Theophylline Improves Early Allograft Function in Rat Kidney Transplantation

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ABSTRACT

Several previous studies have demonstrated a beneficial effect of the adenosine receptor (AdoR) antagonist theophylline in different forms of acute renal failure in laboratory animals and in humans. Therefore, we wanted to test whether theophylline can also improve impaired allograft function following ischemia reperfusion injury in experimental kidney transplantation (KT). Orthotopic transplantation of the left kidney that was performed from Fisher 344 into Lewis rats. All transplanted rats received daily cyclosporine (5 mg/kg). The effect of theophylline treatment (10 mg/kg) on graft function was compared with appropriate controls on day 5 after KT by assessment of glomerular filtration rate (GFR) (inulin clearance). On day 5, GFR of allografts in control rats was 0.23 ± 0.05 ml/min/g kidney weight (n = 10) compared with 0.50 ± 0.09 ml/min/g in rats receiving theophylline (n = 9, p < 0.01), representing a 2-fold increase in GFR. Renal AdoR A1 mRNA content was significantly increased in both KT groups compared with their respective control groups, whereas mRNA of AdoR A2a, A2b, and A3 were found to be unchanged. Theophylline did not affect significantly interstitial infiltration of the graft by monocytes/macrophages and T-cells. Likewise, serum cytokines [interleukin (IL)-2, IL-6, IL-10, tumor necrosis factor-α] and erythropoietin plasma levels were not different among the allograft groups. The present study demonstrates that theophylline remarkably improved early renal allograft function in rats undergoing KT without influencing cytokine serum patterns or tissue inflammation. Since theophylline is a commonly used medication in humans, clinical studies in patients undergoing KT are warranted.

A delayed initial graft function secondary to ischemia-reperfusion injury (IRI) has been shown to have impaired long-term allograft function following kidney transplantation (KT) (Azuma et al., 1997; Tilney and Guttmann, 1997). Furthermore, the increased cell death and cell disintegration in allografts with severe IRI are supposed to increase the risk of early allograft rejection episodes (Shoskes and Halloran, 1996). Several efforts have been made to minimize the unavoidable IRI in organ donation (Perico et al., 2004). Apart from immunologic and surgical complications, alterations in renal hemodynamics are considered to be an independent risk factor for a delayed onset of renal function or initial graft failure (Alejandro et al., 1995).

Theophylline, an unselective competitive adenosine receptor antagonist, has been shown to improve kidney function in different forms of acute renal failure. Adenosine-mediated afferent arteriolar vasoconstriction and a subsequent decrease in glomerular filtration pressure have been identified as a key hemodynamic mechanism, which contributes to acute renal failure following renal ischemia or administration of nephrotoxic substances (Osswald and Vallon, 1998). Theophylline or other adenosine receptor antagonists have been found to improve renal function in the following models of acute renal failure in laboratory animals: 1-h renal artery clamping (Lin et al., 1986; Gouyon and Guignard, 1988), administration of glycerol (Bidani and Churchill, 1983; Yates et al., 1987), uranyl nitrate (Osswald et al., 1979), radio contrast media (Arend et al., 1987; Erley et al., 1997), am-

ABBREVIATIONS: IRI, ischemia-reperfusion injury; KT, kidney transplantation; MAP, mean arterial blood pressure; GFR, glomerular filtration rate; CsA, cyclosporin A; CON-VV, unilateral nephrectomized Lewis rats with vehicle treatment; CON-CV, unilateral nephrectomized Lewis rats with CsA and vehicle treatment; CON-CT, unilateral nephrectomized Lewis rats with CsA and theophylline treatment; KT-CV, kidney-transplanted rats (F → L) with CsA and vehicle treatment; KT-CT, kidney-transplanted rats (F → L) with CsA and theophylline treatment; IL, interleukin; TNF, tumor necrosis factor; EPO, erythropoietin; AdoR, adenosine receptor; PCR, polymerase chain reaction; G, glomerular; T, tubular; TGF, tubuloglomerular feedback; V, vascular.
photerincin B (Heidemann et al., 1983), and cisplatin (Heide-
mann et al., 1989; Nagashima et al., 1995). In humans, theophylline prevented renal functional deterioration follow-
ing application of radio contrast media (Erley et al., 1994; Ix et al., 2004) and cisplatin-containing chemotherapy (Benoehr et al., 2005).

Therefore, we hypothesized that theophylline might be effective to ameliorate early allograft function following KT. Furthermore, theophylline is a well established medication in drug therapy and is thus available for this new indication in human KT.

Materials and Methods

Animals. Male Fisher 344 rats served as kidney donors and male Lewis rats as graft recipients. The animals (240–300 g; Charles River GmbH, Sulzfeld, Germany) were kept on a regular 12-h dark/light cycle with free access to standard rat chow (Altromin 1320; Altromin, Lage, Germany) and tap water. Experiment protocols were approved in accordance with the German Law on the Protection of Animals.

Kidney Transplantation. The left kidneys from male Fisher 344 rats were orthotopically transplanted into male Lewis recipients following ipsilateral nephrectomy under ether anesthesia according to Fandrich et al. (2002). In brief, the graft was slowly perfused in situ with 8 to 12 ml of 4°C University of Wisconsin solution within 5 min, explanted, and stored for 2 h in University of Wisconsin solution (0–4°C). After completing the anastomoses using a continuous technique for the anterior and posterior wall of the vessels, the vein and artery of the transplant were clamped to test the tightness of the anastomoses by removing the cross-clamps on the aorta and vena cava inferior. If bleeding persisted after several minutes, another suture was placed at the bleeding point, otherwise the clamps could be removed directly. The warm ischemic time lasted from 30 to 40 min. Following reperfusion, the ureter was anastomosed by four to five single stitches end-to-end with 10-0 Prolene. Thereafter, the contralateral native kidney was removed without touching the ad-
renal vessels. Rats with unsuccessful surgery, defined as persistent bleeding at the anastomoses or without successful reperfusion of the graft, were excluded (15% of the total). In addition, rats died before day 5, or rats with insufficient blood pressure [mean arterial blood pressure (MAP) < 60 mm Hg] on day 5 during clearance experiments were excluded from evaluation (10% of the total).

Clearance Experiments. On day 5 after KT, rats were anesthe-
tized with an i.p. injection of thiobutabarbital (80–100 mg/kg Inac-
tin; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and pre-
pared for clearance experiments as described previously (Luipold et al., 2001). [3H]Inulin (1 μCi/ml) at a rate of 0.6 ml/h and isotonic (0.85%) NaCl at a rate of 3.0 ml/h were infused via the tail vein for determination of hemoglobin, hematocrit, erythropoietin, cytokines, and electrolyte serum concentra-
tions. Animals were then exsanguinated, and the allograft was re-
moved. Half of the kidney was frozen in liquid nitrogen and stored at −80°C, and the other half was fixated in buffered formalin (4.5%) for immunohistologic and histologic evaluation.

Experimental Groups. Following KT, all rats received low-dose CsA (5 mg/kg/day s.c. diluted in 0.15 ml of NaCl; Sandoz, Basel, Switzerland) everyday until day 5 to suppress an early episode of acute rejection. The first dose of CsA and theophylline (Broncho-
parat, 6 mg/kg in a volume of 1 ml/kg isotonic saline; Klinge Pharma, München, Germany) was administered immediately before KT via the ventral tail vein. During the postoperative course, rats received theophylline twice daily (10 mg/kg s.c., equivalent to 0.5 ml/kg Bronchoparat) until the day of the clearance experiment. The vehicle group was applied 0.85% NaCl. The effect of CsA and theophylline on normal kidney function was assessed in Lewis rats following unilat-
eral nephrectomy.

Treatment Groups. Treatment groups included the following: unilateral nephrectomized Lewis rats with vehicle treatment (CON-
VV) (n = 8), unilateral nephrectomized Lewis rats with CsA and vehicle treatment (CON-CV) (n = 6), unilateral nephrectomized Lewis rats with CsA and theophylline treatment (CON-CT) (n = 6), kidney-transplanted rats (F → L) with CsA and vehicle treatment (KT-CV) (n = 10), and kidney-transplanted rats (F → L) with CsA and theophylline treatment (KT-CT) (n = 9).

Dosing and Drug Interaction. To achieve therapeutic theoph-
ylline plasma levels (9–16 mg/l), we tried different doses of theoph-
ylline and different frequencies and routes (i.p. versus s.c.) of admin-
istration in control rats without unilateral nephrectomy (n = 5 in each group). Rats receiving theophylline at a dose of 6 mg/kg i.v. via the tail vein and subsequently 10 mg/kg s.c. twice daily achieved theophylline plasma concentrations close to the therapeutic range (8–20 mg/l). In another group of control rats, we determined serum CsA and theophylline concentrations and their potential interaction following unilateral nephrectomy. Serum theophylline and blood CsA levels were measured on days 2 and 5. Similar blood CsA and plasma theophylline concentrations were found in all groups, indi-
cating no relevant interactions between both medications (data not shown), which is in concordance with findings in humans (Dai et al., 2004; Faber and Fuhr, 2004).

Histologic Evaluation. For histologic assessment, kidney tis-
ues were fixed in 4.5% buffered formalin, dehydrated, and embed-
ded in paraffin. Sections (3 μm) were stained with H&E and periodic acid-Schiff using standard procedures. Examination and scoring of the whole section of each kidney were carried out at ×200 magnifi-
cation under light microscopy by a renal pathologist who was blinded. The degrees of glomerulosclerosis, vascular lesions, tubulo-
interstitial damage, and inflammatory infiltration were scored from 0 to 4+ using a grading system as described previously (el Nahas et al., 1991; Velasquez et al., 1997).

Immunohistochemistry. Paraffin-embedded tissue (3 μm) was incubated with monoclonal antibodies against monocytes/macroph-
ages (ED1) and CD57 T-lymphocytes (OX19, CD43; Serotec Camon Labor-Service, Wiesbaden, Germany) and the secondary rab-
tbit anti-mouse IgG antibody and the alkaline phosphatase-antialka-
line phosphatase complex (Dukko AS, Hamburg, Germany). Sections without the first or second antibody treatment served as negative controls. The whole section area of each kidney was evaluated at ×400 magnification for monocytes/macrophages and T-lymphocytes and scored from 0 up to 4+ depending on the degree of infiltration: grade 0, normal renal tissue; grade 1, mild localized infiltration; grade 2, moderate infiltration in different areas; grade 3, severe infiltration <50%; and grade 4, severe diffuse infiltration more than 50% of renal tissue.

Serum Cytokine and Erythropoietin Concentrations. Cyto-
kine [interleukin (IL)-2, -6, -10, and tumor necrosis factor α (TNF-α)] and erythropoietin (EPO) concentrations in serum were determined using commercial enzyme-linked immunosorbent assay kits (Bio-
Source International, Camarillo, CA; Medac, Hamburg, Germany).

Adenosine Receptor (AdoR) mRNA Expression in the Kid-
ney Cortex. Relative quantification of AdoR mRNA was performed using two-step reverse transcription-PCR experiments. Total RNA was isolated from renal tissues using peqGOLD RNApure according to the manufacturer’s instructions (Peqlab Biotechnologie GmbH, Erlangen, Germany). RNA (500 ng of each sample) was transcribed.
to cDNA by avian myeloblastosis virus reverse transcriptase (Peqlab) with oligo(dT)15, and random hexamers were used as primers in PCR buffer II (Applied Biosystems, Foster City, CA). PCR was carried out on Lightcycler instrument using the FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Mannheim, Germany). Primer sequences used for amplification are listed in Table 1.

The relative expression ratio of the target genes was computed, based on the crossing point difference (Δ) of an unknown sample versus a control and its real-time PCR efficiency, according to the mathematical model by Pfaffl (2001). Cyclophilin A (peptidyl prolyl isomerase A) was used as the reference gene to account for any variance in the quality of mRNA and the amount of input cDNA. To determine real-time PCR efficiency, standard curves were composed with serial dilutions of cDNA generated for each transcript.

**Analyses and Calculations.** Hematocrit was determined by centrifugation of arterial blood samples. Inulin concentrations in plasma and urine were measured by liquid scintillation (2550 TR; Canberra Packard, Frankfurt, Germany). GFR was the mean value of three clearance periods. Sodium and potassium concentrations in plasma and urine were determined by flame photometry (ELEX 6361; Eppendorf, Hamburg, Germany). Renal excretory and hemodynamic values were calculated as per gram of kidney weight using standard formulas.

**Statistical Methods.** To evaluate the effect of theophylline on GFR, cytokine serum concentrations, and AdoR mRNA expression, the statistical significances of the different experimental groups were assessed by analysis of variance and the unpaired two-sided Student’s t test. Histological scores were compared using the Kruskal-Wallis method. All values presented means ± S.E.M. p < 0.05 was considered to be statistically significant.

**Results.**

**Graft Function.** In control rats following nephrectomy (CON-VV), neither CsA (CON-CV) nor combined CsA with theophylline (CON-CT) treatment significantly altered body weight, kidney weight, hematocrit, or MAP (see Table 2). At the immunosuppressive dosage, CsA as well as additional theophylline treatment in nephrectomized rats did not change GFR, electrolyte excretion, or urinary flow rate on day 5 after nephrectomy compared with rats with vehicle administration (CON-VV; Table 2).

All kidney-transplanted rats received CsA at the standard immunosuppressive dosage with theophylline (KT-CT) or vehicle (KT-CV) administration. The duration of cold ischemia for renal allografts stored in University of Wisconsin solution as well as the body weight and kidney weight of the rats were similar in both groups (Table 2). A similar significant decrease of MAP and hematocrit were observed in kidney-transplanted rats of both groups compared with their respective nephrectomized controls (KT-CV versus CON-CV, KT-CT versus CON-CT, p < 0.05; Table 2). Plasma sodium and potassium concentrations were similar in transplanted rats and their respective controls (Table 2).

The GFR of allografts was decreased by 80% in the KT-CV group on day 5 compared with the CON-CV group. However, theophylline administration resulted in a markedly attenuated decrease of GFR by only 56% in the KT-CT group compared with the CON-CT group (Table 2). Therefore, the GFR was 2-fold higher in the KT-CT group compared with the KT-CV group (p = 0.0075; Table 2).

Urinary flow rate and fractional sodium and potassium excretion (FeNa and FeK) were increased in KT-CV rats compared with their respective nephrectomized controls (CON-CV, p < 0.05; Table 2), indicating a tubular electrolyte transport deficiency. However, treatment with theophylline (KT-CT) increased the tubular electrolyte transport capacity (FeNa and FeK) up to the levels of their respective controls (CON-CT).

**Cytokine and EPO Serum Levels.** The serum concentrations of the proinflammatory cytokines increased significantly in rats following KT (KT-CV, KT-CT) compared with their respective control rats (CON-CV, CON-CT): IL-2 (0.7 ± 0.3, 0.5 ± 0.3 versus 0.2 ± 0.1, 0.18 ± 0.18 pg/ml) and IL-6 (100 ± 44, 96 ± 55 versus 52 ± 31, 56 ± 38 pg/ml) and TNF-α (59 ± 14, 44 ± 20 versus 10.4 ± 8.2, 10 ± 10 pg/ml), whereas the serum concentrations of the anti-inflammatory cytokine IL-10 (5.3 ± 1.8, 6 ± 2 versus 5.2 ± 1.1, 4.5 ± 2 pg/ml) were not significantly changed. Thus, theophylline administration influenced neither serum concentrations of inflammatory nor anti-inflammatory cytokines in KT rats. EPO serum concentrations were 8.2 ± 0.7 mU/ml in CON-CV and 6.8 ± 0.2 mU/ml in CON-CT nephrectomized controls, and no significant change was observed in kidney-transplanted rats with (9.5 ± 1 mU/ml) or without (7.6 ± 0.8 mU/ml) theophylline administration.

**Histologic Assessments.** KT rats with theophylline (KT-CT) or vehicle (KT-CV) administration showed a significant glomerular (G), tubular (T), and vascular (V) damage (G: 1.44 ± 0.29 versus 1.3 ± 0.26, T: 1.22 ± 0.15 versus 1.7 ± 0.45, V: 1.67 ± 0.67 versus 2.0 ± 0.58) compared with their respective controls (CON-CV, CON-CT).

<table>
<thead>
<tr>
<th>Gene (RefSeq Accession No.)</th>
<th>Primers (Nucleotide Position)</th>
<th>Amplicon Size bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdoR A1 (NM_017155)</td>
<td>5’-694CTCCAGTCTGCTCTGCTCG713-3’ Forward 5’-900CACTGCGCTGGCTCTCC482-3’ Reverse</td>
<td>207</td>
</tr>
<tr>
<td>AdoR A2a (NM_053294)</td>
<td>5’-159GCTGCTGCGCTAGAAAGTG1216-3’ Forward 5’-208TACCCCTGTAAGATGCGAT2777-3’ Reverse</td>
<td>201</td>
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<tr>
<td>AdoR A2b (NM_017161)</td>
<td>5’-139GCGCCGCTGCCGCTTCTTA156-3’ Forward 5’-249CAAAGGCGGCGGAAAG1481-3’ Reverse</td>
<td>160</td>
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<tr>
<td>AdoR A3 (NM_012896)</td>
<td>5’-217CCCTGCTGCTGCTGCTTT138-3’ Forward 5’-302GACATGCAACCGAGG023-3’ Reverse</td>
<td>186</td>
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<tr>
<td>Cyclophilin A (NM_017101)</td>
<td>5’-146GAGAGAAGAAGATTGCGTATA165-3’ Forward 5’-257CACTGCCGTTGCCCAAGC634-3’ Reverse</td>
<td>257</td>
</tr>
</tbody>
</table>
TABLE 2
Summary of functional characteristics of rats in the different groups: CON-VV, CON-CV, CON-CT, KT-CV, and KT-CT

<table>
<thead>
<tr>
<th>Variables</th>
<th>CON-VV (n = 8)</th>
<th>CON-CV (n = 6)</th>
<th>CON-CT (n = 6)</th>
<th>KT-CV (n = 10)</th>
<th>KT-CT (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>254 ± 9</td>
<td>276 ± 11</td>
<td>254 ± 10</td>
<td>265 ± 15</td>
<td>283 ± 12c</td>
</tr>
<tr>
<td>Kidney wet weight (g/100 g BW)</td>
<td>1.11 ± 0.04</td>
<td>1.10 ± 0.02</td>
<td>1.03 ± 0.03</td>
<td>1.03 ± 0.03</td>
<td>1.10 ± 0.03</td>
</tr>
<tr>
<td>CIT (min)</td>
<td></td>
<td></td>
<td></td>
<td>113 ± 14</td>
<td>101 ± 9</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>105.5 ± 3.2</td>
<td>98.1 ± 3.8</td>
<td>106.5 ± 6.9</td>
<td>81.0 ± 3.2d</td>
<td>81.7 ± 3.0b</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>45.8 ± 0.8</td>
<td>46.8 ± 1.2</td>
<td>46.3 ± 1.4</td>
<td>40.4 ± 1.7d</td>
<td>43.7 ± 2.3</td>
</tr>
<tr>
<td>Na&lt;sub&gt;int&lt;/sub&gt; (mmol)</td>
<td>136.3 ± 2.1</td>
<td>139.3 ± 2.7</td>
<td>143.4 ± 3.4</td>
<td>138.9 ± 5.3</td>
<td>139.6 ± 4.7</td>
</tr>
<tr>
<td>K&lt;sub&gt;int&lt;/sub&gt; (mmol)</td>
<td>4.5 ± 0.2</td>
<td>5.2 ± 0.2a</td>
<td>5.0 ± 0.2d</td>
<td>5.3 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>GFR (ml/min/g KW)</td>
<td>1.12 ± 0.04</td>
<td>1.10 ± 0.07</td>
<td>1.13 ± 0.07</td>
<td>0.23 ± 0.05d</td>
<td>0.50 ± 0.08bc</td>
</tr>
<tr>
<td>Urinary flow rate (μl/h/g KW)</td>
<td>10.05 ± 2.23</td>
<td>7.07 ± 1.11</td>
<td>8.51 ± 3.43</td>
<td>19.20 ± 3.9d</td>
<td>11.80 ± 3.65</td>
</tr>
<tr>
<td>U&lt;sub&gt;Na&lt;/sub&gt;V (μmol/min/g KW)</td>
<td>0.71 ± 0.21</td>
<td>0.50 ± 0.22</td>
<td>0.59 ± 0.46</td>
<td>0.56 ± 0.09</td>
<td>0.30 ± 0.14</td>
</tr>
<tr>
<td>FE&lt;sub&gt;Na&lt;/sub&gt; (%)</td>
<td>0.43 ± 0.12</td>
<td>0.28 ± 0.13</td>
<td>0.31 ± 0.22</td>
<td>2.95 ± 0.77d</td>
<td>0.62 ± 0.34b</td>
</tr>
<tr>
<td>U&lt;sub&gt;K&lt;/sub&gt;V (μmol/min/g KW)</td>
<td>1.37 ± 0.27</td>
<td>1.18 ± 0.25</td>
<td>1.33 ± 0.46</td>
<td>0.49 ± 0.07d</td>
<td>0.39 ± 0.15c</td>
</tr>
<tr>
<td>FE&lt;sub&gt;K&lt;/sub&gt; (%)</td>
<td>32.88 ± 6.38</td>
<td>20.44 ± 4.44</td>
<td>23.67 ± 9.00</td>
<td>53.69 ± 9.78d</td>
<td>18.92 ± 9.34b</td>
</tr>
</tbody>
</table>

KW, kidney weight; BW, body weight; CIT, cold ischemic time; U<sub>Na</sub>V, urinary sodium excretion; U<sub>K</sub>V, urinary potassium excretion; FE<sub>Na</sub>, fractional urinary sodium excretion.

*p < 0.05 versus CON-VV.

*p < 0.05 versus KT-CV.

*p < 0.05 versus CON-CT.

*p < 0.05 versus CON-CV.

Discussion

Experimental and clinical studies have shown that AdoR antagonists such as xanthine derivatives (i.e., theophylline) reduce or prevent the severity of acute renal failure. Based on these observations, we hypothesized that the beneficial effect of theophylline in different models of acute renal failure can be extended to the ischemia reperfusion injury following KT. Our study demonstrates in an allogeneic rat model of KT that administration of theophylline during transplantation and in the postoperative course improves GFR on day 5. This effect of theophylline was not accompanied by differences in histological signs of inflammation, as well as in serum cytokine patterns. The increase in AdoR A<sub>1</sub> mRNA expression might present an important pathophysiological factor contributing to delayed allograft function.

The well established model of KT in Fisher 344 to Lewis rats was chosen to study the functional and immune response of the graft to IRI with and without theophylline. We selected a 2-h cold ischemia period followed by a 30- to 45-min warm ischemia period. Earlier studies by Dragun et al. (2001) have shown that long-time cold ischemia (12–24 h) did not induce further functional deterioration compared with short-time cold ischemia (2–6 h). The same research group has also shown that the peak infiltration of immune competent cells into the allograft occurred between 48 h and 1 week after transplantation. Renal function started to recover during the same interval (Dragun et al., 2001). Therefore, day 5 after transplantation was selected as a critical time period to assess kidney function (GFR and electrolyte excretion) as well as cell infiltration into the graft. We measured CsA and theophylline blood levels in control rats to determine possible drug interactions. In humans, no interactions have been reported for cytochrome P450-dependent metabolism of CsA and theophylline (Dai et al., 2004; Faber and Fuhr, 2004).

The major finding of this study is that theophylline showed a 2-fold increase in GFR in theophylline-treated rats compared with the vehicle-treated group on day 5 following transplantation. Munger et al. (1993) reported a similar reduction of GFR by 80%, as found in the present study, in an allogeneic model of KT in rats without CsA treatment. These authors assume that this fall in GFR resulted from pregglomerular vasoconstriction (Munger et al., 1993). Since adenosine induces vasoconstriction of the afferent glomerular arterioles in the kidney in vivo, leading to a fall in glomerular hydrostatic pressure and consequently a fall in GFR (Osswald et al., 1978; Haas and Osswald, 1981), it is conceivable that the hemodynamic action of theophylline is mainly located at the vasoconstriction sites. The half-maximal inhibition of adenosine-induced renal vasoconstriction by theophylline is observed at 2 to 5 μM (Osswald, 1975). This concentration is within the therapeutic range of theophylline plasma levels. It is therefore unlikely that theophylline at this micromolar concentration inhibits phosphodiesterases
to elicit its renal effects. This conclusion is further supported by the observation that the renal actions of theophylline are absent in AdoR A1 knockout mice (Rieg et al., 2005). In addition, theophylline and AdoR A1 antagonists also block tubuloglomerular feedback (TGF) response of the nephron (Osswald et al., 1997). The TGF describes the sequence of signaling processes in which the single nephron glomerular filtration rate decreases when the electrolyte concentration (Na, Cl, K) in the tubular fluid passing the macula densa segment is increased. Adenosine is considered to be the main mediator of the TGF (Osswald et al., 1991; Thomson et al., 2000; Sun et al., 2001). Furthermore, the TGF is considered to be an important element in the pathophysiology of several types of acute renal failure (Osswald and Vallon, 1998). Thus, it is likely that the improvement of GFR by theophylline in rats following kidney transplantation is mediated by AdoR A1 antagonism. However, we cannot exclude additional mechanisms of action for the beneficial effect of theophylline in the allograft.

Concomitant with the elevated GFR, theophylline increased the electrolyte reabsorption capacity of the nephron as indicated by a fall of fractional electrolyte excretion (Table

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**Fig. 1.** Immunostaining of renal allografts 5 days after transplantation. A, moderate interstitial infiltration of ED-1-positive monocytes/macrophages (score 2) in vehicle-treated rats (KT-CV). B, same staining as in A but in theophylline-treated rats (KT-CT). C, immunostaining of T-lymphocytes in vehicle-treated rats (KT-CV) showing moderate interstitial infiltration (score 2). D, same staining as in C showing mild infiltration (score 1) in theophylline-treated KT rats (KT-CT).

**Fig. 2.** AdoR A1, A2a, A2b, and A3 receptor mRNA expression (arbitrary units) under different experimental conditions. Expression of AdoR A1 mRNA was significantly ($p < 0.05$) enhanced after transplantation, whereas the difference of the other AdoR mRNA did not reach significance. Expression of the four AdoRs were unaffected by theophylline treatment in all groups.
and IL-1 receptor antagonist, and TGF-
CsA.

However, could be masked by the immunosuppressive effect of
theophylline treatment compared with the vehicle-treated group.

Adenosine can modulate the release of cytokines from inflam-
matory cells as well as tissue endothelial cells by decreasing the release of proinflammatory cytokines and by increasing the release of anti-inflammatory cytokines (Bouma et al., 1996). Anti-inflammatory action of adenosine via the AdoR A1 was suggested to be protective against reperfusion injury by reducing neutrophil accumulation in the kidneys subjected to IRI (Okusa et al., 2000). Furthermore, it was shown that AdoR A2a activation of bone marrow-derived cells reduces IRI-mediated elevation of IL-6, IL-1β, and IL-1 receptor antagonist, and TGF-β mRNA in the kidney (Okusa et al., 1999). Based on these observations, one would expect that antagonism of AdoR A2a by theophylline should lead to a proinflammatory reaction. However, in the experiments, we could not detect an enhancement of allograft inflammation with theophylline. The absence of an additional inflammatory tissue response by theophylline, however, could be masked by the immunosuppressive effect of CsA.

Our finding, of an up-regulation of AdoR A1 mRNA in allografts, further supports a role of adenosine in the pathophysiology of IRI and KT. These findings can account for an increased preglomerular vasoconstriction by adenosine and could explain the beneficial effects of adenosine antagonists, such as theophylline in different models of acute renal impairment (Osswald and Vallon, 1998).

Considering clinical use of theophylline, its side effects are well known and documented. Theophylline is widely used in drug therapy, mainly to treat obstructive lung diseases. Since theophylline is eliminated mainly by hepatic metabolism, renal functional impairment does not affect theophylline plasma concentrations. Theophylline-induced severe side effects are unlikely to occur within plasma levels of 5 to 16 μM (DrugDex database search).

In summary, these results demonstrate the first time that theophylline remarkably improved allograft function at day 5 following KT without significantly altering inflammatory processes in a rat KT model. Furthermore, all allografts showed an enhanced expression of AdoR A1 mRNA. We suggest that the beneficial effect of theophylline is based mainly on the reduction of AdoR A1-induced preglomerular vasoconstriction. Since theophylline treatment would result in a cost-effective prevention of IRI and since theophylline is a commonly used medication in humans, clinical studies in patients undergoing KT appear to be warranted.

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