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

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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 cytotoxic 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 administered 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.
**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 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
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 \( \mu \text{mol/l} \) and the highest concentration of any patient having a pharmacokinetic study was 126 \( \mu \text{mol/l} \); 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 \( \pm \) 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 \( \pm \) 4.2 versus 13.4 \( \pm \) 1.1, mean \( \pm \) SD, \textit{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, 0.9 and 1.5 g/kg ascorbic acid. Each point represents the mean value for either 5 or 6 patients.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>No. in cohort</th>
<th>Adverse event</th>
<th>Number affected</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>5</td>
<td>Abdominal cramps</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diarrhea</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nausea</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vomiting</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.6</td>
<td>6</td>
<td>Headache</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.9</td>
<td>7</td>
<td>Dizziness</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fatigue</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Facial flushing</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perspiration</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nausea</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weakness</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1.5</td>
<td>6</td>
<td>Dizziness</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Facial flushing</td>
<td>1</td>
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<td>Headache</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nausea</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

![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.](image-url)
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 $[D - D/4]/0.2W = 3.75D/W$, 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 h/l) can be predicted with acceptable clinical accuracy by multiplying the dose in g/kg by 62.

Table 3. Ascorbic acid plasma concentrations and urinary excretion

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

Values are expressed as the mean ± SD.

$^a$(Dose – urinary excretion)/estimated extracellular volume.

$^b$(Dose – 0.25 dose)/estimated extracellular volume.

**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
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**

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**references**


