Abstract
We previously developed a model of acute cyclosporine A (CsA)-induced vasomotor nephrotoxicity in rabbits. In the present study, we evaluated the role of endothelin (ET), angiotensin II (AII) and adenosine in this experimental model. All animals received CsA (25 mg/kg/day) for 5 days. Renal function parameters were first measured in a 30-min period, showing renal insufficiency in all animals. Then, rabbits were administered bosentan (10 mg/kg; antagonist of ET \(_{AB}\) receptors), perindopril (20 µg/kg; angiotensin-converting enzyme inhibitor), or theophylline (1 mg/kg; adenosine receptor blocker at micromolar concentrations). After a 40-min equilibration period, renal function was assessed again for 30 min. Bosentan, perindopril and theophylline significantly reduced renal vascular resistance (–28±5%, –39±7% and –8±3%, respectively), and improved renal blood flow (+38±15%, +66±16% and +20±5%), glomerular filtration rate (+33±9%, +52±13% and +50±8%) and diuresis (+48±9%, +76±19% and +73±14%). Filtration fraction was unchanged with bosentan, decreased with perindopril (–10±9%) and increased with theophylline (+24±5%). The overall results suggest that ET, AII and adenosine are involved in the acute renal failure induced by CsA. We conclude that CsA administration for 5 days induced a vasomotor nephropathy with ET- and adenosine-mediated afferent arteriolar constriction as well as ET- and AII-mediated efferent arteriolar constriction.

Key words
Acute nephrotoxicity · Adenosine · Angiotensin II · Cyclosporine A · Endothelin · Rabbit

Introduction
Cyclosporine A (CsA) remains one of the most effective immunosuppressive drugs used in the management of organ transplantation and autoimmune diseases. Despite its great benefits, CsA is associated with nephrotoxicity that is either acute and reversible (hemodynamic) or chronic and irreversible (structural). Acute CsA administration has been shown to induce renal vasoconstriction in experimental studies as well as in humans.

We previously developed a rabbit model of CsA-induced acute nephrotoxicity [1]. The rabbit was chosen because of the close similarities of the long-term CsA-induced renal side effects with those in humans [2, 3]. Compared to untreated animals, administration of CsA 25 mg/kg/day for 5 days induced decreases in renal blood flow (RBF) and glomerular filtration rate (GFR) and increases in renal vascular resistance (RVR). The unchanged filtration fraction (FF) in this model indicated a contraction of both afferent and efferent arterioles, which has scarcely ever been described in the literature: CsA has usually been reported to induce contraction and hyperplasia of afferent arterioles [4]. Morphological studies of our animal kidneys showed normal arteries, indicating a vasomotor origin for these CsA-induced renal function changes.

Therefore, we were interested in evaluating some of the putative mediators in this model since the mechanisms of this vasomotor nephropathy remain controversial.

Renal hemodynamic effects of exogenous endothelin (ET), angiotensin II (AII) and adenosine administration are close to the changes observed in the early stage of CsA-induced nephrotoxicity [5].

In each species examined, the i.v. administration of ET was shown to cause a dose-dependent and long-lasting decrease in RBF, GFR and urine volume as a result of increased RVR, mesangial cell contraction and decreases in the ultrafiltration coefficient [6, 7, 8, 9]. The
Plasma proteins (g/l) 42.7±1.3 38.0±0.5 a 39.5±1.2 34.5±1.2 a 38.8±1.6 34.2±1.9 a 39.1±2.1 36.5±1.9 a

Intravenous (i.v.) bolus. This group acted as a control study, they were randomly divided into four groups (see Fig. 1). Malmaison, France) for 5 days. Then, on the day of the renal clearance experiments and maintained on a standard diet (C15, Piétrement, France) and tap water ad libitum.

Materials and methods

Experiments were performed on 39 male New Zealand white rabbits weighing 3110±33 g (2460–3570 g), housed in individual cages and maintained on a standard diet (C15, Piétrement, France) and tap water ad libitum.

All rabbits were treated with CsA as reported previously [1]. They received subcutaneous injections of 25 mg/kg/day (0.25 ml/kg/b.i.d.) Sandimmun (CsA 50 mg/ml, Novartis, Rueil-Malmaison, France) for 5 days. Then, on the day of the renal clearance study, they were randomly divided into four groups (see Fig. 1).

In group 1, CsA-water-treated animals (n=6) were administered 5 ml sterilized water for injections (Aguettant, Lyon, France) as an intravenous (i.v.) bolus. This group acted as a sham-surgery group and was designed to ensure that the changes in renal function parameters induced by CsA were stable throughout the experiment.

In group 2, CsA-bosentan-treated animals (n=11) were administered 10 mg/kg bosentan (Hoffmann-La Roche, Basel, Switzerland) dissolved in 5 ml sterilized water as an i.v. bolus. Bosentan (Ro 47-0203) is a mixed and competitive antagonist of the three ET receptors ET_{A}, ET_{B1} and ET_{B2}. It was shown to selectively inhibit vasoconstriction in response to ET-1 with no agonist activity [16]. The dose used in our study (10 mg/kg i.v.) was shown to be effective in Wistar rats at inhibiting the decrease in blood pressure induced by the ET_{B} agonist sarafotoxin S6C [16].

In group 3, CsA-perindopril-treated animals (n=11) were administered 20 µg/kg perindopril (IRIS, Courbevoie, France) as an i.v. bolus dissolved in 5 ml sterilized water. Perindopril, a clinically used drug, has been described as one of the most potent angiotensin-converting enzyme (ACE) inhibitors. It is a prodrug des-ethylated in body fluids into an active diacid, perindoprilat. The dose used in this study was shown to induce a significant increase in plasma renin activity in rabbits, from 2.2±0.7 to 15.5±5.5 ng angiotensin II/ml/h in the 60 min following perindoprilat administration, indicating an ACE inhibition [17].

In group 4, CsA-theophylline-treated animals (n=11) were administered 1 mg/kg theophylline (aminophylline, Sigma Chemicals, St. Quentin, France; aminophylline is theophylline complexed with ethylenediamine, which dissociates into theophylline in body fluids) as an i.v. bolus dissolved in 5 ml sterilized water. The low dose used in this study was shown to reach micromolar serum levels, which are sufficient to competitively antagonize adenosine interaction with its surface cellular membrane receptors [18].

Table 1 Influence of intravenous infusion of water 5 ml (group 1), bosentan 10 mg/kg (group 2), perindopril 20 µg/kg (group 3), or theophylline 1 mg/kg (group 4) on physiological parameters of rabbits pretreated with 25 mg/kg/day cyclosporine A (CsA) for 5 days (values are means ± SEM; MBP mean blood pressure, Hct hematocrit)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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</thead>
<tbody>
<tr>
<td>CsA</td>
<td>+ Water</td>
<td>CsA</td>
<td>+ Bosentan</td>
</tr>
<tr>
<td>Period I</td>
<td>Period II</td>
<td>Period I</td>
<td>Period II</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>75.5±5.3</td>
<td>74.5±4.2</td>
<td>78.1±3.2</td>
</tr>
<tr>
<td>PaO_{2} (mmHg)</td>
<td>168±5</td>
<td>164±7</td>
<td>148±5</td>
</tr>
<tr>
<td>pH</td>
<td>7.64±0.03</td>
<td>7.67±0.01</td>
<td>7.58±0.03</td>
</tr>
<tr>
<td>Plasma proteins (g/l)</td>
<td>42.7±1.3</td>
<td>38.0±0.5</td>
<td>39.5±1.2</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>33.2±0.8</td>
<td>31.8±0.8</td>
<td>35.5±1.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> P<0.05 when period II is compared with period I by Wilcoxon’s non-parametric test

<sup>b</sup> P<0.05 when periods I of the four groups are compared by the Kruskal-Wallis non-parametric test.