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The safety and pharmacokinetics of high dose intravenous ascorbic acid synergy with modulated electrohyperthermia in Chinese patients with stage III-IV non-small cell lung cancer

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ABSTRACT

Ascorbic acid (AA) infusion and modulated electrohyperthermia (mEHT) are widely used by integrative cancer practitioners for many years. However, there are no safety and pharmacokinetics data in Chinese cancer patients. We carried out a clinical trial to evaluate the safety and pharmacokinetics of those methods in patients with stage III-IV non-small cell lung cancer (NSCLC). Blood ascorbic acid in the fasting state was obtained from 35 NSCLC patients; selecting from them 15 patients with stage III-IV entered the phase I study. They were randomized allocated into 3 groups, and received doses 1.0, 1.2, 1.5 g/kg AA infusions. Participants in the first group received intravenous AA (IVAA) when mEHT was finished, in the second group IVAA was administered simultaneously with mEHT and in the third group IVAA was applied first, and followed with mEHT. Pharmacokinetic profiles were obtained when they received solely IVAA and when IVAA in combination with mEHT. The process was applied 3 times a week (every other day, weekend days off) for 4 weeks. We found that fasting plasma AA levels were significantly correlated with stage of the disease. Peak concentration of AA was significantly higher in the simultaneous treatments than in other combinations with mEHT or in solely IVAA-managed groups. IVAA synergy with simultaneous mEHT is safe and the concomitant application significantly increases the plasma AA level for NSCLC patients.

1. Introduction

Lung cancer is the most common cancer type and the leading cause of cancer mortality in China, accounting for 19.59% of all newly diagnosed cancer cases (Chen et al., 2015). Nearly 80% of lung cancers are non-small cell lung cancer (NSCLC). The majority of patients diagnosed with advanced NSCLC are not suitable for surgery. Chemotherapy and tyrosine kinase inhibitors (TKIs) are the first recommendations for these patients. However, side effects including diarrhea, neuropathy, neutropenia, skin reaction, and weight loss are frequently accompanied by chemotherapy and TKIs, which are the major reasons for patients stopping treatments.

Ascorbic acid (AA) infusion and modulated electrohyperthermia (mEHT) are widely used by integrative and complementary cancer practitioners for many years. High-doses ascorbic acid infusion means the use of ascorbate, administered IV, to achieve plasma levels of ascorbate on the order of 100–1000 times that of healthy nutritional levels (Welsh et al., 2013). A Phase I study showed the safety of high dose AA (Kawada et al., 2014), and the safety of mEHT has also been shown for most heat-sensitive organs, such as the brain (Wismeth et al., 2010). High dose AA might have a prophylactic effect against lung cancer, as suggested by a meta-analysis (Luo et al., 2014) of 18 articles, including 21 studies involving 8938 lung cancer cases. Estimates based on dose-response analysis (Luo et al., 2014) show a risk reduction in lung cancer by 7% for every 100 mg/day increase in the intake of AA. The refractory cases may also be re-sensitized by AA (Chiang et al., 1994). The high dose intravenous AA (IVAA) produces high blood-plasma concentrations that could be 70- to 100-fold higher than the maximally tolerated oral doses (Creagan et al., 1979). Only IVAA produces high plasma and urine concentrations of ascorbates (Padayatty et al., 2004). The expected peak of 60 g IVAA administration is about 25 mmol/L in the plasma at tmax ≈ 90 min (Duconge et al., 2008).

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Laboratory studies (Chen et al., 2008) showed that pharmacological concentrations achieved by high-dose AA have redox properties and can decrease cell proliferation in lung cancer cell lines. Different studies have reported the anticancer mechanisms induced by IVAA, which include apoptosis (Carosio et al., 2007), DNA damage and ATP depletion (Ma et al., 2014), and cell cycle arrest (Chen et al., 2005). Human studies (Hoffer et al., 2008; Riordan et al., 2005; Stephenson et al., 2013) indicated that high-dose IVAA infusions are safe and well tolerated, and can modulate inflammation, improving outcomes for cancer patients (Mikirova et al., 2013). Clinical studies (Ma et al., 2014; Monti et al., 2012; Welsh et al., 2013) have suggested that large doses of IVAA can increase its efficacy or reduce toxic side effects from chemo drugs when used in synergy with chemotherapy. The action of AA was a mystery for a long time (Naidu, 2003) due to its declared anti-oxidant effects, but it also shows pro-oxidant behavior as well (Podmore et al., 1998; Putchala et al., 2013). Its real action depends on the physiological conditions and plasma-concentration (Carr and Frei, 1999).

Synergy of hyperthermia with high-dose AA has been shown in vitro (Saitoh et al., 2015), expecting the same clinical results. mEHT has long been used in clinical practice for various malignant diseases (Baronzio et al., 2014). It is based on the modulated electric field effect, which works in synergy with heat (Andocs et al., 2009). The key advantage of this method is the nano-range energy liberation instead of overall heating of the target (Andocs et al., 2015). It is a descendant of hyperthermia initially based on nano-thermal but not temperature-dependent effects of electromagnetic fields and special fractal modulation, whose effect could exceed the effect of the overall heating, the macroscopic temperature elevation, by 3–4 times (Szasz, 2012). mEHT does not require homogenous hyperthermia-range temperatures in the tumor; it selects the malignant cells by heterogenic heating.

Previous hyperthermia studies showed the benefits of hyperthermia applied complementary to chemo and radio-therapies (Falk and Issels, 2001). A previous study indicated the linear increase in the cytotoxic effect of alkylating agents (e.g. ifosfamide) and the platinum chemo drugs when temperatures are elevated from 37 °C to over 40.5 °C (Hildebrandt et al., 2002). The hyperthermia method mEHT has shown its capability for NSCLC in clinical studies (Szasz, 2014). Consequently, the treatment could be performed safely without invasive thermal control. These facts motivated our team to conduct a Phase I clinical trial to evaluate the safety and pharmacokinetics of high dose intravenous ascorbic acid synergy with mEHT in patients with NSCLC.

2. Patients and methods

2.1. Study population & eligibility criteria

Patients were required to have histologically confirmed non-small cell lung cancer, diagnosed with primary lung cancer, stage III and IV. Patients were progression after radio and/or chemotherapy, or fail to respond to other conventional therapies. When they receive IVAA and mEHT, patients cannot simultaneously receive anticancer therapies during treatment period (for almost 2 months). Patients who can tolerate the above treatments and with the ECOG performance status of 0 to 2 were enrolled into the study. Patients were excluded if they had G6PD deficiency or a history of oxalosis by urinalysis, since high dose ascorbic acid infusions may cause red blood cell hemolysis in G6PD deficient candidates and kidney stones in patients with a preexisting risk of oxalate stones. Patients with a co-morbid condition that would affect survival such as end stage congestive heart failure, unstable angina, myocardial infarction within the past 6 weeks of the study were excluded as well. Other exclusion criteria included metallic implants or replacements in the treatment area and electronic implanted devices anywhere in the body. Metallic implants that are located in or on or attached to the patient's body may be excessively (and preferentially) heated or may contribute to excessive heating of the tissues contacting the metal implant or the electrical conductors and this may cause severe burns and patient injury. The radio frequency power from mEHT System and applicators can interfere with active device implants, it could present a risk to patients who have a pacemaker or other electronic device implants if they receive this treatment. Detail information of inclusion criteria and exclusion criteria were recorded in the study protocol (Supplementary Clinical Trial Protocol).

The protocol and consent form were approved by the Ethics Committee of the Clifford Hospital.

2.2. Study design

The study is a single center, Phase I, Single Blind, Randomized clinical trial. Trial Registration: ClinicalTrials.gov, NCT02655913, Registration date: 7th Jan, 2016. Date of enrolment of the first participant to the trial: 17th Jan, 2016. All participants were recruited by Clifford Hospital.

The preparative end-point of the study was to find out whether there is a relationship between fasting blood ascorbic acid and degree of tumor burden in NSCLC patients. No mEHT and IVAA were applied to study this point.

The primary endpoint of the treatment trial was to evaluate the safety, tolerability and pharmacokinetics of high dose intravenous ascorbic acid synergies with mEHT for patients with stage III-IV NSCLC.

Based on previous studies, the interaction of heat with different kind of drugs and different drug-heat sequence can achieve different clinical results (Hildebrandt et al., 2002). However, there's no relevant research study for the interaction effect of heat with intravenous ascorbic acid. Thus, the third end-point was to identify the best pattern and sequence of intravenous AA synergies with mEHT for the preparation of a phase II study, and to evaluate the maintenance or improvement in quality of life by using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (QLQ-C30).

2.2.1. Treatment protocol

Blood ascorbic acid in the fasting state was obtained from all participants. Patients with stage III-IV satisfied the inclusion and exclusion criteria, and those entered the treatment phases of the present study. They were randomized into 3 groups, denoted A, B and C. Each group contained 5 patients for dose escalation. Prior to the study, they received a test dose of 15 g IVAA over 30 min. If tolerated, a second dose of 1 g/kg would be administered in the next treatment. Participants in Group A received intravenous ascorbic acid (IVAA) when mEHT was finished, participants in Group B had IVAA administered simultaneously with mEHT and in Group C, IVAA was applied first, followed with mEHT. In group A and group C, mEHT was applied in conjunction with IVAA within 5 min interval of time. The completion of escalation to the higher dose was 4 weeks' treatment without dose-limiting toxicity (DLT).

Pharmacokinetic profiles were obtained when they received solely IVAA at concentrations of 1 g/kg, 1.2 g/kg, and 1.5 g/kg, and when IVAA in combination with mEHT. The process was applied 3 times a week (every other day, weekend days off) for 4 weeks. All patients started the trial doses of 1 g/kg for 4 treatments. When there was no DLT observed, the test dose for patients increased to 1.2 g/kg continuously for 4 treatments, and then the test dose for patients was escalated to 1.5 g/kg continuously for 4 treatments. DLT was defined as any reversible grade ≥ 3 adverse events, whether haematological or non-haematological.

Ascorbic acid injection was a gift from Biological Therapies (Australia) Ltd. as sterile solution of ascorbic acid for parenteral use. Each milliliter contains 3 g sodium ascorbate and water for injection with pH 6.5–8.0 adjusted with sodium bicarbonate. The infusion solution was diluted in sterile water to achieve an osmolarity of approximately 900 mOsm/L. All dosages of ascorbic acid were infused in 120 min. Patients can consume water freely during the treatment process.

Table 1
Patient demographics and disease status.

Characteristics	No. of patients	
	Preparative study	Phase I study
	35	15
Age, years	Value	
Median	63	60
Range	46–72	46–72
Sex		
Male	25	7
Female	10	8
Weight(kg)		
Median	57.39	57.6
Range	40–69.9	40–69.9
ECOG performance status		
0	15	2
1	10	7
2	10	6
Received anticancer therapies in the past 6 months	30	15
Stage at study entry		
I	6	
II	7	
III	5	5
IV	17	10
Pathology		
Squamous cell carcinoma	9	3
Adenocarcinoma	26	12
Gene mutation		
EGFR(+)	10	8
ALK(+)	3	1

EGFR:Epidermal growth factor receptor
ALK:Anaplastic lymphoma kinase
ECOG:Eastern Cooperative Oncology Group

The treatment regime of mEHT was 60 min/session; the power of mEHT was gradually increased from 135 W to 150 W depending on the patient's actual tolerance. The applicator used was 7.1 dm^2. The applied energy range in one session was between 486 kJ and 540 kJ. The patient was lying in prone position and the treatment covered the complete lung (30 cm diameter circle). The temperature of treatment area is about 40–42 °C indirectly calculated by the treatment device. Monitoring conditions of the participants, quality of life assessment, and statistic method were shown in the study protocol (Supplementary Clinical trial Protocol).

3. Results

The characteristics of the participants are collected in Table 1. Thirty-five NSCLC patients were studied in a preparative study. Thirty participants received prior conventional anticancer therapies, with 18 experiencing severe adverse events leading to cessation of those treatments. Blood ascorbic acid in the fasting state was obtained from all 35 participants. 15 patients with stage III-IV satisfied the inclusion and exclusion criteria, and those entered the treatment phases of the present study. The characteristics of the participants are collected in Table 1. Patients who were eligible for the study (n = 15) are identified in Table 2.

3.1. Pharmacokinetics

We collected information including the stage of disease, pathology and gene mutation status and we examined fasting plasma ascorbic acid level and tumor markers of C-reactive protein (CRP) in 35 NSCLC patients. We found that fasting plasma AA levels were significantly correlated with the stage of the disease. Supplementary Fig. S1 shows the tendency of lower level of fasting ascorbic acid at late stage of the disease. Other markers such as CRP, CEA, CA153, NSE, CYFRA21-1, SCC, pathology, and gene mutation status had no relationship with fasting ascorbic acid levels.

We found that fasting plasma ascorbic acid in these patients (n = 35) was significantly lower than in healthy people (0.05 ± 0.04 vs. 0.09 ± 0.03, [mmol/L] $p < 0.05$). The fasting AA concentration of the selected eligible group of patients (n = 15) is shown in Supplementary Table S1 and used as a baseline. The average AA concentrations in the plasma were 0.048 and 0.040 mmol/L in stage III and IV patients, respectively. The concentration of fasting AA in selected cohort (n = 15) does not significantly differ from the concentration of fasting in the investigated group of patient (n = 35) ($p > 0.18$).

The complete averages of measured data are collected in Supplementary Table S1.

The dose escalation well distinguishes the plots for different doses (Fig.1). The pharmacokinetic curves show the well-unified plots of sole AA treatment in all the three groups (confirmed by Mann-Whitney test). The A and C groups in the combined treatment shows also indistinguishable plots with the sole curves (Fig.1), (confirmed by the Wilcoxon pair-test).

The conventional pharmacokinetic parameters for all the 15 patients in the selected cohorts is shown in Table 3. Group B has special behavior compared to the other two groups (Fig.1). The C_{max} of AA were significantly higher in group B than in the other two groups, while other plasma AA concentration increased from < 0.15 mmol/L to peak concentration up to higher than 20 mmol/L at the end of the infusion. The pharmacokinetic behavior of ascorbic acid concentration in other groups did not show any significant differences.

3.2. Measurement of oxalic acid in urine

The urinary OA excretion and AA excretion expected to be dependent on OA is the major end metabolite of AA. OA could be dangerous at high values (Cameron and Campbell, 1974), causing necrosis and acute tumor-haemorrage, but such effects of OA were not observed in our study. We measured the oxalic acid (OA) in the urine of patients to indicate the metabolism of AA. Urine was taken twice for every treatment duration, after the first 2 h (end of the IV process) and 4 h. The total OA in urine is shown in the Supplementary Table S2. No significant difference can be observed between the groups and treatments.

3.3. Adverse effects and toxicity

The overall toxicity was marginal. Thirstiness was the major symptom during all of the treatments and this was observed in both the IVAA and combined treatment groups. It was grade 1 for the dose of 1 g/kg, and grade 2 for the 1.2 and 1.5 g/kg doses of AA for all patients in all groups. Thirstiness symptoms were released when treatment was finished. Symptoms disappear when the treatments were finished, except for two patients, feeling fatigue on the complete day of the treatment. Coenzyme Q10 helped to relieve symptoms. One patient (1/15) experienced serious diarrhea. This patient was withdrawn from the study in the stage when he finished the second combined treatment at a dosage of 1.5 g/kg. Serious toxicity was not observed in other patients in any stage of the treatments, nor in sole AA or the combined groups. All of the observed adverse effects were continued in the complete IV process or longer, not only for the duration of mEHT treatment.

Some mild side effects occurred (Table 4). No significant differences were registered in full blood count, biochemical and hematologic profiles before and after every escalation dosage cycles. We did not find any hematologic and serum creatinine abnormalities during the whole course of the trial (shown in Supplementary Table S3).

3.4. Quality of life

The QLQ-C30 scores were registered over the full cycle of the study. The average scores for the functioning scales continuously increased, so that the quality of life (QoL) improved. The physical improvement was

Table 2
Identification of the patients involved in phase I study (n = 15).

Patient #	Gender	Age (y)	Weight (kg)	Stage	Pathology	ECOG Status	ALK	EGFR
1	Male	64	44.20	IIIb	squamous cell	2	−	−
2	Female	65	54.90	IV	adenocarcinoma	1	−	+
3	Female	56	57.60	IV	adenocarcinoma	2	−	+
4	Male	72	57.50	IV	adenocarcinoma	1	−	+
5	Female	55	68.10	IV	adenocarcinoma	2	−	+
6	Female	46	52.60	IIIb	adenocarcinoma	1	+	−
7	Male	58	62.30	IIIb	adenocarcinoma	0	−	+
8	Female	54	61.90	IV	adenocarcinoma	0	−	+
9	Female	58	61.40	IV	adenocarcinoma	1	−	+
10	Male	62	69.90	IV	adenocarcinoma	1	−	−
11	Male	62	40.00	IIIb	squamous cell	1	−	−
12	Female	56	55.00	IV	adenocarcinoma	2	−	+
13	Female	63	58.30	IV	adenocarcinoma	2	−	−
14	Male	72	59.50	IIIb	squamous cell	1	−	−
15	Male	57	49.00	IV	adenocarcinoma	2	−	−

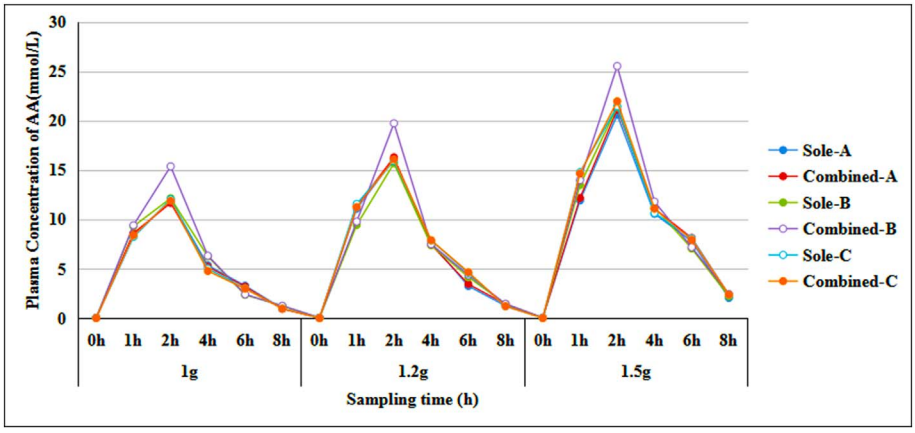

Fig. 1. Pharmacokinetic plots of each treatment in the six groups by increasing dosage.

Table 3
Pharmacokinetic parameters of the measured patients.

Pharmacokinetic parameters of treatments																		
Group	Pts. no.	Sole IVAA									Combined IVAA & mEHT								
		1 g/kg·d			1.2 g/kg·d			1.5 g/kg·d			1 g/kg·d			1.2 g/kg·d			1.5 g/kg·d		
		C_{max}	AUC	$t_{1/2}$	C_{max}	AUC	$t_{1/2}$	C_{max}	AUC	$t_{1/2}$	C_{max}	AUC	$t_{1/2}$	C_{max}	AUC	$t_{1/2}$	C_{max}	AUC	$t_{1/2}$
A	1	2.47	41.00	1.32	2.81	57.00	1.44	3.11	89.00	2.11	2.43	42.00	1.32	2.83	57.00	1.48	3.06	87.50	2.08
	2	2.46	45.00	1.35	2.81	60.50	1.64	3.01	78.00	2.10	2.48	45.50	1.33	2.73	58.00	1.55	3.03	80.50	2.17
	3	2.51	39.50	1.10	2.80	58.00	1.43	3.00	75.50	1.94	2.41	37.00	1.07	2.82	61.00	1.60	3.07	81.00	2.05
	4	2.39	43.00	1.28	2.68	57.00	1.72	3.02	87.50	2.10	2.40	42.00	1.21	2.74	58.00	1.75	3.09	90.00	2.17
	5	2.54	52.50	1.51	2.79	58.00	1.42	2.97	80.00	1.96	2.56	53.50	1.49	2.82	60.00	1.49	3.00	85.00	2.04
	Mean	2.47	44.20	1.31	2.78	58.10	1.53	3.02	82.00	2.04	2.46	44.00	1.28	2.79	58.80	1.57	3.05	84.80	2.10
	CV%	2.30	11.50	11.22	1.99	2.46	9.15	1.74	7.25	4.13	2.68	13.89	12.14	1.75	2.79	6.97	1.16	4.84	3.04
B	6	2.47	42.00	1.09	2.80	60.50	1.44	3.15	92.00	2.12	2.65	48.00	1.15	3.02	66.00	1.95	3.28	98.00	2.14
	7	2.49	46.50	1.49	2.71	54.50	1.64	3.23	101.00	2.25	2.70	51.00	1.53	2.99	63.00	1.53	3.34	107.00	2.31
	8	2.51	49.00	1.50	2.80	60.00	1.43	2.89	72.00	1.97	2.74	50.50	1.51	3.01	69.00	1.74	3.11	82.50	2.14
	9	2.43	42.50	1.36	2.68	53.00	1.72	2.94	72.50	1.89	2.79	51.00	1.47	2.97	62.50	1.53	3.16	82.50	1.99
	10	2.58	50.00	1.21	2.77	59.50	1.42	3.06	85.00	2.03	2.77	55.50	1.33	2.91	62.00	1.63	3.29	95.00	2.09
	Mean	2.43	42	1.09	2.68	53	1.42	2.89	72	1.89	2.65	48	1.15	2.91	62	1.53	3.11	82.5	1.99
	CV%	2.22	7.95	13.42	1.98	6.06	9.15	4.63	14.84	6.77	2.06	5.29	11.38	1.46	4.59	10.5	2.98	11.35	5.43
C	11	2.42	42.00	1.34	2.82	65.00	1.65	3.13	86.00	2.12	2.40	42.00	1.30	2.73	62.50	1.62	3.15	89.50	2.16
	12	2.52	44.50	1.34	2.67	54.50	1.45	3.00	87.00	2.15	2.53	42.50	1.27	2.76	55.50	1.52	3.07	88.50	2.15
	13	2.47	45.50	1.32	2.76	59.50	1.53	2.98	76.50	1.93	2.49	46.50	1.32	2.69	57.50	1.51	3.00	89.00	1.96
	14	2.48	41.50	1.25	2.79	42.00	1.71	3.09	95.00	2.22	2.45	41.00	1.26	2.85	67.00	1.73	3.09	92.00	2.18
	15	2.56	43.50	1.22	2.79	65.00	1.78	3.12	89.50	2.03	2.51	48.00	1.25	2.86	66.50	1.80	3.14	92.00	2.07
	Mean	2.49	43.40	1.29	2.77	57.20	1.62	3.06	87.00	2.09	2.48	44.00	1.28	2.78	61.80	1.64	3.09	90.20	2.10
	CV%	2.13	3.86	4.29	2.09	16.71	8.23	2.27	8.92	5.38	2.09	6.96	2.28	2.69	8.4	7.81	1.96	1.86	4.31

Cmax - the maximum concentration recorded, AUC (Area Under the Curve) - a measure of the exposure to the drug; $t_{1/2}$ (elimination half-life) - the time taken for the plasma concentration to fall by half of its original value; The tmax - the time taken to reach Cmax is not shown, due to it being a constant of 2 h in all cases.

Table 4
Adverse effects of the treatment counting all of the subgroups (n = 15).

Adverse events(n = 15)				
AA dose	Adverse event	Number affected	%	Grade
1.0 g/kg	Fatigue	1	6.7	1
	Nausea	1	6.7	1
	Vomiting	1	6.7	1
1.2 g/kg	Fatigue	2	13.3	1
	Facial flushing	1	6.7	1
1.5 g/kg	Nausea	1	6.7	1
	Fatigue	3	20	1
	Diarrhea	2	13.3	3
	Headache	1	6.7	1

significant; others were not so (shown in Supplementary Tables S4 & 5). The overall average improvement in functions was 2.0 (0.6—3.2). The symptoms gradually decrease (Fig. 2) despite the advanced NSCLC and the short (four weeks) period of study. The overall average change in symptoms was − 3.71 (− 8.81 — 0.83) (negative, corresponding to the decrease in symptoms). We counted the financial difficulties of the patients as a special “symptom”, which influenced patients' QoL and social behavior directly. Note, no significant difference between the groups was observed by the QoL changes.

4. Discussion

Our data from the preparation of the study with a larger number of patients (n = 35) show that tumor burden can affect fasting plasma AA level. Fasting plasma AA was significantly correlated with the stage of disease. The lower the level of plasma AA, the more advanced stage of the disease. In comparison with healthy people, fasting plasma AA was significantly lower in stage III-IV NSCLC patients. Cancer cells produce a significant amount of reactive oxygen species (Sundaresan et al., 1996), which can accelerate cell growth and proliferation. Oxygen radicals promote cancer cell migration and invasion. Higher levels of oxygen radicals produced in the more advanced stage of the disease. This may contribute to lower fasting plasma AA concentration by the depletion of AA in cancer patients. Another possible cause of depletion may be due to the structure of dehydroascorbic acid (DHA), the oxidised form of ascorbic acid, similar to glucose (Rumsey et al., 1997). The glucose transporter 1 (GLUT1) is highly expressed in cancer cells (Cao et al., 2005). It facilitates the transport of glucose and DHA across the plasma membranes of mammalian cells (Cao et al., 2005; Lee and Kim, 2015), which causes an accumulation of AA in cancer cells, directly decreasing the level of plasma AA in late stage cancer patients. However, unlike in the previous study (Riordan et al., 2005), we did not find any correlation of CRP and tumor markers with fasting plasma AA in this trial.

The pharmacokinetic parameters (C_{max}, AUC and $t_{1/2}$) all were significantly higher in combined treatment in Group B compared to its sole AA treatment counterpart ($p < 0.05$, Wilcoxon test), (Supplementary Table S1 & Fig. 1). At the same time, there was no significant difference between the sole IVAA and the combined treatment in Groups A and C. (Supplementary Table S1)·These significant investigations prove the unity of all sole IVAA applications, independent of the Groups, but also shows the only efficacy of Group B, when IVAA and mEHT were concomitantly applied. The IVAA before or after mEHT shows the same results and does not differ from the sole IVAA treatment. We conclude that the concomitant application of mEHT with IVAA increases the plasma concentration of AA compared to the sole or non-concomitant application of the treatments. Note, unlike the previous study (Welsh et al., 2013), we found plasma AA levels that were higher than 20 mmol/L only at a dosage of 1.5 g/kg, but not at 1 g/kg or 1.2 g/kg. It might be because the patients are in late stages of the NSCLC, which significantly decreases the plasma AA level, as shown above (Fig.1). A limitation of the study is that some of the patients who had very advanced disease (who has liver metastasis, and/or brain metastasis) did not entered our trial. The participants enrolled in this study cannot be considered representative of all late stage NSCLC

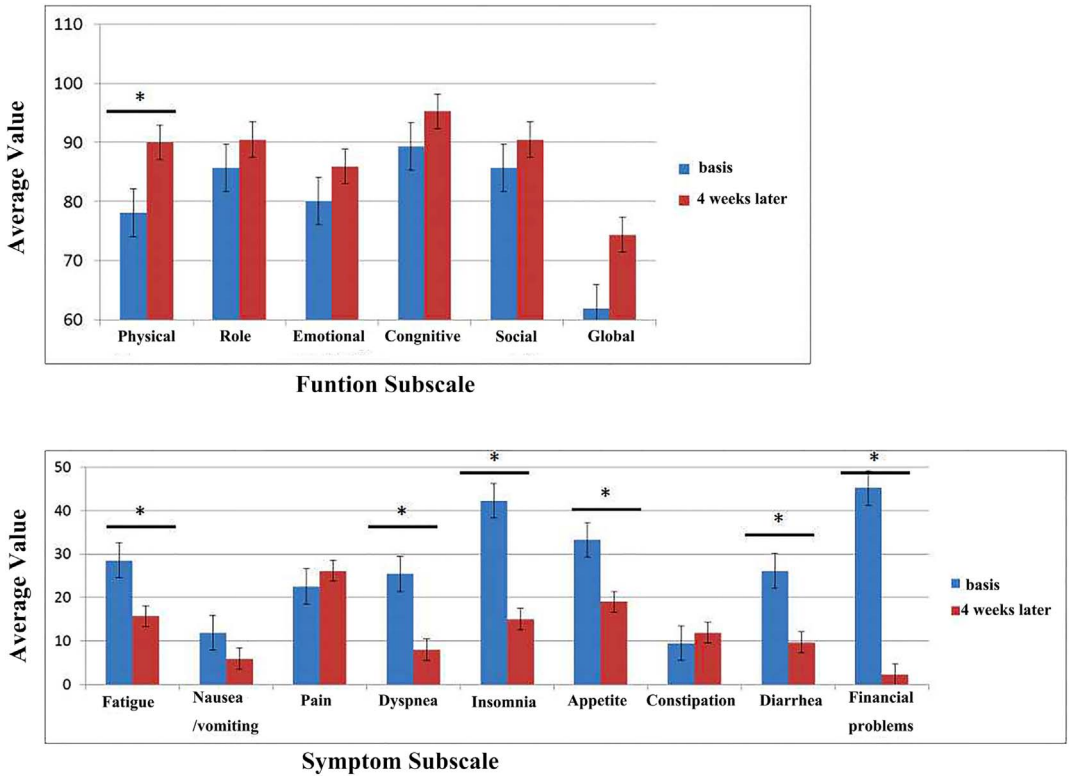

Fig. 2. The improvement in function and symptoms (negative value) of the QLQ-C30 scores. The significant changes ($p < 0.05$) are shown with * above the columns.

patients.

To summarize, among the 3 groups, peak concentration of AA (end of infusion) was significantly higher in group B than in the other two groups, while other AA concentration-time profiles showed no significant differences between the three groups. This may be due to localised heat improving the circulation of the medication. Similar definite significant increases of C_{max} were observed by mEHT in the study of Nefopan (active substance is fentanyl) in healthy subjects (Lee and Kim, 2015).

5. Conclusion

The goals of this trial were to evaluate the safety, tolerability and pharmacokinetics of high dose intravenous AA synergies in mEHT patients with stage III-IV NSCLC, and to identify the best pattern of intravenous AA synergies with mEHT for a phase II study to monitor the maintenance or improvement in quality of life by using QLQ-C30. AA concentrations safely reached a peak concentration and only mild adverse events were observed in this study. There was one patient who suffered severe diarrhea at dosage of 1.5 g/kg·d. Overall, AA synergies with mEHT are safe and well tolerated. The average scores for the functioning scales continuously increase, and the average values for the symptoms gradually decrease, which indicates that quality of life is improved when patients receive the above treatments. An important conclusion is that there was a measured effect in pharmacokinetic data only when the mEHT was administered concomitantly with IVAA.

This trial has 3 characters: the first time to report the pharmacokinetics of large dose IVAA combined with mEHT patients; the first time to evaluate the safety and tolerability of IVAA synergy with mEHT in the treatment of cancer patients; and the first time to focus on the clinical safety of IVAA methods on NSCLC patients.

We proved in this trial that large doses of IVAA simultaneously with mEHT are safe and well tolerated for NSCLC patients. This motivated us to carry out a Phase II study by administering mEHT simultaneously with IVAA, which can produce higher peak concentrations of AA in plasma.

Abbreviations

AA	Ascorbic acid
mEHT	modulated electrohyperthermia
NSCLC	non-small cell lung cancer
TKIs	tyrosine kinase inhibitors
IVAA	intravenous AA
G6PD	Glucose-6-phosphate dehydrogenase deficiency
DLT	dose-limiting toxicity
QLQ-C30	Quality of Life Questionnaire
QoL	quality of life

Ethical approval and consent to participate

The protocol and consent form were approved by the Ethics Committee of the Clifford Hospital. Approval code: 2/2015-10

Informed consent was obtained from all participants. There was no identifying information relating to participants.

Availability of data and materials

All data generated and analyzed during this study are included in this published article and in its supplementary data files.

Competing interests

The authors declare that they have no competing interests.

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Trial registration

ClinicalTrials.gov, NCT02655913, Registration date: 7th Jan, 2016. Date of enrolment of the first participant to the trial: 17th Jan, 2016. No changes to the methodology occurred following trial commencement. We confirmed that all methods were performed in accordance with the approved guidelines and regulations. We report and present data according to the CONSORT statement.

Author contributions

JO designed the whole protocol, enrolled the patients, and wrote the manuscript text; XYZ followed up with the patients, analyzed the samples and collected data; YL and JW applied mEHT treatment on participants; CZ and XG randomized the patients, XTZ and TZ analyzed the data; HZ and XW monitored the status of the participants. CP gave suggestions of full text writing. All authors reviewed the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ejps.2017.08.011.

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