original article

Annals of Oncology 19: 1969–1974, 2008
doi:10.1093/annonc/mdn377
Published online 9 June 2008

Phase I clinical trial of i.v. ascorbic acid in advanced malignancy

L. J. Hoffer[1]*, M. Levine[2], S. Assouline[1], D. Melnychuk[1], S. J. Padayatty[2], K. Rosadiuk[1], C. Rousseau[1], L. Robitaille[1] & W. H. Miller, Jr[1]

[1]*Montreal Centre for Experimental Therapeutics in Cancer, Lady Davis Institute for Medical Research, McGill University and the Jewish General Hospital, Montreal, Quebec, Canada;* [2]*Molecular and Clinical Nutrition Section, Digestive Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA*

Received 28 March 2008; revised 25 April 2008; accepted 7 May 2008

Background: Ascorbic acid is a widely used and controversial alternative cancer treatment. In millimolar concentrations, it is selectively cytotoxic to many cancer cell lines and has *in vivo* anticancer activity when administered alone or together with other agents. We carried out a dose-finding phase I and pharmacokinetic study of i.v. ascorbic acid in patients with advanced malignancies.

Patients and methods: Patients with advanced cancer or hematologic malignancy were assigned to sequential cohorts infused with 0.4, 0.6, 0.9 and 1.5 g ascorbic acid/kg body weight three times weekly.

Results: Adverse events and toxicity were minimal at all dose levels. No patient had an objective anticancer response.

Conclusions: High-dose i.v. ascorbic acid was well tolerated but failed to demonstrate anticancer activity when administered to patients with previously treated advanced malignancies. The promise of this approach may lie in combination with cytotoxic or other redox-active molecules.

Key words: antioxidants, vitamin C

introduction

The possibility of a role for ascorbic acid in cancer therapy emerged ~30 years ago when objective responses to its i.v. and oral administration were reported in a large consecutive series of patients with advanced cancer [1]. Oncologists discarded the concept when two randomized clinical trials with ascorbic acid, consumed by mouth, failed to demonstrate any benefit [2]; ascorbic acid nevertheless continues to be widely used by alternative medicine practitioners [2, 3]. Scientific interest in the interaction between ascorbic acid and cancer has increased in recent years with evidence that in millimolar concentrations—which are attainable only after parenteral administration—it is selectively cytotoxic to many neoplastic cell lines [4–6], potentiates cytoxic agents [7–11] and demonstrates anticancer activity alone and in combination with other agents in tumor-bearing rodents [12–14]. Simultaneously, theoretical interest has arisen in the potential of redox-active molecules like menadione, trolox and ascorbic acid to modify cancer biology [13, 15, 16], especially when administered together with cytotoxic drugs [17–19].

Information about the safety and pharmacokinetics of high-dose i.v. ascorbic acid is crucial for the proper design of clinical trials, but is currently lacking in the peer-reviewed literature. In high doses, ascorbic acid can trigger hemolysis in some variants glucose-6-phosphate dehydrogenase deficiency, especially in the presence of infection and fever [20]. Because oxalic acid is a major end metabolite of ascorbic acid oxidation, even limited oxidation of a large i.v. dose of ascorbic acid to oxalic acid could be dangerous. Acute tumor hemorrhage and necrosis have been reported within days after starting i.v. ascorbic acid in patients with advanced cancer [1].

Virtually alone among alternative practitioners, Riordan et al. [21] have described a clinical protocol to administer i.v. ascorbic acid, and published preliminary pharmacokinetic data [22], case reports [23] and a phase I clinical trial in which patients were administered 0.15 to 0.7 g/kg/day of ascorbic acid as a continuous infusion for up to 8 weeks. However, in that trial plasma ascorbic acid concentrations did not exceed 3.8 mmol/l [24]. The aim of the present study was to document the safety and clinical consequences of i.v. ascorbic acid administrated in a dose sufficient to sustain plasma ascorbic acid concentrations >10 mmol/l for several hours, in line with emerging concepts regarding its potential *in vivo* anticancer activity [6, 25]. The adoption of a broad pharmacokinetic criterion for selection of the recommended phase II dose anticipated the possibility that the toxic dose could greatly exceed the effective dose or a dose that would be clinically practical to administer.

Correspondence to: Dr L. J. Hoffer, Lady Davis Institute for Medical Research, Jewish General Hospital, 3755 Cote-Ste-Catherine Road, Montreal, Quebec H3T 1E2, Canada. Tel: +1-514-340-8222; Fax: +1-514-340-7502; E-mail: l.hoffer@mcgill.ca

patients and methods

eligibility

Patients were eligible for study if they had a histologically documented solid tumor or hematological malignancy with locally advanced, metastatic or recurrent disease that was refractory to standard therapy; an Eastern Cooperative Group performance status of zero to two and a life expectancy of 8 or more weeks; clinically and/or radiographically documented and evaluable disease; serum creatinine ≤175 μmol/l, fasting serum glucose <10 mmol/l, serum-corrected calcium ≤2.65 mmol/l, blood hemoglobin ≥7 g/l and prothrombin time and partial thromboplastin time <1.5 times the upper limit of normal. All patients were biochemically tested to exclude glucose-6-phosphate dehydrogenase deficiency and underwent an abdominal X-ray examination to screen for radio-opacities suggestive of silent urolithiasis. Other exclusion criteria were a history of renal calculi, current treatment with vitamin K antagonists, pulmonary disease severe enough to cause dyspnea at rest, pleural effusions of more than moderate size and a history of heart failure due to myocardial disease, or, if known, a cardiac ejection fraction <40%. There was no limit to the number of prior treatment regimens, but no anticancer therapy was allowed within 4 weeks of study entry. The protocol and consent form were approved by the Research Ethics Committee of the Jewish General Hospital of Montreal.

study design

This was a single-center phase I dose-escalating trial of i.v. ascorbic acid whose primary objective was to determine a recommended phase II dose. Secondary objectives were to define any toxic effects, detect any preliminary antitumor effects, monitor for preservation of or improvement in quality of life using the Functional Assessment of Cancer Therapy — General (FACT-G) questionnaire and determine the effects of different i.v. doses on the plasma ascorbic acid profile. Each cohort included five to seven patients with escalation to the next dose level upon completion of one 4-week treatment cycle without dose-limiting toxicity. The criteria for discontinuance were unacceptable toxicity and disease progression after a minimum of two treatment cycles. Ascorbic acid was administered three times per week at fixed doses of 0.4, 0.6, 0.9, and 1.5 g/kg assigned at registration. For patients registered at the two lower doses, a test dose of 0.1 g/kg was administered to screen for unforeseen toxicity; for the next infusions, the dose was 0.2 g/kg followed by the target dose. When there was no toxicity at these target doses, the test dose for patients entering the 0.9-g/kg cohort was 0.4 g/kg followed immediately by 0.6 g/kg and 0.9 g/kg thereafter, and the test dose for patients entering the 1.5-g/kg cohort was 0.6 g/kg followed immediately thereafter by 1.5 g/kg. The infusates were prepared from ascorbic acid for injection (Canadian drug identification number 02245214) provided in single-use 50-ml glass ampules as a gift from Bioniche Pharma (Canada) Ltd. Each milliliter of this product contains 500 mg ascorbic acid (2.84 mmol), edetate disodium 0.025% and water with the pH adjusted to neutral with sodium bicarbonate; the theoretical osmolarity was 5.7 mOsm/ml. Doses <15 g were diluted in Ringer's lactate to achieve physiological osmolarity. Doses >15 g were diluted in sterile water to achieve a theoretical osmolarity between 500 and 900 mOsm/l. The infusates were delivered immediately to the bedside covered by an opaque bag allowed to warm to ambient temperature and administered by calibrated infusion pump within 1 h of preparation. Doses up to 90 g were infused at a constant rate >90 min; doses >90 g were infused over 120 min. Water and soft drinks were provided and the patients were encouraged to consume them freely before, during and after the ascorbic acid infusions. All patients were provided with a daily multivitamin tablet (Centrum Select, Wyeth) and 400 IU d-α-tocopherol twice daily with meals, and, on noninfusion days, 500 mg ascorbic acid twice daily to obviate large shifts in plasma ascorbic acid concentrations.

monitoring

At study entry and at the beginning of each 4-week cycle, patients underwent a complete physical examination and laboratory evaluations including complete blood count, serum chemistries, coagulation profile, tumor markers and urinalysis. Computerized tomographic examinations for staging and tumor response were carried out at baseline and at the end of every second cycle. Participants completed the FACT-G quality-of-life questionnaire at baseline, after 2 weeks on protocol and every month thereafter. All patients were seen 4 weeks after the termination of the protocol. Toxicity assessments were carried out continuously while on the trial. On treatment days during the first cycle, the patient arrived in the morning and voided. The patient was weighed and fitted with a peripheral or central i.v. catheter. On the days of pharmacokinetic studies, a blood sample was drawn between 1.5 and 6 h after the end of the infusion (mean 4.3 h) to examine for Heinz bodies.

pharmacokinetics

Formal pharmacokinetic studies were carried out in 18 patients administered doses of 0.1, 0.2, 0.4, 0.6, 0.9 and 1.5 g/kg. Blood was drawn from an antecubital vein or central venous catheter distant from the infusion catheter just before and for up to 6 h after the end of the infusion. After clearing the catheter and discarding 1 ml of blood, 4 ml was drawn into a chilled EDTA test tube, kept on ice for <2 h, centrifuged at 4°C for 2 min, 0.2 ml of the resulting plasma mixed immediately with 0.8 ml of ice cold 90% methanol 10% 1 mmol/l EDTA, centrifuged at 4°C for 10 min and the supernatant was analyzed immediately or frozen at −80°C until analysis. Patients emptied their bladders to provide a urine sample just before the start of the ascorbic acid infusion, then voided again and began a fresh collection as soon as the infusion finished. They then provided urine samples at least every 2 h for the following 6 h. The sample volume was measured and 0.2 ml mixed with 0.8 ml of ice cold 90% methanol 10% 1 mmol/l EDTA and treated like a plasma sample. The analysis was carried out on site by high performance liquid chromatography separation with electrochemical detection using an ESA Coulochem III detector equipped with a 5011A analytical cell [26]. Urinary ascorbic acid was measured as the sum of ascorbic acid and dehydroascorbic acid by reducing all dehydroascorbic acid present to ascorbic acid with dimercaprol before analysis [26]. Plasma ascorbic acid was analyzed as ascorbic acid because dehydroascorbic acid was undetectable (<5%) in plasma.

An important parameter for dose selection with i.v. ascorbic acid is the peak plasma concentration achieved. We tested the hypothesis that this concentration can be predicted from the dose alone with the following assumptions: (i) ascorbic acid does not bind to plasma proteins [27]; (ii) a large i.v. dose rapidly and evenly distributes throughout the extracellular volume, which comprises 20% of normal body weight [28] and (iii) exit from the extracellular space is mostly via urinary excretion.

results

The characteristics of the participants are presented in Table 1. Every patient had received at least one prior conventional treatment, and 16 of them (33%) had been heavily treated with three or more prior treatment regimens. The average duration of participation was 10 weeks with a maximum of 30 weeks (92 treatments). Despite the requirement for frequent visits, the patients were strongly committed to the study and missed doses only on hospital holidays or as needed to manage complications of their cancers. Only mild clinical toxic effects occurred (Table 2). Heinz bodies (particles of denatured hemoglobin caused by hydrogen peroxide) were not

Table 1. Patient characteristics ($N = 24$)

Characteristics	Value	No. of patients
Age, years		24
Median	61	
Range	21–88	
Sex		
Male		16
Female		8
Weight (kg)		
Median	68	
Range	37–106	
Performance status		
0		4
1		14
2		6
Received chemotherapy in the past 2 years		23
Type of cancer		
Urothelial		3
Head and neck		3
Sarcoma		3
Lymphoma		
Follicular		2
Cutaneous T cell		1
Hodgkin's		1
Prostate		2
Epidermoid		2
Breast		1
Hepatocellular		1
Ovarian		1
Pancreatic		1
Renal		1
Lung		1
Unknown origin		1

Table 2. Adverse events

Cohort	No. in cohort	Adverse event	Number affected	Grade
0.4	5	Abdominal cramps	1	1
		Diarrhea	2	1
		Nausea	1	1
		Vomiting	1	2
0.6	6	Headache	1	2
0.9	7	Dizziness	1	1
		Fatigue	1	1
		Facial flushing	1	1
		Perspiration	1	1
		Nausea	1	1
		Weakness	1	1
1.5	6	Dizziness	1	2
		Facial flushing	1	2
		Headache	1	1
		Nausea	1	1

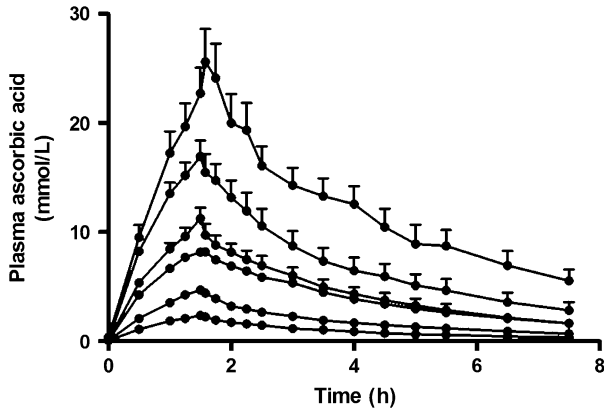

Figure 1. Mean plasma ascorbic acid concentrations ± SEM during and following infusions of 0.1, 0.2, 0.4, 0.6, 0.9 and 1.5 g/kg ascorbic acid. Each point represents the mean value for either 5 or 6 patients.

detected in blood cells following i.v. ascorbic acid infusions. Because high plasma ascorbic acid concentrations can interfere with several biochemical analyses, samples other than for the Heinz body preparation were taken before the ascorbic acid infusions. No unusual biochemical or hematologic abnormalities were observed. The median serum creatinine concentration of all the participants was 72 μmol/l and the highest concentration of any patient having a pharmacokinetic study was 126 μmol/l; there was no trend for the serum creatinine concentration to increase during the course of the study. Patients in the 1.5-g/kg cohort excreted 81.3 ± 18.8 mg of oxalic acid during and over the 6 h following the infusion. Oxalic acid excretion normally ranges from 10 to 60 mg/24 h [29]. The methodology developed to analyze oxalic acid in the presence of extremely high ascorbic acid concentrations and the urinary oxalic acid excretion profiles that resulted from the ascorbic acid infusions are reported separately. Unlike in an earlier Scottish case series [1], acute tumor hemorrhage and necrosis were not observed in this study.

Three patients failed to complete two cycles and hence could not be evaluated for response. None of the other patients had an objective tumor response, and all of them eventually experienced progression. Two patients at the 0.6-g/kg dose (one with prostate cancer and the other with epidermoid carcinoma) received greater than six cycles of ascorbic acid with stable disease (less than a 20% reduction and less than a 20% increase in the sum of the two perpendicular diameters of the target lesion and the appearance of no new lesions). As assessed by the FACT-G questionnaire, patients in the 0.4-g/kg cohort experienced a significant deterioration in physical function over the course of the study (5.4 ± 4.2 versus 13.4 ± 1.1, mean ± SD, $P < 0.01$ by Mann–Whitney test), but there was no deterioration in physical function among patients in the higher dose cohorts. There were no changes in the social, emotional or functional parameters of quality of life in any cohort.

pharmacokinetics

Pharmacokinetic profiles were obtained for six patients infused with 0.1, 0.2, 0.4, 0.6 and 0.9 g/kg and five patients infused 1.5 g/kg. During the continuous infusion, plasma ascorbic acid concentrations rose from normal values (<0.1 mmol/l)

Table 3. Ascorbic acid plasma concentrations and urinary excretion

Dose (g/kg)	0.1	0.2	0.4	0.6	0.9	1.5
Peak plasma concentration (mmol/l)	2.4 ± 0.3	4.7 ± 0.5	8.5 ± 0.6	11.3 ± 2.4	17.0 ± 3.6	26.2 ± 4.9
Percent of dose excreted during infusion	20 ± 6	23 ± 8	24 ± 5	27 ± 8	25 ± 8	25 ± 11
Peak concentration predicted from dose and excretion[a]	2.5 ± 0.4	4.6 ± 0.6	9.2 ± 0.9	12.3 ± 1.2	19.1 ± 1.9	28.0 ± 4.9
Peak concentration predicted from dose alone[b]	2.4 ± 0.2	4.4 ± 0.3	9.0 ± 0.4	12.8 ± 0.8	19.2 ± 0.5	28.2 ± 3.0

Values are expressed as the mean ± SD.

[a](Dose − urinary excretion)/estimated extracellular volume.

[b](Dose − 0.25 dose)/estimated extracellular volume.

to concentrations which peaked at the end of the infusion (Figure 1). As shown in Table 3, the peak ascorbic acid concentrations predicted from dose, volume of distribution and excretion were within 10% of the actually measured concentrations. Upon noting that ~25% of the ascorbic acid was excreted over the course of the infusion irrespective of dose, we tested the even simpler prediction that the peak ascorbic acid concentration (g/l) is equal to $[D - D/4]/0.2\ W = 3.75\ D/W$, where D is the ascorbic acid dose (g) and W is body weight (kg). This formula provided equivalent information (see Table 3).

The ascorbic acid concentration–time product over the entire 7.5–8 h measurement period was directly proportional to the dose (see Figure 2). A given dose–time product (mmol hr/l) can be predicted with acceptable clinical accuracy by multiplying the dose in g/kg by 62.

Figure 3 illustrates the number of hours during which plasma ascorbic acid concentrations exceeded 5, 10 and 15 mmol/l for different ascorbic acid doses. When the recommended phase II dose was administered, plasma ascorbic acid concentrations exceeded 5 mmol/l for ~7 h and exceeded 10 mmol/l for ~4.5 h. The length of time plasma ascorbic acid concentrations exceeded 15 mmol/l was brief and variable.

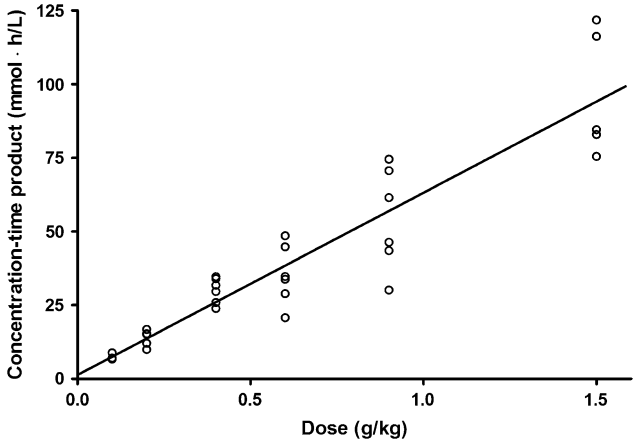

Figure 2. Ascorbic acid concentration–time product (mmol h/l) for each ascorbic acid infusion. The concentration–time product is directly proportional to the dose per kilogram body weight. $y = (61.85 \pm 4.15)\ x + 1.35 \pm 3.10$; $r^2 = 0.870$, $P < 0.0001$.

discussion

Intravenous ascorbic acid, administered in a dose of 1.5 g/kg three times weekly, appears to be safe and free of important toxicity in appropriately screened patients with advanced untreatable malignancies, and sustains plasma ascorbic acid concentrations >10 mmol/l for >4 h in patients with normal renal function. In part, because apoptotic cell death occurs in many cancer cell lines exposed to ascorbate concentrations >5 mmol/l for <1 h [6], 1.5 g/kg was adopted as the recommend dose for future phase II trials. This dose cannot be considered the maximum tolerable dose, for larger and more frequent doses might also have been tolerated. However, higher doses would have been problematic because the sodium load, plasma osmolality and duration of the infusion increases in proportion to the administered dose. The recommended phase II dose of 1.5 g/kg was therefore selected because of its adequate pharmacokinetic profile and clinical practicality.

It became clear during the conduct of the study that virtually all the side-effects that occurred were consistent with the side-effects attending the rapid infusion of any high-osmolarity solution. The symptoms were preventable by encouraging patients to drink fluids before and during the infusion. Indeed, rather than provoking fluid overload, ascorbic acid acted like an osmotic diuretic which could induce volume depletion if patients do not compensate by increasing their voluntary fluid intake. Contraindications to the infusion of very high osmolarity ascorbic acid infusions would be the same as for other osmotic diuretics: anuria, dehydration, severe pulmonary congestion or pulmonary edema and a fixed low cardiac output.

Patients whose ascorbic acid dose was ≥0.6 g/kg maintained their physical quality of life throughout the trial, whereas those at the lowest dose did not. The potential clinical significance of this observation is unclear because it has no defined biological rationale, and we cannot exclude that it may be a chance occurrence.

The simple noncompartmental pharmacokinetic analysis carried out for this report provides useful guidelines for dose selection in future clinical trials. However, even though the peak plasma ascorbic acid concentration and the plasma concentration–time product can be accurately predicted using the simple formulas developed from our data, the peak concentration will be less than predicted in people whose extracellular volume is expanded and higher than predicted in very obese patients whose extracellular fluid concentration is a smaller fraction of their total body weight. Plasma ascorbic

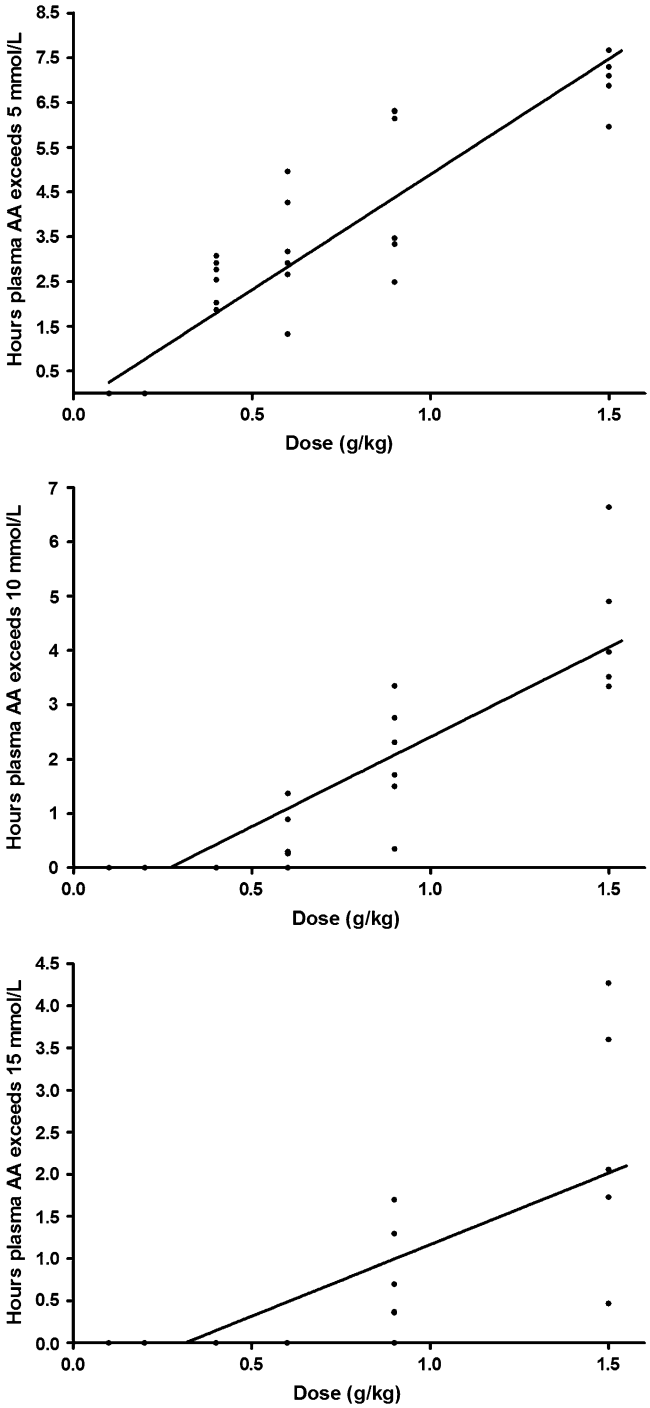

Figure 3. Number of hours during which the plasma ascorbic acid concentration exceeded 5, 10 and 15 mmol/l for different ascorbic acid doses.

acid concentrations can be dramatically reduced following some chemotherapy regimens [30] and in critical illness [31], so the plasma and urinary profiles observed in this study are not likely to apply to those situations. Finally, it should be borne in mind that the median serum creatinine concentration of the participants in this study was 72 μmol/l, and the highest concentration during a pharmacokinetic study was 126 μmol/l. Patients with impaired renal function will have higher and more sustained plasma ascorbic acid concentrations.

In summary, this study shows that 1.5 g/kg ascorbic acid infused >90–120 min three times weekly is essentially free of risk and important side-effects when simple precautions are taken. In people with normal renal function, this dose achieves a plasma ascorbic acid concentration >10 mmol/l for several hours. No patient experienced an objective anticancer response, although two patients at the 0.6-g/kg dose received greater than six cycles of ascorbic acid with stable disease. Even though only six patients received the recommended phase II dose, our results suggest that the likelihood of an objective anticancer response to i.v. ascorbic acid alone is slight in unselected patients with multiply treated advanced cancer. The promise of ascorbic acid in the treatment of advanced cancer may lie in combination with cytotoxic agents, where high concentrations of this redox-active compound might modify either toxicity or response [19, 32, 33]. We are currently planning a phase I–II clinical trial that will combine i.v. ascorbic acid with chemotherapy as first-line treatment in advanced stage non-small-cell lung cancer, using the dose determined from this study.

funding

Lotte and John Hecht Memorial Foundation.

references

1. 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.
2. Block KI, Mead MN. Vitamin C in alternative cancer treatment: historical background. Integr Cancer Ther 2003; 2: 147–154.
3. Padayatty SJ, Riordan HD, Hewitt SM et al. Intravenously administered vitamin C as cancer therapy: three cases. CMAJ 2006; 174: 937–942.
4. Bram S, Froussard P, Guichard M et al. Vitamin C preferential toxicity for malignant melanoma cells. Nature 1980; 284: 629–631.
5. Sestili P, Brandi G, Brambilla L et al. Hydrogen peroxide mediates the killing of U937 tumor cells elicited by pharmacologically attainable concentrations of ascorbic acid: cell death prevention by extracellular catalase or catalase from cocultured erythrocytes or fibroblasts. J Pharmacol Exp Ther 1996; 277: 1719–1725.
6. Chen Q, Espey MG, Krishna MC 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 USA 2005; 102: 13604–13609.
7. Song EJ, Yang VC, Chiang CD, Chao CC. Potentiation of growth inhibition due to vincristine by ascorbic acid in a resistant human non-small cell lung cancer cell line. Eur J Pharmacol 1995; 292: 119–125.
8. Kurbacher CM, Wagner U, Kolster B et al. Ascorbic acid (vitamin C) improves the antineoplastic activity of doxorubicin, cisplatin, and paclitaxel in human breast carcinoma cells in vitro. Cancer Lett 1996; 103: 183–189.
9. Kassouf W, Highshaw R, Nelkin GM et al. K3 sensitize human urothelial tumors to gemcitabine. J Urol 2006; 176: 1642–1647.
10. Grad JM, Bahlis NJ, Reis I et al. Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. Blood 2001; 98: 805–813.
11. Abdel-Latif MM, Raouf AA, Sabra K et al. Vitamin C enhances chemosensitization of esophageal cancer cells in vitro. J Chemother 2005; 17: 539–549.
12. Sarna S, Bhola RK. Chemo-immunotherapeutical studies on Dalton's lymphoma in mice using cisplatin and ascorbic acid: synergistic antitumor effect in vivo and in vitro. Arch Immunol Ther Exp 1993; 41: 327–333.

13. Verrax J, Stockis J, Tison A et al. Oxidative stress by ascorbate/menadione association kills K562 human chronic myelogenous leukaemia cells and inhibits its tumour growth in nude mice. Biochem Pharmacol 2006; 72: 671–680.
14. Taper HS, Jamison JM, Gilloteaux J et al. Inhibition of the development of metastases by dietary vitamin C:K3 combination. Life Sci 2004; 75: 955–967.
15. Lim D, Morgan RJ Jr, Akman S et al. Phase I trial of menadiol diphosphate (vitamin K3) in advanced malignancy. Invest New Drugs 2005; 23: 235–239.
16. Verrax J, Vanbever S, Stockis J et al. Role of glycolysis inhibition and poly(ADP-ribose) polymerase activation in necrotic-like cell death caused by ascorbate/menadione-induced oxidative stress in K562 human chronic myelogenous leukemic cells. Int J Cancer 2007; 120: 1192–1197.
17. Tetef M, Margolin K, Ahn C et al. Mitomycin C and menadione for the treatment of lung cancer: a phase II trial. Invest New Drugs 1995; 13: 157–162.
18. Diaz Z, Colombo M, Mann KK et al. Trolox selectively enhances arsenic-mediated oxidative stress and apoptosis in APL and other malignant cell lines. Blood 2005; 105: 1237–1245.
19. Doroshow JH. Redox modulation of chemotherapy-induced tumor cell killing and normal tissue toxicity. J Natl Cancer Inst 2006; 98: 223–225.
20. Levine M, Rumsey SC, Daruwala R et al. Criteria and recommendations for vitamin C intake. JAMA 1999; 281: 1415–1423.
21. Riordan NH, Riordan HD, Meng X et al. Intravenous ascorbate as a tumor cytotoxic chemotherapeutic agent. Med Hypotheses 1995; 44: 207–213.
22. Riordan HD, Hunninghake RB, Riordan NH et al. Intravenous ascorbic acid: protocol for its application and use. P R Health Sci J 2003; 22: 287–290.
23. Gonzalez MJ, Miranda-Massari JR, Mora EM et al. Orthomolecular oncology review: ascorbic acid and cancer 25 years later. Integr Cancer Ther 2005; 4: 32–44.
24. Riordan HD, Casciari JJ, Gonzalez MJ et al. A pilot clinical study of continuous intravenous ascorbate in terminal cancer patients. P R Health Sci J 2005; 24: 269–276.
25. Chen Q, Espey MG, Sun AY et al. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. Proc Natl Acad Sci USA 2007; 104: 8749–8754.
26. Levine M, Wang Y, Rumsey SC. Analysis of ascorbic acid and dehydroascorbic acid in biological samples. Meth Enzymol 1999; 299: 65–76.
27. Dhariwal KR, Hartzell WO, Levine M. Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. Am J Clin Nutr 1991; 54: 712–716.
28. Hamadeh MJ, Robitaille L, Boismenu D et al. Human extracellular water volume can be measured using the stable isotope $Na_2{}^{34}SO_4$. J Nutr 1999; 129: 722–727.
29. Painter PC, Cope JY, Smith JL. Reference information for the clinical laboratory. In Burtis CA, Ashwood ER (eds): Tietz Fundamentals of Clinical Chemistry. Philadelphia: Saunders 2001; 955–1028.
30. Marcus SL, Petrylak DP, Dutcher JP et al. Hypovitaminosis C in patients treated with high-dose interleukin 2 and lymphokine-activated killer cells. Am J Clin Nutr 1991; 54: 1292S–1297S.
31. Schorah CJ, Downing C, Piripitsi A et al. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. Am J Clin Nutr 1996; 63: 760–765.
32. Drisko JA, Chapman J, Hunter VJ. The use of antioxidant therapies during chemotherapy. Gynecol Oncol 2003; 88: 434–439.
33. Fang J, Nakamura H, Iyer AK. Tumor-targeted induction of oxystress for cancer therapy. J Drug Target 2007; 15: 475–486.