Original Article

Prevention of cyclosporin nephrotoxicity with a platelet-activating factor (PAF) antagonist

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Abstract

Background. Cyclosporin (CsA) is a potent immunosuppressive drug whose main side-effect is nephrotoxicity. In the kidney, CsA induces vasoconstriction with a decrease in renal blood flow (RBF) and glomerular filtration rate (GFR) and a significant increase in renal vascular resistance (RVR). CsA enhances platelet-activating factor (PAF) synthesis in mesangial cells in vitro. PAF, a secondary mediator of anaphylaxis and inflammation, exhibits vasoactive properties in the kidney similar to those of CsA.

Methods. The in situ autoperfused rat kidney model was used to investigate whether PAF plays a role in the haemodynamic injury induced by CsA.

Results. In this model, CsA (40 mg/kg and 20 mg/kg i.v.) induced a significant decrease in RBF and in GFR and an increase in RVR. BN 52021, a potent and specific PAF antagonist (20 mg/kg i.v. bolus dose) induced a significant increase in GFR (137 ± 32% of initial value, p < 0.05). BN 52021 (20 and 10 mg/kg) also significantly prevented the decline in RBF and GFR induced by CsA.

Conclusions. We have demonstrated that the PAF antagonist BN 52021 can minimize the alteration of renal function induced by CsA.

Key words: cyclosporin; platelet-activating factors; haemodynamics

Introduction

Cyclosporin (CsA) is a potent and widely used immunosuppressive drug whose main side-effect is nephrotoxicity [1]. Intrarenal vasoconstriction, characterized by a fall in renal blood flow (RBF) and glomerular filtration rate (GFR) and an increase in renal vascular resistance (RVR) is the hallmark of the acute renal effect of cyclosporin [2–4]. Although widely studied in animals and humans [5–7], the pathophysiology of the renal haemodynamic impairment induced by CsA is still unclear. An imbalance between endothelium-derived relaxing and contracting factors induced by CsA could contribute to the changes of the renal circulation which are observed.

PAF is a phospholipid that exhibits numerous biological activities as a secondary mediator of inflammation and anaphylaxis [8]. This potent autacoid is released by various kidney cell types, including glomeruli, mesangial, and medullary cells [9]. In the kidney, PAF causes a decrease in renal blood flow and contraction of glomerular mesangial cells [10], thereby reducing glomerular filtration [11].

Recent studies demonstrated that CsA induces an increase in PAF synthesis in mesangial cells in vitro [12]. In animal models the injection of PAF into the kidney induces similar effects to that observed with CsA [13]. It was thus tempting to speculate that PAF could be involved in CsA acute nephrotoxicity.

The study was conducted with continuous monitoring of haemodynamics parameters in the in-situ autoperfused rat kidney model. Rapid changes in RBF or MABP and renal haemodynamic effects of CsA were precisely monitored with and without administration of a PAF antagonist.

Subjects and methods

Experimental animals

Eighty Sprague-Dawley male rats (Charles River, Saint Aubin les Elbeuf, France) weighing 468 ± 9 g were divided into six groups. All animals were fed a standard rat chow (Extralabo, Sainte Colombe, France) ad libitum, allowed free access to water, and were housed at 22°C for at least 2 days before the experimental procedure.
First set of experiments

The animals were divided into six groups:

Group 1, Control group (n = 12). These rats were intravenously infused with isotonic saline.

Group 2, cremophore (40) group (n = 5). These rats received 0.8 ml/kg intravenous (i.v.) bolus of cremophore.

Group 3, CsA (40) group (n = 10). 40 mg/kg CsA was administered intravenously as a bolus.

Group 4, Anti-PAF (20) group (n = 5). The rats received a 20 mg/kg i.v. bolus of BN 52021 injected into the jugular vein. Group 5, cremophore (40) + anti-PAF (20) (n = 5). In this group a 20 mg/kg bolus of BN 52021 was injected 30 min before a 0.8 ml/kg bolus of cremophore.

Group 6, CsA (40) + anti-PAF (20) group (n = 7). These rats received a 20 mg/kg i.v. bolus of BN 52021 30 min before a 40 mg/kg i.v. bolus of CsA.

Second set of experiments

Five additional groups were studied:

Group 7, CsA (20) group (n = 10). Rats were administered a 20 mg/kg bolus of cyclosporine.

Group 8, cremophore (20) group (n = 5). An intravenous dose of cremophore 0.4 ml/kg was administered.

Group 9, anti-PAF (10) group (n = 5). These rats received a 10 mg/kg i.v. bolus of BN 52021 injected into the jugular vein.

Group 10, CsA (20) + anti-PAF (20) (n = 8). These rats received a 20 mg/kg i.v. bolus of BN 52021 30 min before a 20 mg/kg i.v. bolus dose of CsA.

Group 11, CsA (20) + anti-PAF (10) (n = 8). These rats received a 10-mg/kg i.v. bolus of BN 52021 30 min before a 20-mg/kg i.v. bolus of CsA.

Experimental drugs

CsA and cremophore were kindly provided by Sandoz (Sandoz, Basle, Switzerland). The commercially available intravenous form of CsA was administered at a dose of 40 mg/kg. Cremophore was intravenously infused at a dose of 0.8 ml/kg.

BN 52021 was a gift from Beaufour (Institut Henri Beaufour Labs, Les Ulis, France). The lyophilisat of BN 52021 was dissolved in saline, as a 20 mg/ml solution, and administered as a 20 mg/kg i.v. bolus dose.

In preliminary experiments we have shown that treatment with BN 52021, before or after a lethal dose of PAF (2 mg/kg i.v. bolus dose), provides protection against its harmful effects for up to 8 h after administration.

*In-situ* autoprefused rat kidney preparation

The experimental model has been previously described in detail elsewhere [14].

Experimental protocol

Sixty minutes after the completion of surgery, the animals were randomized and the experimental protocol was initiated. The first 30-min clearance period was used as the control period. At the beginning of the second 30-min clearance period, a bolus of BN 52021 was administered in groups 4, 5, 6, 9, 10 and 11. At the beginning of the third period, a bolus of either cremophore (in groups 2, 5 and 8) or CsA (in groups 3, 6, 7, 10 and 11) was given. A 60-min clearance period was then observed.

Renal clearance studies and analytical procedure

Inulin clearance was used to estimate GFR. After a priming dose of 0.5 ml Inulin, all rats received a continuous intravenous infusion of a 16.6% inulin solution diluted in isotonic saline at the rate of 3 ml/h. A 60-min equilibration period was allowed after surgery, and the infusion was initiated in order to attain constant plasma levels of Inulin. Samples were collected for two 30-min and one 60-min clearance periods. At the midpoint of each period, 400 μl arterial blood was obtained from the extracorporeal shunt and the plasma was separated by centrifugation. Urine samples were collected through a ureteral catheter in preweighed tubes. Plasma and urine inulin concentrations were determined by the method of Galli and Jeanmaire [15]. Renal vascular resistance (RVR) was calculated as the quotient of mean arterial blood pressure (MABP) and renal blood flow. MABP and pulse rate were continuously monitored with the pressure transducer connected to a physiograph.

Blood concentration of CsA was measured with a radioimmunoassay using a specific monoclonal antibody (Incast Corporation, Stillwater, Minnesota, USA).

Statistical analysis

Statistical analysis was performed using the Statview program installed on a Macintosh LCIII computer. All values are expressed either as mean ± SEM or % of initial value and were analysed by parametric test (paired or unpaired t test and ANOVA). P values equal to or less than 0.05 were considered as statistically significant.

Results

First set of experiments

No statistical differences were observed in the six experimental groups for RBF, MABP, GFR or diuresis, during the first 30-min control clearance period (Table 1).

Control group. No statistical differences were observed among the three experimental periods in the control group for RBF (2.4 ± 0.1, 2.5 ± 0.2 and 2.7 ± 0.2 ml/min/100 g, NS), MABP (87 ± 2, 88 ± 3 and 90 ± 4 mmHg, NS), RVR (7.7 ± 0.3, 7.3 ± 0.3 and 7.4 ± 0.5 mmHg × min/ml, NS), GFR (3.20 ± 0.01, 0.15 ± 0.02 and 0.15 ± 0.02 ml/min/100 g, NS) and diuresis (4 ± 1.71 ± 1 and 7.1 ± 1 μl/min, NS), which demonstrates the stability of the model.

Effect of BN 52021 (20 mg/kg). Groups 4 and 5 were exposed to identical experimental conditions prior to administration of BN 52021. This allowed us to combine the data from these animals in order to analyse the effect of BN 52021 on renal haemodynamics.

BN 52021 induced no change in RBF and a significant increase in MABP (102 ± 3 versus 91 ± 3 mmHg, P < 0.05 versus control period), RVR (9.3 ± 0.7 versus 8.3 ± 0.5 mmHg × min/ml, P < 0.05 versus control period), GFR (0.26 ± 0.03 versus 0.13 ± 0.02 ml/min/100 g, P < 0.05 versus control period) and diuresis.
Effect of BN 52021 on CsA nephrotoxicity

Table 1. RBF, MABP, GFR and diuresis for the different experimental groups during the first control period (30 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>RBF (ml/min/100 g)</th>
<th>MABP (mmHg)</th>
<th>GFR (ml/min/100 g)</th>
<th>Diuresis (ul/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>2.4 ± 0.1</td>
<td>87 ± 2</td>
<td>0.15 ± 0.01</td>
<td>6.4 ± 1</td>
</tr>
<tr>
<td>Cremophore group</td>
<td>2.6 ± 0.2</td>
<td>91 ± 4</td>
<td>0.16 ± 0.02</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>CsA group</td>
<td>2.2 ± 0.1</td>
<td>93 ± 4</td>
<td>0.14 ± 0.01</td>
<td>6.3 ± 2</td>
</tr>
<tr>
<td>Anti-PAF group</td>
<td>2.6 ± 0.3</td>
<td>97 ± 5</td>
<td>0.13 ± 0.03</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Anti-PAF + CsA group</td>
<td>2.4 ± 0.2</td>
<td>87 ± 3</td>
<td>0.13 ± 0.02</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Anti-PAF + cremophore</td>
<td>2.1 ± 0.3</td>
<td>92 ± 3</td>
<td>0.22 ± 0.02</td>
<td>4.8 ± 0.5</td>
</tr>
</tbody>
</table>

(11 ± 2 versus 4 ± 0.6 µl/min, P < 0.05 versus control period).

Effect of CsA (40 mg/kg) and cremophore with or without pretreatment with BN 52021 (20 mg/kg) (Figures 1–5). Continuous recording of RBF and MABP allowed us to study the acute effects of CsA and cremophore with, and without BN 52021, during the first 15 min after its administration (as a percentage of initial value). The main effect was observed at the end of the intravenous infusion (4th minute). All parameters were then analysed at the end of the experiment.

Cremophore group. A significant decrease in RBF to 91 ± 2% of its initial value during the control period (P < 0.05), and a significant increase in MABP to

![Fig. 1. Effect of CsA (40 mg/kg) and cremophore on RBF (% of initial value) with or without BN 52021. *P<0.05 versus control period.](image1)

![Fig. 2. Effect of CsA (40 mg/kg) and cremophore on MABP (% of initial value) with or without BN 52021. *P<0.05 versus control period.](image2)

![Fig. 3. Effect of CsA (40 mg/kg) and cremophore on RVR (% of initial value) with or without BN 52021. *P<0.05 versus control period.](image3)

![Fig. 4. Effect of CsA (40 mg/kg) and cremophore on GFR (% of initial value) with or without BN 52021. *P<0.05 versus control period.](image4)

![Fig. 5. Effect of CsA (40 mg/kg) and cremophore on diuresis (% of initial value) with or without BN 52021. *P<0.05 versus control period.](image5)
130 ± 8% of its initial value ($P < 0.05$) were observed 4 min after the administration of cremophore. No significant change was observed in RVR (148 ± 15% of its initial value, NS).

At the end of the experimental period, cremophore induced a significant decrease in RBF (66 ± 6% of its initial value, $P < 0.05$ versus control group), and a significant increase in RVR (143 ± 7% of its initial value, $P < 0.05$ versus control group). No significant change was observed for MABP, GFR and diuresis.

**CsA group.** A decrease in RBF (64 ± 5% of its initial value, $P < 0.05$ versus control group) and an increase in MABP (150 ± 4% of its initial value, $P < 0.05$ versus control group) and RVR (260 ± 34% of its initial value, $P < 0.05$ versus control group) were observed 4 min after infusion.

At the end of the experimental period, CsA induced a significant decrease in RBF (66 ± 6% of its initial value, $P < 0.05$ versus control group), and a significant increase in RVR (143 ± 7% of its initial value, $P < 0.05$ versus control group). No significant change was observed for MABP, GFR and diuresis.

**BN+ CsA group.** Four minutes after CsA administration we observed a significant fall in RBF (74 ± 6% of its initial value, $P < 0.05$ versus control group) and a significant increase in MABP (162 ± 9% of its initial value, $P < 0.05$ versus control group) and RVR (232 ± 33% of its initial value, $P < 0.05$ versus control group).

At the end of the experiment, RBF (82 ± 7% versus 61 ± 7% of its initial value, $P < 0.05$), GFR (134 ± 15% versus 58 ± 7% of its initial value, $P < 0.05$), and diuresis (288 ± 70% versus 130 ± 43% of its initial value, $P < 0.05$) were significantly higher than in the group treated with CsA alone. No difference was observed between these two groups for MABP and RVR.

CsA levels did not differ significantly between CsA and BN 52021 + CsA groups (92 ± 9 and 80 ± 8 µg/ml respectively, NS).

**BN 52021 + cremophore.** Four minutes after cremophore administration RBF decreased significantly (82 ± 6% of its initial value) ($P < 0.05$ versus control group).

MABP and RVR increased significantly (136 ± 5 and 175 ± 23% of its initial value, respectively, $P < 0.05$ versus control group).

At the end of the experiment, RBF decreased significantly (73 ± 9% of its initial value, $P < 0.05$ versus control group) and RVR increased significantly (158 ± 18% of its initial value, $P < 0.05$ versus control group). No change was observed in MABP, GFR or diuresis.

**Second set of experiments (Figures 6—11)**

We only present the results obtained 60 min after CsA administration.

**Effect of a low dose of CsA.** At the end of the experiment, CsA (20 mg/kg) induced a significant decrease in RBF (70 ± 7%, $P < 0.05$ versus control group, NS versus CsA (40 mg/kg) group), and GFR (59 ± 14, $P < 0.05$ versus control group, NS versus CsA (40 mg/kg) group), and a significant increase in RVR (150 ± 9, $P < 0.05$ versus control group, NS versus CsA (40 mg/kg) group). No change in MABP (100 ± 5, NS) or diuresis (85 ± 23, NS) were observed.
Effect of BN 52021 on CsA nephrotoxicity

Effect of a low dose of BN 52021 (10 mg/kg). BN (10 mg/kg) induced a significant decrease in RBF (85 ± 5%, P < 0.05 versus control group), a significant increase in RVR (129 ± 9%, P < 0.05 versus control group) and had no effect on MABP (108 ± 2%, NS), GFR (104 ± 16, NS) or diuresis (127 ± 18%, NS).

Effect of a low dose of CsA on a low dose of CsA. Administration of 10 mg/kg BN before CsA (20 mg/kg) resulted in a significant fall in RBF (75 ± 7%, P < 0.05 versus control group, NS versus group 10), a significant increase in RVR (148 ± 9%, P < 0.05 versus control, NS versus group 10), but no change in GFR (72 ± 15%, NS versus control group, NS versus group 10, MABP (105 ± 6%, NS) or diuresis (160 ± 21%, NS).

Discussion

Our in-situ autoperfused rat kidney model was validated by in vivo electromagnetic [16,17] and microsphere techniques [17]. Contrary to clearance techniques which require a long urine collection period, or to methods involving radiolabelled microspheres, which measure flow only during the instant in which the indicator enters the kidney, transient modifications in RBF may be appreciated in our model. The construction of an electromagnetic flow probe sufficiently small to fit around the renal artery is possible, but requires extensive dissection of renal artery with hormonal modifications, whereas our model preserves all neural and hormonal afferents. Extracorporeal autoperfusion appears to offer significant advantages for the analysis of renal haemodynamics.

Many authors have shown that CsA, even at low doses, exhibits renal toxicity with significant fall in RBF and GFR and a marked increase in RVR in animals [2] and humans [19]. The pathophysiology of CsA renal toxicity is not completely understood.

In the kidney, PAF induces a fall in renal blood flow and glomerular filtration rate [11] and seems to be implicated in the pathogenesis of immunological [20,21], ischaemic [22], or toxic [10,23] glomerular injury. Badr et al. [13] demonstrated that intrarenal PAF reduced glomerular plasma flow rate by increasing both afferent and efferent arteriolar resistance and that the decline in single-nephron filtration rate was predominantly due to a decline in the glomerular filtration coefficient. Thus, CsA and PAF obviously share haemodynamic effects in the kidney.

Several mediators which may play a role in CsA nephrotoxicity may also mediate renal PAF properties. In isolated glomeruli, PAF stimulates the biosynthesis of thromboxane B2 in a dose-dependent manner [24]. Formation of PAF is initiated by activation of phospholipase A2 which depends on an increase in local calcium concentration. The effect of PAF in the kidney...
can be reversed by a specific thromboxone receptor antagonist [13,25,26]. In addition, PAF antagonist induces a fall in the intraglomerular synthesis of thromboxane B2 in the rabbit [27] suggesting that thromboxane could at least partially mediate the haemodynamic effect of PAF [25–27]. Therefore it was tempting to speculate that PAF could be involved in CsA acute nephrotoxicity.

Beneficial effects of platelet activating factor antagonists on glycerol, gentamicin, cis-platin or ischaemic induced acute renal failure have already been demonstrated in experimental studies [10,23,28,29]. Few studies are available on the potential beneficial effect of PAF antagonists on CsA toxicity. In isolated human and rat glomeruli, Lamas et al. [30] speculated that PAF could be involved in CsA acute nephrotoxicity.

Numerous drugs modify CsA pharmacokinetics and our results may be attributed to a decrease in blood CsA levels. However, plasma circulating levels of CsA were not modified by anti-PAF perfusion.

The usefulness of BN 52021 in the field of transplantation is already outlined by the recent double-blind, randomized study conducted in kidney transplant patients by Grino et al. [42], who showed that BN 52021 may prevent ischaemia–reperfusion injury in clinical transplantation.

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References

Effect of BN 52021 on CsA nephrotoxicity


