

Elimination of Ascorbic Acid After High-Dose Infusion in Prostate Cancer Patients: A Pharmacokinetic Evaluation

Torben K. Nielsen^{1,2}, Martin Højgaard¹, Jon T. Andersen^{2,3,4}, Henrik E. Poulsen^{2,3,4}, Jens Lykkesfeldt² and Kári J. Mikkelsen^{1,2}

¹Department of Urology, Copenhagen University Hospital Herlev, Herlev, Denmark, ²Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, ³Laboratory of Clinical Pharmacology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark and ⁴Department of Clinical Pharmacology, Copenhagen University Hospital Bispebjerg, Copenhagen, Denmark

(Received 19 June 2014; Accepted 2 September 2014)

Abstract: Treatment with high-dose intravenous (IV) ascorbic acid (AA) is used in complementary and alternative medicine for various conditions including cancer. Cytotoxicity to cancer cell lines has been observed with millimolar concentrations of AA. Little is known about the pharmacokinetics of high-dose IV AA. The purpose of this study was to assess the basic kinetic variables in human beings over a relevant AA dosing interval for proper design of future clinical trials. Ten patients with metastatic prostate cancer were treated for 4 weeks with fixed AA doses of 5, 30 and 60 g. AA was measured consecutively in plasma and indicated first-order elimination kinetics throughout the dosing range with supra-physiological concentrations. The target dose of 60 g AA IV produced a peak plasma AA concentration of 20.3 mM. Elimination half-life was 1.87 hr (mean, S.D. \pm 0.40), volume of distribution 0.19 L/kg (S.D. \pm 0.05) and clearance rate 6.02 L/hr (100 mL/min). No differences in pharmacokinetic parameters were observed between weeks/doses. A relatively fast first-order elimination with half-life of about 2 hr makes it impossible to maintain AA concentrations in the potential cytotoxic range after infusion stop in prostate cancer patients with normal kidney function. We propose a regimen with a bolus loading followed by a maintenance infusion based on the calculated clearance.

In the 1970s, Cameron, Campbell and Pauling reported prolonged overall survival after treatment with high-dose ascorbic acid (AA) in patients with advanced cancer disease [1,2]. The methodology in these two studies was criticized, and the results were not confirmed in two subsequent randomized, controlled trials with oral AA [3,4]. Treatment with intravenous (IV) AA has continued in complementary and alternative medicine and is widely used today [5].

Cell culture studies and animal models have shown that AA in millimolar extracellular concentrations are cytotoxic to cancer cells without apparent toxicity for normal cells [6–11]. From oral intake, plasma AA concentrations are tightly controlled by a saturable gastrointestinal absorption and a saturable renal tubular reabsorption. Despite daily oral gram doses of AA, the upper plasma level is \sim 220 μ M [12,13]. To achieve the >100 times higher concentrations required for potential cytotoxicity to cancer cells, AA must be administered intravenously. Very little data have been published on the pharmacokinetics of high-dose AA, although AA infusions have been used in alternative medicine for decades. One phase I trial was designed as a dose-escalation trial with 5–7 patients in 4 groups [14], and within the last year, two additional studies have been published with 14 and 17 patients, respectively [15,16], with only one presenting pharmacokinetic data like half-life ($T_{1/2}$) and clearance [16]. The purpose of this study was to evaluate the basic pharmacokinetics of high-dose AA.

Author for correspondence: Torben K. Nielsen, Copenhagen University Hospital Herlev, Department of Urology HA54F1, Herlev Ringvej 75, 2730 Herlev, Denmark (e-mail torben.kjaer@dadlnet.dk). Clinicaltrials.gov: NCT01080352

Material and Methods

Study design. Patients were consecutively recruited from an ongoing non-comparative, single-centre, phase II trial, investigating efficacy and safety of IV AA in patients with metastatic castration-resistant prostate cancer. Patients were recruited from the outpatient urology clinic at Copenhagen University Hospital Herlev, Copenhagen, Denmark.

Patient eligibility. All patients signed informed consent forms. The trial was approved by the Regional Ethics Committee (H-C-2009-018) and the Danish Health and Medicines Authority (2612-3978), registered (Eudra-CT 2008-008692-33/NCT01080352), the Danish Data Protection Agency (2007-58-0015/750.19-15) and followed the current Guideline for Good Clinical Practice issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Declaration of Helsinki.

Eligibility criteria included adenocarcinoma of the prostate with a least one metastatic lesion on bone- or CT scan; ongoing androgen deprivation therapy with a castration level of testosterone (<1.7 nmol/L); disease progression (prostate-specific antigen or on imaging) as defined by the Prostate Cancer Working Group 2 (PCWG-2) [17]; no prior chemotherapy; and Eastern Cooperative Oncology Group (ECOG) performance 0–2. Key exclusion criteria were significant renal impairment (creatinine >200 μ mol/L); significant cardiac disease (NYHA >2 , CSS >2 , recent myocardial infarction (less than 6 months)); and haemochromatosis, glucose-6-phosphate dehydrogenase deficiency or history of oxalate renal stones.

Patient characteristics. Twelve consecutive patients from the main trial were invited to participate in this study. Two did not wish to participate due to the time burden. All patients ($n = 10$) were male Caucasians diagnosed and followed in our clinic. Detailed baseline characteristics are listed in table 1. Patient characteristics were

Table 1.

Patient characteristics measured/collected with a full medical examination in the week prior to week #1. Estimated glomerular filtration rate (eGFR) was calculated with the Modification of Diet in Renal Disease (MDRD) equation [20].

	Median	Range
Age [years]	72.8	54.2–87.2
Weight [kg]	83	63–104
BMI [kg/m ²]	27.8	21.3–33.2
Body surface area (DuBois) [m ²]	2.01	1.75–2.20
eGFR [L/hr/1.73]	4.62	3.58–5.18
GFR estimate (creatinine clearance) with body surface correction [L/hr/1.73 m ²]	5.14	1.43*–6.59

*One extreme outlier with very small urine sample, eGFR normal = 4.96 L/hr.

collected during a full medical examination 1 week prior to study week #1. Body surface area (BSA) was calculated with the DuBois formula [18], and extracellular volume (ECV) was calculated with the Bird formula [19] and estimated glomerular filtration rate (eGFR) was calculated with the 'MDRD formula' [20]. Creatinine clearance was measured from plasma creatinine concentration and 24-hr urinary creatinine excretion.

Intervention. Patients received three different doses of AA in the 4-week study period. Doses, infusion rates and volumes are shown in table 2. The main trial target dose of 60 g was reached in week 3 and continued once a week until initial efficacy evaluation after twelve weeks; hence, the interventions in week 3 and 4 were identical. AA was administered via an infusion pump to an IV catheter in the proximal forearm. AA for infusion was batch-produced by the 'Capitol Region Pharmacy' (Good manufacturing practices (GMP) certified governmental pharmacy). Each mL of infusion concentrate contained 562.4 mg sodium ascorbate (equal to 500 mg of ascorbic acid). Just before infusion, the concentrate was dissolved in 5% isotonic glucoses or sterile water. Osmolarity was measured to 337 and 623 mOsm, respectively. Drug stability was checked once in the study period.

Patients were under observation by study personnel during and after the infusion. Thirty minutes after the infusion, vital signs were measured and blood was drawn to screen for haemolysis. All adverse events (AE) were scored by Common Terminology Criteria for Adverse Events v4.03 (CTCAE) [21].

Patients were provided a daily 500 mg oral dose of AA starting the day after the first infusion to avoid a suggested rebound deficiency after high-dose AA infusions [22].

Sample collection and analysis. Blood samples were collected from the cubital vein in the contra lateral arm of the infusion. Samples

Table 2.

Treatment scheme. Dose of ascorbic acid, solvent and infusion rate/time.

Week	AA [g]	Vehicle	Total volume [mL]	Infusion rate [g/min]	Infusion time [min]
#1	5	5% isotonic glucose	510	0.17	30
#2	30	Sterile water	500	1	30
#3	60	Sterile water	1000	1	60
#4	60	Sterile water	1000	1	60

were collected at the following times: (i) before infusion (–60/–30 min.); (ii) at 50% of volume infused (–30/–15 min.); (iii) at infusion stop (0 min.); and (iv) after infusion stop (+30, 60, 120, 180 and 240 min.). Samples to be analysed for AA were taken and pre-treated as follows: whole blood was collected using a 6-mL 'GBO Vacuette' K₃EDTA-coated blood collection tube [23]. Immediately after collection, 1.5 mL of blood was transferred to a microcentrifuge tube and centrifuged at 16,000 × g for 2 min. at 4°C. Plasma (500 µL) was then transferred into a microcentrifuge tube containing 500 µL of cold (4°C) 10% metaphosphoric acid containing 2 mM EDTA. The mixture was vortexed for 10 sec. and centrifuged at 16000 × g for 1 min. at 4°C. The protein-free supernatant was collected and stored at –80°C until analysis. Stability of the samples under these conditions is at least 5 years [24]. All samples from each individual were analysed together using high-performance liquid chromatography with coulometric detection as described previously [25]. The within- and between day coefficients of variation are less than 1.5 and 3.5%, respectively, and detection limit less than 1 µmol/L. AA samples were stored for 5.9 months (mean; range 1.8–9.9) before analysis.

Haemoglobin (Hb) was measured at each time-point to correct for haemodilution during the infusion, and the plasma AA measured was multiplied with the ratio (Hb_{time} × Hb_{baseline}). Two measurements of AA and three of haemoglobin were not included in the analysis due to sample coagulation or analytical error.

Modelling. The AA plasma concentration versus time curve showed exponential decay after infusion was stopped (fig. 1), indicating first-order elimination kinetics. It was therefore possible to calculate elimination, elimination half-life (T_{1/2}), volume of distribution (V_d) and clearance of plasma AA. AUC was calculated using the trapezoidal rule. We used the ratio of AUC in the post-infusion phase over the total AUC, to compensate for elimination prior to infusion stop. V_d was calculated as AUC-ratio adjusted dose over modelled C_{max}.

Results

All ten patients received the planned four doses of AA without any severe adverse events. One episode of transient arterial hypertension immediately after the infusion (CTCAE Grade 2) and two unrelated AEs (CTCAE Grade 1) were observed. The maximal infused dose of 60 g was equal to 723.3 mg/kg (median, range: 576.9–952.4) and 29.79 g/m² (median, range: 27.21–34.38). The infusion of 5, 30 and 60 g

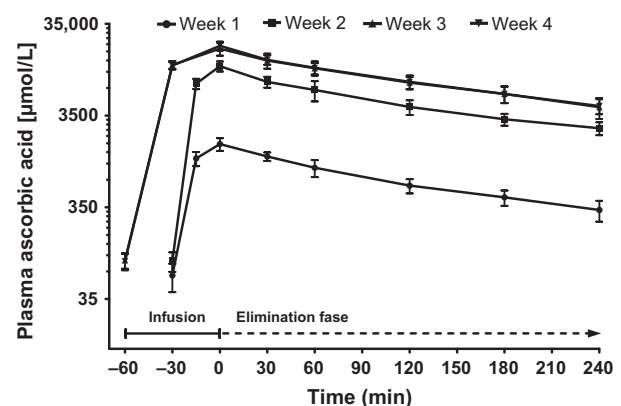


Fig. 1. Concentration of ascorbic acid in plasma during infusion and elimination.

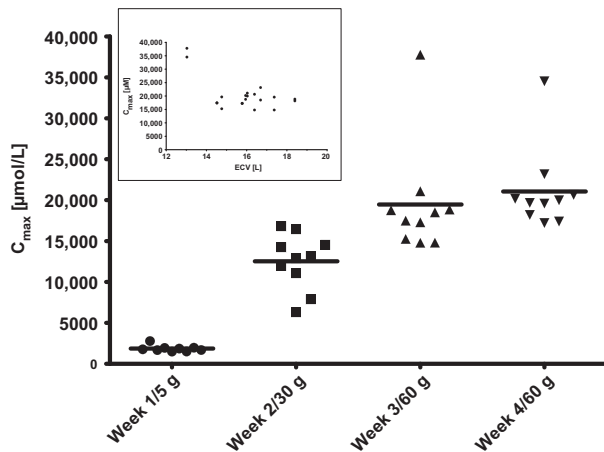


Fig. 2. Peak plasma ascorbic acid concentration (C_{\max}). Inset: C_{\max} after the 60-g target dose (week 3 + 4) correlated to extracellular volume (ECV) in litre.

of AA resulted in mean peak AA concentration of 1.9, 12.5, 19.5 and 21.0 mmol/L, respectively, as shown in fig. 2. Without correction for haemodilution, the mean peak concentrations were 1.8, 11.7, 18.2 and 19.3 mmol/L, respectively. Pre-infusion baseline AA showed a non-significant rise from week 1 of 63.2–90.5 μM in week 4 ($p = 0.18$).

The C_{\max} , AUC, elimination $T_{1/2}$ and clearance are presented in table 3. Elimination $T_{1/2}$ was 1.87 hr (mean, S.D. ± 0.40) and V_d was 0.19 L/kg (S.D. ± 0.05). Mean ECV was 15.9 L (S.D. ± 1.5) which was similar to V_d multiplied by weight with a mean of 15.6 L (S.D. ± 4.2). Clearance was 6.02 L/hr (S.D. ± 1.91) and standardized 5.20 L/hr/1.73 m^2 (S.D. ± 1.59). There were no significant differences in the pharmacokinetic parameters between weeks including the AUC/dose ratio.

Discussion

The primary objective of our study was to collect pharmacokinetic information on high-dose AA infusion. The trial had a target dose of 60 g AA, which produced a peak plasma AA concentration of 20.3 mM. We found first-order elimination kinetics with a very short half-life of 1.87 hr (112 min), a V_d of 0.19 L/kg which was similar to ECV and an AA clearance rate of 6.02 L/hr (100 mL/min).

After visual examination of each individual concentration versus time curve, it was concluded that the pattern justifies the use of one compartment first-order kinetics in the calculations because of exponential decay as demonstrated by linearity of the log plasma concentration versus time curves. Urine samples were not collected during infusion and elimination, and consequently, the actual excretion was not assessed, which should be performed in future studies. All patients received fixed doses of 5, 30 and 60 g of AA. Three existing studies have used either a fixed dose [15] or dose adjusted by weight [14] or surface [16]. In the present study, V_d was found to be similar to ECV, while dose scaling for ECV size is not supported by our data as shown in the inset in fig. 2. Thus, in a linear regression model, ECV was a significant predictor of C_{\max} , but the model was completely dependent on data from one outlier, and removal of those from the model resulted in a C_{\max} independent of ECV volume which confirms the visual impression. Androgen deprivation therapy induces metabolic changes, which is also seen by the relatively high mean BMI of the patients. The outlier had the lowest body mass and BMI of all individuals, so it is not possible to determine whether the very high concentrations achieved are due to a different body composition or due to other factors unique to the patient.

Stephenson *et al.* have reported a half-life between 1.7 and 2.5 hr after 30–110 g IV AA/ m^2 [16], which is comparable to

Table 3.

Calculated pharmacokinetic data by week number.

	Week 1 (5Gr)	Week 2 (30Gr)	Week 3 (60Gr)	Week 4 (60Gr)
C_{\max} [$\mu\text{mol/L}$]				
Mean \pm SD	1852 \pm 386	12,525 \pm 3404	19,456 \pm 6745	21,055 \pm 5039
n, range	(n = 9), 1495–2775	(n = 10), 6271–16,814	(n = 10), 14,789–37,767	(n = 10), 17,206–34,524
Dose/kg [mg/kg]				
Median	60.3	361.6	723.3	723.3
Range	48.1–79.4	288.5–476.2	576.9–952.4	576.9–952.4
Dose/ m^2 [g/ m^2]				
Median	2.48	14.90	29.79	29.79
Range	2.27–2.87	13.61–17.19	27.21–34.38	27.21–34.38
$T_{1/2}$ [min.]				
Mean \pm SD	99.6 \pm 21.0	114.7 \pm 27.1	118.1 \pm 24.0	117.1 \pm 21.3
AUC [hr mM]				
Mean \pm SD	3.59 \pm 1.05	24.75 \pm 8.29	48.38 \pm 16.7	49.76 \pm 14.8
% of AUC before infusion stop	15.7	15.0	24.0	24.3
V_d [L/kg]				
Mean \pm SD	0.19 \pm 0.03	0.18 \pm 0.09	0.19 \pm 0.03	0.18 \pm 0.03
Clearance [L/hr]				
Mean \pm SD	6.84 \pm 1.67	5.56 \pm 2.11	5.92 \pm 1.85	5.75 \pm 2.00

C_{\max} , peak ascorbic acid concentration; $T_{1/2}$, half-life; AUC, area under the curve. V_d , volume of distribution.

the findings of the present study. Graumlich *et al.* [26] studied AA pharmacokinetics within the normal physiological range and reported a clearance rate of 10.9 L/hr with net tubular excretion as a contributor in the AA concentration range just above physiological level. When plasma concentration approaches physiological levels, the elimination follows a multi-compartmental model [26]. In the present study, the calculated creatinine clearance is nearly identical to the AA clearance. The interpretation is therefore that renal filtration accounts for most of the AA clearance, and other elimination mechanisms apparently play a minor role in the supra-physiological plasma range. Although $T_{1/2}$ was similar, the clearance found in the present study normalized to surface area but was slightly higher than the clearance observed by Stephenson *et al.* [16]. Apparently, their calculation does not correct for AA lost during the infusion phase and thereby underestimating clearance, while the present study may slightly overestimate clearance when correcting for AA elimination during infusion. Using the actual measured peak AA instead of model C_{\max} in clearance, the calculations result in a non-significant reduction in clearance of about 1 L/hr (data not shown).

Graumlich *et al.* [26] estimated the volume of the central compartment to 11.8 L in their model, which is comparable to V_d of 15.6 L found in the present study, also taking into account the nearly ten kilogram larger body-weight of the individuals in our study.

Efficacy of AA in clinical medicine has not been established. AA has been reported as an effective *ex vivo* cytotoxic substance in a large panel of tumour cell lines [8–11], although the high inhibitory concentration may make it less probable to succeed through the drug developmental phases [27]. Cytotoxicity was not evident in normal cells at ascorbate concentrations exceeding 20 mM [8], in contrast to numerous cancer cell lines, which leaves as possible therapeutic window to be investigated further. The concentration of ascorbic acid varies between tissues [28]. Endothelial cells [29] and white blood cells have been shown to be able to accumulate AA in concentrations above 1 mM, but the uptake mechanism saturates above 100 mg of AA daily [30]. The consequence of this accumulation in relation to antitumour activity is unknown.

The detailed mechanism by which AA exerts its cytotoxicity remains to be established. Data from the NCI60 panel does not provide a good fingerprint of leads to follow in order to unveil the mechanism of action [11]. Chen *et al.* [31] proposed that the cytotoxic effects may be mediated through the reactive oxygen species hydrogen peroxide (H_2O_2) formation rather than by AA itself. Thus, they suggested that H_2O_2 is dose dependently generated in the extracellular environment from the ascorbyl radical and a protein-centred metal [32], which could be ferritin secreted by the tumour cells [33]. H_2O_2 generation displayed a linear relationship with the formation of the AA radical. Although H_2O_2 might be able to reach the cancer cells by diffusion, in order to reach intracellular targets of the cancer cell, it has to pass the cell membrane. Simple passive diffusion of the polar H_2O_2 across the lipid bilayer membranes should be limited as for water, but the

transfer might be facilitated by and dependent on aquaporins [34].

In addition to the concentration of AA, the tumour cell count and exposure time also play an important role for the cytotoxicity of AA. H_2O_2 degradation rate and toxicity of AA are dependent on the number of cells in *in vitro* experiments [33], which could imply a lower penetration of AA into cell formations or the generation of a protective microenvironment when cell count (or tumour size) reaches a certain level [35]. Only a limited number of *in vitro* studies have used short AA exposure time, which can be comparable to the setting in human beings after a bolus of intravenous AA. Venugopla *et al.* have reported an inverse relationship between cytotoxic AA concentration levels and incubation time in urologic cancer cell lines [36], and similar trends have been reported by Chen *et al.* [37]. Exposure time for cytotoxicity to be effective *in vivo* needs to be evaluated further in future studies.

Three studies have given some implications of efficacy *in vivo*, with doses of daily 1 g/kg [10] and 4 g/kg [7,8] intraperitoneally, although the latter by far exceeds what has been used in human phase I trials. AA was shown to reduce tumour size in xenograft models (athymic, nude mice) of three different cell lines [8] and to induce some degree of cytostasis in a syngenic rat prostate cancer model [7] and a syngenic hepatocarcinoma mouse model [10]. Only one of the studies provides data to calculate valid response parameters of efficacy (change in tumour volume in treated *versus* controls; %T/C), but from figures in the papers, it does not appear to be <40% which is a traditionally used cut-off for efficacy [38].

Data from the present study can be used to calculate a bolus-loaded target plasma concentration, which can be maintained during a defined time frame using a calculable maintenance infusion. If the AA bolus is not delivered in a central venous catheter, the osmotic activity of AA will require it to be combined with a large fluid load. Dissolved in a suitable vehicle to around 600 mOsm, a bolus infusion rate of 1 g/min is reasonable with the aim of producing high AA plasma concentrations and concurrently avoiding local vascular reactions due to osmolality. The maintenance infusion rate and elimination rate are equal in steady-state, which makes it possible to calculate maintenance dose: $\text{dosing rate} = \text{clearance} \times \text{target concentration}$ [39]. Based on our data, a bolus infusion of 60 g (at 1 g/min) followed by a maintenance infusion of 21.1 g/hr will achieve a plasma steady-state concentration of AA above 20 mM, continuing to a specified time. No trial has used a bolus maintenance regimen, and despite the apparent atoxic profile of AA in previous trials, it has not been examined if the drug itself or volume-induced electrolyte disturbances may produce adverse events over time.

We conclude that with constant infusion of AA in prostate cancer patients with normal kidney function, it is possible to achieve a desired steady-state plasma concentration of AA in the range of 1.5–37.8 mM from doses up to 60 g as long as the infusion lasts. The relatively fast first-order kinetics, demonstrated by an elimination half-life of about 2 hr, makes it impossible to maintain such high AA concentrations after infusion stop.

Acknowledgements

The study was founded by the Kirsten and Freddy Johansen Foundation.

References

- Cameron E, Campbell A. The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem Biol Interact* 1974;**9**:285–315.
- Cameron E, Pauling L. Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. *Proc Natl Acad Sci U S A* 1978;**75**:4538–42.
- Creagan ET, Moertel CG, O'Fallon JR, Schutt AJ, O'Connell MJ, Rubin J *et al.* Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. *N Engl J Med* 1979;**301**:687–90.
- Moertel CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ, Ames MM. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. *N Engl J Med* 1985;**312**:137–41.
- Padayatty SJ, Sun AY, Chen Q, Espey MG, Drisko J, Levine M. Vitamin C: intravenous use by complementary and alternative medicine practitioners and adverse effects. *PLoS ONE* 2010;**5**: e11414.
- Kim J, Lee S-D, Chang B, Jin D-H, Jung S-I, Park M-Y *et al.* Enhanced antitumor activity of vitamin C via p53 in cancer cells. *Free Radic Biol Med* 2012;**53**:1607–15.
- Pollard HB, Levine MA, Eidelman O, Pollard M. Pharmacological ascorbic acid suppresses syngeneic tumor growth and metastases in hormone-refractory prostate cancer. *In Vivo* 2010;**24**:249–55.
- Chen Q, Espey MG, Sun AY, Pooput C, Kirk KL, Krishna MC *et al.* Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. *Proc Natl Acad Sci U S A* 2008;**105**:11105–9.
- Frömberg A, Gutsch D, Schulze D, Vollbracht C, Weiss G, Czubyko F *et al.* Ascorbate exerts anti-proliferative effects through cell cycle inhibition and sensitizes tumor cells towards cytostatic drugs. *Cancer Chemother Pharmacol* 2011;**67**:1157–66.
- Verrax J, Calderon PB. Pharmacologic concentrations of ascorbate are achieved by parenteral administration and exhibit antitumoral effects. *Free Radic Biol Med* 2009;**47**:32–40.
- National Institutes of Health - U.S. Department of Health and Human Service. Developmental Therapeutics Program. <http://dtp.nci.nih.gov/> 2014 (last accessed 1st May 2014).
- Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A *et al.* Vitamin C pharmacokinetics: implications for oral and intravenous use. *Ann Intern Med* 2004;**140**:533–7.
- Blanchard J, Tozer TN, Rowland M. Pharmacokinetic perspectives on megadoses of ascorbic acid. *Am J Clin Nutr* 1997;**66**:1165–71.
- Hoffer LJ, Levine M, Assouline S, Melnychuk D, Padayatty SJ, Rosadiuk K *et al.* Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. *Ann Oncol* 2008;**19**:1969–74.
- Monti DA, Mitchell E, Bazzan AJ, Littman S, Zabrecky G, Charles J *et al.* Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *PLoS ONE* 2012;**7**:e29794.
- Stephenson CM, Levin RD, Spector T, Lis CG. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. *Cancer Chemother Pharmacol* 2013;**72**:139–46.
- Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci Ma *et al.* Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol* 2008;**26**:1148–59.
- Wang Y, Moss J, Thisted R. Predictors of body surface area. *J Clin Anesth* 1992;**4**:4–10.
- Bird NJ, Henderson BL, Lui D, Ballinger JR, Peters AM. Indexing glomerular filtration rate to suit children. *J Nucl Med* 2003;**44**:1037–43.
- Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S *et al.* Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006;**145**: 247–54.
- National Cancer Institute - U.S. Department of Health and Human Services. Common Terminology Criteria for Adverse Events v4.0. National Cancer Institute - U.S. Department of Health and Human Services. vol. **2009**. 2010.
- Tsao CS, Salimi SL. Evidence of rebound effect with ascorbic acid. *Med Hypotheses* 1984;**13**:303–10.
- Lykkesfeldt J. Ascorbate and dehydroascorbic acid as biomarkers of oxidative stress: validity of clinical data depends on vacutainer system used. *Nutr Res* 2012;**32**:66–9.
- Lykkesfeldt J. Ascorbate and dehydroascorbic acid as reliable biomarkers of oxidative stress: analytical reproducibility and long-term stability of plasma samples subjected to acidic deproteinization. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:2513–6.
- Lykkesfeldt J. Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: reliable reduction with tris[2-carboxyethyl]phosphine hydrochloride. *Anal Biochem* 2000;**282**: 89–93.
- Graumlich JF, Ludden TM, Conry-Cantilena C, Cantilena LR, Wang Y, Levine M. Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. *Pharm Res* 1997;**14**:1133–9.
- Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S *et al.* Relationships between drug activity in NCI pre-clinical *in vitro* and *in vivo* models and early clinical trials. *Br J Cancer* 2001;**84**:1424–31.
- Omaye ST, Schaus EE, Kutnink Ma, Hawkes WC. Measurement of vitamin C in blood components by high-performance liquid chromatography. Implication in assessing vitamin C status. *Ann N Y Acad Sci* 1987;**498**:389–401.
- May JM, Qu Z. Transport and intracellular accumulation of vitamin C in endothelial cells: relevance to collagen synthesis. *Arch Biochem Biophys* 2005;**434**:178–86.
- Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR *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.
- Chen Q, Espey MG, Krishna MC, Mitchell JB, Corpe CP, Buettner GR *et al.* Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc Natl Acad Sci U S A* 2005;**102**:13604–9.
- Chen Q, Espey MG, Sun AY, Lee J-H, Krishna MC, Shacter E *et al.* Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid *in vivo*. *Proc Natl Acad Sci U S A* 2007;**104**:8749–54.
- Deubzer B, Mayer F, Kuçi Z, Niewisch M, Merkel G, Handgretinger R *et al.* H(2)O(2)-mediated cytotoxicity of pharmacologic ascorbate concentrations to neuroblastoma cells: potential role of lactate and ferritin. *Cell Physiol Biochem* 2010;**25**:767–74.
- Bienert GP, Möller ALB, Kristiansen Ka, Schulz A, Möller IM, Schjoerring JK *et al.* Specific aquaporins facilitate the diffusion of

- hydrogen peroxide across membranes. *J Biol Chem* 2007;**282**: 1183–92.
- 35 Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. *Nature* 2013;**501**: 346–54.
- 36 Venugopal M, Jamison JM, Gilloteaux J, Koch JA, Summers M, Giammar D *et al.* Synergistic antitumor activity of vitamins C and K3 on human urologic tumor cell lines. *Life Sci* 1996;**59**: 1389–400.
- 37 Chen P, Yu J, Chalmers B, Drisko J, Yang J, Li B *et al.* Pharmacological ascorbate induces cytotoxicity in prostate cancer cells through ATP depletion and induction of autophagy. *Anticancer Drugs* 2012;**23**:437–44.
- 38 Genzyme Corporation. Principles of Anticancer Drug Development. New York, NY: Springer New York; 2011.
- 39 Katzung BG, Masters SB, Trevor AJ. Basic & Clinical Pharmacology, 11th edn. McGraw-Hill Medical, New York, 2009.