Original Article

Vitamin C improves endothelial dysfunction in renal allograft recipients

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Abstract

Background. Endothelial function is impaired in renal allograft recipients but the effects of antioxidant vitamin therapy on endothelial function in such patients is unknown.

Methods. Thirteen renal allograft recipients were randomized to vitamin C or placebo in a double blind cross-over study design. Flow-mediated endothelium-dependent dilation and glyceryltrinitrate-induced endothelium-independent dilation of the brachial artery were assessed before and 2 h after oral administration of 2 g vitamin C or placebo.

Results. Plasma vitamin C levels increased from 33.5 ± 17.0 μmol/l to 98.8 ± 60.2 μmol/l after treatment (P = 0.0001). Endothelium-dependent dilation improved (from 1.6 ± 2.6 to 4.5 ± 2.5%) after vitamin C administration but was unchanged after placebo (1.9 ± 1.5 to 1.8 ± 2.5%; P = 0.003 for vitamin C vs placebo). There was no significant change in endothelium-independent dilation in response to vitamin C. Vitamin C was also associated with a significant increase in the lag time in dilute serum oxidation (P = 0.001).

Conclusions. Vitamin C acutely improves flow-mediated, endothelium-dependent dilation and increases the resistance of lipoproteins in dilute serum to oxidation in renal transplant recipients.

Keywords: antioxidants; ascorbic acid; endothelium; transplantation

Introduction

Despite the major advances in the management of patients with end-stage renal disease, these individuals still have an approximately threefold higher risk of dying from coronary heart disease (CHD) compared with age-matched healthy subjects [1]. The prevalence of established risk factors for CHD, including hyperlipidemia and hypertension, remains elevated after transplantation. Also, there is evidence of increased oxidative stress [2,3] and enhanced susceptibility of low density lipoproteins (LDLs) to oxidation [4] in renal allograft recipients. These factors have been associated with endothelial dysfunction, which is regarded as an important early event in atherogenesis. Recent studies have shown impaired endothelium-dependent vasodilation in patients with renal allografts [5,6]. However, this abnormality is apparently independent of hypercholesterolemia, hypertension, uraemia and treatment with cyclosporine [5]. The mechanism underlying endothelial dysfunction in renal allograft recipients is unclear.

Increased oxidative stress is thought to play a role in the development of endothelial dysfunction. Hypercholesterolemia, hypertension and atherosclerotic disease impair endothelial function in part by increasing vascular production of superoxide anions, which inactivate the major endothelial relaxing factor nitric oxide (NO) [7]. Oxidized LDL accumulates in vascular lesions, inhibits NO release from endothelial cells and may inactivate NO directly [8]. There is evidence that oxidation of LDL is increased in renal allograft recipients [4]. Vitamin C scavenges superoxide anions and improves endothelial dysfunction in patients with risk factors for CHD [9] and in those with proven coronary artery disease [10]. Whether oxidative mechanisms, including lipoprotein oxidation, contribute to impaired endothelium-dependent vasodilation in renal allograft recipients has not been widely studied. The purpose of the present study was therefore to determine the effect of vitamin C on endothelium-dependent vasodilation and copper ion-catalysed serum lipoprotein oxidation in renal allograft recipients.

Subjects and methods

Patients

The study group consisted of 13 (nine male, four female) renal allograft recipients. The subjects were recruited from
the regional nephrology service. Inclusion criteria included stable graft function and serum creatinine <210 μmol/l. Patients were at 78 ± 59 months (range 6–188 months) post-transplantation. None of the patients was diabetic or a smoker, and none had clinical evidence of cardiovascular disease. Twelve of the 13 patients were treated with cyclosporine (whole blood trough levels 263 ± 69 μmol/l, range 181–453 μmol/l); nine with prednisone and azathioprine, and three with mycophenolate. Antihypertensive treatment consisted of angiotensin converting enzyme inhibitor in 10 patients, loop diuretics in one patient, calcium antagonists in eight patients, and beta blockers in three patients. Four patients were receiving treatment for hypercholesterolemia with a statin drug. Patients’ drug therapy remained unaltered during the study.

All subjects gave written informed consent and the study was approved by the regional health authority ethics committee. Studies were performed on the arm that had not previously had an arteriovenous fistula or vascular surgery. All vasoactive medications were withheld for at least 24 h prior to the study. Patients treated with cyclosporine took their evening dose the day prior to the study, but the morning dose was withheld.

**Study design**

A randomized, double blind, placebo-controlled cross-over study design was used. Subjects were randomized to receive either a single oral dose of Vitamin C capsules (2 g) or similar appearing placebo. One week later the study was repeated with the alternate treatment. After a 12 h overnight fast, subjects reported to the study centre in the early morning. Height, body weight and blood pressure in the subjects were recorded. Brachial artery reactivity was measured at baseline and 2 h after treatment. At these times, venous blood was drawn into tubes containing disodium EDTA and into a plain tube. Plasma lipids, lipoproteins, vitamin C levels and copper ion oxidation of dilute serum were measured.

Brachial artery ultrasound studies were performed in a quiet, temperature-controlled laboratory. The subjects rested in the supine position for 10 min before the study. Studies were performed using a Hewlett Packard Sonos 2000 ultrasound machine and a high resolution 7.5 MHz linear array transducer. Longitudinal scans of the right brachial artery were obtained proximal to the antecubital fossa, positioning the probe so that the best images were obtained. Operating variables of the machine were kept constant during the study. The transmit focus zone was set at the depth of the anterior wall. Images were magnified and gain settings optimized to optimize the vessel wall and its interface with the vessel lumen. After obtaining adequate images the arm was marked and kept in a constant position for the remainder of the study.

The method of assessing endothelium-dependent and -independent dilation was as described previously [11]. Flow-mediated, endothelium-dependent dilation was assessed by measuring the arterial diameter of the brachial artery before and during reactive hyperaemia induced by deflation of a blood pressure cuff, previously inflated to 300 mmHg around the forearm for 5 min. Arterial flow velocity was measured using a pulsed Doppler signal at an angle of 60° in the centre of the vessel at baseline and during the first 15 s of reactive hyperaemia. The artery was allowed to recover for a period of 10 min and a second baseline scan of brachial artery diameter was performed. The endothelium-independent response was assessed as the change in artery diameter after a 400 μg spray of sublingual glyceryl trinitrate (GTN).

**Data analysis**

Maximum arterial diameter after reactive hyperaemia was assessed 50 to 60 s after cuff deflation, and between 3 and 4 min after GTN administration. The ultrasound images were recorded on Super-VHS videotape for subsequent analysis. The recorded images were then digitized and stored using a Macintosh 8500 computer with built-in digitizer. Four images were digitized from each stage at end diastole, indicated by the R wave on the continuously recorded ECG. Subsequent analysis was performed using NIH Image 1.60, a public domain measurement program. The diameter of the vessel was measured by two independent investigators without knowledge of the scan sequence or treatment assignment. Arterial diameter was measured from a fixed anatomic marker in all scans of the same patient. Measurements were taken from the anterior ‘m’ line (media-adventitia interface) anteriorly to the leading edge of the intima-lumen interface of the posterior wall. The four measurements for each stage were then averaged. The percentage change in the brachial artery diameter was then calculated in response to reactive hyperaemia and GTN by each observer, and the average results of the two observers recorded.

Blood flow at baseline and during reactive hyperaemia was calculated by multiplying the velocity–time integral of the Doppler flow signal by the heart rate and cross-sectional area of the blood vessel. Reactive hyperaemia was calculated as the maximal flow in the first 15 s after cuff deflation divided by baseline flow. As the flow velocity was measured in the centre of the vessel, absolute flow may be overestimated, but relative flow values before and after cuff inflation are accurate [11]. The inter-observer variability for measurement of flow-mediated dilatation is 1.3% (mean difference in endothelium-dependent dilatation (EDD) of 0.2%) and EDD for normal subjects is routinely 5.3 ± 2.3% in our laboratory.

**Laboratory methods**

Plasma and serum were separated by low-speed centrifugation at 4°C. Plasma high density lipoprotein (HDL) cholesterol was isolated in the supernatant after treatment of plasma with dextran sulphate/magnesium chloride. Cholesterol and triglycerides were measured in plasma and plasma fractions by enzymatic methods using commercial kits (Boehringer Mannheim, Germany). Plasma LDL cholesterol was calculated using the Friedewald formula. Plasma vitamin C levels were measured by a fluorometric method [12]. The lag time in dilute serum oxidation was measured as described previously [13].

**Statistical analysis**

The subject number for the study was calculated to have an 80% power at the 5% level of detecting a 2% improvement in arterial reactivity after vitamin C using a cross-over study design. Descriptive data are presented as means ± SD. A two-way repeated measures analysis of variance was performed with correction for baseline values for all comparisons in the study. Spearman’s rank correlation analysis was used to test for a relationship between changes in endothelial function and vitamin C concentration, and lag time in dilute serum
oxidation. Statistical significance was defined as a two-sided P value of <0.05.

Results

The 13 subjects had a mean age of 48 ± 12 years (range 30–64 years). Blood pressure was 141 ± 15 mmHg (systolic)/87 ± 8 mmHg (diastolic) and was not different by treatment allocation. Body mass index for the group was 27.7 ± 3.3 kg/m² and mean serum creatinine was 126 ± 40 μmol/l. The effect of treatment on vitamin C, serum lag time, and lipids is shown in Table 1. Plasma vitamin C levels increased significantly 2 h after oral vitamin C administration, as did the serum lag time. Subjects had similar baseline levels of lipoprotein and triglycerides before and after vitamin C or placebo administration.

The baseline heart rate, brachial artery size, baseline vessel flow and hyperemic flow after cuff deflation were similar for each of the four arterial studies (Table 2). There was a significant increase (P = 0.003) in endothelium-dependent dilation after oral Vitamin C (1.6 ± 2.6 vs. 4.5 ± 2.5%) compared with placebo (1.9 ± 1.5 vs. 1.8 ± 2.5%), as shown in Figure 1. The significance of the increase in endothelium-dependent dilation after vitamin C was unchanged when variation in baseline artery size, baseline flow and percentage hyperemic flow were taken into account. The GTN-induced response was not significantly (P = 0.46) different before and after vitamin C.

![Figure 1](image)

**Fig. 1.** Effect of vitamin C on EDD- and GTN-induced dilation in 13 renal allograft recipients. Subjects had brachial ultrasound examinations at baseline and 2 h after a 2 g oral dose of vitamin C or placebo. Compared with placebo, vitamin C improved endothelium-dependent dilation (P = 0.003). Vitamin C had no effect on GTN-induced dilation. Data are presented as mean ± SEM.

Table 1. Effect of treatment on vitamin c, serum lag time and lipids

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin C</th>
<th>P*</th>
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<tbody>
<tr>
<td></td>
<td>Baseline 2 h</td>
<td>Baseline 2 h</td>
<td></td>
</tr>
<tr>
<td>Vitamin C (μmol/l)</td>
<td>36.9 ± 20.4</td>
<td>33.5 ± 17.0</td>
<td>0.0001</td>
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<td>Serum lag time (min)</td>
<td>68 ± 15</td>
<td>65 ± 16</td>
<td>0.0001</td>
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<td>Total cholesterol (mmol/l)</td>
<td>6.12 ± 1.38</td>
<td>6.22 ± 1.48</td>
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<td>LDL cholesterol (mmol/l)</td>
<td>3.75 ± 1.32</td>
<td>3.71 ± 1.47</td>
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<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.27 ± 0.29</td>
<td>1.26 ± 0.33</td>
<td>0.10</td>
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<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.44 ± 1.22</td>
<td>2.74 ± 1.41</td>
<td>0.78</td>
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*P values are for comparisons by repeated measures analysis of variance. HDL = high density lipoprotein, LDL = low density lipoprotein. Data are presented as mean ± SD.

Table 2. Heart rate and arterial variables in 13 renal allograft recipients

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vitamin C</th>
<th>P*</th>
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<tr>
<td></td>
<td>Baseline 2 h</td>
<td>Baseline 2 h</td>
<td></td>
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<tr>
<td>Heart rate (bpm)</td>
<td>67 ± 6</td>
<td>66 ± 10</td>
<td>0.43</td>
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<tr>
<td>Vessel size (mm)</td>
<td>4.5 ± 0.9</td>
<td>4.5 ± 0.9</td>
<td>0.35</td>
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<tr>
<td>Flow (ml/min)</td>
<td>235 ± 164</td>
<td>176 ± 88</td>
<td>0.75</td>
</tr>
<tr>
<td>Hyperemic flow (%)</td>
<td>493 ± 187</td>
<td>627 ± 222</td>
<td>0.85</td>
</tr>
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</table>

*P values are for comparisons by repeated measures analysis of variance. Data are presented as mean ± SD.
(10.5 ± 4.8 vs 11.4 ± 6.6%) and placebo (9.9 ± 5.9 vs 12.2 ± 5.2%) administration. The increase in plasma vitamin C was correlated significantly with the increase in lag time of dilute serum oxidation \( (r = 0.60; P = 0.03) \).

**Discussion**

This randomized, double-blind, placebo-controlled cross-over trial indicates that the antioxidant vitamin C (2 g) improves brachial artery flow-mediated, endothelial-dependent vasodilation and does not alter endothelial-independent dilation in renal allograft recipients. Thus, increased oxidative stress may contribute to endothelial dysfunction in conduit arteries in these individuals.

The factors responsible for endothelial dysfunction in renal allograft recipients are not entirely clear. Long-term immunological reaction to the graft, and renal and systemic effects of immunosuppressive therapy may increase oxidative metabolism [2]. For example, cyclosporine increases the generation of reactive oxygen species [14,15]. Hypercholesterolemia and hypertension are common in renal transplant recipients and may increase vascular oxidative stress and impair endothelial function [9]. In the present study, about half the subjects had plasma cholesterol levels in the range previously associated with endothelial dysfunction [9], and most were receiving antihypertensive medications. However, evidence that hypertension, hypercholesterolemia and cyclosporine mediates impaired endothelium-dependent vasodilation in renal transplant recipients is limited [5,16].

Endothelium-dependent vasodilation at baseline in the present study was lower than normal values in our laboratory and was comparable with the impaired levels reported previously in renal allograft recipients [5,6]. Acute administration of vitamin C increased flow-mediated vasodilation three-fold to levels close to those seen in healthy subjects in our laboratory. A number of mechanisms have been proposed to account for improved endothelial function induced by a high dose of vitamin C. It has been suggested that vitamin C increases endothelium-dependent NO by scavenging superoxide anions and preventing inactivation of NO, improving cellular redox status, increasing the activity of nitric oxide synthetase or inhibiting LDL oxidation. Although scavenging superoxide anions can not be discounted as a potential explanation for the effect of vitamin C on endothelial function, it has been calculated that very high, supraphysiological levels may be required to preserve endothelium-dependent NO [10]. The effect of vitamin C on cell redox status is not yet clear [10]. Nevertheless, there is evidence that vitamin C may spare intracellular levels of glutathione that can improve flow-mediated, endothelium-dependent dilatation [17]. A recent study has reported a vitamin C-induced increase in the activity of NO synthase, which is the enzyme responsible for the formation of NO [18]. A link between resistance of isolated LDL to oxidation and endothelium-dependent vasodilation has been reported [19]. Also, oxidized LDL damages endothelial cells, inhibits release of NO from these cells and may directly inactivate NO in vitro [8]. Vitamin C in the aqueous milieu may counteract these changes by inhibiting LDL oxidation [8]. In the present study, increased plasma vitamin C levels markedly delayed the oxidation of lipoproteins in dilute serum (serum lag time) in renal transplant recipients after a dose of vitamin C. Thus, it is conceivable that increased vitamin C levels may delay lipid and lipoprotein oxidation in the artery wall and thereby improve vascular endothelial cell function. Oxidation of dilute serum may be a relevant model of lipoproteins oxidation in environments such as the arterial wall, where interstitial fluid with its variety of antioxidants is present.

The present study has limitations. The number of subjects studied was relatively small. Thus, care should be taken in extrapolating the findings to other populations of renal allograft recipients. It is possible that routine medications taken by the participants influenced our data. We attempted to minimize this effect by withholding vasoactive medications > 24 h prior to each study visit. The numbers of subjects are too small and the numbers of drugs too large for reliable use of multivariate techniques to adjust data for the effect of medications. Finally, we measured endothelium-dependent dilation at only one time point following the dose of vitamin C, when plasma levels have been shown to plateau [20].

It is possible the acute improvement in endothelial function observed in this study may be maintained during regular supplementation with vitamin C. A recent study has reported improved endothelium-dependent vasodilation maintained during long-term ascorbic acid treatment in patients with coronary artery disease [10]. The long-term effect of vitamin C supplementation on endothelial function in renal allograft recipients remains to be tested.

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**References**


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