Original Article

Effect of 6 weeks of vitamin E administration on renal haemodynamic alterations following a single dose of neoral in healthy volunteers

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Abstract

**Background.** A single oral dose of cyclosporin-A (CsA) transiently reduces renal plasma flow (RPF) and glomerular filtration rate (GFR) in transplant patients and, in some patients, chronic administration of CsA leads to renal impairment and fibrosis. Based on experimental studies, several mediators including free radicals have been proposed to account for CsA-nephrotoxicity. We have previously reported that administration of the antioxidant vitamin E in a rat model of chronic CsA-nephrotoxicity reduces renal fibrosis and maintains renal function.

**Methods.** In the present study, the effect on renal haemodynamics of a single dose of the new oral formulation of CsA (neoral) was assessed before and after 6 weeks of vitamin E (800 IU/day, 2-fold increase in serum vitamin E). GFR (inulin clearance) and RPF (para-hippuric acid clearance) were measured before and after a single dose of 5 mg/kg of neoral in 12 healthy subjects under standardised conditions.

**Results.** Although the mean area under the curve of the CsA levels was 21% lower after the vitamin E period, the peak CsA level at 120 min after neoral was similar both before and after vitamin E administration. At 120 min after neoral, a transient reduction in RPF and GFR was noted both before and after vitamin E administration. The nadir of the reductions in RPF (−81 ± 27 ml/min) and GFR (−14 ± 6 ml/min) at 120 min compared with baseline tended to be lower before than after the treatment with vitamin E (−51 ± 33 ml/min of RPF and −12 ± 8, ml/min of GFR, respectively). Plasma and urinary levels of F2-isoprostanes (free radical-catalysed vasoconstrictive prostanooids (F2-isos) at 120 min after the administration of neoral were not different from the pre-neoral levels.

**Conclusion.** The findings demonstrate that a single oral dose of neoral causes transient, yet significant, reductions in RPF and GFR, and suggest that F2-iso might not be involved in the CsA-induced acute renal vasoconstriction. The tendency for a lower reduction in RPF and GFR following CsA during the vitamin E period in healthy humans warrants additional studies in transplant patients.

**Keywords:** cyclosporin A; glomerular filtration rate; renal plasma flow; vitamin E; tocopherol

Introduction

Cyclosporin A (CsA) is a potent and selective immunosuppressive agent and its introduction has permitted a steady increase in kidney and other organ transplantation. However, CsAs full clinical use has not been fully realized because of its frequent and at times severe toxicity involving the kidneys [1]. Lowering the doses of CsA may not be the answer, because that does not completely spare the kidneys and can promote chronic rejection [2]. Furthermore, although the short-term survival of the cadaveric renal transplants has increased impressively with the advent of CsA, its use has not reduced the prevailing high rates of chronic attrition of cadaveric renal allografts [3]. In this regard, subclinical CsA nephrotoxicity, which could be difficult to differentiate from chronic rejection, has been thought in part to contribute to the chronic loss of renal allograft. In patients treated with CsA for reasons other than renal transplantation, long-term treatment with CsA is associated with impaired function of the native kidneys [4]. Thus, CsA-nephrotoxicity continues to remain a clinically important problem and despite considerable research, the exact pathogenetic mechanism of CsA nephrotoxicity remains unclear.

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Two main components have been recognized in CsA nephrotoxicity: (i) an acute and reversible renal vasoconstriction leading to acute reductions in renal blood flow and glomerular filtration rate (GFR) and (ii) a chronic and irreversible tubulo-interstitial fibrosis leading to chronic renal failure. A number of mediators and mechanisms have been proposed to account for the adverse effects of CsA on the kidneys including a role for reactive oxygen species (ROS) [5–8]. ROS, in addition to their direct toxicity on cells and tissues, can directly catalyse the production of F2-isoprostanes (F2-iso) through the non-enzymatic pathway [9]. F2-iso have been shown to be very potent renal vasoconstrictors [10]. A consistent finding in this regard is the effect of CsA in inducing excessive renal production of the vasoconstrictor thromboxane [6].

That CsA induces oxidative stress has been demonstrated in several experimental studies. Administration of a number of different types of antioxidants attenuates CsA-induced acute and chronic adverse effects on the kidneys [7,11–14]. Clinical studies are lacking to verify a role of antioxidants in CsA-induced nephrotoxicity. In an earlier experimental study, administration of vitamin E along with CsA in the chronic rat model was associated with marked suppression of CsA-induced lipid peroxidation, renal dysfunction and tubulo-interstitial fibrosis [12]. The present study was undertaken as an initial step towards testing whether the beneficial effect of vitamin E in the animal models is demonstrable in humans. For this, the effect of short-term vitamin E on a single dose of CsA-induced renal haemodynamic alteration in healthy volunteers was determined. Neoral, the relatively new oral formulation of CsA, was used at a clinically relevant dose of 5 mg/kg. Plasma and urine levels of F2-iso were also measured at the nadir of renal plasma flow (RPF) reduction to determine whether F2-iso played any role in CsA-induced renal vasoconstriction.

**Patients and methods**

Twelve healthy males (33 ± 2 years; range 23–50 years) were studied. They were all normotensive (126 ± 3/81 ± 2 mmHg) and with a normal weight (BMI 23.6 ± 0.5 kg/m²; range 20.8–26.0 kg/m²). None of the subjects were on any medication. The study was approved by the Ethics Committee of the Karolinska Institutet, Stockholm, Sweden. All subjects gave informed consent to participate in the study. The subjects were studied on the two occasions (i.e. under basal conditions without any medications and after 6 weeks of E-vitamin supplementation). On the morning of investigation the subjects drank 500 ml of water. At about 08.00 h, infusions of 20% para-amino hippuric acid (PAH, Merck Sharp & Dohme, West Point, USA; 0.2 ml/kg body weight, rate 0.5 ml/min) and 25% inulin (Intest®, Kemiflor, Stockholm, Sweden; 0.2 ml/kg body weight, rate 0.5 ml/min) were started. After an equilibration period of 1 h, timed urine collections were started. To ensure adequate diuresis 300 ml of tap water was given orally each hour. During the study period the subjects were in the supine position except when voiding urine. The subjects voided urine every 60 min and urine samples were analysed for inulin and PAH. Blood samples for inulin, PAH and CsA were taken each hour. Basal blood samples were taken for analysis of vitamin E. F2-iso were analysed in blood and urine samples at basal (collected between −60 min to time zero) and 120 min (collected between 60–120 min after time zero) following the administration of CsA which were given in a dose of 5 mg/kg at basal (time zero). All subjects were re-investigated following 6 weeks of oral supplementation (800 mg/day) of E-vitamin (ACO, Stockholm, Sweden). CsA was measured by EMIT 2000 (Behring Diagnostics). Vitamin E was determined by high-performance liquid chromatography with UV-detection using vitamin E-acetate as internal standard.

The plasma and urine F2-iso levels were analysed by GC-MS method as described in samples collected at the peak of reduced RPF, i.e. 120 min post-CsA [15]. The samples were kept frozen and transported frozen on dry ice.

**Statistical methods**

Results are presented as mean ± SEM. Parametrical statistical methods (analysis of variance and student’s t-test) were employed. A P-value of < 0.05 was considered significant.

**Results**

Following 6 weeks of E-vitamin supplementation (800 mg/day) plasma vitamin E levels rose in each of the 12 study subjects (Figure 1). The mean plasma E-vitamin level increased from 26.5 ± 2.1 to 55.7 ± 0.8 pmol/l. The plasma CsA levels peaked at 120 min (Figure 2) following oral administration of CsA. The mean area under the curve of the CsA levels were 21% lower after the vitamin E period (P < 0.001). The CsA levels checked at 60, 180, 300, and 360 min after neoral intake were significantly lower in the post-vitamin E period compared with the pre-vitamin E period. However, the peak level of CsA that corresponded with the maximum fall in RPF and GFR in the pre- and post-vitamin E periods was similar (Figure 3).

Transient but significant reductions in RPF and GFR were noted after the administration of neoral (Figure 3). The baseline values and the curves after intake of neoral for RPF and GFR between pre- and post-vitamin E periods were similar (ANOVA n.s.; Figure 3). In the pre-vitamin E period, the reductions in RPF and GFR at 120 min post-CsA compared with baseline were −81 ± 27 ml/min and −14 ± 6 ml/min, respectively (Figure 3). The reductions in the post-vitamin E period of RPF and GFR were
Fig. 1. Fasting plasma concentrations of vitamin E in 12 healthy males before and after 6 weeks of oral supplementation with 800 mg/day of vitamin E.

Fig. 2. Blood levels of CsA in response to oral administration of neoral before (basal) and after 6 weeks of supplementation with vitamin E. The area under the curve is lower after vitamin E administration (P<0.001).

Fig. 3. Glomerular filtration rate (GFR) (A) and renal plasma flow (RPF) (B) in response to oral administration of neoral (CsA) before (basal) and after 6 weeks of supplementation with vitamin E. Values are expressed as per cent of mean GFR (basal mean 106 ml/min, E-vitamin mean 104 ml/min) and RPF (basal mean 551 ml/min, E-vitamin mean 529 ml/min) at time zero (before administration of neoral).

Table 1. Plasma and urine isoprostanes (F2-is) before and 120 min after intake of CsA (5 mg/kg) at basal and after 6 weeks of supplementation with E-vitamin

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<th>Basal</th>
<th>E-vitamin</th>
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<tr>
<td></td>
<td>P-F2-is (ng/ml)</td>
<td>U-F2-is (ng/ml)</td>
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<tr>
<td>0 min</td>
<td>1.56 ± 0.39</td>
<td>2.12 ± 0.35</td>
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<tr>
<td>120 min</td>
<td>1.51 ± 0.42</td>
<td>1.87 ± 0.13</td>
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−51 ± 33 ml/min and −12 ± 8 ml (Figure 3). The systolic blood pressure during the pre- and post-vitamin E clearance periods following CsA were similar, without significant changes during the experiment (systolic blood pressure 128 ± 2 mmHg before and 127 ± 3 mmHg after vitamin E, mean of five measurements during the experiment). A slight, but significant decrease in diastolic blood pressure was noted (81 ± 1 before vs 76 ± 1 mmHg after vitamin E, P<0.05, mean of five measurements).

Plasma and urine levels of F2-is collected at 120 min after the administration of neoral were not different from the pre-neoral levels and levels did not differ between the basal values of pre- and post-vitamin E periods (Table 1).
Discussion

In this study, administration of a single and clinically relevant dose of neoral caused a transient but significant reduction in RPF and GFR in healthy volunteers. Administration of vitamin E did not significantly alter the RPF and GFR during the experiment, although a tendency towards a lower reduction of the nadir values 120 min after neoral administration was observed. Administration of vitamin E and neoral at the dose employed in this study had little effect on the plasma and urinary levels of vasoconstrictive prostanoids, F2-iso.

Neoral is a relatively new formula of CsA based on microemulsion technology that enhances CsA bioavailability and improves dose linearity compared with the original CsA formulation. The faster absorption of neoral than the old CsA formulation may lead to higher peak concentration and hypothetically increase the risk for acute nephrotoxicity. Our sequential plasma levels including the timing of the peak value after neoral agree with the earlier pharmacokinetic studies of neoral in healthy volunteers [16]. A significantly lower value for the mean area under the curve of blood CsA levels following vitamin E supplementation was unexpected and cannot be readily explained. In fact, earlier studies reported enhanced and not reduced CsA absorption in the presence of water-soluble vitamin E (d-alpha-tocopheryl polyethylene glycol 1000 succinate) [17,18]. It should be noted that the vitamin E and CsA formulation used in our study were different from the above studies. The lower CsA levels in the post-vitamin E period cannot be explained on the basis of systematic laboratory error in the measurement since these pre and post-vitamin E levels for CsA have been measured during a single batch run. Clearly additional studies are required to verify the possibility of vitamin E delaying the absorption of neoral and to delineate the mechanism.

In our study, the timing and the concentration of peak CsA levels in the pre- and post-vitamin E study periods were similar. The vasoconstrictive effect of CsA on renal vasculature is dose-related and immediate as has been shown in experimental models, which also included direct application of CsA on renal microvasculature in the hydronephrotic model [11]. One can argue that the early lower levels of CsA in the vitamin E period could have influenced the tendency to a lower reduction in GFR and RPF during the vitamin E period.

The lack of any increase in F2-iso following CsA administration suggests that F2-iso might not be an immediate mediator of CsA-induced vasoconstriction. F2-iso is also a sensitive marker for free radical-catalysed lipid peroxidation and absence of its increase raises the possibility that at least in the acute setting of CsA-induced reduction in RPF and GFR, lipid peroxidation might not be occurring. It remains, however, possible that excessive generation of superoxide, purportedly during the metabolism of CsA through the cytochrome P-450 system [19], may in term scavenge endothelial nitric oxide, leading to renal vasoconstriction. A consistent finding following the administration of CsA is the excessive renal production of the vasoconstrictive thromboxane. Free radicals by generating lipid hydroperoxide even at trace quantities can catalyse the formation of thromboxane through the cyclooxygenase pathway. Speculatively, vitamin E can suppress any increase in CsA-induced lipid hydroperoxide and in theory, limit increased renal production of thromboxane.

Vitamin E therapy had no significant effect against the CsA-induced reductions in RPF and GFR. It should be noted that our study provides data in this regard on healthy volunteers, given a single dose of CsA and, arguably, the protective vitamin E effect may be different in renal transplant patients with lower GFR receiving chronic daily administration of CsA. Consistent with this is the study by Perico et al. [20], in which GFR decreased by 63% following the intake of 5 mg/kg CsA dose in renal transplant patients. It is thus possible that vitamin E administration in transplant patients might be associated with attenuation in CsA-induced renal haemodynamic alterations. While vitamin E is widely recognized mainly for its lipid peroxidation inhibiting property, it has also a number of additional properties. Its ability to suppress the pleotropic nuclear factor NF-kB may be important in the CsA-induced activation of TGF-β and osteopontin [8], and thus, could be partly responsible for the characteristic tubulointerstitial fibrosis of chronic CsA-nephrotoxicity. Recent preliminary studies in a chronic CsA-nephrotoxicity model support the hypothesis that vitamin E reduces CsA-induced renal fibrosis at least in part through the suppression of fibrogenic factors [21]. Thus, chronic therapy with vitamin E in CsA treated patients could have benefits on the kidneys over and above the reduction of acute renal haemodynamic effects.

In summary, our data do not support a significant acute effect of vitamin E on the CsA-induced reductions in renal RPF and GFR. The lack of increase in F2-iso following CsA makes lipid peroxidation and the lipid peroxidation product, F2-iso, unlikely involved in the CsA-induced acute renal vasoconstriction. Still, further experiments in healthy subjects and transplant patients receiving CsA are needed to elucidate the possible renal protective effect of vitamin E administration.

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