Effects of oral vitamin C supplementation on oxidative stress and inflammation status in haemodialysis patients

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Abstract

Background. There is increasing evidence for the presence of oxidative stress and vitamin C deficiency in dialysis patients. Limited data, however, are available regarding the effects of vitamin C supplementation on oxidative stress and inflammation markers in such patients.

Methods. We ran a prospective, randomized, open-label trial to assess the effects of oral vitamin C supplementation (250 mg three times per week) for 2 months on well-defined oxidative and inflammatory markers in 33 chronic haemodialysis (HD) patients.

Results. Normalization of plasma total vitamin C and ascorbate levels by oral vitamin C supplementation did not modify plasma levels of carbonyls, C-reactive protein and albumin, or erythrocyte concentrations of reduced and oxidized glutathione.

Conclusion. Short-term oral vitamin C supplementation did not modify well-defined oxidative/antioxidative stress and inflammation markers in HD patients. Whether a higher oral dose or the intravenous route can modify these markers remains to be determined.

Keywords: dialysis; inflammation; oxidative stress; vitamin C

Introduction

Evidence has accumulated that oxidative stress is present in haemodialysis (HD) patients [1]. The susceptibility to oxidative stress in HD patients is mediated by abnormal oxidant and defective antioxidant production [1]. In unsupplemented HD patients, several deficiencies in various components of the antioxidant defence mechanisms have been demonstrated, including reduced plasma total vitamin C concentration [2]. Vitamin C is an effective water-soluble antioxidant in human plasma against lipid peroxidation, and also has hypochlorous acid-scavenging ability [3]. This latter action is of major importance in HD patients, since recent studies suggested that chlorinated stress played a central role in this setting [4].

Vitamin C deficiency in dialysis patients is primarily due to dietary restriction of fresh fruits and vegetables to avoid hyperkalaemia, and loss of the vitamin during dialysis sessions [5]. Furthermore, it has been demonstrated that not only the total vitamin C concentration, but also the reduced form of vitamin C (i.e. the active form or ascorbate) is decreased [6,7]. Although the mechanisms underlying ascorbate deficiency have not been characterized, a possible impairment of enzymatic or non-enzymatic recycling of ascorbate from dehydroascorbate (DHA; i.e. the oxidized form of vitamin C) is suspected, since the recycling is largely reduced glutathione (GSH) dependent [8], and dialysis patients have a marked GSH deficiency [6]. These latter data suggest that the observed deficiency of vitamin C is not only quantitative but also qualitative. Currently, 1–1.5 g of oral ascorbate/week, or 300 mg of parenteral ascorbate/dialysis session, are recommended to compensate for subclinical deficiency, although evidence for such recommendations is scarce [5]. To the best of our knowledge, the effects of vitamin C supplementation on plasma total and reduced form of vitamin C, and on oxidative stress markers have not yet been evaluated.

Several recent studies suggested that oxidative stress and inflammation might be linked in dialysis patients. First, we suggested that the presence of inflammation...
and the duration of dialysis are the most important determinants of oxidative stress in such patients [6]. Secondly, an association between F2-isoprostanes and C-reactive protein (CRP) levels has also been reported in these patients [9]. If oxidative stress and inflammation are linked in this condition, one could speculate that antioxidative treatment strategies should be beneficial and decrease the degree of inflammation. A recent preliminary report showed that short-term administration of γ-enriched tocopherols led to a decrease in median CRP level in HD patients [10]. However, there is no controlled study evaluating the effects of regular vitamin C intake on the inflammatory response of HD patients.

The primary aim of the present study was to evaluate the effects of regular vitamin C intake on well-defined oxidative and inflammatory markers in HD patients.

Patients and methods

Study design

The study was a prospective, randomized, open-label trial. The trial was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. After screening assessment, all patients included entered a 2 month washout phase (phase 1). At the end of the washout period, patients were randomly assigned to receive, or not to receive, oral vitamin C supplementation for 2 months (phase 2). Vitamin C (Laroscorbine®, Roche Nicholas SA, Gaillard, France), 250 mg three times per week, was given orally after each dialysis session for 2 months. The dose was selected according to a previous study which showed that the plasma ascorbate concentration increased significantly, whereas the plasma oxalate level did not increase after 8 weeks of intravenous vitamin C (300 mg after dialysis session three times per week) [11]. The dose of 250 mg has also been selected for practical reasons, since the oral pharmaceutical brands available in France are 500 mg capsules, which can be easily divided into two parts of 250 mg each.

Patients

We included study patients, male or female, aged between 18 and 80 years, on intermittent HD for at least 6 months and who gave their written informed consent. We excluded from the study patients who had undergone renal transplantation <1 year previously and had been back on HD, and patients with systemic disease, on immunosuppressive agents, on lipid-lowering drugs or on supplementation with vitamin C and/or E during the 3 months before the beginning of the study. A total of 40 patients (20 patients in each group) fulfilled these inclusion and exclusion criteria, and agreed to participate in the study.

Blood samples were obtained at the end of months 2 and 4. Blood (10 ml) was drawn from the arteriovenous fistula just before the dialysis session and was collected in two types of Vacutainer® tubes (Becton Dickinson) either containing or not containing sodium heparinate as anticoagulant. Following centrifugation (600 g for 10 min at 4°C), samples of plasma and serum were stored at −80°C until analysis. Erythrocytes were washed three times with isotonic saline solution before storage at −80°C until analysis. Laboratory data were analysed within 1 month.

Biochemical determinations

Plasma levels of albumin and high-sensitive CRP (hsCRP) were determined using routine methods on a Cobas Integra 800 analyser (Roche, Meylan, France), serum levels of iron and transferrin on a Hitachi 911 analyser (Roche), and serum ferritin levels on an Elecsys 2010 (Roche). Haemoglobin concentration was determined by colorimetry using Gen’s analyser (Beckman-Coulter, Villepinte, France). Plasma levels of protein carbonyls were measured as previously described [12]. The normal carbonyls were 0.76 and 0.91 nmol/mg of protein, respectively.

Plasma antioxidant system

Plasma vitamin C concentrations (ascorbate, the reduced form, and DHA, the oxidized form) were determined by high-performance liquid chromatography (HPLC) with coulometric detection of ascorbate. Separation was performed on a Satisfaction® column using 30 mM phosphate-buffered saline (PBS) pH 2.8 as the mobile phase delivered at a flow rate of 1 ml/min. Analytical cell potentials of the detector Coulchem 5100A (Eurosep, France) were set at E1 = −300 mV and E2 = 100 mV. Retention times of ascorbate and 3,4-dihydroxybenzylamine, as internal standard, were 5.6 and 6.6 min respectively. The plasma concentrations of total vitamin C were obtained after sample treatment by dithioerythritol (40 mM), while those of ascorbate were obtained without this reduction. The DHA concentrations were therefore calculated by the difference between total vitamin C and ascorbate. The mean and the upper limit of normal plasma ascorbate were 60 and 84 μM, respectively, and of total vitamin C were 72 and 101 μM, respectively. The detection limit of the assay was <5 μM.

Erythrocyte antioxidant system

Erythrocyte glutathione content was simultaneously determined in both reduced and oxidized form by HPLC using coulometric detection as previously described [6]. Briefly, after deproteinization by 5% metaphosphoric acid, separation was performed on an Uptisphere® column (ODB, 250 × 4.6 mm, 5 μm; Interchim, Montluçon, France) using 10 mM PBS, pH 2.7, containing 2% methanol and delivered at a flow rate of 1 ml/min. Analytical cell potentials were set at E1 = 400 mV and E2 = 800 mV. Retention times of reduced (GSH) and oxidized (GSSG) glutathione and N-acetylcysteine, as internal standard, were 6, 15 and 12 min, respectively. The intracellular redox potential was measured by the red blood cell (RBC) concentration ratio of GSSG and GSH, the GSSG concentration being expressed as GSH equivalents (1 mol of GSSG corresponding to two GSH equivalents). The mean and the upper limit of the normal GSSG/GSH ratio were 0.12 and 0.24, respectively.

Statistical methods

Results have been expressed as means ± SD. The difference between the groups at different times was assessed using
Seven patients withdrew from the study. Two withdrew before study start, one due to renal transplantation and the other for personal reasons. The remaining five (four in the group without vitamin C and one in the group with vitamin C) had to leave the study after its start because of a de novo inflammatory syndrome (n = 3), renal transplantation (n = 1) and transfer to another centre (n = 1). Complete data were obtained for 33 HD patients (20 male, 13 female) with a mean age of 52 ± 14 years and a mean time on dialysis of 6.1 ± 6.8 years. Nine patients had prior cardiovascular disease and five had diabetes. Initial kidney disease was vascular renal disease in seven, diabetic nephropathy in four, glomerulonephritis in 15, polycystic kidney disease in two and interstitial nephropathy in five patients. The 33 patients were dialysed using a diacetate cellulose membrane or a polyethersulfone polysulfone membrane. Mean duration of dialysis sessions was 4.2 h. The clinical characteristics of patients according to vitamin C supplementation are listed in Table 1. Both groups, with and without vitamin C supplementation, were similar concerning age, mean time on dialysis, sex ratio and main clinical characteristics. The mean Kt/V was 1.18 ± 0.2 for patients without vitamin C supplementation and 1.17 ± 0.2 for patients with vitamin C supplementation (P = NS).

As shown in Table 2, HD patients exhibited no significant difference between the two groups at baseline regarding oxidative stress markers and the antioxidant system. Oral vitamin C supplementation led to a normalization of plasma total vitamin C and ascorbate levels in the treated group. Plasma DHA concentrations increased slightly, but significantly, leaving the DHA/total vitamin C ratio unchanged. Vitamin C supplementation did not modify the levels of plasma or erythrocyte oxidative/antioxidative stress markers. A tendency towards an elevation of the GSSG/GSH ratio was observed after treatment, but the difference did not reach the level of statistical significance (Table 2). There were also no changes of albumin and CRP levels in response to oral supplementation of vitamin C.

Since vitamin C supplementation may affect iron status and erythropoietin needs in HD patients [15], we checked these parameters before and after treatment. We found no significant difference regarding haemoglobin levels, iron status or erythropoietin between the two groups, before vs at the end of the 2 months of vitamin C treatment.

### Table 1. Clinical characteristics of patients according to oral supplementation of vitamin C

<table>
<thead>
<tr>
<th></th>
<th>Without vitamin C (n = 14)</th>
<th>With vitamin C (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.3 ± 14.8</td>
<td>51.8 ± 13.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mean time on dialysis</td>
<td>5.1 ± 4.7, 3.28</td>
<td>6.8 ± 8.0, 2.12</td>
<td>NS</td>
</tr>
<tr>
<td>Male % (n)</td>
<td>71.4 (10)</td>
<td>56.6 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>Active smoker % (n)</td>
<td>7.1 (1)</td>
<td>15.8 (3)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes % (n)</td>
<td>21.4 (3)</td>
<td>10.5 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiovascular disease % (n)</td>
<td>28.6 (4)</td>
<td>26.3 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Dry weight (kg)</td>
<td></td>
<td></td>
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<tr>
<td>Phase 1</td>
<td>69.4 ± 12.1</td>
<td>62.9 ± 13.1</td>
<td>NS</td>
</tr>
<tr>
<td>Phase 2</td>
<td>69.2 ± 12.0</td>
<td>63.2 ± 13.2</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Phase 1</td>
<td>139 ± 29</td>
<td>141 ± 15</td>
<td>NS</td>
</tr>
<tr>
<td>Phase 2</td>
<td>133 ± 23</td>
<td>143 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 1</td>
<td>83 ± 13</td>
<td>82 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Phase 2</td>
<td>79 ± 15</td>
<td>80 ± 10</td>
<td>NS</td>
</tr>
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</table>

Data are expressed as mean ± SD, except for time on dialysis which is also given as median and range. Phase 1 = 2 month washout phase; phase 2 = 2 months treatment.

### Discussion

Our findings demonstrate that the normalization of plasma total vitamin C and ascorbate levels by oral vitamin C supplementation did not modify the levels of well-defined oxidative/antioxidative stress and inflammation markers in HD patients.

The lack of efficacy of oral vitamin C supplementation in terms of chlorinated and carbonylated stress markers was unexpected in view of the intensity of both oxidative stress in HD patients [4,13] and the hypochlorous acid scavenging ability of vitamin C in vitro [3]. One possible explanation could be the relatively short treatment time period (2 months), which may not be sufficient for complete protein turnover. In healthy volunteers, vitamin C supplementation for 15 weeks was necessary to reduce carbonyl levels [14]. A difference of oxidative stress marker detoxification and/or repair could also explain the discrepancy between the present finding and a recent comparable study in which 8 weeks of intravenous vitamin C supplementation at 300 mg three times a week significantly increased plasma ascorbate concentration, and reduced lymphocyte 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels [20]. The reduction of the latter was associated with an upregulation of steady-state mRNA levels of 8-oxoguanine-DNA glycosylase 1 (8-OHdG repair enzyme), which could suggest an increase of this enzyme activity [15]. Unfortunately, there is no information regarding circulating serum 8-OHdG concentrations in the latter report, which have been reported to be elevated after vitamin C therapy in healthy volunteers [16]. The increase of serum 8-OHdG concentrations is usually corrected in healthy volunteers by a concomitant
increase of urinary excretion, whereas in HD patients it could lead to accumulation of 8-OHdG in serum. Of interest, increased serum 8-OHdG concentrations have been reported in HD patients, and were associated with erythropoietin resistance in such patients [17].

It should be noted that oral vitamin C supplementation leads to a higher intracellular concentration of vitamin C in lymphocytes (mM) than in plasma (µM) [18]. These high intracellular concentrations could affect lymphocyte oxidative stress markers, while the low equivalent plasma concentrations remain without effects on plasma markers (e.g. F2-isoprostane) [18]. However, we found that oral vitamin C supplementation did not decrease the erythrocyte GSSG/GSH ratio, another intracellular marker, which argues against the latter hypothesis.

An additional difference between the present study and that of Tarng et al. [11] could be related to the route of vitamin C administration (oral vs intravenous, respectively). Indeed, intravenous administration produces higher plasma vitamin C peak and area under the curve concentrations than those observed with similar doses of oral vitamin C supplementation in healthy volunteers [19]. In contrast to intravenous administration, the elevation of vitamin C plasma concentrations after oral vitamin C is tightly controlled by the intestine in healthy volunteers [19]. Therefore, one could anticipate higher plasma vitamin C concentrations in HD patients after intravenous administration for at least these two reasons, although pharmacokinetic data are lacking. In line with this observation, a higher mean plasma total vitamin C concentration was observed in the study of Tarng et al. than in the present study (88.4 vs 65.5 µM, respectively), although the difference in administered doses was small (300 vs 250 mg x 3 per week, respectively) [11]. However, it remains to be seen whether intravenous vitamin C administration is efficacious with respect to
oxidative stress markers other than intra-lymphocyte markers, and whether it is safe after long-term administration. Clearly, pharmacokinetic and pharmacodynamic studies of vitamin C supplementation in HD patients are warranted to clarify these issues.

Under vitamin C supplementation, we observed a mild increase of plasma DHA levels. To the best of our knowledge, this is first report regarding the levels of DHA under vitamin C supplementation in HD patients. This increase could be attributed to a lack of erythrocyte regeneration of ascorbate from DHA, because most of this regeneration in whole blood takes place in erythrocytes mediated by intracellular GSH [8], and erythrocyte GSH concentrations were low in HD patients. Of note, GSH concentrations were not increased in HD patients taking vitamin C, in contrast to increased GSH levels observed in healthy volunteers [20]. We could not exclude a possible pro-oxidant effects of vitamin C, since in addition to a mild increase of plasma DHA levels, we also observed a tendency to an elevation of the GSSG/GSH ratio in HD patients treated with vitamin C. The lack of simultaneous vitamin E supplementation with vitamin C could explain this latter phenomenon. Indeed, vitamin C in the absence of vitamin E can mediate oxidative reactions [21]. However, antioxidant therapy with vitamins C and E was not associated with changes in plasma biomarkers of oxidative stress or inflammation in hyperlipidaemic children [22]. On the other hand, the presence of both vitamin E and vitamin C in the dialysate led to the most effective overall antioxidant production in ex vivo haemolipodialysis of blood, while vitamin C alone had a marked pro-oxidant effect [23]. Whether a similar response could be observed in HD patients remains an open issue.

Several recent clinical studies suggested a link between oxidative stress and inflammation in HD patients [6,9]. A preliminary study reported that a 14 day administration of γ-enriched tocopherols led to a decrease in median CRP in such patients, but the authors did not explore whether this effect was mediated by a decrease of oxidative stress [10]. We were unable to demonstrate any difference regarding albumin and CRP levels in response to oral supplementation of vitamin C. These results are in line with a previous study, in which the authors failed to observe a significant effect of a 6 month vitamin C supplementation on CRP and albumin levels [24]. Since oral vitamin C supplementation did not modify the levels of well-defined oxidative/antioxidative stress markers, it is not surprising to see no effects on inflammatory status. On the other hand, it is possible that uraemia-induced oxidation is a post-inflammation secondary phenomenon; however, this theory requires additional evaluation.

Previous studies explored the effects of vitamin C supplementation in HD patients on iron store mobilization and erythropoietin response [11,24]. In the present study, we observed no significant difference regarding haemoglobin levels, iron status or need for erythropoietin between the two groups, before and after treatment with vitamin C. However, patients in the present study had a mean transferrin saturation coefficient >25%, which is a predictive threshold for the lack of response to vitamin C [11].

A first limitation of the present study may be an inadequate final number of patients, with a possible underpowered sample size. However, post hoc sample size estimation (type I error = 0.05; type II error = 0.20), based on carbonyl lowering in healthy volunteers with low baseline vitamin C levels (A carbonyls = 30%) [14] and carbonyl concentrations in dialysis patients, revealed sufficient statistical power in the present study. Another possible limitation is the lack of plasma oxalate level measurements. However, as mentioned above, we selected the dose of vitamin C according to a previous study which showed that the plasma ascorbate concentration increased significantly, whereas the plasma oxalate level did not increase after 8 weeks of intravenous vitamin C (300 mg after the dialysis session three times per week) [11]. It should be noted that Canavese et al. recently reported that long-term intravenous supplementation was associated with a significant risk of oxalate supersaturation [25]. Whether this is also the case after long-term oral vitamin C supplementation remains to be determined.

In conclusion, short-term oral vitamin C supplementation did not modify well-defined oxidative/antioxidative stress and inflammation markers in HD patients. Whether a higher oral dose or the intravenous route can modify these markers remains to be determined.

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References

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