Telmisartan attenuates chronic ciclosporin A nephrotoxicity in a pig model

Donata Cibulskyte, Michael Pedersen, Arne Hørlyck, Niels Marcussen, Hans Erik Hansen, Jørgen Frøkiær, Melvin Madsen and Jens Mortensen

Abstract

Background. We have previously demonstrated renal enlargement in pigs treated with ciclosporin A (CsA) 10 mg/kg/day orally for 6 months. The aim of the study was to investigate the effect of oral CsA (10 mg/kg/day) for 12 months on kidney structure and function and the potential renoprotective role of angiotensin II (Ang II) receptor blocker telmisartan on chronic CsA nephrotoxicity in pigs.

Methods. Fourteen Göttingen minipigs aged 12–14 months were included: pigs received either CsA 10 mg/kg/day (n = 7) or CsA 10 mg/kg/day + telmisartan 40 mg/day (n = 7) orally for 12 months. At week 0, 12, 31, 38, 47 and 54, we measured body weight, mean arterial blood pressure (MAP), serum creatinine, whole blood trough CsA, plasma Ang II, haemoglobin and liver function parameters. Magnetic resonance imaging was used to estimate kidney length, volume, relative glomerular filtration rate (rGFR) and renal blood flow (RBF). Kidney tissue biopsies were used for conventional histological examination.

Results. Plasma Ang II levels were significantly higher during telmisartan treatment. Interstitial fibrosis and glomerulosclerosis occurred in both groups, but were attenuated in the telmisartan-treated pigs (P = 0.064). A significant increase in renal volume was seen in both groups, but tended to be lower in the CsA + telmisartan pigs at 54 weeks (P = 0.097). Telmisartan did not reduce MAP, RBF or rGFR.

Conclusions. Long-term CsA treatment causes histopathological changes in the porcine kidney similar to those observed in humans and results in renal enlargement. Telmisartan attenuates the CsA-induced histopathological changes and enlargement in the pig kidney.

Keywords: angiotensin II receptor blocker; ciclosporin A; nephrotoxicity; pig; renal enlargement; telmisartan

Introduction

Despite the introduction of novel immunosuppressants, ciclosporin A (CsA) remains a gold standard in the management of organ transplantation and some immunemediated diseases. The major limitation of long-term CsA use is chronic nephrotoxicity. The pathogenesis of CsA-induced nephropathy is multifactorial. Both in vivo and in vitro studies indicate that the predominant mediators are an altered release of vasoactive substances such as angiotensin II (Ang II), endothelin-1, prostaglandins and tromboxanes, nitric oxide, increased sympathetic tone, as well as stimulation of cytokines and growth factors such as transforming growth factor β1 (TGF-β1) and osteopontin [1].

The renin–angiotensin system (RAS) in CsA-induced nephropathy has previously been addressed and it is well accepted that CsA activates the RAS [2]. Blockade of the RAS with angiotensin-converting enzyme inhibitors (ACEI) or Ang II receptor blockers (ARB) can prevent CsA-induced interstitial fibrosis in rats [3,4]. In addition, the ARB losartan reduces both tubular and interstitial cell apoptosis which correlates with interstitial fibrosis [5], and it has also been demonstrated that losartan decreases plasma levels of TGF-β1 in renal transplant patients [6]. Recently, it has been shown that Ang II is involved in inducing oxidative stress in CsA nephropathy, as it can be ameliorated by the ARB irbesartan [7]. Thus, these studies suggest that Ang II plays an important role for the renal pathophysiological changes in response to CsA treatment.

Previously, we have investigated chronic CsA nephrotoxicity in Göttingen minipigs given 10 mg/kg/day orally for 6 months [8]. Consistent with the study by Frey et al. [9], we found that the
whole blood trough CsA levels were lower than in humans, and no signs of toxic CsA effects on kidney function or histology were observed. Surprisingly, CsA treatment was associated with a significant increase in GFR [8], and we also demonstrated that renal volume increased in response to 10 mg/kg/day CsA [10]. We hypothesized that these findings might be an early stage of CsA nephrotoxicity similar to those observed in diabetic nephropathy.

The aims of the present study were therefore first to investigate the chronic effects of oral CsA treatment with 10 mg/kg/day on renal function and structure in the porcine model for a prolonged time, and second to examine the role of ARB using telmisartan on CsA-induced nephropathy.

Subjects and methods

Experimental animals

Fourteen female Göttingen minipigs weighing 11–27 kg were housed in individual cages. All animals were treated with CsA 10 mg/kg/day for 1 year. Seven pigs were randomized for telmisartan treatment (40 mg/day) for 1 year, blinded to the investigator. The pigs in both groups were initially 12–14 months of age. All animals were fed a standard pig diet on a calorie-restricted feeding schedule. Amount of food was adjusted to the body weight in order to maintain an equivalent weight gain.

Drugs

CsA as Sandimmune Neoral oral solution (Novartis Pharmaceuticals, Basel, Switzerland) was given in two oral doses daily after baseline study programme. Telmisartan (Micardis, Boehringer Ingelheim Danmark A/S, Copenhagen, Denmark) was given orally once a day after baseline investigations. Both drugs were added to the food, which was readily consumed within minutes.

Experimental protocol

The following study programme was performed at baseline and then after 12, 31, 38, 47 and 54 weeks.

From the evening before the experimental procedure, the pigs were abstained from food but had free access to tap water. In the morning, they were premedicated with 4 mg/kg azaperone (Stresnil; Janssen Cilag, Beerse, Belgium) and 0.5 mg/kg midazolam (Dormicil; Hameln Pharmaceuticals, Hameln, Germany) intramuscularly. Anaesthesia was induced by 0.25 mg/kg etomidate (Hypnomidate; Janssen Pharmaceutica NV, Beerse, Belgium) intravenously and maintained with 10 mg/kg/h ketamine (Ketaminol Vet; Intervet, Skovlunde, Denmark) intravenously and 0.5–0.75% isoflurane (Forene; Abbott Scandinavia AB, Solna, Sweden) in a volumetric mixture of O2 and N2O at a ratio of 1:1. The pigs were orotracheally intubated and ventilated using a respirator (Siemens, Servo 900D). Isotonic saline (sodium chloride, 9 g/l) alternating with glucose 5% were given through an ear vein, 10 ml/kg/h, to maintain hydration during anaesthesia. Ampicillin (Pentrexyl; Bristol-Myers Squibb AB, Bromma, Sweden) 1 g was given intravenously at the beginning and at the end of the study programme. A 12-F catheter was placed transurethrally in the bladder for urine collection. The femoral artery was cannulated for blood sampling and measurements of blood pressure. The animals were weighed at arrival. Urine was examined for protein and glucose. Blood samples were drawn to measure concentrations of serum creatinine, sodium, potassium, albumin, triglycerides, cholesterol, bilirubin, alkaline phosphatase, alanine aminotransferase, blood glucose, haemoglobin and whole blood levels of CsA. In addition, plasma Ang II and aldosterone levels were estimated. Blood pressure was measured using a pressure transducer (Statham Gould #4523551) attached to a monitor (CardioMed CM-4008; Medistem, Oslo, Norway).

Analytical methods

The animals received CsA in the afternoon before the day of investigation, and blood samples for determination of whole blood CsA levels were taken about 18 h after the latest dosage. For analysis the Emit 2000 Ciclosporin Specific Assay (Dade Bering, Marburg, Germany) was used.

Magnetic resonance imaging (MRI)

MRI was employed using a 1.5T clinical system (Philips Medical, Best, The Netherlands). Kidney length was estimated by a gradient-echo sequence in the long-axis plane of the kidneys. The total kidney volume was calculated by manual segmentation. Relative single-kidney glomerular filtration rate (rskGFR) was derived from the time-activity curve in response to an intravenous bolus of Gd-DPTA (0.05 mmol/kg) combined with deconvolution analysis based on the indicator dilution theory using the two-compartment model by Lawrence and Lee analysis [11]. A recent study in humans has demonstrated that relative glomerular filtration rate (rGFR) is reproducible within 7% using this technique [12]. Single-kidney renal blood flow (skRBF) was measured by a velocity-sensitive gradient-echo MRI sequence [13].

Kidney histopathology

Two 18G renal biopsies were drawn guided by ultrasonography from the lower pole of the kidney alternating between the right and the left kidney. The paraffin-embedded biopsies were cut at a thickness of 3.4 μm and stained with haematoxylin and eosin and periodic acid Schiff. Changes in the biopsies were recorded and semiquantitative scoring from 0 to 3+ was done for glomerulosclerosis, arteriolar hyalinosis, interstitial fibrosis and tubular atrophy. Complications after renal biopsies were recorded.

On the final study day, the right kidney was perfusion-fixed with 4% paraformaldehyde in situ before removal and examined for histopathological changes.

Ethical aspects

Principles of Laboratory animal care (NIH publication No. 86-23, revised 1985) were followed and the study protocol was approved by the Animal Ethics Council, Copenhagen.
Alkaline phosphatase (mmol/l) 62
Alanine aminotransferase (mmol/l) 30
Bilirubin (mmol/l) 2.3
Aldosteron (pg/ml) 28
Angiotensin II (pg/ml) 12
Whole blood trough CsA levels (ng/ml) 15
Body weight (kg) 18

From week 31 onwards, definite interstitial fibrosis and glomerulosclerosis occurred in the kidney biopsies (Figure 1). Most prominent changes were

Histopathology

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Statistical analysis

The data are expressed as mean ± SD, unless otherwise stated. Comparisons within groups were performed using one-way repeated measures analyses of variance (ANOVA) followed by Holm–Sidak multiple comparison method (vs baseline values). Comparisons between the groups (changes between baseline values and values at all later times) were statistically evaluated by an unpaired Student’s t-test. If normality and equal variance test failed, Friedman repeated measures analysis of variance on ranks and Mann–Whitney test were used. A significance level of P < 0.05 was chosen.

Results

All treatments and investigations were well tolerated by all pigs during the study. In all pigs, two biopsies of sufficient size were successfully drawn at each visit without complications. At 12 weeks only four of seven animals in the CsA + telmisartan underwent the MRI studies, otherwise all parameters were obtained each time.

A significant increase in body weight was observed both in the CsA-treated pigs (P < 0.001) and CsA + telmisartan group (P = 0.023), especially during the first 12 weeks (Table 1). Whole blood CsA trough levels are shown in Table 1. CsA trough levels were significantly higher in the telmisartan group at 47 weeks (P = 0.041) and borderline significant at 38 weeks (P = 0.071).

Plasma Ang II levels were significantly higher in the CsA + telmisartan pigs than in the CsA-treated animals (P < 0.05) (Table 1), whereas plasma aldosterone concentrations did not differ between the two groups (P > 0.05) (Table 1).

Histopathology

From week 31 onwards, definite interstitial fibrosis and glomerulosclerosis occurred in the kidney biopsies (Figure 1). Most prominent changes were

Fig. 1. Normal glomerulus, arteriole and tubules at baseline. PAS, 20× objective (A). Focal glomerulosclerosis and patchy interstitial fibrosis in the pig kidney at 54 weeks of ciclosporin A treatment. No significant arteriolar changes. PAS, 20× objective (B).
demonstrated at weeks 47 and 54 (Table 2). However, even at these time points the fibrosis was only slight and focal, and sclerosis only present in few glomeruli. The histopathological changes were less pronounced in the CsA + telmisartan group, although the difference was only borderline significant (P = 0.064).

**Dimensional parameters**

Renal length remained constant in both groups (P > 0.05) (Figure 2A). However, a significant increase in kidney volume was observed during the whole study, both in the CsA and CsA + telmisartan group (P < 0.001 and P = 0.014, respectively) (Figure 2B). In the CsA-treated pigs, a maximal increase in volume of 175% (79 cm³) was seen at the end of the study compared with baseline value of 100% (46 cm³), whereas in the CsA + telmisartan group a maximal value of 151% (87 cm³) occurred at 12 weeks compared with baseline value of 100% (62 cm³). The difference in increase between the groups was borderline significant at 54 weeks (P = 0.097).

**Functional parameters**

Figure 3A depicts rGFR. The rGFR remained unchanged in the CsA group during the study (P > 0.05), whereas in the CsA + telmisartan group a slight but significant increase was observed at 38, 47 and 54 weeks. The difference between the groups was not significant (P > 0.05). Renal blood flow (RBF) was stable in the CsA-treated pigs (P = 0.626), whereas a slight but significant elevation in RBF was found in the CsA + telmisartan group at 38, 47 and 54 weeks (P < 0.001) (Figure 3B). However, no significant difference between the groups was found (P > 0.05).

### Table 2.

Patchy interstitial fibrosis (fibrosis) and glomerulosclerosis (sclerosis) in the kidney biopsies of pigs treated with ciclosporin A (CsA) or CsA + telmisartan taken at week 0, 12, 31, 38, 47 and 54. The difference between the two groups at 54 weeks was borderline significant (P = 0.064).

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Fig. 2. Changes in kidney length (A) and kidney volume (B) vs time for the ciclosporin (CsA) and the CsA + telmisartan-treated pigs measured at week 0, 12, 31, 38, 47 and 54. Significant increase (P < 0.05) in kidney volume vs baseline values were found in both groups at all times. The difference in increase between groups was borderline significant at 54 weeks (P = 0.097). (Baseline values equal 100% for each animal).
Serum creatinine increased significantly reaching maximum at 31 weeks in the CsA-treated pigs (103 ± 18 vs. 74 ± 8 μmol/l at baseline, $P < 0.05$) and at 38 weeks in the CsA + telmisartan group (94 ± 6 vs. 74 ± 9 μmol/l at baseline, $P < 0.05$). Thereafter, creatinine decreased reaching baseline levels at the end of the experiment (Figure 4A). The difference between the two groups was not significant ($P > 0.05$).

Correspondingly, mean arterial blood pressure (MAP) demonstrated a rising tendency to a borderline significant level at 31 weeks ($P = 0.060$ in the CsA group) and at 38 weeks in the CsA + telmisartan group ($P = 0.051$) in the CsA + telmisartan group and then decreased towards baseline level again (Figure 4B).

Liver function parameters remained normal during the study, except for an increase in alanine aminotransferase in the CsA + telmisartan-treated pigs ($P = 0.004$) (Table 1).

No significant changes were found in serum sodium, potassium, albumin, triglycerides, cholesterol, blood glucose or haemoglobin in both groups.

Discussion

The present study was designed to investigate the renal side effects of oral CsA 10 mg/kg/day in a pig model for 12 months as well as to elucidate the role of telmisartan on CsA-induced nephropathy. The major results were development of patchy interstitial fibrosis and glomerulosclerosis in the pig kidney similar to those observed in humans and reproduction of the previously reported renal enlargement during CsA administration. Importantly, telmisartan attenuated development of histopathological changes and kidney enlargement.

To our knowledge, the present study, for the first time, demonstrated renal histopathological changes in
pigs similar to those observed in humans. Thus, our results indicate that the pig may be a useful model of chronic CsA nephrotoxicity. In the present study we used minipigs which were fed a standard pig diet on a calorie-restricted feeding schedule in order to prevent obesity, but at the same time supplying all essential nutrients. This feeding does not result in nutritional deficiency which might interfere with CsA effects on kidney function and structure. To our knowledge there is no literature concerning food restriction and CsA nephrotoxicity in humans and the impact of food restriction is unclear. Previous studies from our group, in which minipigs received CsA 10 and 20 mg/kg/day for 6 months [14], failed to demonstrate histological changes in the pig kidney, and the present study indicates that this may be caused by too short exposure to CsA. The long-term effects of CsA treatment in a porcine model are not well described. Vaden and Riviere [15] reproduced mild renal tubular dilatation, which might be a precursor of interstitial fibrosis, but they only treated the animals for 4 weeks. No morphometric analyses were performed in the present study, which focused on development of interstitial fibrosis during long-term CsA treatment to prove the usefulness of the pig model. In a coming study, more detailed histological evaluation of kidney biopsies will be performed.

Another interesting finding in this study was the increase in kidney volume despite a stable kidney length. CsA is known to induce vasoconstriction in the short term and fibrosis or shrinkage in the long term [1]. Our finding is therefore surprising, but recently renal enlargement was observed in rats in response to CsA treatment [16]. Dieperink et al. [17] also found increased kidney weight in the rat during ongoing CsA intake with concomitant presence of interstitial fibrosis and decreased renal function. Interestingly, the weight increase was reversible after CsA withdrawal in some rats. This indicates that CsA causes the renal enlargement. A significant increase in kidney volume occurred in pigs treated with CsA 10 and 20 mg/kg/day for 6 months, whereas volume remained stable in control animals [14]. In addition, a statistically significant difference in volume between the CsA-treated and control groups was found in both studies (at 25 weeks during CsA 10 mg/kg/day administration and at 5 and 20 weeks during 20 mg/kg/day treatment). It may be argued that the renal enlargement follows the increase in body weight in the present study, but the stable kidney length opposes this. Although being of the same age, the two groups initially had different body weights and the increase in body weight in the CsA-treated animals was higher than in the telmisartan group. Thus, it would be tempting to speculate whether the beneficial effect of telmisartan on volume increase might partially be attributable to the differential changes in body weight. Pair-feeding would have been appropriate in this case, but kidney weight was not the end point of the present study, contrary to a previous study [14]. If we correct kidney volume maximally for body weight, a significant increase in volume is seen at 12, 47 and 54 weeks in the CsA group, and the increase is prevented by telmisartan [Figure 5]. In addition, it is known that in Large White strain pigs, a gradual increase in renal weight is observed until the age of 12 months, after which renal weight remains relatively unchanged [18]. This indicates that the importance of increased body weight on kidney volume in our study is minor. A control group would have been of interest, but due to capacity this was not possible in this study. Besides, no kidney enlargement was observed in the control group in the previous studies [14]. We believe that the increase in renal volume may be early stage chronic CsA nephrotoxicity, although kidney enlargement has never been described in clinical studies.

![Figure 5. Changes in kidney volume corrected for body weight vs time for the ciclosporin (CsA) and the CsA + telmisartan treated pigs measured at week 0, 12, 31, 38, 47 and 54. Significant differences (P < 0.05) vs baseline values were found in the CsA group at 12, 47 and 54 weeks. No significant differences between the groups were observed. (Baseline values equal 100% for each animal).](https://academic.oup.com/ndt/article-abstract/22/2/369/1890137)
Effects of telmisartan on chronic ciclosporin A nephrotoxicity in pigs

The rat model of CsA nephrotoxicity, where a discrepancy between ACEI and ARB effects on renal functional impairment and structural damage were reported [4,5].

Most studies on chronic CsA nephrotoxicity have been performed in rodents, but the pig has several advantages as an experimental model due to similarity to the human with regard to renal and cardiovascular anatomy and physiology [19]. Development time of interstitial fibrosis in the pig kidney resembles the human, in whom renal histological changes may be observed after 6–12 months of CsA administration [20,21]. In contrast, salt-depleted rats can develop interstitial fibrosis after 21–35 days [3,4]. In addition, the pig can be used for serial procedures including renal biopsies. In the present study, the animals tolerated CsA treatment and investigations well, and all pigs fulfilled the study without complications.

The best CsA dose for long-term studies in a pig model has not been determined yet as our findings after 12 months of CsA treatment with 10 mg/kg/day are sparse. Long-term studies are expensive, but with the optimal dosage results similar to clinical practice may be obtained in a pig model after 12 months of treatment, and therefore become more relevant compared with results after short-term treatment in the salt-depleted rat model.

In conclusion, CsA treatment 10 mg/kg/day orally for 12 months results in development of histopathological changes in the porcine kidney similar to those observed in humans, and causes kidney enlargement. Telmisartan attenuates these changes. The pig is a suitable model of chronic CsA nephrotoxicity.

The perspectives of the present study are first to examine further the renoprotective role of ARB on chronic CsA nephropathy, and secondly to study the relation between renal enlargement, fibrosis and function in the porcine model during long-term CsA treatment. This will initiate clinical studies of the beneficial effects of ARB treatment on chronic CsA nephrotoxicity.

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Conflict of interest statement. None declared.

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