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Early postoperative substitution procedure of the antioxidant ascorbic acid

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Abstract

Background: Postoperatively reduced concentration of ascorbic acid (AA) in plasma (≤45.5 μmol/l (≤800 μg/dl)) is commonly interpreted as increased metabolic requirements, but it is not shown yet that the patient benefits from a substitution toward normal levels of AA. This is due to the missing knowledge on how to substitute AA effectively to normal plasma values in postoperative patients. Therefore, a postoperative AA substitution procedure "overnight" to normal values in plasma was investigated on a postoperative intensive care unit (ICU) in a university hospital.

Material and Methods: Fifty-seven operated patients were randomly assigned to a control- or intervention group (CG and IG, respectively). In all patients, the AA plasma concentration was analysed preoperatively and on the first three postoperative days. Patients of the IG received AA intravenously up to four times within 12 h depending upon the initial AA concentration (<34.1 μmol/l (4×500 mg AA); ≤56.8 μmol/l (2×500 mg AA); ≤68.2 μmol/l (1×500 mg AA)).

Results: The preoperative and early postoperative AA values did not differ between the groups. On the first postoperative day in both groups the plasma concentration was lowered (≤45.5 μmol/l) in 23 of all patients (CG: 85.18%; IG: 82.14%). In the IG, the dosage regime increased the AA plasma concentration to ≥45.5 μmol/l in 26 of 28 (92.86%) patients overnight.

Conclusion: The investigated substitution procedure is sufficient to increase AA plasma concentration overnight to normal or high normal values in postoperative ICU patients.

Keywords: Ascorbic acid; Substitution; Postoperative; ICU; Antioxidant; Oxidative stress

1. Introduction

A decreased postoperative ascorbic acid (AA) concentration in plasma is common with a tendency to reach normal values within 5–8 days [1–3]. Combined with the onset of postoperative complications, the level of AA is further reduced and therefore Borrelli et al. [4] attributed AA deficiency to the risk of postoperative complications. Together with the known influence of AA on tissue- or ischemia–reperfusion damage, immune function, etc., a postoperative supplementation of the antioxidant AA can be considered useful. To investigate this further, an efficient substitution regime is necessary but not available yet. This is due to the missing knowledge on how to substitute AA rapidly to normal plasma values in postoperative patients. A supplementation of 100–300 mg AA per day added to the clinical nutrition prevents patients from hypovitaminosis, but has been shown to be ineffective in the prevention or therapy of lowered AA plasma concentration [5–8].

Therefore, we investigated a postoperative AA substitution procedure "overnight" to normal values in plasma.

2. Methods

2.1. Patients

All patients provided written informed consent to participate in this investigation, which was carried out according to the recommendations for clinical trials in humans in the Declaration of Helsinki and was approved by the Ethics Committee of Rhineland-Palatinate in Mainz.

Criteria for the inclusion of patients to the study were elective operation with a planned postoperative stay on the

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Table 1
Ascorbic acid substitution procedure "overnight" depending on the measured AA concentration

AA concentration	AA substitution
<34.1 μmol/l (600 μg%)	500 mg (16°°+20°°+24°°+4°°)
≥34.1 μmol/l (600 μg%)< 56.8 μmol/l (1000 μg%)	500 mg (16°°+24°°)
≥56.8 μmol/l (1000 μg%)< 68.2 μmol/l (1200 μg%)	500 mg (16°°)
≥68.2 μmol/l (1200 μg%)	no substitution

AA was given intravenously in 100 ml 5% glucose for 30 min.

ICU and given written informed consent. Patients with at least one of the following criteria were excluded from the study: pregnancy, mental incapacity, a known history of allergic reactions to one of the substances of AA vials, elevated creatinine concentration in serum, urethro-/ureterolithiasis, glucose-6-phosphate dehydrogenase deficiency, haemosiderosis, haemochromatosis, thalassaemia, any other predisposing factor for haemolytic reactions or reoperation within the study period. The following factors led to the exclusion from the study group during the investigation: withdrawal from the given written informed consent, occurrence of allergic reactions or incomplete postoperative investigation.

Data of 11 patients were disregarded because of missing postoperative values (no operation, withdrawal from the given written informed consent, reoperation within the study period), and values of four patients due to analytical problems. With respect to the intention-to-treat agreement, the data of one patient was kept in the intervention group (IG) without postoperative treatment. The data of 27 patients in the control group (CG) and 28 patients in the IG were analysed.

The age of the patients of the CG (IG) was 58±10 (57±11) years old; body mass index was 22±4 (21±2.4) kg/m^2. The patients were hospitalised for neuro- (astrocytoma, meningioma, aneurysm) (30 patients), maxillofacial- (modified neck dissection, acusticus tumour) (5 patients), orthopaedics- (endoprothetics) (2 patients), abdominal- (liver resection, lung- and vascular-) (18 patients) surgery. The average duration of the surgical procedure was 198±114 (216±90) min. In addition, APACHE II (CG: 6.8; IG: 7.2) and SAPS II scores on the second and third postoperative days (CG: 11.9/11.8; IG: 11.8/11.7) were not different between groups.

2.2. Material

The following AA preparation was used: Ascorell (Sanorell Pharma, Bühl, Germany). Ascorell contains 100 mg AA/ml stabilized in sodium hydrogen carbonate.

2.3. Experimental procedure

All patients were randomly distributed into either the CG or IG. Blood samples were taken for analyzing AA on the day before the operation and on the first three postoperative days. All IG patients were substituted on the second and third postoperative day in dependence on the actual AA concentration (Table 1) on a day-to-day basis. Ascorbic acid was given intravenously in 100 ml 5% glucose for 30 min. Control group patients were not substituted.

On the day of admission to the ICU, the APACHE II score and, on the following day, the SAPS II score were applied.

For analysing AA in plasma, the previously published method was used [9]. In brief, all specimens were collected in tubes with 1.6 mg/ml ethylenediaminetetraacetic acid as anticoagulant. During the transport of the specimen to the laboratory (transport time: max. 10 min) they were stored on ice in darkness. Plasma was obtained by centrifugation; cold 10% perchloric acid containing 1% metaphosphoric acid in a brown microcentrifuge tube was added for deproteinization (1:1; v/v). Hemolyzed samples were rejected because hemoglobin may react with AA. All samples were kept in darkness at 4°C for 60 min to complete the deproteinization process, followed by centrifugation. Subsequently, a mobile phase was added (1:1; v/v). The mobile phase contained 20 mM ammonium dihydrogen phosphate with 0.015% (w/v) mPA, adjusted to pH 3.5. The specimens were centrifuged, filtered and stored in liquid nitrogen until the analysis was carried out. All specimens were analysed in duplicate by high-performance liquid chromatography (HPLC) employing ultraviolet detection.

2.4. Statistical analysis

The parameters of AA concentration in plasma were evaluated for normality using the Kolmogorov–Smirnov test. Differences in all measured parameters between the groups were evaluated using the two-sided (unpaired) *t* test for parametric data, Mann–Whitney rank sum test (unpaired) and the Wilcoxon signed rank test (paired) for skewed data. The Sigma Stat (Jandel Scientific, Corte Madera, CA) and an SPSS statistics program were used for statistical analysis. In order to increase the probability that

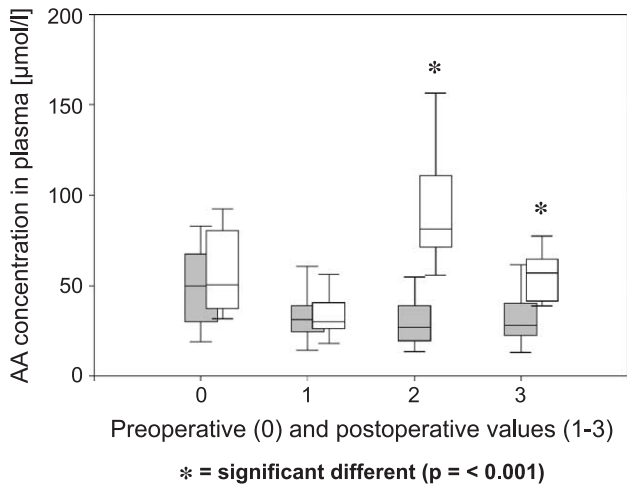
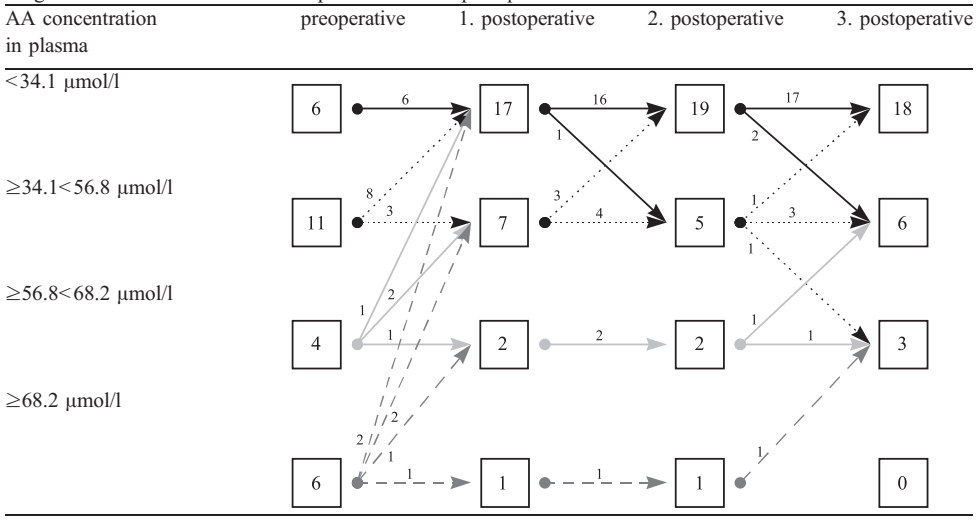


Fig. 1. Ascorbic acid concentration in plasma in CG- (gray boxplots) and IG patients (white boxplots). The concentration significantly increased in IG compared to the CG.

Table 2
Progression of AA concentration in patients without postoperative AA substitution



Number of patients with different AA concentration preoperative and on the first to the third postoperative day in rectangles. No spontaneous normalisation of AA plasma concentration was seen within the third postoperative day.

the relative difference is clinically relevant, a difference (ΔAA concentration on the second postoperative day) of 56.8 µmol/l (1000 µg%) and a standard deviation of 56.8 µmol/l (1000 µg%) were calculated for sample size determination. The level of probability was set at 5% and β-error at 10%.

3. Results

Data are presented as median/25%/75% percentile for skewed data.

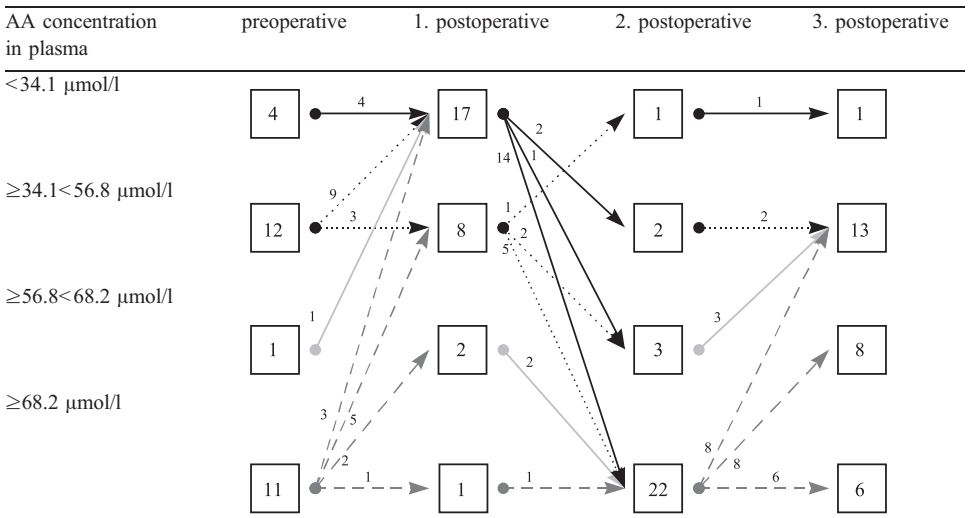
3.1. Ascorbic acid in plasma

The preoperative values of AA in plasma (CG: 49.81/29.98/67.58 µmol/l; IG: 50.79/37.3/80.66 µmol/l) were not significantly different (Mann–Whitney rank sum test) in both groups (Fig. 1).

On the first postoperative day (CG: 31.35/24.21/29.10 µmol/l; IG: 30.06/25.76/40.55 µmol/l) in both groups, the AA plasma concentration was significantly ($P \leq .001$) lowered (Wilcoxon signed rank test) compared to the preoperative values. In the CG (IG), in 17 (17) patients the AA concentration was ≤34.1 µmol/l (600 µg/dl); in 7 (8) patients, ≤56.8 µmol/l (1000 µg/dl); and in 2 (2) patients, ≤68.2 µmol/l (1200 µg/dl) (Tables 2 and 3).

After overnight substitution in the IG, the AA concentration significantly ($P \leq .001$) rose (Mann–Whitney rank sum test) on the second postoperative day to 81.22/71.16/110.85 µmol/l compared to the untreated CG (26.70/19.53/38.76 µmol/l). On the second postoperative

Table 3
Postoperative AA substitution overnight in dependence to the measured AA plasma concentration



Number of patients with different AA concentration preoperative and on the first to the third postoperative day in rectangles. No spontaneous normalisation of AA plasma concentration was seen within the third postoperative day.

day in the IG, six patients (≤68.2 μmol/l) received a second time a dosage-dependent supplementation of AA (Table 1) overnight.

At the end of the study period, the AA concentration in plasma was significantly ($P \leq .001$) different (CG: 27.92/22.36/40.31 μmol/l; IG: 56.88/41.27/64.75 μmol/l). In the CG (IG), the AA concentration in plasma in 18 (1) patients was <34.1 μmol/l; in 6 (13) patients, ≤56.8 μmol/l; and in 3 (8) patients, ≤68.2 μmol/l. No patient of the CG and six patients of the IG had an AA concentration >68.2 μmol/l (Tables 2 and 3).

4. Discussion

Postoperatively, an imbalance of pro- and antioxidants (oxidative stress [10]) occurs. In addition, the plasma concentration of AA, which is a major antioxidant of the plasma, is often lowered. Recently, the positive effects of AA substitution on ischemia–reperfusion damage [11,12], acute respiratory distress syndrome (ARDS) [13,14], systemic inflammatory response syndrome (SIRS) and sepsis [15] were shown. These effects are ascribed to the radical scavenger activity of AA.

For further investigations on the effect of AA substitution on the recovery of postoperative ICU patients, a rapid substitution procedure to normal values in plasma is necessary and not available yet. We investigated the efficiency of a postoperative substitution regime to upper normal values within 12 h. The actually recommended AA amount of 100–300 mg/day during clinical nutrition is not sufficient to sustain normal AA plasma concentration in postoperative patients in the ICU [5–8]. As a basis of our investigation, we hypothesise that a plasma concentration of 56.8–96.6 μmol/l would be helpful. From a previous investigation, we know that the maintenance dosage of AA is more than 800 mg/day on the first postoperative day. Together with an average loading dosage to increase the AA concentration to normal values, up to 1151 mg AA/day is required [16].

To avoid unclear postoperative bioavailability, an intravenous substitution procedure was chosen. A continuous infusion over 12 h is not recommended due to the known instability of AA in solutions, nor is a bolus injection because of high amounts of AA excretion in the urine [17]. Therefore, keeping the conditions of a daily routine in mind, we substituted 500 mg AA in a solution of 100 ml glucose 5% over 30 min up to four times in 12 h.

For the applied analysis method, the normal plasma concentration of AA is 45.5–68.2 μmol/l (800–1200 μg/dl). In the CG (IG), in 85.18% (82.14%) the AA plasma concentration was less than 45.5 μmol/l on the first postoperative day. After substitution, the AA concentration increased to ≥45.5 μmol/l in 26 of 28 (92.86%) patients. In 22 patients, the AA concentration was ≥68.2 μmol/l (Table 3). Due to our dosage-dependent substitution procedure, 25 patients were substituted once a day or no further on the second postoperative day. Subsequently, the AA concentration decreased in 19 patients on the third postoperative day (Table 3). We interpret this as an indication for the need for further substitution of AA.

One patient of the IG did not receive any AA supplementation. The data of this patient are part of the IG data (intention-to-treat agreement) (Table 3). One patient of the CG had an AA concentration in plasma of >68.2 μmol/l for two postoperative days. This patient was a young female athletic who substituted multiple vitamins daily. The oral AA supplementation was 1–2 g AA/day (Table 2).

No side effects (e.g., allergies) were observed in the patients. Therefore, this dosage regime can be considered safe. It was previously shown that an intravenous substitution of 3–6 g AA/day for up to 48 h [15,18] or 3 g AA for multiple weeks [19] produces no side effects.

In general terms, we do not recommend higher dosage regimes as described here. A disadvantage of a high amount is reduced efficiency of AA due to increased excretion of AA in urine [17]. Theoretically, an oversubstitution of AA could result in imbalance of high antioxidants and low oxidants. Extremely high dosage of AA (500 mg/kg body weight) was shown to damage DNA in animal studies [20]. However, the clinical relevance of this finding is not known; oversubstitution might suppress the cell membrane transport system for AA [21] and influence the immune function of granulocytes in the postoperative period, which produces radicals to destroy bacteria, etc. Consequently, free radicals could partly be scavenged before the defence has reached optimal levels. On the other hand, a diminished AA concentration increases the autoxidation of granulocytes [3]. The AA supplementation is part of the idea to "balance" antioxidants and oxidants.

Case reports of renal failure due to oxalosis during oral [22,23] or parenteral [24–28] AA supplementation are rare. These patients, who had no preexisting chronic renal insufficiency, tend to increase the oxalate concentration in plasma and formate oxalate stones [29] after intake of relatively small amounts of AA. These are a few patients compared to the number of individuals who had taken AA supplements daily; but for those, the consequences are serious and no reliable predictor for the development of renal impairment in these persons is known.

Known contraindications for the dosage regime are renal insufficiency, a history of urethro-/uterolithiasis [30], hemosiderosis/hemochromatosis, thalassemia [31] or glucose-6-phosphate dehydrogenase deficiency [32].

Besides our study design, no hospital laboratory provides HPLC measurements of plasma AA within 24 h. Over 80% of the patients had lower AA concentration in plasma on the first postoperative day, and more than 90% of them had an adequate response to the substitution procedure. In the interpretation of our findings, and taking into account that AA has a wide therapeutic range, we do not recommend a postoperative AA substitution that is dependent on the actual postoperative plasma concentration.

Observing the contraindications, we suggest that a postoperative AA substitution of initially 500 mg, four times in 12 hours followed by 500 mg AA twice a day during the next days might be effective for the investigation of potential benefits. This procedure is the "hand tool"; the clinical relevance of a postoperative AA substitution still has to be proven.

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References

[1] McGinn FP, Hamilton JC. Ascorbic acid levels in stored blood and in patients undergoing surgery after blood transfusion. Br J Surg 1976; 63:505–7.

[2] Louw JA, Werbeck A, Louw MEJ, Kotze TJvW, Cooper R, Labadarios D. Blood vitamin concentrations during the acute-phase response. Crit Care Med 1992;20:934–41.

[3] Maderazo EG, Woronick CL, Hickingbotham N, Mercier E, Jacobs L, Bhagavan H. Additional evidence of autoxidation as a possible mechanism of neutrophil locomotory dysfunction in blunt trauma. Crit Care Med 1990;18:141–7.

[4] Borrelli E, Roux-Lombard P, Grau GE, et al. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. Crit Care Med 1996;24:392–7.

[5] Schorah CJ, Downing C, Piripitsi A, Gallivan L, Al-Hazaa AH. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. Am J Clin Nutr 1996;63:760–5.

[6] Metnitz PG, Bartens C, Fischer M, Fridrich P, Steltzer H, Druml W. Antioxidant status in patients with acute respiratory distress syndrome. Intensive Care Med 1999;25:180–5.

[7] Baines M, Shenkin A. Lack of effectiveness of short-term intravenous micronutrient nutrition in restoring plasma antioxidant status after surgery. Clin Nutr 2002;21:145–50.

[8] Long CL, Maull KI, Krishnan RS, et al. Ascorbic acid dynamics in the seriously ill and injured. J Surg Res 2003;109:144–8.

[9] Rümelin A, Fauth U, Halmágyi M. Determination of ascorbic acid in plasma and urine by high performance liquid chromatography with ultraviolet detection. Clin Chem Lab Med 1999;37:533–6.

[10] Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. Philos Trans R Soc Lond B Biol Sci 1985;311:617–31.

[11] Ozaki M, Fuchinoue S, Teraoka S, Ota K. The in vivo cytoprotection of ascorbic acid against ischemia/reoxygenation injury of rat liver. Arch Biochem Biophys 1995;318:439–45.

[12] Bushell A, Klenerman L, Davies H, Grierson I, Jackson MJ. Ischemia–reperfusion-included muscle damage. Acta Orthop Scand 1996;67:393–8.

[13] Brown LAS, Harris FL, Jones DP. Ascorbate deficiency and oxidative stress in the alveolar type II cell. Am J Physiol 1997; 273:L782–8.

[14] Dwenger A, Pape HC, Bantel C, et al. Ascorbic acid reduces the endotoxin-induced lung injury in awake sheep. Eur J Clin Invest 1994;24:229–35.

[15] Galley HF, Davies MJ, Webster NR. Ascorbyl radical formation in patients with sepsis: effect of ascorbate loading. Free Radic Biol Med 1996;20:139–43.

[16] Rümelin A, Humbert T, Lühker O, Fauth U. Postoperative increased metabolism of ascorbic acid. Intensive Care Med 2003; 29:S103.

[17] Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A 1996;93:3704–9.

[18] Vallance BD, Hume R, Weyers E. Reassessment of changes in leucocyte and serum ascorbic acid after acute myocardial infarction. Br Heart J 1978;40:64–8.

[19] Nathens AB, Neff MJ, Jurkovich GJ, et al. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. Ann Surg 2002;236:814–22.

[20] Paolini M, Pozzetti L, Pedulli GF, Marchesi E, Cantelli-Forti G. The nature of prooxidant activity of vitamin C. Life Sci 1999;64: 273–8.

[21] MacDonald L, Thumser AE, Sharp P. Decreased expression of the vitamin C transporter SVCT 1 b ascorbic acid in a human intestinal epithelial cell line. Br J Nutr 2002;87:97–100.

[22] Mashour S, Turner JF, Merrell R. Acute renal failure, oxalosis, and vitamin C supplementation. Chest 2000;118:561–3.

[23] Auer BL, Auer D, Rodgers AL. Relative hyperoxaluria, crystalluria and haematuria after megadose ingestion of vitamin C. Eur J Clin Invest 1998;28:695–700.

[24] Swartz RD, Wesley JR, Somermeyer MG, Lau K. Hyperoxaluria and renal insufficiency due to ascorbic acid administration during total parenteral nutrition. Ann Intern Med 1984;100:530–1.

[25] Lawton JM, Conway LT, Crosson JT, Smith CL, Abraham PA. Acute oxalate nephropathy after massive ascorbic acid administration. Arch Intern Med 1985;145:950–1.

[26] Ramaswamy CR, Williams JD, Griffiths DF. Reversible acute renal failure with calcium oxalate cast nephropathy—possible role of ascorbic acid. Nephrol Dial Transplant 1993;8:1387–9.

[27] Wong K, Thomson C, Bailey RR, McDiarmid S, Gardner J. Acute oxalate nephropathy after a massive intravenous dose of vitamin C. Aust NZ J Med 1994;24:410–1.

[28] Alkhunaizi AM, Chan L. Secondary oxalosis: a cause of delayed recovery of renal function in the setting of acute renal failure. J Am Soc Nephrol 1996;7:2320–6.

[29] Honow R, Simon A, Hesse A. Interference-free sample preparation for the determination of plasma oxalate analyzed by HPLC-ER: preliminary results from calcium oxalate stone-formers and non-stone-formers. Clin Chim Acta 2002;318:19–24.

[30] Wandzilak TR, D'Andre SD, Davis PA, Williams HE. Effect of high dose vitamin C on urinary oxalate levels. J Urol 1994;151: 834–7.

[31] Halliwell B. Vitamin C: antioxidant or pro-oxidant in vivo? Free Radic Res 1996;25:439–54.

[32] Udomratn T, Steinberg MH, Campbell GD, Oelshlegel FJ. Effects of ascorbic acid on glucose-6-phosphate-dehydrogenase-deficient erythrocytes: studies in an animal model. Blood 1977;49:471–5.