Abstract

Background. Cyclosporin A (CsA) causes renal magnesium wasting, hypertension, and occasionally irreversible renal damage. We examined the effect of dietary sodium and magnesium on renal histology in spontaneously hypertensive rats (SHR) receiving CsA.

Methods. Forty-six 8-week-old SHR were divided into six groups and given different dietary levels of sodium (low 0.3%, high 2.6%) and magnesium (low 0.2%, high 0.6%). Low-dose CsA (5 mg/kg/d) was given subcutaneously for 6 weeks in four groups. Systolic blood pressure, serum creatinine, degree of proteinuria, and renal tissue CsA and calcium concentrations were determined. Kidney wet weight to total body-weight ratio was calculated as an index of renal hypertrophy. Renal histological alterations were scored according to glomerular changes: 100 glomeruli were assigned for severity of change a score from 0 to 3. The number of affected glomeruli was multiplied by the damage score to obtain a damage index.

Results. In the CsA-treated high-sodium diet group systolic blood pressure and glomerular damage index were increased, and renal hypertrophy was the most common. These changes were prevented by oral magnesium supplementation. The glomerular damage index correlated positively with increases in systolic blood pressure, serum creatinine, proteinuria, and renal calcium concentration.

Conclusions. Dietary sodium enhanced CsA-induced functional and morphological renal changes in SHR and aggravated hypertensive renal arteriolar and glomerular lesions. Dietary magnesium supplementation protected against the deleterious effects of sodium and CsA.

Key words: calcium; cyclosporin side-effects; functional and histological renal changes; magnesium; sodium; spontaneously hypertensive rats; tissue calcium

Introduction

Cyclosporin A (CsA) is a potent immunosuppressive agent used to prevent rejection after organ transplantation and also as a therapy in autoimmune diseases. Side-effects of CsA such as magnesium wasting [1,2], hypertension [3,4], and nephrotoxicity have been described both experimentally and clinically [1,5]. Milder changes in kidney function, i.e. decreased glomerular filtration rate and decreased magnesium reabsorption, are reversible and dose-dependent [6]. Infrequently, changes in vascular structure are seen. They occur at higher doses of CsA and are irreversible once established [7].

Both in epidemiological and experimental studies a positive correlation between magnesium deficiency and rise in blood pressure has been found [8]. Magnesium deficiency will lead to an elevated blood pressure because the calcium antagonistic vasodilating effect of magnesium is lacking [9].

In the average human diet, the energy-adjusted quantity of salt (sodium) as mmol of Na\(^+\) per 100 kcal is approximately 10 times higher than in the conventional rat chows. The low dietary levels of sodium in animal models seem to explain the failure of previous attempts to reproduce the toxicity, characteristic of CsA in man, in animal experiments [4]. We recently found that CsA, at a relatively low daily dose, 5 mg/kg, induced a marked rise of blood pressure in spontaneously hypertensive rats, but in the presence of additional dietary salt only [4].

The aim of the present study was to examine whether the renal structural damage, seen as a side-effect of CsA therapy in man, is also produced in SHR by a low-dose CsA treatment combined with a high-salt diet of sodium.
diet. Since dietary magnesium supplementation was able to block the CsA-induced rise in blood pressure and serum creatinine [4], we also examined the possible protective effect of magnesium against renal lesions.

**Subjects and methods**

**Animals**

Forty-six 8-week-old male spontaneously hypertensive rats (SHR) (Harlan–Sprague–Dawley, Indianapolis, IN, USA) were used. The rats were housed three to four animals per cage in a standard experimental animal laboratory (illuminated from 6.30 a.m. until 6.30 p.m., room temperature 22 ± 1°C).

**Groups and diets**

In the beginning of the study, the blood pressure- and body weight-matched SHR were divided into six experimental groups (n=7–8 in each group) to receive different diet and drug regimens for 6 weeks: (1) control group (C) received a relatively low-sodium standard rat chow (R36, Finnewos Aqua, Helsinki, Finland) (Na 0.3%, Mg 0.2%, K 0.8%, Ca 1.0%, P 0.75% of the dry weight of the chow); (2) salt group (Na) on the high-sodium (2.6%) diet; (3) cyclosporin A (CsA) group on a low-sodium (0.3%) diet; (4) CsA group on the high-sodium (2.6%) diet; (5) CsA group on the high-sodium/high-magnesium (Mg 0.6%) diet (CsA + Na + Mg); and (6) CsA group on the low-sodium/high magnesium diet (CsA + Mg). The high-sodium diet was produced by adding sodium chloride (Merck, Darmstadt, Germany) to the control chow and the high-magnesium diet by adding MgCl₂ (Merck, Darmstadt, Germany) to the control chow. The rats had free access to tap water and chow during the experimental period.

**Drugs**

Cyclosporin A (Sandimmun® infusion concentrate 50 mg/ml, Novartis Ltd., Basel, Switzerland) was diluted in a 20% lipid solution (Intralipid®, Kabi Pharmacia, Stockholm, Sweden) to produce a 25-mg/ml solution, which was administered subcutaneously at a daily dose of 5 mg/kg for 6 weeks. The control rats received the same volume of the vehicle.

**Measurement of systolic blood pressure**

Systolic blood pressure was measured in the beginning and at the age of 13 weeks, the rats were housed individually in metabolic cages and they had free access to tap water and chow. Urine was collected over a 24-h period. Urine volumes were measured and the urine samples stored at −80°C until protein determination was performed.

At the end of the experimental period the animals were decapitated 20 h after the last CsA administration. Blood samples for determination of serum creatinine were drawn into glass tubes without an anticoagulant. The wet weight of kidney samples (about 100 mg) were minced and homogenized in buffer (10 mM PBS, 50 mM Tris–HCl, 0.5% Triton). The volume of the homogenate was adjusted with a buffer to give a final tissue concentration of 100 mg/ml. The CsA concentration of kidney tissue was determined by radioimmunossay (Abbott TDX cyclosporin monoclonal whole blood method, Abbott Laboratories, Abbott Park, IL, USA) using a monoclonal antibody specific to the parent molecule.

**Histology**

For histological evaluation, half of the right kidney was fixed in 4% formaline for at least 3 days. A sagittal and a longitudinal piece were embedded in paraffin. Sections 3 µm thick were cut and stained with H&E and Van Gieson. The control chow. The rats had free access to tap water and chow during the experimental period. Interstitial, tubular, glomerular and vascular changes were looked for. Because most changes were detected in the afferent arterioles associated with corresponding glomeruli and a few in the other elements, the slides were scored according to glomerular changes: 100 consecutive glomeruli from each *kidney slide* were assigned for severity of change using scores from 0 to 3.

(0) Normal arteriologlomerular unit with open capillary lumina and a normal afferent arteriole (Figure 8a).

(1) Slight thickening of the media of the afferent arteriole. Slight proliferation of the mesangial cells and a slight increase in the mesangial matrix. Open capillary lumina in the glomerulus (Figure 8b).

(2) More severe medial thickening than in score (1). Narrowed capillary lumina. Necrosis of the media of the wall of the afferent arteriole and partly collapsed capillaries in the glomerulus. Sometimes segmental necrosis of the glomerular tuft (Figure 8b).

(3) Fibrinoid necrosis of the arteriolar wall, haemorrhagic necrosis of the glomerular tuft with some plump, but still persisting mesangial cells (Figure 8c).

In order to emphasize the degree of changes, we used a damage index which was calculated by assessment of 100 consecutive glomeruli in each kidney and counting the number of affected glomeruli in each score group, e.g. a × 0 + b × 1 + c × 2 + d × 3 = 4 (a + b + c + d = 100 = glomeruli, 0–3 = the degree of change, i.e. score).
Correlations between the damage index and the changes in blood pressure, serum creatinine, proteinuria, and the concentrations of calcium and CsA in the kidney tissue were drawn. For statistical analysis, one-way analysis of variance (ANOVA) was used. The data were analysed using Stat View II Abacus concepts Inc® (Brainpower®, Calabasas CA, USA). P values less than 0.05 were considered statistically significant. The data are presented as mean ± SEM.

Results

In all groups receiving added sodium (Na, CsA + Na, CsA + Na + Mg), the systolic blood pressure was significantly higher than in the control group (P < 0.001) and also higher than in CsA and CsA + Mg groups (P < 0.05). The combination of CsA and Na produced a significant increase in the systolic blood pressure when compared to other treatment groups (CsA, Na, CsA + Mg, CsA + Na + Mg) (P < 0.05) (Figure 1).

The glomerular damage index was significantly higher in the CsA-treated high-sodium diet group than in the other groups (P < 0.001) (Figure 2). No glomeruli were affected in the control group. In rats receiving the high-salt diet, 0.5% of the glomeruli were affected. In the CsA-treated rats receiving the low-sodium diet 5% of the glomeruli were affected, while in the CsA-treated rats receiving the high-sodium diet the proportion of affected glomeruli was as high as 36%. When magnesium was added to the high-sodium diet of the CsA-treated rats, only 10% of the glomeruli were affected. In the CsA-treated rats receiving the low-sodium diet and magnesium, as few as 2% of the glomeruli were affected. No interstitial or tubular changes were noted in any group.

The glomerular damage index correlated positively with increases in blood pressure (Figure 3; r = 0.6, P < 0.001), serum creatinine (Figure 4; r = 0.7, P < 0.001), proteinuria (Figure 5; r = 0.8, P < 0.001), and the calcium concentration of the kidney tissue (Figure 6; r = 0.7, P < 0.001). The glomerular damage index did not correlate with the CsA concentration in the kidney tissue (r = 0.3, n.s.). Renal CsA concentrations did not differ between the groups (Figure 7). The typical histological changes are presented in Figure 8a–c. The combination of CsA and the high-sodium diet aggravated renal hypertrophy and also urinary excretion of protein over 24 h when compared with the other groups. The rats on either the high-sodium diet or magnesium supplementation showed a high urinary excretion of these elements. The concentration
Detrimental effect of dietary sodium in cyclosporin-A-treated spontaneously hypertensive rats

Discussion

Cyclosporin A (CsA) toxicity, comprising elevation of blood pressure and kidney damage, is an important clinical problem. No effective method to prevent these deleterious side-effects of CsA therapy has been available. Earlier attempts to produce an animal model with a good correlation with the clinical CsA-induced condition have been disappointing. Even in the present study using spontaneously hypertensive rats (SHR), CsA-treatment only induced minor toxicity when given in combination with the conventional low-sodium rat diet. However, during a high-sodium diet resembling the high-sodium average diets of man much more closely, CsA produced marked histological vascular and glomerular lesions in the kidneys of SHR and also raised serum creatinine levels. The most severely affected glomeruli were necrotic and the number of functioning glomeruli in the kidney was decreased. This suggests that the present dietary sodium intakes, which exceed four-to tenfold [12] the recommended dietary allowance of 0.5 g/day for sodium [13], may also play an important role in CsA-induced nephrotoxicity in man. Magnesium supplementation almost completely prevented the renal vascular changes during the high-salt diet. This suggests that the loss of magnesium into urine induced by CsA [1,2] is also an important mechanism in the development of the harmful effects of CsA.

It has been emphasized that renal side-effects caused by CsA should be classified as tubular or vascular as well as functional or structural [6]. The changes in tubular function are common but they are not clinically very important. Furthermore, they are reversible. The alterations in tubular structure occur at higher doses. No significant lesions are seen at doses of less than 10 mg/kg in normotensive rats [14]. Functional vascular changes are also common, notable clinically, and reversible in nature. Changes in vascular structure are clinically infrequent but very important, and they are irreversible once established [6]. Vascular changes are seen in the afferent arteriole entering the glomerulus. Damage in endothelial and smooth muscle cells can lead to occlusion of the arteriole. Collapse and ultimate sclerosis of the associated glomerulus will follow [6]. Nizze et al. [15] found that in organ transplant patients CsA-induced vascular changes in combination with interstitial fibrosis did not occur until 6 months after

Fig. 4. Scatter plots showing a positive linear correlation between the serum creatinine concentration and the glomerular damage index in spontaneously hypertensive rats receiving different diet and drug regimens for 6 weeks as explained in the legend of Figure 1 ($r = 0.7$, $P < 0.001$).

Fig. 5. Scatter plots showing a positive linear correlation between proteinuria and the glomerular damage index in spontaneously hypertensive rats receiving different diet and drug regimens for 6 weeks as explained in the legend of Figure 1 ($r = 0.8$, $P < 0.001$).

Fig. 6. Scatter plots show a positive linear correlation between renal calcium concentrations and the glomerular damage index in spontaneously hypertensive rats receiving different diet and drug regimens for 6 weeks as explained in the legend of Figure 1 ($r = 0.7$, $P < 0.001$).
the transplantation. The time of occurrence of arteriolar changes ranged from 30 to 90 days and that of interstitial fibrosis from 6 to 12 months. In our model it is possible that interstitial fibrosis could also appear after a longer period of treatment.

In normotensive rats on a low-sodium diet, CsA will only produce minor morphological changes in the kidneys, even when given at high doses [14]. In Sprague–Dawley rats, receiving a low-salt diet and a 5-month treatment with CsA at the daily dose of 20 mg/kg, only mild tubular and no vascular lesions could be detected [16]. In other studies in Sprague–Dawley rats with the dose of 15 mg/kg and a low-salt diet, both vascular, tubular and and interstitial changes were detected. In contrast to our study, the blood pressure decreased during those trials [17,18]. In accordance with these earlier findings, CsA alone did not in our study produce any serious structural changes in SHR, since only 5% of the glomeruli were affected. Necrotic glomeruli, dilated arterioli with splitting of wall elements, thrombi in the lumina and red blood cells in all layers of the arteriolar wall could be seen in the most seriously affected kidneys. Large arteries were spared. Because we used low-dose (5 mg/kg) CsA treatment, tubular or interstitial lesions would not be expected. Therefore, high dietary salt with a low-dose CsA treatment in SHR may correspond to the situation in humans on CsA treatment after organ transplantation.

A high intake of sodium has an important role in the development of arterial hypertension in humans [12,19]. Hypertension related to CsA therapy has been reported in every clinical condition in which CsA is used. Hypertension induced by CsA has been proposed to be sodium-dependent and shows features similar to essential hypertension including renal histological changes [20]. Of cardiac transplant patients before CsA treatment 10% had hypertension, but 71–100% during CsA treatment [21]. In bone-marrow-transplant patients, the blood pressure begins to rise within the 1st week of CsA therapy and approximately 50% of the patients become hypertensive [3]. In liver-transplant patients up to 85% have been reported to have hypertension when CsA is used as immunosuppressant [22].

We found in our earlier study that salt markedly aggravated the CsA-induced hypertension in SHR [4].

Fig. 8a–c. Histological findings in spontaneously hypertensive rats receiving different diet and drug regimens for 6 weeks as explained in the legend of Figure 1. (a) Normal slender arteriole (arrow) gives score 0. H&E stain, original magnification ×200. Length of bar in the lower left corner is 25 μm. (b) Glomerulus on the left (whole arrow) gives score 1. The glomerulus on the right side (arrow head) has segmental necrosis and some media necrosis in the arteriole and gives score 2. H&E stain, original magnification ×200. Length of bar in the lower left corner is 25 μm. (c) Glomerulus is totally destroyed. Significant haemorrhage and some mesangial cells can be seen. Arrow points at the arteriole with fibrinoid necrosis in the wall. Arrow shows a preserved, probably an efferent arteriole. H&E stain, original magnification ×200. Length of bar in the lower left corner is 25 μm.
Several studies have shown that CsA causes magnesium wasting through the kidneys [1,2]. It has been proposed that there could be an association between hypomagnesaemia and development of hypertension in CsA-treated patients [5]. A severe dietary magnesium deficiency caused vasoconstriction in the intestinal capillary bed of the rat in vivo. A progressive, quantitative reduction in lumen size could be measured as the magnesium depletion increased. The changes were more severe in the capillaries than in the terminal arteries [9]. June et al. [2] have proposed that a possible mechanism for vascular vasoconstriction in smooth muscular cells and also in mesangial cells in CsA-treated rats is an increase in intracellular calcium. We found that calcium accumulated in damaged kidney cells in the high-salt CsA group whereas neither salt nor CsA alone caused this effect. An exogenous calcium antagonist or magnesium as an endogenous calcium antagonist could prevent this. Magnesium has been shown to cause vasodilatation by blocking the calcium influx through voltage- and receptor-operated channels [23]. Interestingly, in our study calcium was richly excreted into the urine of the animals with CsA treatment and magnesium supplementation but only modest damage was seen in the kidney preparations. The impact of this finding remains unexplained.

In rat models for CsA toxicity, renal vascular lesions have been seen in the SHR strain. The nature of the arteriolar changes did not differ from the spontaneously occurring hypertensive arteriolar changes of this strain. With a daily dose of 20 mg/kg/day no correlation has been found between arteriolar changes and blood pressure [24]. In another study, SHR were treated with CsA at a daily dose of 25 mg/kg from 4 to 8 weeks. The blood pressure rose without any histological changes [25]. In the present study, practically no lesions were detected with low-dose CsA during 2. June CH, Thompson CB, Kennedy MS, Nims J, Thomas D. Lab Clin Med. 1985; 39: 620–624. Contrary to findings of Ryffel et al. [24], we found a positive correlation between histological changes and blood pressure. The differences in the results between the two studies [24,25] and the present study are probably due to the different salt contents of the diet.

In two patients with autoimmune disease receiving CsA, severe nephrosclerotic changes have been seen in kidney biopsies even after a short period of hypertension [26]. This finding suggests that kidneys initially damaged by an autoimmune disease are especially vulnerable to CsA toxicity. In heart transplantation patients during low-dose CsA therapy, obliterator arteriopathy and ischaemic changes with thickening and wrinkling of the glomerular capillary wall were detected [27]. These findings are very similar to our findings. Therefore, further clinical studies are warranted to determine the roles of dietary salt and calcium in the pathogenesis of CsA toxicity.

**Clinical significance**

More than half of all transplantation patients receiving CsA develop hypertension. The present data emphasise the importance of restriction of sodium intake towards the recommended daily dietary allowance of 0.5 g. An increased oral intake of magnesium also appears to prevent from CsA nephrotoxicity. As dietary magnesium may act as an endogenous calcium-channel blocker, an increase in oral intake of magnesium should protect against hypertension in patients on CsA treatment.

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### References


### Table 1. Renal hypertrophy index expressed as the wet weight of both kidneys-to-total body weight ratio (mg/g) of spontaneously hypertensive rats (SHR) on different diet and drug regimens

<table>
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<tr>
<th>Diet/Drug</th>
<th>Controls</th>
<th>Na</th>
<th>CsA</th>
<th>CsA + Na</th>
<th>CsA + Mg</th>
<th>CsA + Na + Mg</th>
<th>ANOVA</th>
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<td>dU-protein/KW (mg protein/g)</td>
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<td>6.2 ± 0.1</td>
<td>7.1 ± 0.1</td>
<td>6.7 ± 0.1</td>
<td>8.2 ± 0.6</td>
<td>6.4 ± 0.1</td>
<td>6.8 ± 0.2</td>
<td>0.0002</td>
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<td>dU-Mg/KW (mol/day × g)</td>
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<td>0.8 ± 0.039</td>
<td>5.5 ± 0.1</td>
<td>0.7 ± 0.034</td>
<td>5.6 ± 0.7</td>
<td>0.8 ± 0.05</td>
<td>7.1 ± 1.1</td>
<td>0.0001</td>
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<td>dU-Ca/KW (mol/day × g)</td>
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<td>0.0081 ± 0.001</td>
<td>0.044 ± 0.011</td>
<td>0.029 ± 0.005</td>
<td>0.1 ± 0.023</td>
<td>0.1 ± 0.01</td>
<td>0.2 ± 0.029</td>
<td>0.0001</td>
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Mean ± SEM. *P < 0.001 vs CsA + Na + Mg; \( P < 0.001 \) vs C, Na, and CsA + Mg; \( P < 0.001 \) vs C, Na, CsA, and CsA + Na; \( P < 0.001 \) vs C, Mg, and CsA + Mg; \( P < 0.001 \) vs C, CsA, CsA + Mg, and CsA + Na + Mg; \( P < 0.001 \) vs all the other groups. Urinary excretion rates of protein and various mineral elements-to-wet weight of both kidneys (KW). Abbreviations are explained in the legend of Figure 1.
5. June CH, Thompson CB, Kennady MS, Loughran TP Jr, Deog HJ. Correlation of hypomagnesemia with the onset of cyclosporine-associated hypertension in marrow transplant patients. Transplant Int 1986; 41: 47–51

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