Lausanne, Switzerland
e-mail: prevotanne@hotmail.com
Tel.: +41-21-3143614, Fax: +41-21-3143664

A. Prévot · J.-M. Liet · D.S. Semama · E. Justrabo · J.-B. Gouyon
Laboratoire de Néphrologie-Hémaphérèse-Transplantation
(UPRES EA 563) and Service de Pédiatrie 2,
Centre Hospitalier Universitaire,
10 Bd Maréchal de Lattre de Tassigny, 21034 Dijon Cedex, France

Present address:
J.-M. Liet, Réanimation Pédiatrique Néonatologie,
Hôpital Mère-Enfant, CHU de Nantes,
44035 Nantes Cedex 01, France

Introduction

Cyclosporine A (CsA) remains one of the most effective immunosuppressive drugs used in the management of organ transplantation and autoimmune diseases. However, its use is still impaired by two major side effects, namely systemic hypertension and nephrotoxicity [1, 2].

Chronic CsA nephrotoxicity has been widely studied. Some studies were performed in the rabbit, a good animal model showing close similarities with chronic CsA nephrotoxicity recorded in humans [3, 4, 5]. Because acute CsA administration is now frequently used, acute CsA nephrotoxicity needs closer attention. Indeed, Bisogno et al. [6] recently reported the use of short-term (3 days), high dose (15 and 30 mg/kg per day) intravenous CsA combined with etoposide to treat recurrent or refractory solid tumours in 18 children. Creatinine and/or urea were elevated in 14 of 32 courses of treatment (44%) [6], thus indicating a fall in glomerular filtration rate (GFR).

Vasoactive substances mediate the acute renal vascular effects of CsA in both experimental models and humans. Among these mediators are angiotensin II, endothelin or adenosine [7]. Guieu et al. demonstrated that chronic administration of CsA to kidney transplant recipients was accompanied by high plasma adenosine levels [8]. This increase in adenosine plasma levels was attributed to CsA administration because: (1) CsA inhibited adenosine uptake by red blood cells and increased its half-life in vitro; (2) CsA blood levels were correlated with adenosine plasma levels; (3) adenosine plasma levels of non-CsA-treated kidney transplant recipients were

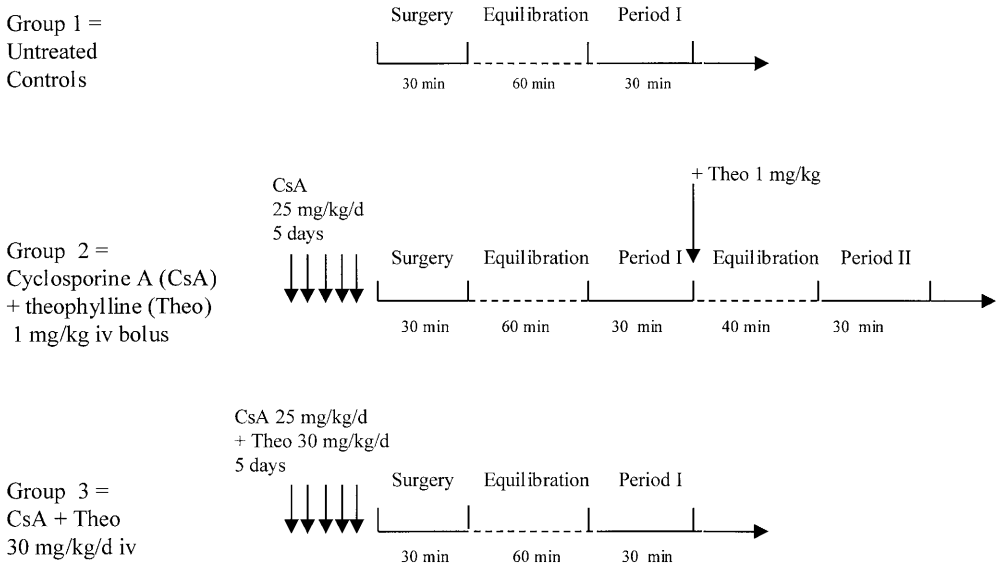

Fig. 1 Experimental protocol in the three groups

in the same range as controls [8]. Likewise, Halimi et al. demonstrated a CsA-induced increase in renal artery adenosine concentration in rats, leading to renal vasoconstriction by action on the adenosine A_1 receptors [9].

Theophylline is an alkylxanthine adenosine receptor antagonist commonly used in humans. It attenuates radiocontrast-induced intrarenal vasoconstriction [10]. A randomized study on 93 patients showed that four injections of theophylline (2.88 mg/kg) every 12 h totally prevented the reduction in creatinine clearance induced by low osmolarity radiocontrast products and partially reduced the acute renal failure (ARF) induced by high osmolarity radiocontrast products [11]. More generally, adenosine receptor antagonists were shown to have beneficial effects in several experimental models of ARF, both nephrotoxic and haemodynamic [12, 13, 14, 15]. Theophylline also was shown to improve acute CsA nephrotoxicity by inhibiting the CsA-induced contraction seen in mesangial cells in culture and in isolated glomeruli [16, 17]. However, disappointing results were obtained when theophylline was given to CsA-treated animals. Gerkens and Smith found no effect of theophylline on the CsA-induced renal function impairment in rats [18]. Moreover, Churchill et al. found that not only did theophylline fail to block the CsA-induced decreases in renal plasma flow and GFR, if anything, it potentiated them [19].

Considering the overall conflicting results on the use of theophylline in CsA-induced nephrotoxicity, additional studies seem to be necessary.

We previously developed a rabbit model of acute CsA-induced nephrotoxicity [20]: administration of 25 mg/kg per day CsA for 5 days induced a significant decrease in diuresis (*V*) which had a glomerular origin. The decrease in GFR was associated with an increase in renal vascular resistance (RVR) and a significant drop in renal blood flow (RBF). Filtration fraction (FF) did not vary significantly. The overall results suggest vasoconstriction of both pre- and postglomerular arteries in this model.

The present study was designed to test the hypothesis that endogenous adenosine mediates the reduction in GFR seen in our model and if so, whether theophylline administered simultaneously with CsA could blunt this reduction.

Materials and methods

Animals and treatment

Experiments were performed on 33 male New Zealand rabbits weighing 2,874±530 g (range 2,390–3,420), housed in individual cages and maintained on a standard diet (C15; Piétrement, France) and tap water ad libitum. The animals were randomly allocated into three groups (see Fig. 1).

In group 1, control animals (n=10) received no treatment before the day of the renal clearance studies.

All CsA-treated animals received subcutaneous injections of 25 mg/kg per day Sandimmun (cyclosporine A 50 mg/ml, 0.25 ml/kg b.i.d.; kindly provided by Novartis Pharma S.A., Rueil-Malmaison, France) for 5 days. In group 2, CsA/theophylline 1-treated animals (n=11) received CsA. Then, on the day of the renal clearance study (see Fig. 1), they were given 1 mg/kg of theophylline dissolved in 5 ml of sterilized water for injection as an i.v. bolus [as aminophylline (Sigma Chemicals, St Quentin Fallavier, France); aminophylline is theophylline complexed with ethylenediamine, which dissociates to theophylline in body fluids]. This group was designed to see whether adenosine was involved in the acute renal failure seen with CsA administration for 5 days [20].

In group 3, CsA/theophylline 30-treated animals (n=12) were treated with CsA and concomitantly infused through the marginal ear vein with theophylline 30 mg/kg per day b.i.d. diluted in 10 ml of saline. The dose of theophylline used in this group had been defined from pre-study experiments (data not shown) in order to obtain trough concentrations comparable with the peak concentrations obtained after the acute administration in group 2. This group was designed to see whether theophylline administered with CsA could have a protective effect on the CsA-induced ARF.

Surgical preparation

All methods have been repeatedly described and thus will only be summarized [7, 20]. Briefly, food was withheld 12 h before the

study with tap water remaining available ad libitum. Animals were anaesthetized with sodium pentobarbital. The trachea was cannulated to allow mechanical ventilation with fractional inspiratory oxygen concentration 0.30. The left carotid artery and right jugular vein were catheterized for blood sampling, continuous monitoring of mean blood pressure (MBP) and solute infusion. The urinary bladder was catheterized for urine collection. Body temperature was maintained in the range of physiological values for rabbits (~39°C) using a heated operating table.

Two millilitres of blood was then taken to measure CsA whole blood trough levels and the animals received the last half-dose of CsA. In group 3, 0.8 ml blood was taken to measure theophylline trough serum concentrations. The animals then received the last half-dose of theophylline (15 mg/kg) over 10 min. Twenty minutes after completion of theophylline infusion, 0.8 ml of blood was again taken to measure theophylline peak serum concentrations.

After completion of the surgical procedures, priming doses of inulin and *p*-aminohippurate (PAH) were administered through the jugular vein, with serum concentrations being maintained by a continuous i.v. infusion at a rate of 0.1 ml/min. A sterile solution of Ringer-mannitol containing NaCl (100 mM), KCl (6 mM), $NaHCO_3^-$ (50 mM) and mannitol (274 mM) was infused through the jugular vein at a rate of 1 ml/min.

Approximately 30 min were spent in animal preparation, followed by 60 min of equilibration.

Experimental procedures (Fig. 1)

In all 3 groups, each rabbit underwent an initial renal clearance study of 30 min (period I), with blood sampling (5 ml) at the midpoint. A sample of 0.9 ml whole blood was immediately used for blood gas and haematocrit determination; the remainder was centrifuged. The red blood cells were reconstituted in macromolecules (Plasmion; Bellon, Antony, France) and immediately returned to the animal. Plasma and urine samples were stored at –20°C for subsequent analysis.

In group 2, theophylline 1 mg/kg was then injected as an i.v. bolus through the jugular vein. A blood sample of 0.8 ml was taken 20 min after theophylline administration to measure its peak serum concentrations. After a 40-min equilibration period, each rabbit underwent a second renal clearance study of 30 min (period II), with another 5 ml blood sampling at the midpoint of the period.

The renal PAH extraction ratio (E_{PAH}) was assessed at the end of the experiment in all rabbits: following a small laparotomy, a fine needle was inserted into the left renal vein and venous blood was slowly withdrawn. Arterial blood was concomitantly taken from the carotid artery. The left kidney was then removed for blinded histological study (by EJ), and the animals were administered a lethal i.v. dose of pentobarbital.

Analytical methods

Urine volume was calculated from the change in weight of preweighed tubes without correction for specific gravity. The analysis of sodium and proteins was performed using a Vitros 250/750 (Ortho Diagnostics, Raritan, N.J., USA). Arterial blood gas determinations were made using a pH/blood gas analyzer (Blood gas system 168; Ciba-Corning Co., Melfield, Mass., USA).

Wright's automatic anthrone [21] and the Bratton and Marshall [22] methods were used for the determination of inulin and PAH concentrations, respectively (Autoanalyzer II; Technicon Instrument Corporation, Tarrytown, N.Y., USA).

Renal clearances of inulin (C_{In}) and PAH (C_{PAH}) were calculated from standard formulae and used as indices of GFR and renal plasma flow, respectively. Mean values of E_{PAH} (0.86±0.03) were not significantly different from 0.92 as previously determined in our laboratory on eight untreated animals [15]. Thus, we used a value of 0.92 to calculate RBF in the three groups.

E_{PAH}, plasma/urine (P/U) inulin ratio, RBF, RVR, FF, fractional excretion of sodium (FE_{Na}) and sodium excretion rate ($U_{Na}V$) were derived from standard equations as previously reported [20].

Whole blood cyclosporine concentrations were measured using the enzyme multiplied immunoassay test (EMIT-Green Liquid; Dade-Behring, Germany) on a Cobas Mira analyzer (Roche Diagnostic Systems, Meylan, France).

Theophylline was measured by immunofluorescence (TDX Abbott, Rungis, France).

For light microscopy, fragments were stained with Masson's trichrome.

Statistical analysis

All data were expressed as mean ±SEM.

Inter-group comparisons were performed using the non-parametric Mann-Whitney *U* test, a Bonferroni adjustment being used so as to assure an overall type I error rate of <0.05. Intra-group comparisons within periods I and II in group 2 were performed using the non-parametric Wilcoxon signed-rank test; each animal thus acted as its own control. *P* values <0.05 were considered statistically significant.

Results

CsA blood concentrations

CsA whole blood trough concentrations were 808±92 and 1343±218 ng/ml ($P<0.05$) in groups 2 and 3, respectively.

Effects of CsA administration (group 2, period I; Tables 1 and 2)

Compared with group 1, CsA administration in group 2 significantly decreased MBP, *V*, GFR, RBF, plasma Na, $U_{Na}V$ and FE_{Na} with a concomitant increase of RVR. The FF and P/U inulin ratio remained unchanged.

Effects of acute theophylline administration (1 mg/kg) on CsA-pretreated rabbits (group 2, period II; Tables 1 and 2)

Theophylline peak serum concentrations were 13.0± 1.2 µmol/l (range 8.8–18.8) 20 min after administration.

There was a slight but significant decrease in protein levels (–6.7±1.4%) from period I to period II, probably as a result of repeated blood sampling. PaO_2 decreased slightly but significantly (–5.3±3%), no animal being hypoxic.

MBP (+11±3%), urine flow rate (+73±14%), GFR (+50±8%), RBF (+20±5%) and FF (+24±5%) all increased significantly, whereas RVR significantly decreased (–8±3%). The P/U inulin ratio significantly increased.

The i.v. infusion of theophylline induced a slight but significant rise in plasma Na (+1.5±0.4%) and a dramatic increase in $U_{Na}V$ (+443±160%) and FE_{Na} (+236±91%).

Table 1 Values of physiological parameters in control animals (group 1) and influence of intravenous infusion of theophylline acutely (1 mg/kg, group 2, *Theo 1*) or theophylline chronically (30 mg/kg per day for 5 days, group 3, *Theo 30*) in cyclosporine A-pretreated rabbits (25 mg/kg per day for 5 days, *CsA*). Values are means ±SEM. Comparisons between groups were performed using non-parametric Mann-Whitney *U* test, a Bonferroni adjustment being used so as to assure an overall type I error rate of <0.05. Intra-group comparisons within periods I and II in group 2 were performed using the Wilcoxon non-parametric test. (*MBP* mean blood pressure, *Hct* haematocrit)

Parameter	Group 1	Group 2		Group 3
	Controls	CsA 25 Period I	+ Theo 1 Period II	CsA 25 + Theo 30
MBP (mmHg)	93.1±4.4	74.8±4.8*	82.4±4.6†	77.9±2.8#
pH	7.54±0.01	7.58±0.03	7.61±0.02	7.46±0.02#,¶
Proteins (g/l)	42.2±1.1	39.1±2.1	36.5±1.9†	36.2±1.2#
Hct (%)	34.9±1.0	34.5±0.9	34.0±1.0	32.1±0.6#

*P<0.05, group 2, period I versus group 1
#P<0.05, group 3 versus group 1
¶P<0.05, group 3 versus group 2, period I
†P<0.05 for period II versus period I

Table 2 Values of renal function and renal haemodynamic parameters, water excretion and sodium handling in control animals (group 1) and influence of intravenous infusion of theophylline acutely (1 mg/kg, group 2, *Theo 1*) or theophylline chronically (30 mg/kg per day for 5 days, group 3, *Theo 30*) in cyclosporine A-pretreated rabbits (25 mg/kg per day for 5 days, *CsA 25*). Values are means ±SEM. Comparisons between groups were performed using non-parametric Mann-Whitney *U* test, a Bonferroni adjustment being used so as to assure an overall type I error rate of <0.05. Intra-group comparisons within periods I and II in group 2 were performed using the Wilcoxon non-parametric test. (*V* urine flow rate, *GFR* glomerular filtration rate, *RBF* renal blood flow, *FF* filtration fraction, *RVR* renal vascular resistances, $U_{Na}V$ natriuresis, FE_{Na} fractional excretion of sodium)

Parameter	Group 1	Group 2		Group 3
	Controls	CsA 25 Period I	+ Theo 1 Period II	CsA 25 + Theo 30
V (ml/kg per min)	0.339±0.028	0.131±0.011*	0.215±0.010†	0.195±0.025#,¶
GFR (ml/kg per min)	5.29±0.37	2.96±0.46*	4.31±0.63†	4.52±0.68
P/U inulin ratio	0.068±0.009	0.050±0.005	0.059±0.008†	0.065±0.024
RBF (ml/kg per min)	28.1±2.6	17.0±2.4*	20.0±2.6†	16.0±3.5#
FF (%)	30.5±2.7	26.8±2.0	32.4±1.6†	59.9±11.5#,¶
RVR (mmHg/ml per kg per min)	3.61±0.29	5.15±0.57*	4.72±0.55†	9.24±2.15#
Plasma Na (mmol/l)	135.8±0.9	132.2±0.8*	134.2±0.6†	132.8±1.0
$U_{Na}V$ (µmol/kg per min)	44.63±4.85	3.98±1.18*	10.11±1.18†	11.20±2.86#,¶
FE_{Na} (%)	6.66±1.08	0.91±0.17*	1.94±0.31†	3.19±1.83#

*P<0.05 for group 2, period I versus group 1
#P<0.05 for group 3 versus group 1
¶P<0.05 for group 3 versus group 2, period I
†P<0.05 for period II versus period I

Effects of chronic theophylline administration (30 mg/kg per day) on CsA-treated rabbits (group 3; Tables 1 and 2)

Theophylline trough serum concentrations were 15.4± 1.9 µmol/l (range 8.3–25.4). Theophylline peak serum concentrations were 133.1±7.8 µmol/l (range 88.3–170.0) 20 min after administration.

CsA + theophylline treatment in group 3 was associated with a significant decrease in blood pH, plasma protein levels and haematocrit compared with untreated controls (group 1, period I).

Compared with groups 1 and 2 (period I), concomitant administration of theophylline with CsA induced a significant rise in FF and an additional rise in RVR, although not significant. Decreases of MBP, RBF and FE_{Na} were not statistically different from those seen with CsA administration alone.

Diuresis and $U_{Na}V$ were significantly higher than in group 2 (period I), but did not reach the values of group 1.

The decrease in GFR seen with CsA alone (group 2, period I) was blunted by concomitant theophylline administration.

The P/U inulin ratio remained unchanged.

Microscopy observations (Fig. 2)

It is noteworthy that all renal structures in group 1 appear histologically normal.

The kidneys of 5 and 12 rabbits of groups 2 and 3, respectively, were used for histological and cytological study.

In group 2, one could see microvacuolization of the proximal epithelial cells of the deep cortex and outer medulla in all sections. The proximal tubule brush border was preserved in the five kidneys studied. There were no changes in the morphology of the glomeruli, arteries or interstitial space which showed no lymphocyte infiltration.

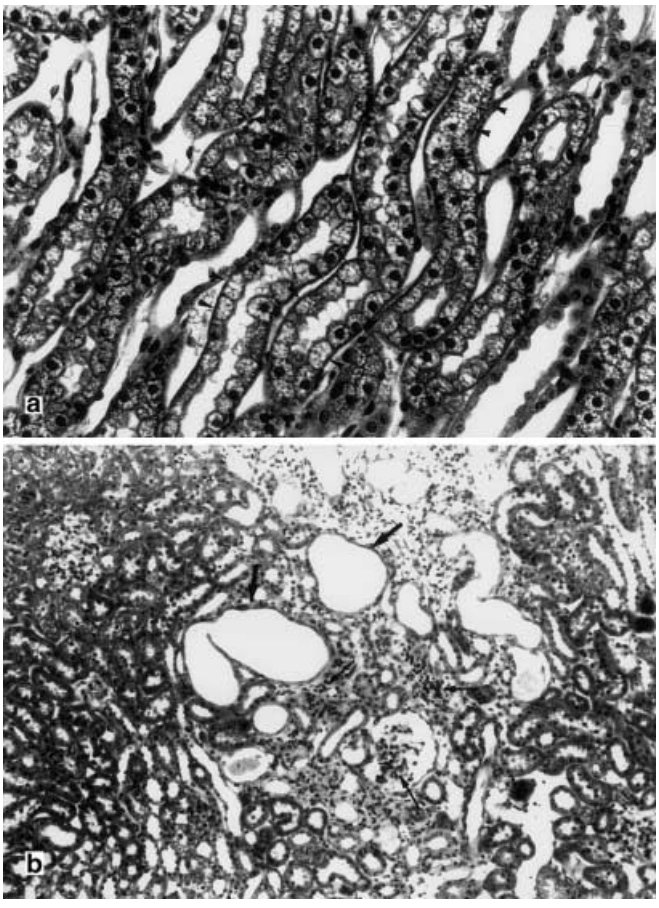

Fig. 2a,b Light microscopy of kidney sections. **a** Rabbit from group 2 treated with CsA (25 mg/kg per day s.c.) for 5 days and theophylline (1 mg/kg as an i.v. bolus). Note the intense cytoplasmic microvacuolization of the proximal tubule epithelial cells (*arrows*). **b** Rabbit from group 3 treated with CsA (25 mg/kg per day s.c.) and theophylline (30 mg/kg per day i.v.) for 5 days. Note the central area with dilated tubules (*big arrows*), lymphocyte infiltration (*small arrows*) and mild interstitial fibrosis. (Masson's trichrome; **a, b** ×230)

In group 3, one could see microvacuolization of the proximal and distal tubules in all sections; one section showed macrovacuolization as well. Shedding of the proximal tubule brush border occurred in 4 of 12 kidneys. In one section, one could see two cortico-medullary strands with interstitial fibrosis and lymphocyte infiltration surrounding some obsolescent glomeruli, and tubules with a thickened basement membrane. Outside these areas, glomeruli and interstitium were normal. In one case, few obsolescent glomeruli were detected (3%). Eight of the ten remaining observations showed dilated glomerular capillaries with neutrophil and red blood cell infiltration. There were no changes in the morphology of the arteries and interstitium, which showed no lymphocyte infiltration.

Discussion

We previously demonstrated that adult rabbits given CsA (25 mg/kg per day for 5 days) presented with acute vasomotor renal failure (decreased GFR and RBF with an increased RVR; stable FF indicating both afferent and efferent vasoconstriction) [20].

In the present study, we infused theophylline, a nonspecific adenosine receptor antagonist, to test the hypothesis that endogenous adenosine mediates the changes in renal function parameters seen after treatment with CsA. Acute administration of theophylline 1 mg/kg in group 2 (reaching micromolar plasma concentrations consistent with adenosine receptor antagonism) increased GFR by 50% and significantly decreased RVR with a consequent increase in renal perfusion in period II. The increase of both FF and RBF indicated a predominant vasodilatation of the afferent arteriole. Therefore, we can assume that the CsA-induced ARF was associated with an adenosine-mediated vasoconstriction of preglomerular arterioles. Even though two in vivo studies failed to disclose a role of adenosine in the CsA nephrotoxicity in the rat [18, 19], our results agree with the beneficial effects of adenosine receptor antagonists, including theophylline, found in other animal models of experimental acute renal failure as well as in humans (hypoxemia, endotoxin administration) [10, 11, 13, 15, 23, 24]. These results also fit well with data obtained in vitro showing that theophylline could ameliorate acute CsA nephrotoxicity by inhibiting the mesangial cell contraction elicited with CsA [17].

The preglomerular vasoconstrictor effect of adenosine could be explained by an adenosine-angiotensin II interaction in the kidney [25, 26], as we have previously reported beneficial effects of perindopril, an angiotensin converting enzyme inhibitor, in this model [7]. This effect could be mediated by A_1 receptors, because adenosine A_1 receptor stimulation in the isolated perfused kidney leads to a decrease in GFR and FF, consistent with afferent arteriolar vasoconstriction [27, 28].

The increase in urine flow rate seen after acute theophylline administration was due to both the increase in GFR and an inhibition of tubular water reabsorption since the P/U inulin ratio increased. Theophylline also induced a dramatic increase in both $U_{Na}V$ (+443%) and FE_{Na} (+236%). Indeed, adenosine antagonists have been shown to induce diuresis and natriuresis even in the absence of detectable increases in RBF or GFR. The localization of A_1 receptors in rabbit cortical collecting tubules would support the assumption of a direct receptor-mediated tubular effect [29]. Peripheral activation of arterial chemoreceptors by adenosine could also lead to antinatriuresis by activation of the sympathetic nervous system with increased renal nerve activity. Therefore, the effect of systemically administered theophylline could also reflect an interaction with arterial chemoreceptors [30]. Finally, we cannot exclude that the natriuretic effect of theophylline could be at least in part pressure-related, since theophylline administration led to a slight (+11%) but significant increase in MBP.

The overall results indicate that acute CsA nephrotoxicity is mediated at least partly via adenosine. Therefore, we wanted to see whether theophylline, a drug commonly used in clinical practice, administered concomitantly with CsA could prevent the CsA-induced ARF.

Administration of theophylline 30 mg/kg per day with CsA was associated with GFR normalization and an increase in FF, but failed to block the CsA-induced decreases in RBF and FE_{Na}. It even potentiated renal vasoconstriction as seen by an increased RVR. These results suggest that chronic administration of theophylline elicited a predominant efferent vasoconstriction, thus promoting enhanced FF and maintaining GFR despite a decreased RBF.

The discrepancy in haemodynamic and histological results between acute and chronic administration of theophylline could be explained by the significant rise in CsA blood levels, by 66%, observed in group 3. We hypothesize that this elevation in CsA blood levels could be the consequence of an action of theophylline on the hepatic metabolism of CsA by cytochrome P450 [18, 31], as has been seen recently in a renal transplant patient treated with theophylline and tacrolimus [32]. The increased efferent renal vasoconstriction could also lead to a decrease in CsA urinary clearance, explaining the elevated CsA blood levels.

The lack of a protective effect of theophylline on the CsA-induced nephrotoxicity could also be explained by the fact that high theophylline dosage has been associated with an augmented release of catecholamines from the sympatho-adrenal system, leading to an α-agonist-mediated renal vasoconstriction [33]. However, this did not seem to be the case in our study because the heart rates were not significantly modified (data not shown, P=0.14).

The mechanism of acute CsA-induced renal vasoconstriction is a complex process involving a cascade of activation of endogenous mediating factors in afferent as well as efferent arterioles. We previously published on the major role of endothelin and angiotensin II in this model [7] and prostaglandins and nitric oxide are currently under evaluation.

The present results are further evidence to suggest that the changes in renal function caused by CsA are also partly due to the mediation of adenosine. However, despite its apparent beneficial effect when used acutely, theophylline should not be used concomitantly with CsA for a long period of time in view of its effects on CsA hepatic metabolism and on renal scarring.

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References

1. Curtis JJ, Luke RG, Dubovsky E, Diethelm AG, Whelchel JD, Jones P (1986) Cyclosporin in therapeutic doses increases renal allograft vascular resistance. Lancet 2:477–479
2. Kaskel FJ, Devarajan P, Arbeit LA, Partin JS, Moore LC (1987) Cyclosporine nephrotoxicity; sodium excretion, autoregulation and angiotensin II. Am J Physiol 252:F733-F742
3. Thliveris JA, Yatscoff RW, Lukowski MP, Copeland KR, Jeffery JR, Murphy GF (1991) Chronic ciclosporin nephrotoxicity: a rabbit model. Nephron 57:470–476
4. Thliveris JA, Solez K, Yatscoff RW (1995) A comparison of the effects of rapamycin and cyclosporine on kidney and heart morphology in a rabbit heterotopic heart transplant model. Histol Histopathol 10:417–421
5. Andoh TF, Lindsley J, Franceschini N, Bennett WM (1996) Synergistic effects of cyclosporine and rapamycin in a chronic nephrotoxicity model. Transplantation 62:311–316
6. Bisogno G, Cowie F, Boddy A, Thomas HD, Dick G, Pinkerton CR (1998) High-dose cyclosporin with etoposide: toxicity and pharmacokinetic interaction in children with solid tumours. Br J Cancer 77:2304–2309
7. Prévot A, Semama DS, Tendron A, Justrabo E, Guignard J-P Gouyon J-B (2000) Endothelin, angiotensin II and adenosine in acute cyclosporine A-induced nephrotoxicity. Pediatr Nephrol 14:927–934
8. Guieu R, Dussol B, Devaux C, Sampol J, Brunet P, Rochat H, Bechis G, Berland YF (1998) Interactions between cyclosporine A and adenosine in kidney transplant recipients. Kidney Int 53:200–204
9. Halimi G, Sampol J, Clot-Faybesse O, Mercier L, Devaux C, Berland Y, Dussol B, Rochat H, Guieu R (1999) Cyclosporine A and purininergic receptors in rat kidney. Life Sci 65:2810–2813
10. Bakris GL, Burnett JC (1985) Theophylline attenuates radiocontrast-induced intrarenal vasoconstriction. Kidney Int 27:227
11. Katholi RE, Taylor GJ, McCann WP, Woods WT, Womack KA, McCoy CD, Katholi CR, Moses HW, Mishkel GJ, Lucore CL, Holloway RM, Miller BD, Woodruff RC, Dove JT, Mikell FL, Schneider JA (1995) Nephrotoxicity from contrast media: attenuation with theophylline. Radiology 195:17–22
12. Heidemann HT, Gerkens JF, Jackson EK, Branch RA (1983) Effect of aminophylline on renal vasoconstriction produced by amphotericin B in the rat. Naunyn Schmiedebergs Arch Pharmacol 324:148–152
13. Lin JJ, Churchill PC, Bidani AK (1986) Effect of theophylline on the initiation phase of postischemic acute renal failure in rats. J Lab Clin Med 108:150–154
14. Bidani AK, Churchill PC, Packer W (1987) Theophylline-induced protection in myoglobinuric acute renal failure: further characterization. Can J Physiol Pharmacol 65:42–45
15. Gouyon J-B, Guignard J-P (1988) Theophylline prevents the hypoxemia-induced renal hemodynamic changes in rabbits. Kidney Int 33:1078–1083
16. Fandrey J, Rob PM, Jelkmann W (1991) Theophylline and magnesium inhibit the contraction elicited with ciclosporin and angiotensin II in mesangial cell cultures. Nephron 57:94–98
17. Potier M, Lakhdar B, L'Azou B, Cambar J (1995) Evidence for a protective effect of theophylline and caffeine on cyclosporine-induced contractions in two in vitro glomerular models. Transplant Proc 27:2492–2494
18. Gerkens JF, Smith AJ (1985) Effect of captopril and theophylline treatment on cyclosporine-induced nephrotoxicity in rats. Transplantation 40:213–214
19. Churchill PC, Rossi NF, Churchill MC, Bidani AK, McDonald FD (1990) Acute cyclosporine-induced renal vasoconstriction: lack of effect of theophylline. Am J Physiol 258:F41–F45
20. Prévot A, Semama DS, Justrabo E, Guignard J-P, Escousse A and Gouyon J-B (2000) Acute cyclosporine A-induced nephrotoxicity: a rabbit model. Pediatr Nephrol 14:370–375

21. Wright HK, Gann DS (1966) An automatic method for determination of inulin in plasma and urine. J Lab Clin Med 67:689–693
22. Bratton AC, Marshall EK (1939) A new coupling component for sulfanilamide determination. J Biol Chem 128:537–550
23. Prada J, Churchill P, Bidani A (1986) Protective effect of theophylline in endotoxin-mediated acute renal failure in rats. Kidney Int 29:308
24. Huet F, Semama D, Grimaldi M, Guignard J-P, Gouyon J-B (1995) Effects of theophylline on renal insufficiency in neonates with respiratory distress syndrome. Intensive Care Med 21:511–514
25. Hall J, Granger JP, Hester RL (1985) Interactions between adenosine and angiotensin II in controlling glomerular filtration. Am J Physiol 248:F340–F346
26. Weihprecht H, Lorenz JN, Briggs JP, Schnermann J (1994) Synergistic effects of angiotensin and adenosine in the renal microvasculature. Am J Physiol 266:F227–F239
27. Murray RD, Churchill PC (1984) Effects of adenosine receptor agonists in the isolated perfused kidney. Am J Physiol 16:H343–H348
28. Agmon Y, Dinour D, Brezis M (1993) Disparate effects of adenosine A_1- and A_2-receptor agonists on intrarenal blood flow. Am J Physiol 265:F802–F806
29. Balakrishnan VS, Coles GA, Williams JD (1993) A potential role for endogenous adenosine in control of human glomerular and tubular function. Am J Physiol 265:F504–F510
30. Fransen R, Koomans HA (1995) Adenosine and renal sodium handling: direct natriuresis and renal nerve-mediated antinatriuresis. J Am Soc Nephrol 6:1491–1497
31. Fuhr U, Doehmer J, Battula N, Wölfel C, Kudla C, Keita Y, Staib AH (1992) Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. Biochem Pharmacol 43:225–235
32. Boubenider S, Vincent I, Lambotte O, Roy S, Hiesse C, Taburet AM, Charpentier B (2000) Interaction between theophylline and tacrolimus in a renal transplant patient. Nephrol Dial Transplant 15:1066–1068
33. Rall TW (1992) Chapter 25. In: Goodman and Gilman's the pharmacological basis of therapeutics, 6th edn. Macmillan Publishing Co, New York, pp 626–628