Phase I Clinical Trial of Intravenous L-ascorbic Acid Following Salvage Chemotherapy for Relapsed B-cell non-Hodgkin's Lymphoma

Hiroshi KAWADA[*1, 2], Masakazu SAWANOBORI[*1], Mitsuyo TSUMA-KANEKO[*1, 2], Izumi WASADA[*1], Mitsuki MIYAMOTO[*1], Hiromichi MURAYAMA[*1], Masako TOYOSAKI[*1], Makoto ONIZUKA[*1, 2], Kosuke TSUBOI[*1], Kei TAZUME[*1], Yukari SHIRASUGI[*1], Ken OHMACHI[*1], Yoshiaki OGAWA[*1], Hiroyuki KOBAYASHI[*3] and Kiyoshi ANDO[*1, 2]

[*1]*Division of Hematology/Oncology, Department of Medicine,* [*2]*Research Center for Regenerative Medicine,* [*3]*Department of Pharmacology, Tokai University School of Medicine*

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Purpose: To determine the safety and the appropriate dose of intravenous L-ascorbic acid (AA) in conjunction with chemotherapy for patients with relapsed lymphoma.
Patients and Methods: Patients with relapsed CD20-positive B-cell non-Hodgkin's lymphoma, who were going to receive the CHASER regimen as salvage therapy, were enrolled and treated with escalating doses of AA administered by drip infusion after the 2nd course of the CHASER regimen. The target plasma concentration immediately after AA administration was >15 mM (264 mg/dl).
Results: A serum AA concentration of >15 mM was achieved in 3 sequentially registered patients, all of whom had received a 75 g whole body dose. No obvious adverse drug reaction was observed in the patients. The trial was therefore successfully completed.
Conclusion: Intravenous AA at a whole body dose of 75 g appears to be safe and sufficient to achieve an effective serum concentration. A phase II trial to evaluate the efficacy of intravenous AA in relapsed/refractory lymphoma patients will now be initiated.

Key words: intravenous L-ascorbic acid, relapsed B-cell non-Hodgkin's lymphoma, phase I clinical trial

INTRODUCTION

The relapse-free survival of patients who are initially diagnosed as having CD20-positive B-cell non-Hodgkin's lymphoma (NHL) has been improved by the addition of rituximab to conventional CHOP chemotherapy [1–3]. However, the management of patients who suffer relapse of this disease requires additional therapeutic strategies.

Recently, intravenous administration of high ascorbic acid (AA) doses (high AA) has been shown to be a potential alternative cancer therapy. AA acts as an antioxidant and plays a key role in protecting cells against oxidative damage. Paradoxically, in the presence of Fe^{3+} or Cu^{2+} ions, AA generates hydrogen peroxide (H_2O_2), a prooxidant [4]. High AA has been shown to exert remarkable anti-cancer effects through generating significant amounts of H_2O_2 in the extracellular fluid of tumor-bearing animals [5, 6], and recent clinical studies have also demonstrated that intravenous high AA has an antitumor effect in patients with a number of different cancers [7, 8]. Different routes of AA administration result in significantly different plasma concentrations, and intravenous administration results in a 70-fold higher plasma concentration than oral administration [9]. Furthermore, the cytotoxic effects of high AA appear to be specific to cancer cells, reflecting their relatively low catalase activity compared to normal cells [6]. We have also demonstrated that high AA effectively induces apoptosis in hematopoietic malignancies and represses hypoxia-inducible factor 1α (HIF-1α) expression in neoplastic cells, but not in normal hematopoietic progenitor and stem cells [10].

High AA is currently used worldwide, mainly by Complementary and Alternative Medicine practitioners [11]. However, most safety and efficacy information concerning high AA has been provided by them in the form of anecdotal accounts and case reports [7, 8, 12, 13]. Very few clinical trials of high AA have been performed to date [14], and, to our knowledge, there have been no clinical trials of high AA in Japan.

Accordingly, we conducted a phase I clinical trial of high AA in patients with relapsed NHL.

PATIENTS AND METHODS

Study population

This study was approved by the Clinical Research Review Committee of Tokai University School of Medicine. Patients with relapsed B-cell NHL expressing the CD20 antigen were enrolled onto this study. The other main inclusion criteria were that patients should be 20–75 years old, have an Eastern Cooperative Oncology Group performance score ≤ 2, an absolute neutrophil count ≥1,000/μL, a platelet count ≥100,000/μL, and adequate pulmonary, cardiac,

Hiroshi KAWADA, Division of Hematology/Oncology, Department of Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan Tel: +81-463-93-1121 ext. 2232 E-mail: hkawada@is.icc.u-tokai.ac.jp

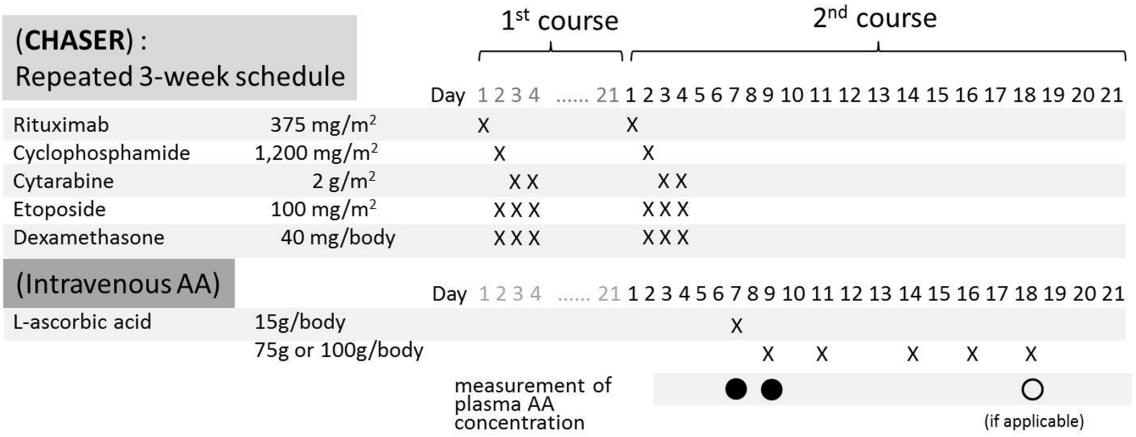

Fig. 1 Study protocol.
The CHASER regimen [25] was performed using 3-week treatment cycles. L-ascorbic acid (AA) was administered intravenously on days 7, 9, 11, 14, 16, and 18 during the 2nd course of the CHASER regimen. A small starting dose of 15 g AA was administered on day 7, and then 75 g or 100 g of AA was administered on the subsequent treatment days. The serum concentration of AA was measured on day 7 immediately prior to administration and on day 9 immediately after administration.

renal, and liver function. Each patient provided signed informed consent before enrollment.

AA preparation

In this study, we used MEGA-C-ACID PLUS® (500 mg/ml, Merit Pharmaceuticals, Los Angeles, California), a preservative-free AA preparation that was imported from the USA after approval by the Ministry of Health, Labour and Welfare, Japan (Yakkan certificate for import of medicines and medical devices). This source of AA was used because other clinical preparations in Japan contained preservatives, and there was no data regarding the safety of these substances when administrated at high doses.

Study design

This was a phase I, open-label, dose-escalation study of AA. AA was given through a central vein catheter on days 7, 9, 11, 14, 16, and 18 during the 2nd course of the CHASER regimen (Fig. 1). A small test dose of 15 g AA in 250 ml Ringer's lactate solution was administered at a rate of 0.5 g/min on day 7, and then 75 g of AA dissolved in 1,000 ml of distilled water was administered at a rate of 1 g/min on each of the other days listed above. Adverse drug reactions (ADRs) were assessed based on ADR grades defined by the Ministry of Health, Labour and Welfare, Japan. The dose-escalation scheme was guided by both the safety evaluation and the serum concentration of AA measured immediately after administration (Fig. 2).

Because hemolysis due to AA administration could occur in patients with a red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency [15], G6PD activity was tested using a G6PD Assay Kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's protocol, on all patients before beginning AA infusion. In order to prevent hypocalcemia due to the chelating effect of AA, 0.5 g magnesium sulfate was added to every 500 ml of AA solution.

Target concentration (TC)

In healthy subjects the plasma AA concentration has been shown to peak immediately after intravenous administration of high AA, and then to decrease to approximately one fifth of this level within 4 hours due to rapid clearance [9]. Based on information supplied by Dr. Jeanne A. Drisko, the University of Kansas Medical Center, we initially decided upon a TC of ≥ 20 mM (350 mg/dl). However, it was previously found that the AA concentration needed to cause a 50% decrease in cell survival (EC50) was less than 5 mM for several cancer cell lines, including the lymphoma cell line JLP119, and was less than 15 mM for most of the other cancer cell lines tested [8]. Therefore, we reduced the TC from ≥20 mM (350 mg/dl) to ≥15 mM (264 mg/dl) after the beginning of this trial, after this change was approved by the Clinical Research Review Committee of Tokai University School of Medicine.

RESULTS

Three patients with relapsed B-cell NHL were enrolled in this study. The patients' characteristics are shown in Table. The CHASER regimen was generally well tolerated, with hematologic toxicities being grade 3 neutropenia and grade 3 anemia and thrombocytopenia requiring transfusion in the 3 patients. Serum AA concentrations were measured just before the start of AA administration on day 7 of the 2nd course of CHASER regimen, and were within the normal range (0.55–1.68 mg/dl) (Table). A small starting dose of 15 g AA was then administered, and no obvious ADRs were observed. Therefore, the AA dose was escalated to 75 g from day 9. Serum AA concentrations immediately after AA administration were measured on day 9, and the TC (≥15 mM) was achieved in all 3 patients (Table). We also measured the AA concentration immediately after AA administration on day 18 in 2 of the 3 patients, and reconfirmed that serum AA reached the TC (Table). There were no obvious adverse reactions caused by AA administration, including exacerbation of myelosuppression after the CHASER

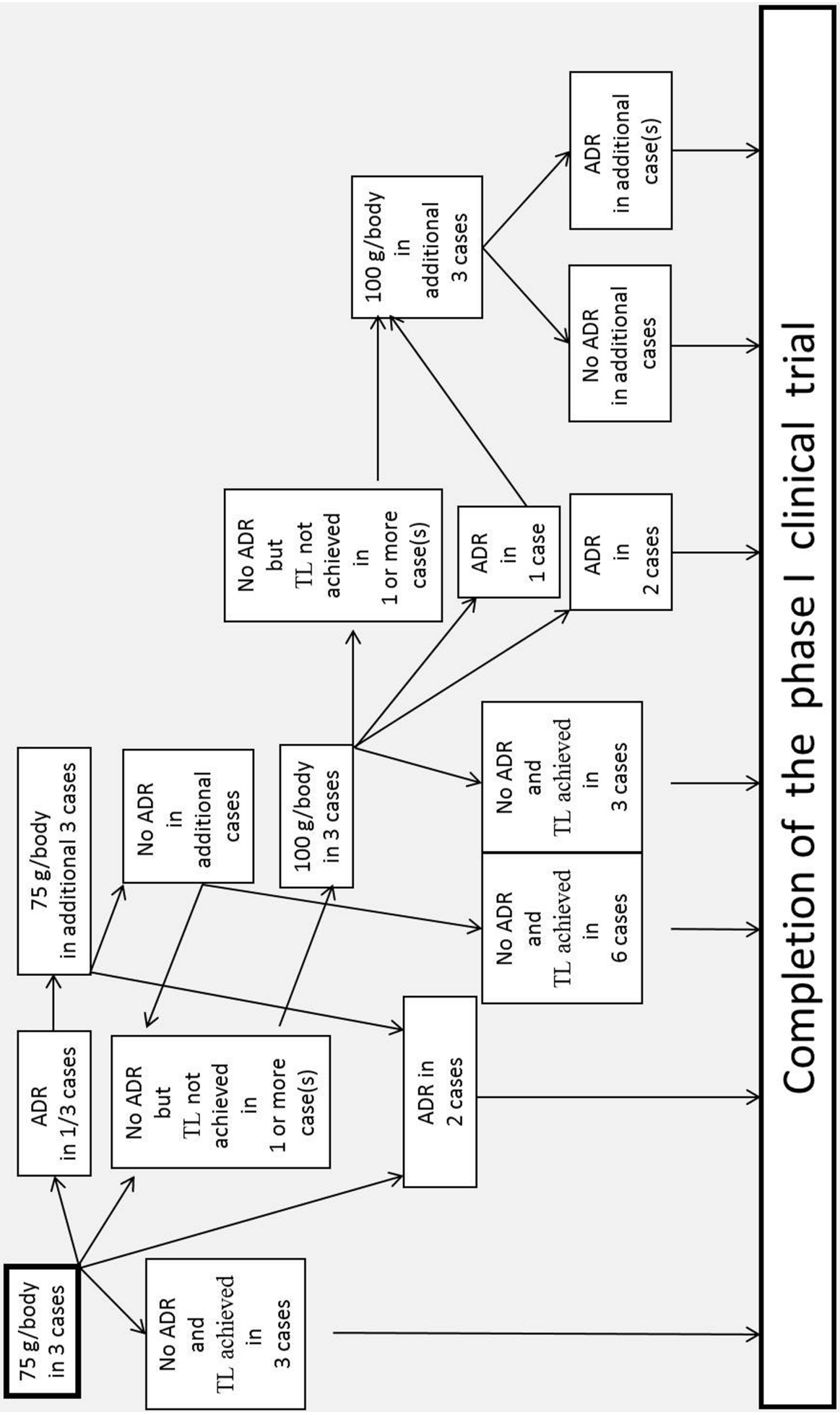

Fig. 2 Trial schematic.
A whole body dose of 75 g L-ascorbic acid (AA) was administered in 3 sequential cases. The serum concentrations of AA measured on day 9 reached the target concentration (TC) and there were no grade 2 or worse adverse drug reactions (ADRs).

Table Patient characteristics and measurement of plasma AA concentration

Patient no.	1	2	3
Age	60	72	57
Sex	Female	Male	Male
Performance status	0	1	1
Histology	MCL	MCL	DLBCL
Stage at study entry	IV_A	IV_A	III_B
Previous therapy	Hyper-CVAD	R-CHOP	R-CHOP+irradiation
Plasma AA concentration*			
On day 7	0.72	1.01	1.16
On day 9	313.5	339.0	302.1
On day 18	447.3	344.1	not measured

MCL, mantle cell lymphoma; DLBCL, diffuse large B-cell lymphoma. Hyper-CVAD consists of cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, and cytarabine, R-CHOP consists of rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisolone. *Plasma L-ascorbic acid (AA) concentration (mg/dl) was measured on day 7 immediately prior to administration, on day 9 immediately after administration, and optionally on day 18.

regimen, in any of the 3 patients.

It was concluded that AA at a dose of 75 g/body was tolerable and suitable for clinical use, and the phase I study was completed.

DISCUSSION

Pauling and Cameron first reported the possibility of a role for AA in cancer therapy more than 30 years ago. When AA was given intravenously to cancer patients for 10 days and then orally in pharmacologic doses of 10 g daily, it was effective in treating some cancers and in improving patient survival [16, 17]. However, the same oral dose had no therapeutic effects on cancer patients in 2 subsequent double-blind placebo-controlled trials [18, 19], and many oncologists therefore dismissed this. However, the efficacy of AA in cancer needs to be reassessed, as the plasma concentrations of AA are severely limited when it is administered orally, even at the highest tolerated dose, and intravenous administration of AA can overcome this limitation [9, 20, 21]. In the present study, the plasma concentrations of AA increased more than 250-fold after intravenous administration of 75 g AA.

Other than the known complications of high AA in those with renal impairment or glucose 6 phosphate dehydrogenase deficiency, i.e. acute renal failure and severe hemolysis, high AA is reported to be remarkably safe [11]. Padayatty *et al.* assessed the ADRs of high AA in the USA in 2006 and 2008, and of 9,328 patients, 101 (1.1%) suffered ADRs, which were mostly minor, including lethargy/fatigue in 59 (0.6%) patients, change in mental status in 21 (0.2%) patients and vein irritation/phlebitis in 6 (0.1%) patients. Other recorded ADRs were kidney stone in 3 patients, hemolysis in 2 patients, elevated blood glucose in 2 patients, and muscle cramps, headache, nausea/vomiting, flu like syndrome, syncope, and pain at the tumor site were each observed in single and separate cases [11]. In this study, we did not observe any obvious ADRs resulting from high AA treatment, including those mentioned above. Because the high AA solutions are hypertonic, we ensured that the infusion rate was low enough to prevent the estimated tonicity exceeding 1200 milliosmal (mOsm) according to the dose conversion table, and hemolysis could be therefore be avoided. Vein irritation/phlebitis was also avoided because high AA was administered through a central venous catheter.

In this study, a whole body dose of 75 g was considered to be sufficient to have an antitumor effect. However, this dose is probably not the maximum tolerable dose, and thus it may be possible to administer even higher doses, although this might be problematic due to the large volume and sodium load. Further, the 75 g whole body dose is very similar to that recommended previously by another group [14]. They reported that, based on a phase I clinical trial in patients with advanced malignancies including 4 lymphoma patients, 1.5 g/ kg body weight of AA was well tolerated and should be adopted in future phase II trials.

We and other investigators previously demonstrated that high AA generated H_2O_2 extracellularly, and that this acted as a prooxidant, which selectively killed a number of different cancer cell types. The basis for this selective cell killing is thought to be the relatively low level of catalase activity in cancer cells compared to normal cells [10, 22, 23]. Furthermore, while HIF-1α plays an important role in the growth and survival of hematopoietic malignancies, we also found that the down-regulation of HIF-1α transcription mediates the growth inhibition of human leukemic cells by high AA, and is specific to malignant cells [10]. Therefore, high AA is considered to be a promising alternative therapy against cancers, including hematopoietic malignancies.

Despite reported successes, the anticancer effects of high AA vary among cancers and patients [5, 8, 14]. The number of neoplastic cells or normal erythrocytes and fibroblasts around neoplastic cells inversely correlates with the efficacy of high AA due to the correspondingly increased catalase activity against H_2O_2 [24]. We have also observed this phenomenon in our experimental model (unpublished observation). Therefore, a combination of high AA and other anticancer drugs that compensate for H_2O_2 decomposition could be a better strategy for eliminating cancer cells. We are currently planning a phase II study to assess the efficacy of this combination therapy in patients with relapsed/refractory lymphomas.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors have no potential conflicts of interest to disclose.

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