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Study of Vitamin C Administration Effect on Postoperative Plasma IL-6 Concentrations in Septic Patients After Abdominal Surgery

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1. Introduction

Satisfactory post-surgical recovery is reflected in a reduction in plasma levels of pro-inflammatory and anti-inflammatory cytokines, which are higher in septic and surgery patients than in healthy individuals [1-4]. The persistence of inflammatory cytokines in the circulation indicates a generalized systemic inflammatory response and is associated with Multiple-Organ Dysfunction Syndrome (MODS) and death in critically ill patients [5].

In the systemic inflammatory response, inflammation and coagulation can be stimulated by microbial invasion (exogenous injury) or direct tissue injury (endogenous injury), when the NF- κ B protein translocates into the nucleus and activates the transcription of both proinflammatory (e.g. IL-6) and anti-inflammatory (e.g. IL-10) cytokines [6].

In the course of an inflammatory process, endothelial cells and polymorphonuclear leukocytes (PMNs) generate elevated amounts of reactive oxygen species (ROS) [7], which act as secondary mediators, inducing chemotactic substances (e.g. cytokines) and adhesion molecules that amplify the inflammatory process. Vitamin C is a hydrosoluble antioxidant molecule that acts as ROS scavenger and vitamin E regenerator [8] and has been shown to reduce cytokine production in different assays [9-12].

The microcirculation is particularly susceptible to oxidative stress which leads to the systemic inflammatory response syndrome, hemodynamic instability, and multiple organ failure [13]. Vitamin C plasma concentrations are strongly altered in patients with sepsis [14]. Restoring antioxidant and endothelial functions in the critically ill patient requires supraphysiologic concentrations of ascorbate [15]. Such concentrations can only be achieved by parenteral administration. In a trial in 14 patients, 2 days on 3000 mg/d given

intravenously were required in critically ill patients to obtain a significant increase of plasma ascorbate concentrations [16]. The rapid replenishment of ascorbate is of special clinical significance in critically ill patients because these patients experience drastic reductions in ascorbate concentrations, and this may be a causal factor in the development of circulatory shock [17]. In a randomized controlled antioxidant trial including 200 patients in the ICU and providing a combination of selenium, zinc, vitamin C, and vitamin B1, it was shown no survival benefit, but antioxidants shortened hospital stay in surviving trauma patients [18]. In a retrospective trial involving 4294 patients, of whom 2272 received antioxidants for 7 days, resulted in a 28% relative risk reduction in mortality and a significant reduction in both hospital and ICU length of stay [19].

The rationale for a benefit in this category of patients with requirements for major wound healing is strong and is reinforced by the beneficial effects shown in the trials that included vitamin C. The optimal duration of supplementation has not yet been determined, but benefits appear after 5 days of supplementation. On the basis of the pharmacokinetic aspects of antioxidants, an intravenous dose ranging between 1 and 3 g/d should probably be considered independent of parenteral nutrition doses in patients with major trauma at least during the first week after injury [17].

Cytokines exert a key role in inflammatory host defense, therefore the study of their levels and their pharmacological modulation is of great interest for the management of septic patients who underwent abdominal surgery.

2. Patients and methods

2.1. Study design and patients: A prospective, pilot study was performed in 20 consecutive septic abdominal surgery patients in our Digestive Surgery Department with postoperative mortality risk of >30 % by POSSUM score [20]. POSSUM was selected to predict death rate more accurately compared to the APACHE II classification [21]. In all cases, sepsis was produced by peritoneal infection. Patient characteristics are listed in Table 1. Ten healthy volunteers were recruited as a control group. Written informed consent was obtained from all patients or their relatives, and the study was approved by the local Clinical Research Ethics Committee. Allocation of patients to experimental (n=10) or placebo (n=10) group was by envelope randomization (designed by a statistician).

2.2. Treatments: Experimental and placebo treatments were started at 12 h post-surgery. Treatments were administered daily on 6 consecutive postoperative days. The experimental (vitamin C) group received 450 mg/day of the vitamin in 5% dextrose administered in three equal doses, and the placebo group received an identical administration of 5% dextrose following previous studies [22,23]. Placebo-treated patients received no supplemental nutrition containing vitamins nor vitamin C.

2.2. Samples: Early-morning peripheral blood samples were drawn for 7-9 a.m. by venipuncture from all patients on every treatment day into a 3-ml sterile EDTA tube. They were immediately centrifuged at room temperature (400 × g for 16 min), and the

supernatant (plasma) was taken for cytokine determination. Sample collection started at 24 h after vitamin C/placebo administration (T1d) and lasted for 6 days (T1d, T2d, T3d, T4d, T5d and T6d).

2.4. Measurements: Plasma IL-6 concentrations were determined in a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) using the BD Cytometric Bead Array (CBA) (Becton Dickinson, San Jose, CA, USA). The CBA technique employs a series of particles with discrete fluorescence intensities to simultaneously detect soluble analytes [24]. The BD CBA system uses the sensitivity of amplified fluorescence detection by flow cytometry to measure soluble analytes in a particle-based immunoassay. Each bead in a BD CBA provides a capture surface for a specific protein and is analogous to an individually coated well in an ELISA plate. The sensitivity of the BD CBA system was 3.0 pg/ml for IL-6.

2.5. Statistical analysis: A two-way analysis of variance was performed between quantitative independent variables (plasma cytokine concentrations) and qualitative dependent variables (placebo/Vitamin C treatment). Because of the small number of patients in each treatment group, a non-parametric test (U Mann-Whitney) was applied to compare differences between the treatment groups and between these groups and the healthy controls. $P < 0.05$ was considered significant. Data are presented as mean values \pm standard error (SE).

3. Results

General characteristics of patients are listed in Table 1. Four (40%) of the ten patients in each group (vitamin C and placebo) developed MODS during the 6 days postoperative period studied. At the end of this period, the postoperative mortality rate was 40% in the placebo group and 60% in the vitamin C group.

Plasma IL-6 concentrations were significantly reduced in experimental group, which received vitamin C, compared with the placebo group during most of the postoperative period, reaching a significant difference on day 2 and day 6 ($p = 0.048$ and $p = 0.033$) (Figure 1). Table 2 shows IL-6 values during 6th the period of study.

	PLACEBO (n=10)	Vitamin C (n=10)
Age (years)	65.1 \pm 3.6	67.8 \pm 4.5
Mortality risk: %	52.0 \pm 4.1	60.5 \pm 8.7
POSSUM: Score	50.4 \pm 1.4	55.0 \pm 3.3
Sex (Men/Women)	6/4	5/5
Diagnoses		
Peritonitis (perforations or inflammations of gallbladder, colon, biliary or cholecystitis and ulcers)	6	7
Complicated intestinal ischemia	1	1
Intra-abdominal abscesses	1	1
Anastomotic leakage after gastrectomy	1	0
Cholangitis	1	1

Surgical Procedure		
Intestinal resection with or without anastomosis	5	3
Perforation suture	3	3
Collection drainage or anastomosis dehiscence	1	1
Cholecystectomy with or without drainage of biliary tract	1	3
Pressors	2/10	3/10
Ventilatory support	2/10	3/10
Oxygenation Parameters PO ₂ (mmHg)	144.7± 47.3	113.9 ±50.5
Oxygenation Parameters PCO ₂ (mmHg)	47.2 ± 7.4	45.7 ± 7.7
Creatinine (mg/dl)	1.99 ± 0.21	2.15 ± 0.12
Lactate (meq/L)	1.97 ± 0.55	3.22 ±1.25
Volume Status (based on the blood sodium level in mmol/L)	137.42 ±7.19	138.07±6.11

Table 1. General characteristics, diagnoses and surgical procedures of abdominal surgery patients studied. Data are presented as mean values ± standard error of mean (SEM).

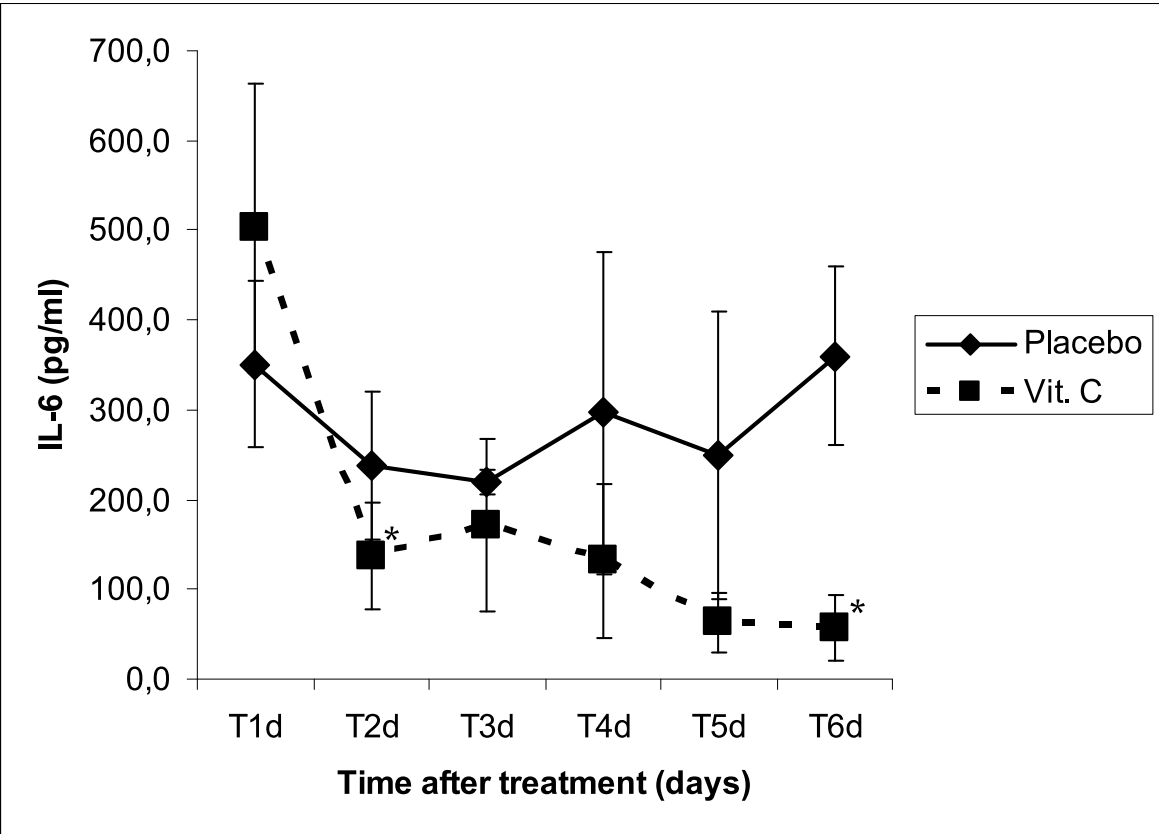


Figure 1. Time course of plasma IL-6 levels (pg/ml) in vitamin C and placebo-treated surgical patients. Statistically significant differences are marked with asterisk (T2d: p=0.048, T6d: p=0.033).

Cytokine	Healthy Control	Group	T1d	T2d	T3d	T4d	T5d	T6d
IL-6 (pg/ml)	9.7 ± 2.4	Placebo	350.5 ± 92.6	237.5 ± 82.3	219.3 ± 13.2	296.3 ± 78.5	248.9 ± 159.6	359.7 ± 99.9
		Vit. C	502.3 ± 161.3	136.4 ± 59.3	171.4 ± 95.5	132.5 ± 85.8	63.0 ± 34.2	57.1 ± 36.2

Table 2. IL-6 values (pg/ml) in placebo and vitamin C groups.

4. Discussion

Plasma IL-6 high concentrations have been documented in high-risk septic abdominal surgery patients [1, 25, 26]. Elevated plasma cytokine levels derive from severe inflammatory processes caused by the disease resulting in an excess of inflammatory mediators. Taking into account the severity of the inflammatory process, plasma cytokine levels have been related to post-surgical complications [4, 25, 26, 27], organ dysfunction development [5, 25, 28, 29] and mortality in critical care, surgery, sepsis and SIRS [1, 3, 29, 30-35]. These events are related to the pro-inflammatory environment that predominates in sepsis, which is responsible for the overactivation of leukocytes and endothelial cells associated with tissue injury. Elevated levels of pro-inflammatory cytokines, notably IL-6, produce early PMN priming and sequestration and are potential mediators of early tissue damage [36,37], exacerbating systemic inflammation through the chronic release of toxic products at inflammation sites [38]. The results presented in our study in patients who underwent abdominal surgery, demonstrate that vitamin C has an inhibitory effect on plasma cytokine concentrations, finding lower IL-6 levels in the vitamin-treated versus placebo-treated patients that reached significance after 2 days and 6 days of treatment. These observations are according with those previously reported for *in vitro* studies by Härtel et al [9] and Bergman et al [10], in studies describing the effect of vitamin C on the intracytoplasmic production of pro-inflammatory cytokines in whole blood samples of healthy individuals, and *in vivo* by Fischer et al [11] reporting that supplementation with antioxidants in healthy volunteers inhibited the IL-6 release from the contracting human skeletal muscle.

The results showed in the present report contribute to clarify the optimal duration of supplementation and reinforce the concept that benefits appear after 5 days of supplementation such as has been noted previously [17].

This inhibitory effect of vitamin C on cytokine production may result from a reduction in the production of ROS, which are at least in part responsible for excessive cytokine production by promoting cell overproduction or by modulating nuclear factors such as NF- κ B, which is involved in cytokine synthesis [7]. The present findings suggest that ROS clearance by scavengers, such as vitamin C, attenuates plasma levels of IL-6, one of the main pro-inflammatory mediators in the acute inflammatory processes observed in septic patients who underwent abdominal surgery and other critically ill patients. A reduction in plasma IL-6 concentrations may improve the prognosis in patients with postoperative persistence of elevated pro-inflammatory mediators, and has been defined as the best parameter for predicting development of MODS as compared to other cytokines [38].

Although different surgical approaches were used in these patients, according to the decision of the surgeons in charge, we emphasize that all patients had a clinical picture of severe peritonitis and a POSSUM score > 50 points. In our view, the differences in surgical procedure would not have influenced results, although a study of a larger sample of patients is warranted to confirm these results.

In conclusion, further studies in larger patient samples are required to establish whether this pharmacological intervention enhances post-surgery recovery, preventing complications and MODS in septic patients who underwent abdominal surgery.

5. Conclusion

Septic patients who underwent abdominal surgery treated with vitamin C for 6 days post-surgery showed a significant reduction in plasma IL-6 levels, which may benefit these patients by downregulating their enhanced systemic pro-inflammatory response.

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