

Efficacy of continuous ascorbate infusion on lymphopenia and neutrophil counts in the supportive care of cancer patients

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Research article

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Abstract

Abnormal blood counts and, in particular, lymphopenia commonly occur in cancer patients and predict poor prognosis. In this study data from a previous Phase 1 clinical trial of continuous vitamin C infusions were analyzed. The trends in the absolute lymphocyte counts (ALC), absolute neutrophil counts (ANC) and neutrophil to lymphocyte ratio (NLR) were evaluated. In addition, pharmacokinetic data on IVC in patients who were treated by continuous infusion were determined for different dosages. During continuous infusion treatment, data for all patients, who completed 6-8 weeks of the treatment, demonstrated improvement of lymphocyte counts in patients with lymphopenia. On average the percentage of improvement in ALC was 22% for ALC <1300 / μ l and 69% for ALC <1000 cells/ μ l. Our data also indicate that lower doses (less than 20 g/70 kg) were more favorable for the improvement of ALC. In addition, the data suggest a normalizing effect of continuous infusions of ANC levels and changes in NLR. In summary, the present analysis demonstrated the normalizing effect of continuous IVC on lymphopenia, ANC and NLR.

Introduction

The use of high dose ascorbate in cancer therapy remains an area of controversy. Currently, there are no clinical data that show the importance of several factors in the treatment schedule using high dose vitamin C, such as dose, frequency and duration of administration on the effectiveness of the cancer patients' treatment [1]. Most practitioners administer IV ascorbate to cancer patients by bolus infusions 2-3 times per week [2]. Bolus infusion of high dose ascorbate (1g/kg) can reach very high concentrations in blood based on the data of pharmacokinetics, but is very quickly eliminated from the body with a half-life of about two hours [3, 4]. Such treatment is generally well tolerated and safe, with few adverse events reported, and there are anecdotal case reports of an anticancer effect of such a regimen [5-7].

There have been two clinical trials that used continuous IVC infusions. Cameron and Pauling performed a clinical trial in 100 terminal cancer patients [8, 9]. The protocol included an initial 10 day course of IV ascorbate, at a relatively low daily dose of 10 g/day given by continuous infusions, followed by daily oral intakes of 10-30 g/day, in divided doses. Their results showed increased survival time and improved the quality of life of the patients compared with patients who had not received IVC.

The ideas of Linus Pauling were extended in the "The dynamic flow model" developed by Dr. Hickey et.al [10, 11].

The second trial of the treatment of cancer patients by continuous infusions was conducted by Dr. Riordan [12]. In this Phase I clinical trial, patients were administered continuous infusions using an infusion pump. The first data analysis published in 2005 was focused on the safety of the treatment. In the next publication [13], we analyzed previously unpublished parameters from the Riordan clinical study, including blood chemistry and blood count parameters that are reportedly related to patient prognosis and degree of inflammation. This included: absolute neutrophil and lymphocyte counts and the neutrophil-to-lymphocyte ratio; lactate dehydrogenase, an enzyme involved in tumor initiation, metastasis, and recurrence; creatinine, the depletion of which is associated with cachexia; and glucose, as hyperglycemia is common in cancer patients. The analysis demonstrated the

regulatory effect of continuous IVC on lactate dehydrogenase enzyme, neutrophil-to-lymphocyte ratios, lymphopenia, neutrophil count and hyperglycemia.

In the present study, we further analyzed the pharmacokinetics of the blood ascorbate levels, effect of the dosages of continuous IVC infusion on absolute lymphocyte counts, and improvement of neutrophil counts and neutrophil to lymphocyte ratios as the result of continuous IVC treatment.

Materials And Methods

A description of how the Phase 1 IVC continuous infusion clinical trial was conducted was given previously [12]. A total of 24 patients with late stage cancer were included in the study. All patients had several rounds of chemotherapy or radiation prior to entering the study. Seventy nine percent of the patients had a metastatic tumor; 17 patients had colon cancer with liver and lung metastasis, three patients had pancreatic or liver cancer and the rest of the patients had esophagus or rectal cancer. Patients were divided into five groups and treated by continuous infusion of 150 mg/kg/day (three patients), 290 mg/kg/day (seven patients), 430 mg/kg/day (six patients), 510 mg/kg/day (three patients) and 710 mg/kg/day (five patients). These doses are equivalent to approximately 10, 20, 30, 35, 50 g per 70 kg person. Sodium ascorbate was diluted in Lactated Ringers solution and infused by continuous infusion pump.

Blood cell counts, blood chemistry parameters, progression or disease, and adverse events were monitored in these patients. Samples were collected one week prior to therapy and at weekly intervals during intervention. White blood cell counts, hemoglobin and hematocrit, red blood cell counts, glucose, lactate dehydrogenase and blood chemistry parameters related to renal function (creatinine, BUN, and uric acid) were determined using standard procedures at the Eppley Institute for Research in Cancer and Allied Diseases at the University of Nebraska Medical Center (Omaha, NE) [12]. Plasma ascorbate concentrations were measured at the Riordan Clinic laboratory by colorimetric method.

The clinical trial was approved by the ethics committee of the Eppley Institute for Research in Cancer and the Institutional Review Board of the Riordan Clinic. Written informed consent was obtained from all patients.

The data were analyzed by Systat software (Systat, Inc, San Jose, USA) and Kaleidagraph software (Synergy software, PA, USA). Statistical significance was evaluated by using a paired, two-tailed, Student's t test where $p < 0.05$ was considered significant.

Results

3.1. *Ascorbate concentrations in blood after continuous infusions.*

During the continuous infusion clinical trial, the levels of ascorbate were measured before intervention, every day for the first four days and then at the end of each week. Pre-treatment measurements demonstrated that patients had hypovitaminosis C, with two thirds of the subjects having levels below the normal range (0.6mg/dL-2mg/dL) [12, 13]. During IVC treatment, ascorbate levels increased, and reached a plateau, but average maximum values did not differ significantly between different doses and were in the range of 1.2 mM -1.5 mM. The ascorbate concentrations in blood for low (150 mg/kg/day) and high (710 mg/kg/day) dosages, averaged for all patients in these dosage groups, are presented in Figure 1.

Figure 1. Time course of the average ascorbate concentrations in blood for continuous infusions with dosages 150 mg/kg/day (black circles) and 710 mg/kg/day (black squares). Data were extrapolated by Michaelis-Menten equation.

3.2. *Effect of continuous infusion on ALC*

The effect of continuous infusions on the absolute lymphocyte count (ALC) was analyzed for 22 patients from 24 terminally ill cancer patients. More than half of the patients enrolled in the study had lymphopenia or absolute lymphocyte counts less than 1300 cells/ μ l (normal range 1300-4000 cells/ μ l). The data of the patients' diagnosis, lymphocyte and neutrophil counts one week before treatments, at the beginning and end of the treatment, and the duration and the dosages of the treatments are shown in Table 1.

Table 1. Characteristics of twenty-four cancer patients (diagnosis, duration/dosages of treatment, and pretreatment, initial and final lymphocyte and neutrophil counts) who participated in a phase I clinical trial of continuous IVC infusions.

| Dosage (mg/kg/d) | Diagnosis | Time (weeks) | ALC pre | ALC initial | ALC post | ANC initial | ANC post |
|------------------|---------------------------------|--------------|---------|-------------|----------|-------------|----------|
| 150 | Colon Cancer/ Liver Mets | 3 | 1230 | 980 | 1056 | 5180 | 7392 |
| 150 | Colon Cancer/ Liver Mets | 8 | | 944 | 1751 | 9417 | 8443 |
| 150 | Colon Cancer/ Wall Mets | 7 | 2162 | 1168 | 1584 | 5480 | 5565 |
| 290 | Colon Cancer/ Liver Mets | 8 | | 1261 | 755 | 7178 | 9887 |
| 290 | Colon Cancer/Liver Mets | 2 | 420 | 518 | 897 | 6052 | 5589 |
| 290 | Appendix/ Carcinomatosis | 7 | 1850 | 1494 | 3843 | 5199 | 7002 |
| 290 | Liver Cancer | 8 | | 552 | 1035 | 5727 | 4913 |
| 290 | Colon Cancer/Liver Mets | 6 | 1045 | 1188 | 573 | 3896 | 4341 |
| 290 | Colon Cancer/ Omentum | 8 | 1170 | 902 | 2058 | 5924 | 6302 |
| 430 | Colon Cancer/Lung Mets | 7 | 2232 | 1971 | 1795 | 3988 | 5341 |
| 430 | Colon Cancer/Lung Mets | 3 | 1296 | 1560 | 1044 | 8748 | 9512 |
| 430 | Colon Cancer/Liver Mets | 8 | 1392 | 1170 | 1365 | 6218 | 7146 |
| 430 | Colon Cancer/Liver Mets | 8 | 4712 | 4712 | 7752 | 4826 | 5515 |
| 430 | Colon Cancer | 6 | 2048 | 2205 | 1610 | 3151 | 3165 |
| 430 | Pancreas Cancer | 8 | 266 | 245 | 299 | 2576 | 3767 |
| 430 | Rectal Cancer/ Liver, Lung Mets | 3 | 1178 | 1311 | 1605 | 4906 | 4051 |
| 570 | Colon Cancer/ Liver Mets | 8 | 1400 | 1590 | 1270 | 2934 | 3124 |
| 570 | Pancreas Cancer/ Liver Mets | 7 | 777 | 663 | 999 | 2969 | 5781 |
| 570 | Colon Cancer/Lung Mets | 8 | 1260 | 355 | 836 | 6048 | 5499 |
| 710 | Colon Cancer/Liver, Lung Mets | 8 | 1512 | 972 | 1055 | 5571 | 6100 |
| 710 | Colon Cancer/Liver, Lung Mets | 8 | | 936 | 915 | 3432 | 4062 |
| 710 | Cholangiocarcinoma/Liver Mets | 0.3 | | 2496* | | 15600 | 9271 |
| 710 | Esophagus Cancer/Liver Mets | 1.5 | | 663* | | 3723 | 6424 |
| 710 | Colon Cancer/Liver Mets | 8 | 496 | 624 | 1075 | 8249 | 5470 |

*Excluded from the data analysis due to lack of post treatment data.

The percentage of change in ALC was calculated based on the pre-treatment ALC (6-12 days before intervention), initial ALC values and the ALC at the end of the treatment. According to our data, the tendency of ALC was to decrease in 56% of patients before treatment. For patients with lymphopenia (15 subjects) the improvement or stabilization of the count was seen in all patients except for one subject. For this group of patients the median values of ALC was 940 cells/ μ l (IQR 588-1168 cells/ μ l) at the beginning of treatment and 1045 (IQR 866-1420 cells/ μ l) at the end of the treatment (p-value = 0.03). On average, there was a 22% of improvement in lymphocyte counts for all patients who completed 6-8 weeks of treatment and had ALC <1300 / μ l (IQR: 89%, -24%). For five patients, the ALC values returned to the normal level (ALC>1300 cells/ μ l) and for five patients the values reached the level of 1000 cells/ μ l. Distribution of the ALC before and after treatment and percentage of ALC improvement for patients with initial ALC less than normal range and in normal range are shown in Figure 2.

Figure 2. Distributions of the percentage of change in absolute lymphocyte counts before and after treatment (A); Percentage of improvement of ALC for patients with initial lymphocyte counts lower than normal range (ALC<1300/ μ l) and in normal range (B).

Severe lymphopenia (ALC<1000 cells/ μ l) was measured in 10 patients. Only six patients with severe lymphopenia completed 6-8 weeks of treatment. On average, for the six patients with the ALC <1000 cells/ μ l,

there was a 69% improvement in the lymphocyte count at the end of the treatment (IQR: 129%, -6%).

For all patients who completed 6-8 weeks of treatment (18 subjects), we analyzed the dosage effect of vitamin C on the improvement in lymphocyte counts. At the low doses of continuous infusions (combined 150 and 300 mg/kg/day) the median increase in lymphocyte counts was 35% (IQR (-11%÷107%)), for high doses (430, 570, and 710 mg/kg/day) the median change in lymphocyte counts were, respectively, 6% (IQR: -22%÷28%), 22% (IQR: 1%÷36%) and -16% (IQR: -25%÷-8%). The dependence of the percentage of ALC change on dosage of continuous infusion is shown in Figure 3. These data indicate that lower doses are more favorable for the improvement of lymphocyte count.

Figure 3. The dosage effect of vitamin C on the change in lymphocyte counts.

3.3 *The effect of continuous infusion on ANC*

As absolute neutrophil counts (ANC) and neutrophil-to-lymphocyte (NLR) ratios are useful prognostic factors in a variety of cancers, with higher values of NLR indicating lower survival times [14], the effect of continuous injection on these parameters was analyzed. First, we compared the initial and final values of ANC for the patients treated by continuous infusion and who completed 6-8 weeks of treatment. The normal range for ANC is 2000-7000 cell/ μ l. During analysis the ANC values were divided into three ranges: ANC less than the middle of the normal range (2000-4500cell/ μ l), higher than the middle of normal range (4500-7000cells/ μ l) and higher than normal range. The distributions of the changes between initial and post ANC are shown in Figure 4. The median percentage change with IQR for these three regions were 22.7% (IQR: 5%-58%), 8% (IQR:-10%-20%) and -22%, respectively.

ANC values were higher than normal range for two patients with metastatic colon cancer (8250 cells/ μ l and 9417cell/ μ l) and decreased after treatment by 10% and 37%. Two patients with pancreatic cancer with metastasis had the initial levels of ANC on the lower level of the normal range (2570 cells/ μ l and 2930 cells/ μ l), which increased by 46% and 94% at the end of the treatment. For the rest of the patients, the tendency was for normalization of the values, i.e. improvement of ANC at the low level of this parameter and decreasing for the higher values.

Figure 4. Dependence of the ANC change on the initial cell counts for continuous treatment. The levels of ANC were divided to three regions: less than the middle of the normal range (2000-4500cell/ μ l, n=6), higher than the middle of normal range (4500-7000cells/ μ l, n=10) and higher than normal range (ANC>7000 cells/ μ l, n=2). The normal range for neutrophil counts is 2000-7000 cells/ μ l.

3.4 *The effect of continuous infusions on NLR*

At the beginning of IVC therapy, 75 % of subjects had NLR levels higher than normal range (0.78 - 3.53). An improvement in the NLR was seen in 36% of the patients. However, for continuous infusions we were able to calculate the tendency in the change of NLR before and during treatment, as the values for ALC and ANC were

measured a week before treatment, at the beginning, and each week during treatment. To calculate the initial Δ NLR (prior to therapy), NLR on day zero was subtracted from NLR measured one week prior to therapy, and this difference was divided by the number of days between the two measurements. The rate of change in this ratio (Δ NLR) for each patient before and after therapy was described in our previous article [13]. The comparison of the trend in the change of NLR measured for periods one week before treatment and during treatment demonstrated that the rate of change was decreased. The average Δ NLR values for the patients who completed 6-8 weeks of treatment were 4.2%/day pre-therapy and 1.0 %/ day post-therapy (Figure 5).

Figure 5. The rate of NLR changes (percentage per day) before and after treatment for patients who completed 6-8 weeks of treatment.

According to our data, the treatment resulted in the suppression or prevention of the progression of the rate of growth of NLR. This improvement of the rate of change of NLR was found for 54% of the patients. For patients with initial NLR higher than the upper level of the normal range of 3.5, improvement was seen in 64% of patients who completed 6-8 weeks of treatment.

Discussion

Vitamin C contributes to immune defense by supporting various cellular functions of both the innate and adaptive immune systems [15]. The role of vitamin C in leukocyte function and its multiple beneficial effects on immune function was summarized in a previous review [15]. In the present study we analyzed the effect of continuous infusions on two immune cell populations, lymphocytes and neutrophils.

IVC treatment has been used for many years as adjuvant therapy for cancer patients, but the relevance of the optimal timing/ length of infusions and the concentrations of the ascorbate reached in the blood on the effectiveness of the treatment were not analyzed and are not clear.

We analyzed the kinetic curves of continuous infusions with dosages in the range 150 mg/kg/day-710 mg/kg/day and the effect of continuous infusions on the ALC, ANC and NLR in 24 terminal cancer patients. Continuous IVC infusion increased plasma levels of 1 mM-1.5 mM at plateau. According to our data of the plasma ascorbate concentrations for different dosages, there was no significant increase of the steady-state ascorbate for high dosages, suggesting that for continuous infusion there exists a saturation point. The leveling off of the ascorbate concentration can be explained by observations that high doses will saturate renal tubular ascorbate reabsorption, leading to increased ascorbate excretion. Recently, the kinetic curves of the continuous infusions for two dosages 2 g/d and 10 g/day were presented in the study [16]. According to this study, the steady-state concentrations of ascorbate were 40-210 μ M for 2 g/d and 230 μ M - 1.470 mM for 10 g/d at 48 h, and vitamin C excretion and renal vitamin C clearance increased with the dosage. IVC high dose bolus infusions can reach much higher concentrations [17], but these peaks are only transient.

In the Riordan Clinic trial [12, 13], patients were treated by continuous infusion, which was administered over much longer periods of time than bolus intermittent treatments. For most patients the duration of the continuous infusion was at least 20 hours, as the duration of bolus infusion is from one hour to three hours depending on the dosage. The present analysis demonstrated the regulatory and normalizing effect of continuous IVC infusions on lymphopenia, neutrophil-to-lymphocyte ratios, and absolute neutrophil counts. In addition, our data also demonstrated the relationship between the survival of patients and the rate of growth of NLR in continuous infusion group [13].

The analyzed data also suggested a benefit of using lower IVC doses in continuous infusions for improvements in immune cell counts, as raising the dose above 20 g/70 kg body weight showed a tendency to decrease improvements in lymphocyte counts and exhibited increased frequency of side effects, the latter of which were discussed in detail in the previous articles [12, 13].

Most practitioners administer IVC to cancer patients by bolus infusions of pharmacological doses of ascorbate several times per week [2]. According to the prevailing hypothesis, pharmacological bolus dosages of 50 - 100 grams have an anti-cancer effect by increasing hydrogen peroxide in the tumor environment [18, 19]. However, ascorbate has multifactorial mechanisms of action. In addition to the proposed mechanism of the pro-oxidant effect of high dose ascorbate, studies have demonstrated that the mechanisms of anti-tumor activity of ascorbate include changes in metabolic activity, stimulation of the 2-oxoglutarate dependent dioxygenase family of enzymes, which regulate the hypoxic response, collagen stabilization, and epigenetic histone and DNA demethylation [20, 21].

There are studies supporting the anti-tumor effect of relatively lower concentrations of vitamin C than pharmacological doses given by bolus IVC. For example, ascorbate can inhibit hypoxia-inducible factor-1 (HIF-1) activation in vitro at intracellular concentrations between 150 and 300 μM [22], and pharmacokinetic data on ascorbate in tumor tissues following vitamin C administration determined an optimal dose for HIF-hydroxylase activity of $\sim 1\text{-}3\text{ mM}$ [23]. The in vitro study of the optimal concentration of ascorbate as a cofactor for hydroxylases that regulate gene transcription and cell signaling pathways shows that ascorbate concentration less than 1,000 μM dose-dependently increases the 5-hmC signal [24].

The frequency of IVC treatment has an effect on mechanisms of tumor suppression. Campbell et al. [25] examined the effects of treatment schedule on the ability of intravenous ascorbate to inhibit HIF-1 expression (and the expression of its target proteins) in tumor bearing mice. It was found that a single bolus injection inhibited expression temporarily while daily injections maintained the inhibition. Increased tumor ascorbate was associated with slowed tumor growth, but alternate day administration of ascorbate resulted in lower tumor inhibition and did not consistently decrease HIF-1 pathway activity compared with daily injections [25]. Our retrospective analysis of prostate cancer patients treated with bolus IVC at the Riordan Clinic (1994-2015) showed that PSA levels increased more slowly in subjects given more frequent IVC treatments [26].

Thus, the paradigm of maximum tolerated dose, or the “more must be better” philosophy, should be reevaluated regarding IVC therapy regimens, since large intermittent doses can be more toxic and less effective than smaller repeated doses. For example, metronomic chemotherapy was proven to be effective in clinical trials in terms of

survival prolongation. Maximum tolerated dose chemotherapy, particularly in the case of solid tumors, kills off chemotherapy-sensitive cancer cell populations, leaving chemo resistant cells behind to re-colonize the tumor, ultimately leading to disease relapse [27]. In addition, recent advances in tumor biology point away from focusing on the cytotoxicity of drugs and focus on the modification of the tumor biology by targeting the tumor microenvironment [28].

Conclusions

In conclusion, continuous low dose IVC infusions exhibited beneficial effect for cancer patients with respect to normalizing leukocyte counts, indicating an improvement in both the innate and adaptive immune systems. This treatment can be effective in supportive and palliative care of cancer patients, as our study [13] demonstrated the regulatory effect of continuous IVC on lactate dehydrogenase, neutrophil-to-lymphocyte ratios, lymphopenia, neutrophil count and hyperglycemia. Further research in this area and clinical studies of the efficacy of continuous intravenous vitamin C infusions are warranted.

Declarations

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Conflicts of Interest: The author declares no conflict of interest.

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Figures

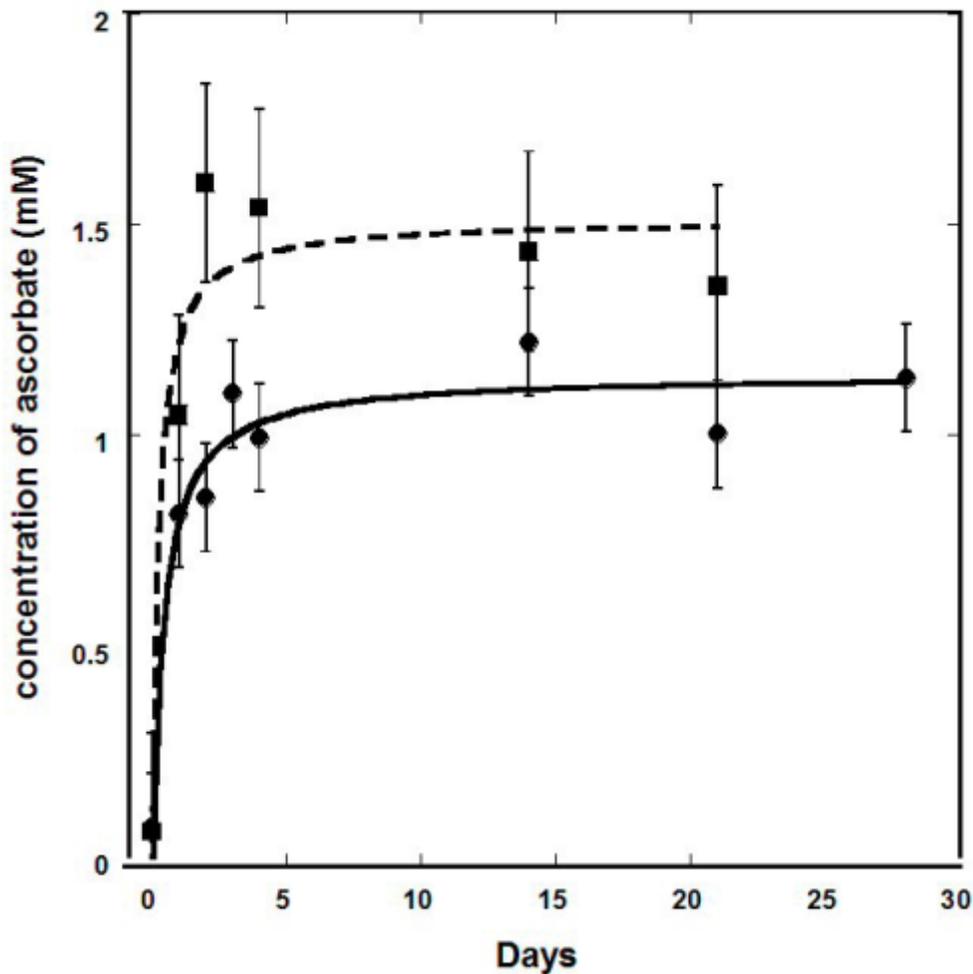


Figure 1

Time course of the average ascorbate concentrations in blood for continuous infusions with dosages 150 mg/kg/day (black circles) and 710 mg/kg/day (black squares). Data were extrapolated by Michaelis-Menten equation.

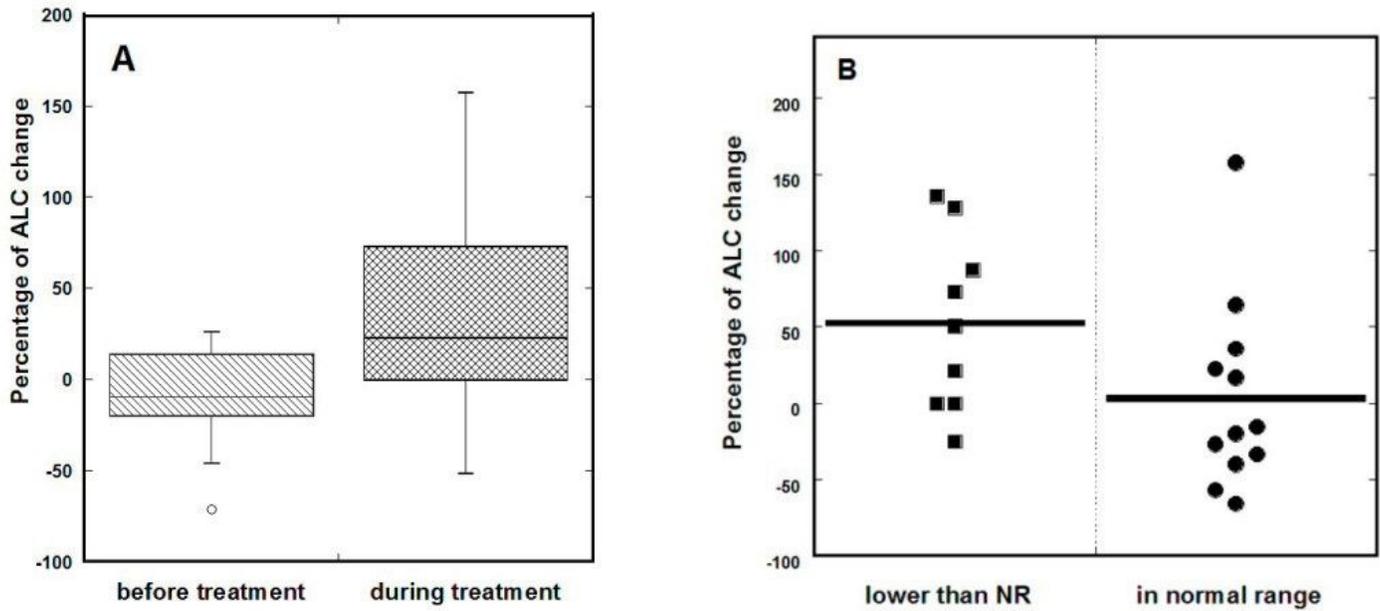


Figure 2

Distributions of the percentage of change in absolute lymphocyte counts before and after treatment (A); Percentage of improvement of ALC for patients with initial lymphocyte counts lower than normal range (ALC<1300/ μ l) and in normal range (B).

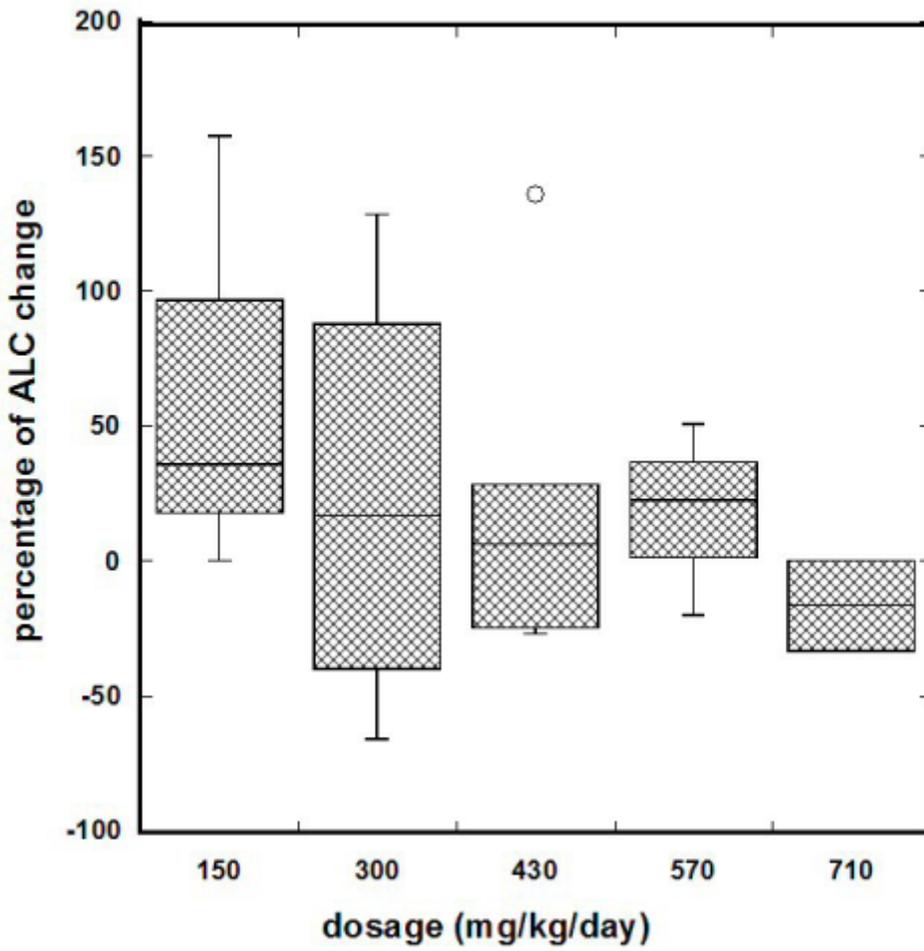


Figure 3

The dosage effect of vitamin C on the change in lymphocyte counts.

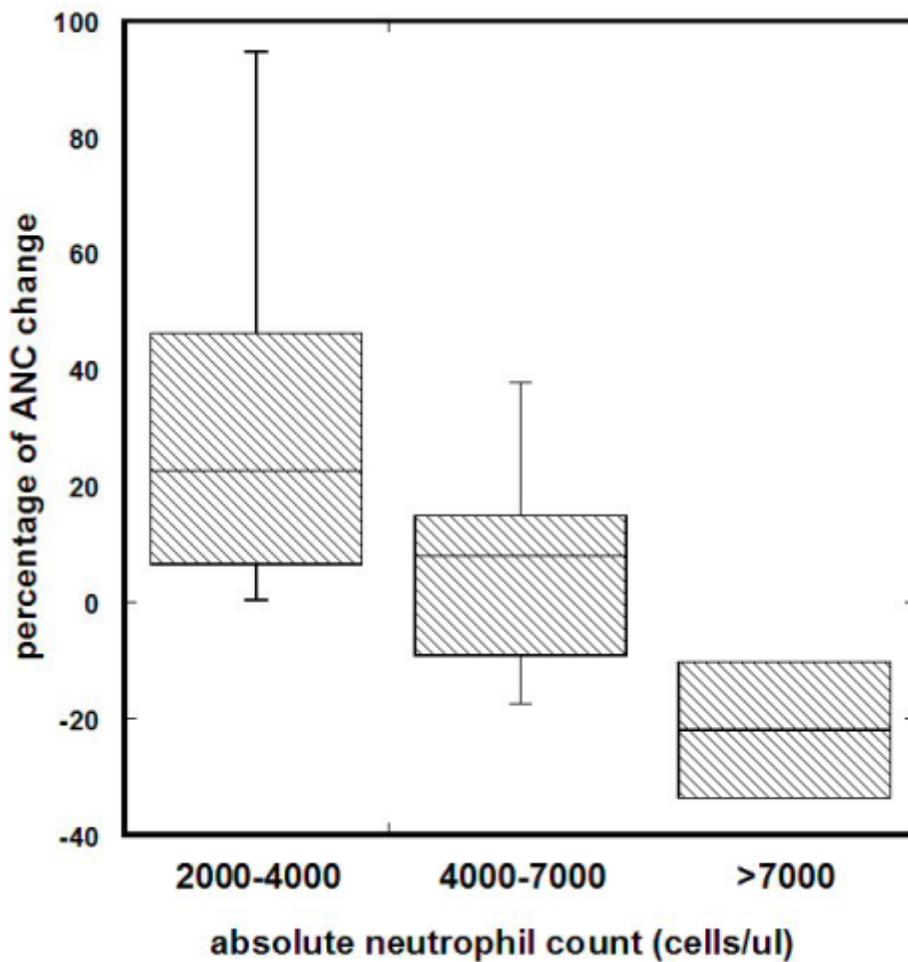


Figure 4

Dependence of the ANC change on the initial cell counts for continuous treatment. The levels of ANC were divided to three regions: less than the middle of the normal range (2000-4500cell/ μ l, n=6), higher than the middle of normal range (4500-7000cells/ μ l, n=10) and higher than normal range (ANC>7000 cells/ μ l, n=2). The normal range for neutrophil counts is 2000-7000 cells/ μ l.

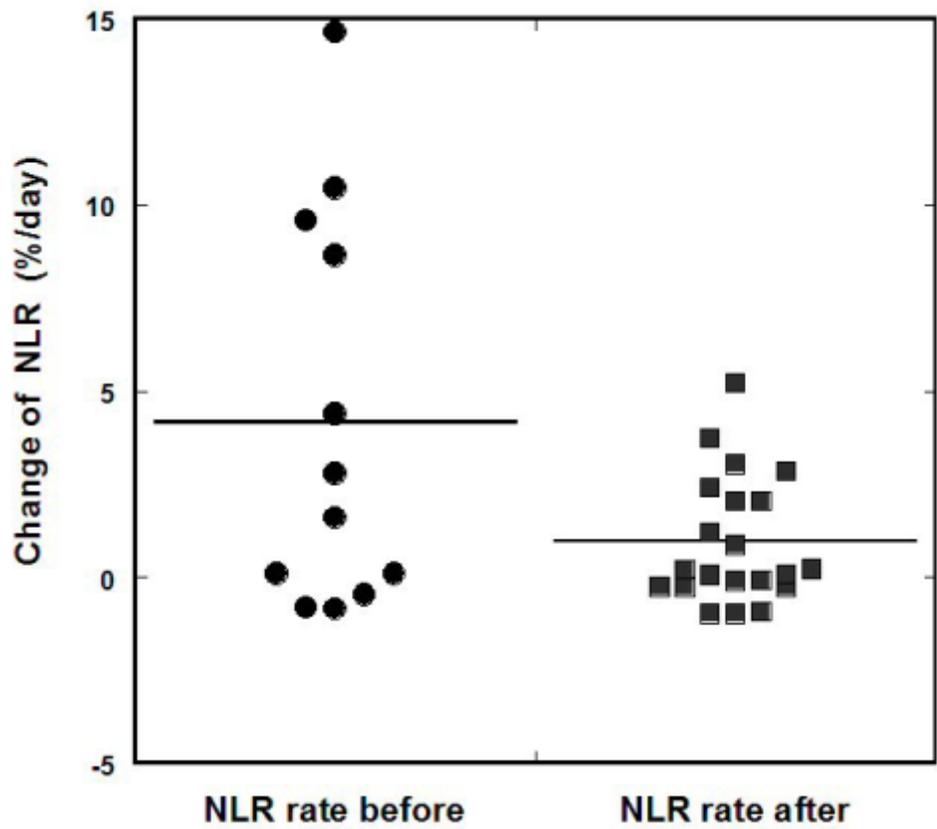


Figure 5

The rate of NLR changes (percentage per day) before and after treatment for patients who completed 6-8 weeks of treatment.