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The Influence of Alpha-Tocopherol on Cytokine Levels and Gastric Intramucosal pH in Severe Sepsis

Alfa-Tokoferol'ün Ağır Sepsiste Sitokin Düzeyleri ve Gastrik İntramukozal pH Üzerine Etkisi

Dilek MEMİŞ[1], Sevgi ESKİOCAK[2], Fatma BAYRAK[3], Emrah BATMAZ[2]

Department of [1]Anesthesiology and Reanimation, [2]Biochemistry, Medical Faculty of Trakya University, Edirne; [3]Department of Anesthesiology and Reanimation, Malatya State Hospital, Malatya

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Objectives: We evaluated the effects of alpha-tocopherol on serum cytokine levels and gastric intramucosal pH in patients with severe sepsis.

Patients ve Methods: Forty patients with severe sepsis were randomized to two groups. Group I (n=20) received 600 mg alpha-tocopherol intramuscularly, and group II (control group, n=20) received the same dose physiological saline solution for three days. The following data were recorded for both groups: hemodynamic parameters, glutathione, catalase levels, nasopharyngeal body temperature, arterial blood gas changes, plasma cytokine levels (interleukin 1≤ and 6), biochemical parameters and intramucosal pH, length of stay in the intensive care unit, duration of mechanical ventilation support, and mortality. All measurements were made at baseline (15 min before alpha-tocopherol administration) and 24, 48, 72, and 96 hours after alpha-tocopherol administration.

Results: None of the parameters evaluated differed significantly between the two groups (p>0.05).

Conclusion: We found that intramuscular alpha-tocopherol 600 mg did not affect hemodynamic and biochemical parameters, gastric intramucosal pH, cytokine levels, or prognosis in patients with severe sepsis.

Key Words: Alpha-tocopherol/therapeutic use; hemodynamics; sepsis; cytokines.

Amaç: Ağır sepsis gelişen olgularda alfa-tokoferol'ün sitokin düzeylerine ve gastrik intramukozal pH üzerine etkisi araştırıldı.

Hastalar ve Yöntemler: Çalışmada ağır sepisli 40 hasta rastgele iki gruba ayrıldı. Grup I'deki (n=20) olgulara üç gün 600 mg alfa tokoferol intramusküler, grup II'deki (n=20) olgulara aynı volümde serum fizyolojik uygulandı. Her iki grupta hemodinamik parametreler, glutatyon, katalaz düzeyleri, nazofarengeal vücut ısısı, arteryel kan gazı değişiklikleri, plazma sitokin düzeyleri (interlökin 1≤ ve 6), biyokimyasal parametreler ve intramukozal pH, yoğun bakım ünitesinde ve mekanik ventilatöre bağlı kalış süresi ve mortalite oranı kaydedildi. Bütün ölçümler başlangıç (çalışmaya başlamadan 15 dakika önce) ve alfa-tokoferol verildikten 24, 48, 72 ve 96 saat sonra yapıldı.

Bulgular: İki grup arasında ölçülen parametrelerin hiçbirinde anlamlı fark saptanmadı (p>0.05).

Sonuç: Ağır sepsisli olgularda üç gün süreyle verilen 600 mg alfa-tokoferol'ün hemodinamik ve biyokimyasal parametreler, gastrik intramukozal pH veya sitokin düzeylerine veya hasta prognozu üzerine herhangi bir etkisini saptamadık.

Anahtar Sözcükler: Alfa-tokoferol/terapötik kullanım; hemodinami; sepsis; sitokin.

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Correspondence (İletişim adresi): Dr. Dilek Memiş. Trakya Üniversitesi Tıp Fakültesi Anesteziyoloji ve Reanimasyon Anabilim Dalı, 22030 Edirne. Tel: 0284 - 235 76 42 Fax (Faks): 0284 - 235 80 96 e-mail (e-posta): dilmemis@mynet.com

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Sepsis is defined as the systemic response to infection.[1,2] The deleterious effects of bacterial invasion of body tissues result from the combined actions of enzymes and toxins, produced by the micro-organisms themselves, and by endogenous cells in response to the infectious process. Patients with severe infections have extremely low concentrations of protective antioxidants and high levels of the metabolic products of free radical damage with the greatest increases seen in the most severely ill patients.[3,4]

Oxidative stress occurs when this balance is disrupted by excessive production of reactive oxygen species (ROS), including superoxide, hydrogen peroxide and hydroxyl radicals, and/or inadequate antioxidant defenses by changes in superoxide dismutase (SOD), catalase, vitamins C and E, and reduced glutathione (GSH).[5,6] The possible sources of ROS during sepsis are: the mitochondrial respiratory chain, the metabolic cascade of arachidonic acid, the protease-mediated enzyme xanthine oxidase, granulocytes and other phagocytes activated by complement, bacteria, endotoxin, lysosomal enzymes, etc., and other oxidases, mainly NADPH oxidase.

The profound oxidative stress that occurs during critical illness leads to early depletion of many endogenous antioxidants. For example, several investigators have documented lower circulating levels of alpha-tocopherol and ascorbate in association with increased levels of oxidized glutathione in the plasma of critically ill patients.[7,8] Given the apparent role oxidative stress and oxidant-mediated tissue injury play in the development of acute respiratory distress syndrome and multiple organ failure (MOF), supplementation with antioxidants may augment endogenous antioxidant defenses and serve to prevent the development of organ dysfunction. Further, there is increasing evidence that antioxidants, particularly ascorbic acid and alpha-tocopherol, may reduce the incidence of infectious complications.[9,10]

Gastric intramucosal pH (pHi) in experimental animals decreases as splanchnic perfusion decreases below the level where local oxygen transport can no longer sustain aerobic energy production.[11] Therefore, it may be possible to utilize pHi as an early and noninvasive index of systemic tissue oxygenation, given that selective reductions in splanchnic perfusion occur with decreases in systemic oxygen transport.[11]

We did not encounter any knowledge in literature, about the effects of alpha-tocopherol on gastric intramucosal pH in severe sepsis. The purpose of the present study was to evaluate the effects of alpha-tocopherol on serum cytokine levels and gastric intramucosal pH in humans suffering from severe sepsis.

PATIENTS AND METHODS

Patient population and study design

The Institutional Committee on Medical Research Ethics approved the study. Written informed consent was obtained from the patients wherever possible, or from the next of kin. Critically ill patients with bacteriologically-documented infections were included in the study as soon as they met at least two of the following criteria of sepsis, defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee:[2] temperature of >38 ºC or <36 ºC; heart rate of >90 beats/min; respiratory of >20 breaths/min or $PaCO_2$ of <32 mmHg; and leukocyte count of $>12x10^9$ cells L^{-1} or $<4x10^9$ cells L^{-1}. In addition, at least one of following conditions was required: hypoxemia (PaO_2/FiO_2 of <250); oliguria (urine output of <0.5 mL/kg^{-1} body weight for 2 hrs); lactic acidosis (lactate concentration of >2 mmol/L^{-1}); thrombocytopenia (platelet count of $<100x10^9$ per L^{-1}) or a recent change in mental status without sedation. Patients who were <18 years of age, pregnant, or receiving corticosteroids, immunosuppressants, or chemotherapy, and those with a known irreversible underlying disease, such as end-stage neoplasm, were excluded.

The acute physiology and chronic health evaluation (APACHE II)[12] was employed to determine the initial severity of illness.

If required, patients underwent surgical procedures before the start of the study. No invasive surgery was performed during the 120-hour

study period. All patients were ventilated in volume-controlled mode (Puritan Bennett 7200, Carlsbad, CA) and received continuous analgesic sedation with midazolam and fentanyl. Ventilator settings, level of positive end-expiratory pressure, and fractional inspired oxygen were kept constant during alpha-tocopherol or placebo infusion intramusculary (im). Antibiotic treatment was adjusted according to bacteriological culture results such as blood culture or samples taken from different sites of the body. In all subjects fluid replacement was administered to keep the central venous pressure between 4-8 mmHg. During the study no inotropic agent was administered. Those patients who met the above criteria for severe sepsis were enrolled into the study within 4 hours of intensive care unit (ICU) admission.

Protocol

Randomization was achieved according to computer-steered permuted block design. The study was prospective, randomized, double-blind, and placebo-controlled. To perform the study in a double-blind protocol, drug solution and infusion was administered to all patients by a nurse without any knowledge about the study protocol. The follow-up was done by an anesthetist without any knowledge of the study protocol. The patients were given alpha-tocopherol (Evigen 300 mg, Eras, İstanbul, Turkey) (n=20, group I) 600 mg i.m. one dose for three days. In the control group (n=20), patients were given serum physiologic same dose i.m. for three days.

A tonometer (TRIP NGS Catheter, Tonometrics, Worchester. MA) was inserted via the nasogastric route before bolus dose. The tonometer was advanced until the balloon was located in the lumen of the stomach. The position of the balloon was confirmed graphically. The silicone balloon of the tonometer was filled with 2.5 mL 0.9% saline. After sufficient time for equilibration of PCO_2 between the saline and the gastric lumen, anaerobic samples of the tonometer saline and of arterial blood were taken simultaneously and analysed with standard pH and blood-gas analysers. pHi was calculated by a modification of the Henderson-Hasselbalch equation:

$$pHi = 6.1 + \log_{10} \frac{\text{arterial bicarbonate concentration}}{F^* \text{x Tonometer Saline } PCO_2}$$

where F is a time-dependent factor for partly equilibrated samples provided by the manufacturer of the device.

Measurements

All patients had arterial catheters (arterial line kit: Abbott, monitoring kit transpac ®IV, Sligo, Ireland) and central venous catheters via subclavian (Braun, Certofix trio V 720 7Fx8", Melsungen, Germany). Arterial blood samples were simultaneously withdrawn for measurements of pH, PO_2, PCO_2 and SaO_2 (Medica EasyBloodGas, Massachusetts, USA). Central venous pressure, mean arterial pressure (MAP), heart rate (HR), nasopharyngeal temperature were continuously monitored (SpaceLabs Inc., Redmond, USA). All measurements were obtained at baseline (15 min before start of the study) and at 24, 48, 72 and 96 hours after alpha-tocopherol administration. Platelets, leukocytes, bilirubin, alanine aminotransferase, and creatinine were determined at the same times (Vitalab Flexor, Dieren, Netherlands).

pHi was measured at baseline (15 min before start of the study) and were repeated immediately after, 24, 48, 72 and 96 hours after alpha-tocopherol administration by the study protocol.

IL-1β and IL-6 levels were measured at the same times. Venous blood was collected into a 10 mL sterile plain tube (without anticoagulant) before administration of any medications and stored at -20 °C. Before assay, all samples were thawed to room temperature and mixed by gentle swirling or inversion. All sera were assayed on the same day to avoid interassay variation. IL-1, IL-6 levels were measured with a solid-phase, two-site chemiluminescent enzyme immunometric assay method (Immulite IL-1β, IL-6 Immulite, EURO/DPC, Llanberis, UK). The antibodies used in this procedure have no known cross-reactivities with other cytokines. The lowest detectable

limits of IL-1β, IL-6, were 1.5 pg.mL^{-1}, 5 pg/mL^{-1}, respectively.

Glutathione level was immediately measured in whole blood samples. Heparinized plasma was separated by centrifugation at 1500 g for 15 min. After the removal of plasma, RBC were washed thrice with normal saline, leukocytes were removed and hemolysed with 1.5 volumes of ice cold distilled water. Erythrocyte catalase activities were measured in hemolysates. Estimation of reduced glutathione was done by the method of Beutler, et al.[13] After lysing red blood cells and removal of precipitate, disodium hydrogen phosphate and DTNB solution were added and color formed was read at 412 nm. Catalase activity was measured according to the method of Aebi by spectrophotometrically following up the decrease in the H_2O_2 concentration at the 240 nm.[14] The results were expressed in U/g Hb. Hemoglobin concentration was determined spectrophotometrically at 540 nm by using Drabkin's reagent.

Duration of mechanical ventilation was recorded. Survival was defined as being alive at hospital discharge.

Statistics

Repeated measures ANOVA test was used to evaluate the differences between and within groups. In case of the presence of any significance, the groups were tested by independent sample t-test to determine which difference was significant. Data were expressed as mean ±SD. A p value of <0.05 was considered significant.

RESULTS

Patient characteristics

Clinical and demographic characteristics of the patients were listed in Table 1. Twenty of 40 patients received alpha-tocopherol (group I) and 20 received placebo (group II). Sixteen patients had septic shock on admission (seven in the alpha-tocopherol group, and nine placebo-treated patients). Baseline APACHE II (18.5±8.8 and 19±6.8, group I and II, respectively), was similar (p>0.05; Table 1). Infection was documented in all patients.

Hemodynamic parameters, oxygen transport variables

There was no significant difference between the groups with respect to pH, PO_2, PCO_2, PaO_2/FiO_2 ratio and SaO_2 (p>0.05). No significant change in MAP and HR was found in either group (Table 2). In biochemical parameters there was no significant difference between the groups (p>0.05) (Table 3).

Outcome

Outcomes were listed in Table 1. The overall ICU mortality rate was three patients (15%) in group alpha-tocopherol, and four patients (20%) in group control (p>0.05). All non-survivors died while being mechanically ventilated. In the alpha-tocopherol and placebo groups, ventilation duration was 9±4 and 10±3 days, respectively (p>0.05). The ICU stay of alpha-tocopherol treated survivors was not significantly different from the placebo-treated survivors (11±3 vs 13±4 days), (p>0.05).

Plasma Cytokine Levels

IL-1β, and IL-6 levels remained unchanged during the study (Table 4).

Glutathione and catalase levels

There was no significant difference between the groups with respect to glutathione and catalase levels (p>0.05; Table 4).

Table 1. Demographic and clinical characteristics of alpha-tocopherol-treated and placebo patients

	Group I (n=20)	Group II (n=20)
Age, years (range)	54 (21-87)	51 (24-82)
Sex, male/female	13/7	9/11
Source of infection		
Respiratory	16	17
Gastrointestinal	1	1
Blood	2	1
Urinary tract	1	1
APACHE II score*	18.5±8.8	19±6.8
Duration of ventilation* (days)	9±4	10±3
Length of stay* (days)	11±3	13±4
Mortality rate, %	15	20

There were no differences between the groups. *: Values are expressed as mean±SD; APACHE II: The acute physiology and chronic health evaluation.

Table 2. Hemodynamic, oxygen and temperature variables

	Baseline	24.h	48.h	72.h	96.h
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Heart rate, beats/min					
Group I	90±34	96±24	98±22	100±20	96±22
Group II	95±27	90±23	100±13	97±26	99±22
Mean arterial pressure, mmHg					
Group I	90±26	92±33	91±13	93±43	93±13
Group II	88±38	93±24	95±22	89±31	89±36
Arterial pH					
Group I	7.34±0.07	7.36±0.04	7.37±0.08	7.36±0.06	7.37±0.06
Group II	7.35±0.05	7.35±0.07	7.32±0.07	7.33±0.05	7.32±0.05
$PaCO_2$,torr					
Group I	35.8±12	34.6±10	35±9	39±6	36±11
Group II	32.6±10	33.5±8	36±13	37±12	34±12
PaO_2/FiO_2, torr					
Group I	192±68	196±46	198±44	193±66	197±76
Group II	194±76	197±35	191±68	195±68	195±35
SaO_2, %					
Group I	97±3	97±3	96±3	95±5	96±4
Group II	96±3	96±3	96±2	95±4	96±49
Temperature, ºC					
Group I	37.5±0.7	37.0±0.6	36.9±0.5	37.1±0.4	37.9±0.4
Group II	37.2±0.5	37.0±0.4	37.2±0.3	37.0±0.5	37.8±0.5

No difference between groups (Data are mean±SD).

Gastric intamucosal pH

There was no significant difference between the groups with respect to gastric intramucosal pH ($p>0.05$; Table 4).

DISCUSSION

Systemic inflammatory response leading to postoperative organ dysfunction and sepsis still remain a formidable clinical challenge and carries a significant risk of mortality. Recent clinical studies suggest that patients with sepsis undergo relative oxidative stress.[15] Overwhelming production of oxygen free radicals is thought to play a central role in the inflammatory process.[3] Reactive oxygen species induce direct oxidative tissue injury by means of peroxidation of cellular membranes, oxidation of critical enzymatic and structural proteins, and induction of apoptosis. Additionally, extensive in vitro data support the relationship between oxidative stress and the induction of genes integral to the systemic inflammatory response, including TNF-α, IL-1, IL-8, and ICAM-1, putatively mediated through activation of the nuclear transcription factor NF-[kappa]B.[16,17] Several in vivo studies document a clear benefit to antioxidant supplementation in animal models of endotoxemia, and reperfusion injury.[17-19]

Oxidative stress, which results from an imbalance between oxidant production and antioxidant defense mechanisms, is cytotoxic to the cells by causing an oxidative damage. It may also act as a regulator of gene expression, promoting the synthesis of proinflammatory proteins.[20] Early work by Takeda et al.[21] found reduced plasma alpha-tocopherol levels accompanied by increased plasma thiobarbituric acid reactive substances levels in critically ill patients compared with controls, suggesting increased lipid peroxidation. Goode et al.[22] investigated antioxidant status in patients with septic shock. They reported reduced plasma concentrations

Table 3. Biochemical parameters

	Baseline	24.h	48.h	72.h	96.h
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Lactate, miligr/dL					
Group I	25.7±4	25±5	26±5.2	24±3.7	25±4
Group II	26.9±8	27±6	27±8	27±3	26±8
Platelets, 10^9/L					
Group I	192±16.5	195±15	188±18	189±15	194±18
Group II	195±14.4	192±15	190±13	190±17	196±13
Leucocytes, 10^9/L					
Group I	14±8	15±7	16±7	16±8	15±8
Group II	15±6	14±7	17±6	18±7	17±6
Bilirubin, mg/dL					
Group I	0.90±0.3	0.90±0.4	0.91±0.3	0.89±0.3	0.92±0.3
Group II	0.90±0.6	0.91±0.3	0.92±0.2	0.93±0.3	0.94±0.2
Alanine aminotransferase IU/L					
Group I	38±5	36±10	35±11	36±8	37±10
Group II	36±7	36±5	36±9	37±4	38±5
Creatinine, mg/dl					
Group I	1±0.9	1.1±0.8	1.2±0.3	1.3±0.8	1.2±0.8
Group II	1±0.9	1.1±0.6	1±0.7	1.2±0.8	1.1±0.6

No difference between groups (Data are mean±SD).

of retinol (vitamin A), vitamin E, β-carotene and lycopene in these patients compared with healthy controls. Cowley et al.[23] described decreased total antioxidant potential in patients with sepsis and secondary organ dysfunction, associated with nonsurvival.

An ideal agent for potential therapeutic consideration is vitamin E. Vitamin E has been described as the major chain-breaking antioxidant in mammalian cellular membranes[24] as a result of its extremely efficient antioxidant capacity interrupting the chain of membrane lipid peroxidations. The ascorbic acid requirement following trauma or infection is not known. Levenson et al.,[25] as early as 1946, reported plasma ascorbic acid levels below normal values in severely injured patients. In some patients, plasma levels of ascorbic acid were immeasurable within a few hours following major injury. They also reported that blood levels of ascorbic acid remained subnormal even with daily 1000-mg intramuscular injections through the eighth day, when excretion normalized. Crandon et al.[26] reported that the daily parenteral administration of 100 mg ascorbic acid to surgical patients had no effect until supplements were increased to 300 mg per day. Even then, the buffy coat ascorbic acid levels still remained borderline. For this reason we used more than 300 mg, lower than 1000 mg, 600 mg of vitamine E. In addition, Long et al.[27] confirmed that maximal early repletion of vitamin E requires rapid pool filling early in the post-injury period using supraphysiologic doses for three or more days. We tried to reach its first effective dosage in three days.

Glutathione metabolism is altered in sepsis. Rapid depletion of intracellular GSH in human and animal endothelial and epithelial cells occurs in response to TNF-α in vitro because of oxidation of GSH to GSSG, followed by rebound increases of GSH synthesis as a result of up-regulation of the enzyme γ-glutamylcysteine synthetase. GSH turnover is increased early in sepsis, with increased GSH synthesis in a number of tissues (especially the liver) but with lower blood GSH concentrations. In children with sepsis, whole blood GSH concentrations and synthesis rates were found to be decreased,[28] while blood GSH

Table 4. Cytokines, gastric intramucosal pH, gastric intramucosal PCO_2, glutathione and catalase levels

	Baseline	24.h	48.h	72.h	96.h
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Il-1β, pg/mL					
Group I	6.4±3.8	6.8±6	6.2±6	6.5±1.8	6.9±6
Group II	6.3±3.4	6.6±4.3	6.3±8.2	6.7±2.7	7.±4.3
IL-6, pg/mL					
Group I	100±58	105±44	108±57	104±58	108±47
Group II	114±38	115±48	113±51	116±38	110±51
Gastric intramucosal pH					
Group I	6.41±0.2	6.43±0.2	6.43±0.1	6.3±0.2	6.4±0.3
Group II	6.26±0.1	6.31±0.1	6.45±0.4	6.1±0.1	6.4±0.5
Gastric intramucosal PcO_2					
Group I	67±20	70±15	69±29	64±15	66±25
Group II	68±28	67±20	68±25	69±20	63±25
Glutathione, mmol/dl RBC					
Group I	4.15±2	3.95±1.8	4.3±2	4±2.3	4±2.5
Group II	4±1.8	4.2±1.9	4.5±1	4.2±3.3	4.5±1.7
Catalase, U/mg Hb					
Group I	25±11	28±8	26±15	29±5	27±10
Group II	29±8	26±12	32±2	28±6	30±2

Data are mean±SD (IL=interleukin).

redox ratios were found to be increased,[29] suggesting increased oxidative stress.

Sepsis and septic shock remain as major causes of death in ICUs. Complications of sepsis have been related to an intense host response based on a delicate equilibrium between various pro- and anti-inflammatory mediators.[30] An overwhelming production of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-2R, IL-6, and IL-8 may induce biochemical and cellular alterations either directly or by orchestrating secondary inflammatory pathways. Cytokine levels in the plasma do not necessarily reflect the local synthesis of cytokines by cells. Many cells have surface receptors for these cytokines with high binding properties, and target cells and soluble receptors trap cytokines. Thus cytokines released at the local level may remain undetected in the plasma. In our study, we found that plasma cytokine levels remained unchanged during a period of 96 hours.

Oxygen radical scavengers, administered before or at the onset of sepsis, were shown to improve the survival rate in animal models of sepsis.[31] The gastric intramucosal pH effects of antioxidant administration has not previously been reported. Nathens et al.[32] found that critically ill surgical patients suggested benefit from the routine, early, prophylactic administration of alpha-tocopherol and ascorbate. In our study, we found that alpha-tocopherol did not affect gastric intramucosal pH and hemodynamic, biochemical and arterial blood gases in severe sepsis in humans. But, our study was designed to assess the effects of alpha-tocopherol treatment given before septic shock but after systemic inflammatory response syndrome. For this reason, there was no effect in our patients.

In summary, we found that intramuscular alpha-tocopherol did not affect hemodynamic and biochemical parameters, gastric intramucosal pH or cytokine levels (IL-1β and IL 6) or patients' outcome in severe sepsis in humans. Effects of alpha-tocopherol in severe sepsis requires further study. However, the findings reported here are disappointing, alpha-tocopherol lack the benefit to patients with severe sepsis.

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