Original Article

Reversal of oliguric tacrolimus nephrotoxicity in children

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Abstract

Background. Acute tacrolimus toxicity is manifest by oliguria and elevated serum creatinine. Various vaso-regulatory molecules have been implicated in calcineurin inhibitor-mediated nephrotoxicity, including calcium, adenosine and endothelin. Theophylline (THEO), a non-specific adenosine-receptor antagonist prevents renal dysfunction from various nephrotoxins which mediate vasoconstriction. In the setting of acute tacrolimus toxicity, we demonstrated that administration of THEO along with a loop diuretic (LD) enhanced diuresis. This randomized, controlled trial was undertaken to confirm these earlier findings under more rigorous conditions.

Methods. Children with non-renal visceral transplant(s) and evidence of tacrolimus nephrotoxicity oliguria with a 25% increase in serum creatinine concentration from baseline, a whole blood tacrolimus concentration > 20 ng/dl and oliguria resistant to therapy with a LD were randomized to receive either THEO (n = 10) or normal saline placebo (n = 8). Using pre and post (6 h) timed urine collections and coincident plasma concentrations the following were measured or calculated: urine flow rate, net fluid balance, creatinine clearance, fractional excretion of chloride, free water clearance and distal delivery of chloride.

Results. These patients had markedly impaired creatinine clearance at the onset of tacrolimus toxicity. Urine flow increased in the LD + THEO group by 110% over baseline, but was unchanged in the LD + NS group. An increase in creatinine clearance did not reach statistical significance (P = 0.09). Fractional excretion of chloride and distal solute delivery increased after THEO treatment.

Conclusions. THEO induced a solute diuresis during furosemide-resistant oliguric tacrolimus toxicity in paediatric patients with a trend towards improved renal function.

Keywords: adenosine; nephrotoxicity; paediatric; tacrolimus; theophylline

Introduction

Activation of FK-binding proteins by the calcineurin inhibitors cyclosporin and tacrolimus (TAC) produces calcium influx into endothelial cells, resulting in vasoconstriction [1]. As with other promoters of renal vasoconstriction, adenosine, a potent vasoconstrictor, has been shown to participate in this phenomenon [2–4]. Plasma adenosine is elevated in patients receiving TAC [5]. In culture, TAC inhibits uptake of adenosine by adenosine kinase in both T lymphocytes and endothelial cells [6,7]. Non-specific adenosine receptor inhibition has been shown to reverse the vasoconstriction induced by calcineurin inhibitors in rats and rabbits [2,3]. These data suggest that adenosine receptor inhibition may be a therapeutic option in patients who manifest calcineurin inhibitor toxicity acutely.

In the clinical setting of acute TAC toxicity, oliguria has been noted to occur in a dose-dependent fashion, particularly at whole blood concentrations > 20 ng/dl [8]. A rise in serum creatinine occurs when there is secondary renal ischaemia. Subsequent fluid retention may lead to pulmonary oedema and respiratory compromise. We previously described a series of children who experienced TAC-induced nephrotoxicity [9]. The addition of a single low dose of aminophylline to a loop diuretic (LD) produced a 2-fold increase in their urine flow. This earlier retrospective study was criticized for lack of a control group, raising the possibility that renal toxicity could be due to aminoglycoside exposure while the improved urine flow could be attributable to a decline in TAC whole-blood concentrations. The primary outcomes compared in the present randomized, placebo-controlled study was urine flow and net fluid balance in response to a LD both with and without low dose aminophylline.

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Secondary outcome measures were creatinine clearance (C_{CR}) as well as free water clearance (C_{H2O}) and calculations of the fractional excretion (FE_{CL}) and distal delivery of chloride (DD_{CL}), in an attempt to characterize the diuresis.

**Subjects and methods**

This study was approved by the Institutional Review Board for Human Subjects at the University of Miami. Patients were eligible for the study if they manifest TAC toxicity following solid organ transplant, defined as a whole blood concentration $\geq 20$ ng/dl, a serum creatinine 25% above the patient's baseline and fluid overload requiring institution of LD therapy as determined by the treating physician. The immunosuppression in these patients was managed by the surgical team. Whole blood TAC concentrations were determined routinely from either trough samples for patients on twice daily enteral dosing or steady-state samples for patients on a continuous infusion. In either case, samples were obtained routinely in the early morning. The results became available in the early afternoon and were used to make decisions regarding the evening dose. The target level for patients who received an intestinal transplant was 15–20 ng/dl and, for liver recipients, the target level was 10–15 ng/dl.

After informed consent was obtained, patients were randomized by the pharmacy department to receive either aminophylline [the soluble salt of theophylline (THEO)] at a dose of 5 mg/kg or an equal volume of normal saline (NS) as placebo. Urine was collected from the time of enrolment until the next scheduled dose of LD. A blood sample was taken for TAC concentration and osmolality immediately prior to administration of LD followed by THEO or NS placebo. Urine was collected for 6 h. At the end of the 6 h period, blood was sampled for osmolality and serum THEO concentration. Both the pre- and post-aminophylline urine collections were assayed for osmolality, creatinine and chloride. The clinical chemistry laboratory performed all assays on a multichannel analyser. Urine osmolality (O) was calculated as ml/min and normalized per m$^2$. The net fluid balance before and after study enrolment was calculated based on the difference between intravenous fluid intake and urine output recorded by the nursing staff for 6 h prior to and 6 h after the intervention. C_{CR}, FE_{CL}, chloride clearance (C_{CL}), C_{H2O} and DD_{CL} were calculated according to the formulae shown in Table 1 [10].

### Table 1. Calculations for renal function parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{CR} (ml/min/1.73 m$^2$)</td>
<td>(U_{CR} \times P_{CR})/(1.73/m^2)</td>
</tr>
<tr>
<td>FE_{CL} (%)</td>
<td>(U_{CL} \times F_{CL})/(P_{CL} \times U_{CR}) \times 100</td>
</tr>
<tr>
<td>C_{CL} (ml/day/m$^2$)</td>
<td>(U_{CL} \times P_{CL})/m^2</td>
</tr>
<tr>
<td>C_{OSM} (ml/day/m$^2$)</td>
<td>U_{OSM} \times V_{OSM}/m^2</td>
</tr>
<tr>
<td>C_{DDO} (ml/day/m$^2$)</td>
<td>V - C_{OSM}</td>
</tr>
<tr>
<td>DD_{CL} (ml/day/m$^2$)</td>
<td>C_{CL}+C_{H2O}</td>
</tr>
<tr>
<td>FDD_{CL} (%)</td>
<td>C_{CL}/C_{OSM} \times 100</td>
</tr>
</tbody>
</table>

V, urine flow rate (ml/time) body surface area (m$^2$); P_{CR}, plasma creatinine; U_{CR}, urine creatinine; P_{CL}, plasma chloride; U_{CL}, urine chloride; C_{OSM}, osmolar clearance; P_{OSM}, plasma osmolality; U_{OSM}, urine osmolality; FDD_{CL}, fractional distal chloride delivery.

**Statistical analysis**

Using the previously determined SD [9] for urine flow rates, it was estimated, based on 80% power, that 8–10 patients per treatment group were needed to detect a 2-fold difference in urine flow rate with an $\alpha$ of 0.05. Data are presented as means±SEM. Categorical data were compared by test of proportions. Continuous data were compared between groups by Student’s $t$-test. Data obtained pre and post THEO or placebo were compared by paired Student’s $t$-test.

**Results**

Nineteen patients were initially enrolled in the study. One patient in whom the osmolality data were outside the normal range was excluded from analysis. This left 18 patients for evaluation, eight in the control group and 10 in the treatment group. As shown in Table 2, no demographic differences between the two groups at the time of randomization were detected. The patients’ median age was 19 months (range: 7–216 months). Thirteen (72%) were male. Six children underwent a liver transplant, 10 children underwent a multivisceral transplant (liver, stomach, intestine and pancreas) and two children underwent an isolated small bowel transplant. Seventeen patients received furosemide at a dose ranging from 0.5 to 2.5 mg/kg (mode 1 mg/kg) one of these patients also received bumetanide (LD+NS). One patient received bumetanide alone (LD+NS).

Data obtained after study enrolment are shown in Table 3. Whole blood TAC concentrations had declined slightly between the time of enrolment and protocol implementation (25.8±3.6 ng/dl for LD+NS; 31.7±4.0 ng/dl for LD+THEO), but remained well above the threshold value of 20 ng/dl. C_{CR} at enrolment was consistent with severely compromised renal function. Patients were studied on average 18±4 h after the last TAC dose.

Urine flow in the LD+THEO group increased 110% from baseline after THEO administration while remaining unchanged in the LD+NS group. There was a trend towards increased C_{CR} in the LD+THEO group, but this did not reach statistical significance ($P = 0.09$). As shown in Figure 1, despite a marked diuresis following THEO, C_{H2O} did not increase significantly. Rather, solute excretion, as measured by C_{OSM} and C_{CL}, more than doubled above the baseline diuresis with the LD alone (Table 3). DD_{CL} tripled and the chloride fraction of distal solute increased 98%,
indicating negligible reabsorption either proximally or distally.

Discussion

This randomized controlled trial confirms the results of a previously published case series in which the addition of aminophylline to LD therapy nearly tripled the urine flow rate for 6 h following the LD dose [9]. Each patient in both studies developed an acute rise in serum creatinine in conjunction with a whole blood TAC concentration ≥20 ng/dl. This study demonstrates a significant compromise of CCR at the time of study enrolment. In contrast to the previous study, no patient received an aminoglycoside, but all patients received liposomal amphotericin and ganciclovir. Thus, differences in exposure to other nephrotoxins cannot account for the differences in LD response between the two groups. Therefore, in the setting of acute TAC toxicity, combination therapy with low dose THEO plus an LD promoted a greater diuretic response than LD alone.
Measurement of $C_{\text{CR}}$ was a secondary end-point in this study. Because we had not previously measured $C_{\text{CR}}$ in this setting we were unable to estimate sample size for an appropriately powered study. Therefore, we were unable to demonstrate a statistically significant change in $C_{\text{CR}}$ in the LD + THEO group. Based on the variance reported here, detection of the 40% increase in $C_{\text{CR}}$ noted in the LD + THEO group would require enrolling approximately 40 patients in each group.

Having previously noted a synergistic diuretic effect between aminophylline and LDs, free water and chloride efflux after a single dose of THEO and LD was measured in an attempt to determine the mechanism of that effect. Our results are consistent with aminophylline’s role as an adenosine receptor antagonist, particularly the A1 subtype receptor, which is linked to inhibition of cAMP, reflex contraction of the afferent arteriole and enhanced distal tubular sodium chloride (NaCl) reabsorption [11]. By these mechanisms, adenosine is believed to mediate tubuloglomerular feedback (TGF) through increased NaCl reabsorption at the macula densa. The solute clearances in our small group of patients are similar to animal studies by micropuncture techniques of a selective adenosine A1 receptor antagonist [11,12]. Blockade of this receptor inhibits tubular fluid reabsorption in the proximal tubule and prevents TGF-mediated reductions in glomerular filtration rate (GFR). This spectrum of actions imparts unique diuretic capabilities that avoids further compromising GFR while perpetuating maximal solute diuresis and overriding resistance to LDs [11–13].

The blinding procedures and randomization of patients in this study were intended to eliminate selection bias. It is possible that additional unexamined factors explain the variation in response to LDs. While there are no statistical differences in examined variables at baseline, the sample size may be too small to detect clinically important differences. Patients may differ in their vasoconstrictor response to calcineurin inhibitors due to bioavailability and receptor binding; however, the enrollment of patients with both an elevated TAC concentration and evidence of nephrotoxicity suggests that this study population was experiencing acute TAC toxicity. Therefore, these data may not be applicable to other clinical settings, such as oliguria not related to TAC toxicity and chronic TAC toxicity.

THEO’s diuretic effect has been studied in other pediatric populations. Mazkereth et al. [14] examined the renal response to THEO in premature infants given THEO for apnoea or to improve diaphragmatic function. Neonates with known renal disease or on diuretic therapy were excluded. Following a loading dose of 6mg/kg, urine output, $C_{\text{H}_{2}\text{O}}$ and osmotic clearance all increased compared with baseline, but this response was not sustained. Similarly, Brater et al. [15] documented no synergy between THEO and furosemide in normal subjects. However, in children undergoing extracorporeal membrane oxygenation, a condition where non-pulsatile blood flow leads to oliguria, Lochan et al. [16] demonstrated a synergistic diuretic effect between furosemide and THEO in a cross-over design study. Bell et al. [17] demonstrated a diuretic effect in critically ill children already receiving LD. These studies suggest that the synergy between the two drugs is measurable only in the setting of tissue ischaemia and local adenosine release. Thus, the renal response to THEO likely depends on the underlying clinical condition.

The increased $FE_{\text{Cl}}$ in the present study suggests that THEO, through its adenosine-antagonist effect, augments renal blood flow and chloride delivery to the distal cortical collecting duct, thereby driving the solute component of the diuresis. During recent years, recognition of the potential clinical benefit of THEO as a treatment for oliguric renal failure in children with TAC toxicity has increased its ‘off-label’ use by paediatric intensivists and transplant surgeons, making enrolment more difficult than predicted. This phenomenon of increased off-label use during a clinical trial was described recently by Clark et al. [18] and was the primary motivation for closing this placebo-controlled trial before reaching the numbers of subjects necessary for statistical significance in several secondary end-points. This study demonstrates that THEO in combination with a LD is superior to a LD alone in promoting diuresis in the setting of acute TAC toxicity. A larger study would be needed to demonstrate an effect on glomerular filtration, as measured by $C_{\text{CR}}$.

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Conflict of interest statement. None declared.

References


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