THE EFFECT OF 3000 mg INTRAVENOUS VITAMIN C ON SERUM NITRIC OXIDE LEVEL OF SEVERE BURNT INJURY PATIENTS

Bobby Swadharma Putra[1], Lobredia Zarasade[2], Iswinarno Doso Saputro[2]
[1]Department of Surgery, Lampung University, Lampung, [2]Department of Plastic & Reconstructive Surgery
Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital, Surabaya

ABSTRAK

*Nitric oxide (NO) berperan menyebabkan hipotensi sitemik dan apoptosis, yang berperan dalam terjadinya kerusakan jaringan dan multiple organ failure. Efek antioksidatif vitamin C menekan pembentukan radikal bebas, menghentikan pelepasan sitokin pro-inflamasi dan menghambat produksi NO. Tujuan penelitian ini adalah mengetahui pengaruh pemberian vitamin C 3000 mg intravena terhadap kadar NO darah pada pasien luka bakar berat setelah resusitasi baxter. Dilakukan uji klinis acak pada 12 pasien luka bakar berat di Unit Luka Bakar Dr. Soetomo Hospital, Surabaya. Sampel dibagi dalam 2 kelompok: Kontrol (K1) diberi vitamin C 2x400 mg/ 24 jam selama 72 jam, kelompok perlakuan (K2) diberi vitamin C 3000 mg/24 jam intravena selama 72 jam. NO diukur dengan metode Griess. Hasil dianalisis dengan Uji t berpasangan (signifikan pada p< 0,05). Setelah penelitian, kadar NO K1 menunjukkan peningkatan namun tidak signifikan dibanding hari ke-1 (p=0,21); kadar NO K2 menunjukkan penurunan namun tidak signifikan dibanding hari ke-1 (p=0,06); terdapat penurunan signifikan kadar NO darah pada K2 dibandingkan dengan K1 (p=0,02). Tidak terdapat perubahan signifikan pada hasil Blood Gas Analisis (BGA), Blood Urea Nitrogen (BUN), dan Serum Creatinine (SC) pada K1. Terdapat penurunan signifikan kadar sel darah putih pada K2 setelah perlakuan (p=0,01). Pemberian vitamin C intravena 3000 mg/24 jam selama 72 jam menunjukkan penurunan signifikan kadar NO darah pada pasien luka bakar berat.**(FMI 2014;50:63-66)***

Kata Kunci: *vitamin c, luka bakar berat, nitric oxide*

ABSTRACT

Nitric oxide (NO) has a potential role to cause systemic hypotension and apoptosis, contributing to tissue damage and multiple organ failure in severe burnt. Antioxidative effect of Vitamin C supreses free radical formation, blocks pro-inflammatory cytokine release and inhibit NO production. The purpose of this research is to review the effect of intravenous vitamin C 3000 mg on serum NO level in severe burnt injury patient post fluid resuscitation. Randomized pre test and post test controlled group design experimental studied on 12 severe burnts patient at the Burn Unit of Dr. Soetomo Hospital, Surabaya. Samples were divided into 2 groups: K1 (control) administered with vitaminC 2x400 mg/24 hour for 72hours; K2, administered with intravenous vitamin C 3000 mg for 72 hours. Serum NO level was re-examined by Griess method. The results were analyzed by paired t-test (significant at p<0.05). After research, serum NO level of K1 showed no significant increase compared to day 1 (p=0.21); serum NO level of K2 showed no significant decrease compared to day 1 (p=0.06); there is a statistically significant decrease of serum NO level in K2 compared to K1 (p=0.02). There was no significant change in Blood Gas Analysis (BGA), Blood Urea Nitrogen (BUN), and Serum Creatinine (SC) on K1. There was significant decreased in White Blood Cell (WBC) on K2 (p=0.01). Administration of intravenous vitamin C 3000 mg/24 hours for 72 hours showed significant decrease in serum NO level of severe burnt patients. ***(FMI 2014;50:63-66)***

Keywords: *vitamin c, severe burnt, nitric oxide*

Correspondence: Bobby Swadharma Putra, Department of Surgery, Lampung University, Jl. Prof. Dr. Sumantri Brojonegoro No. 1, Bandar Lampung 35145, Lampung, Indonesia

INTRODUCTION

The management and treatment of burn injury is still a challenge for professionals of the medical field due to the high number of morbidity and mortality. In The United States in 2011 500,000 cases of burns were reported each year with number of deaths around 3-4 thousand deaths per year. In The Burn Injury Unit of Dr. Soetomo Hospital, Surabaya, the number of cases treated within a year period (January 2011 – December 2011) reached 167, 94 of them (56.29%) were classified as severe burnt injury. The number of deaths was 11 people (19.54%) from all the severe burnt injury cases.

One of the systemic responses caused by severe burnt injury is the activation of nuclear factor κB (NF-κB), a transcription factor protein in macrophages which will increase the production of inflammatory mediators or pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL) and interferon γ (IFN-

γ). Elevating level of inflammatory mediators will induce expression of nitric oxide (NO). NO is a cellular mediator produced by one of three NO synthases: nNOS (neural nitric oxide synthase), eNOS (endothelial nitric oxide synthase) and iNOS (inducible nitric oxide synthase). iNOS is expressed by some types of immune cells, especially macrophages and is a key mediator of several inflammatory responses. In severe burnt injury, high level of NO will be produced by iNOS in the macrophages, liver cells, and endothelial cells of the blood vessels. Increased production of NO in severe burnt injury causes functional changes in the cardiovascular system such as systemic hypotension, vascular hypocontractility, myocardial depression, circulatory failure, and even Multi-system Organ Dysfunction Syndrome (MODS) (Çakir & Yegen 2004).

Vitamin C is an antioxidant capable to react with Reactive Oxygen Species (ROS) produced by neutrophiles and macrophages in inflammatory response phase and converts free radicals into a more inert form. The arachidonic acid cascade activated by ROS could be interrupted by vitamin C activity, resulting in termination of inflammatory response. Thus, the anti-inflammatory effect of vitamin C is thought to be correlated to its anti-oxidative properties (Lima et al 2009). Farris (2005) stated that vitamin C as an anti-inflammatory agent is also capable of suppressing the activation of NF-κB which will consequently inhibit the production of NO (Farris 2005). Therefore, administration of vitamin C to severe burnt injury patients could supposedly lower the level of serum NO. This research was conducted in order to investigate the effect of intravenous administration of vitamin C 3000 mg/24 hours for 72 hours post fluid resuscitation towards serum NO level in severe burnt injury patients.

MATERIALS AND METHODS

The population in this randomized clinical trial is severe burnt injury patients post fluid resuscitation in Dr. Soetomo Hospital, Surabaya. Severe burnt injury patients with multiple trauma, HIV, liver, respiratory, cardiac, and kidney disorders were excluded from the research. History taking and physical examination were conducted. Blood Urea Nitrogen (BUN), Serum Creatinine (SC), and Blood Gas Analysis (BGA) from each patient were also analyzed. Samples were divided into two groups. Control Group (K1) (n=6) consisted of severe burnt injury patients who were given standard vitamin C therapy 2x400 mg/24 hours for 72 hours. Treatment Group (K2) (n=6) consisted of severe burnt injury patients who were given vitamin C therapy 3000 mg/24 hours intravenously for 72 hours. Blood samples were collected from peripheral vein (cubital vein) as many as 3 cc from both groups to examine serum NO level before treatment. After 72 hours, blood samples were recollected to evaluate serum NO level after treatment. Serum NO level was examined with the Griess method using the Cayman Chemical Nitrate tool/nitrite assay. To ensure patient safety, medicines to treat adverse drug reactions were prepared. Patients were discontinued from the research if there occurs conditions related to basic diseases, heart failure, and unexpected drug reactions or by the patient's own will.

RESULTS

Twelve severe burnt injury patients (7 male, 5 female) eligible for this study were divided into two groups: Control Group (K1) and Treatment Group (K2), each consists of 6 patients. Mean age of the total sample was 36.17 ± 12.99 years old. Mean age of K1 was 39.2 ± 15.2 years old, the youngest was 17 and the oldest was 61 years old. Mean age of K2 was 33.2 ± 10.9 years old, the youngest was 20 and the oldest was 51 years old. Mean burn percentage of the total sample was 40.2 ± 18.1%. Mean burn percentage of K2 was 32.8 ± 16.6 and K2 was 47.6 ± 17.7. Data of patient's characteristics are displayed in table 1.

Table 1. Patient's Characteristics

Variables	K1 n=6	K2 n=6	p
Sex			
male	3	4	
female	3	2	
Age	39.2 ± 15.2	33.2 ± 10.9	
Burn %	32.8 ± 16.6	47.6 ± 17.7	0.166

The mean difference of leukocytes, serum creatinine, BUN and blood pH in K1 between day 1 and day 4 was not statistically significant (p>0.05) while albumin level of K1 showed a significant decrease (p=0.04). K2 showed significant decrease of leukocyte level (p = 0.01) from day 1 to day 4 but serum creatinine level, BUN, blood pH and albumin level was not significantly different. To determine normality of data distribution, Kolmogorov-Smirnov normality test was preformed on serum NO level before statistical test and both groups show normal data distribution.

Paired-t test was used to analyze the change in serum NO level before and after treatment in each group. In K1 there was an increase of serum NO level (4.7 ± 3.2 to 6.5 ± 3.3 µM) but was not statistically significant (p = 0.21) (Figure 2). In K2 there was a decrease of serum NO level (6.6 ± 3.1 to 3.8 ± 1.4 µM) but was not statistically significant (p = 0.06) (Figure 3).

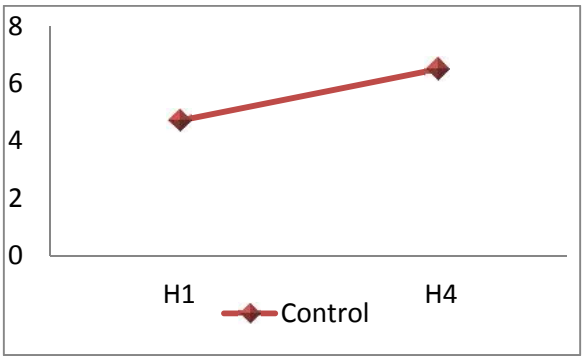

Figure 1. Average serum NO level of K1

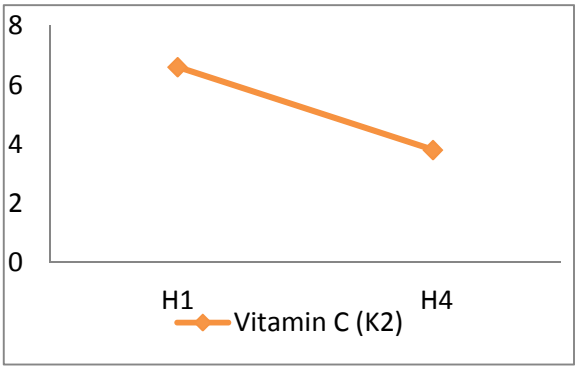

Figure 2. Average serum NO level of K2

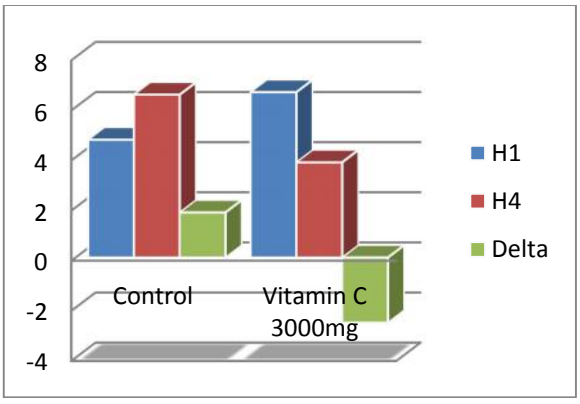

Figure 3. Average Serum NO level of K1 and K2 and difference between day 1 and day 4.

Independent-t test is conducted to analyze mean differences between K1 and K2. K1 showed 1.8 ± 3.1 µM increase of serum NO level from 7 ± 3.2 µM to 6.5 ± 3.3 µM. K2 showed -2.6 ± 2.7 µM decrease of serum NO level from 6.6 ± 3.1 µM to 3.8 ± 1.4 µM. The difference of serum NO level between both groups were statistically significant with p = 0.02 (Figure 2).

DISCUSSION

Local and systemic changes in burn injury patients are caused by inflammatory mediators. Severe burnt injury also causes depression of cellular response, humoral immune response and phagocytic aspects of macrophages. Burn injuries initiate a systemic inflammatory reaction caused by burn injury toxins and oxygen radicals which results in peroxidation. Reactive oxygen metabolites cause destruction on the cell membrane by lipid peroxidation. Association between the amount of oxidative metabolism products and natural scavengers of free radicals determine the outcome of local, systemic tissue damage or the following organ failure in burn injury (Horton 2003). Release of inflammatory cytokines (TNF-α, IL-1 dan IL-6) is an important mechanism in regulation of acute phase response towards burn injury. TNF-α is a mediator cytokine which induces secondary cytokine cascades and humoral factors responsible of causing systemic and local sequel. TNF-α is also involved in development of shock-like conditions related to burn injury and sepsis (Çakir & Yegen 2004). Nitric oxide (NO) is a biological molecule produced by various types of cells with both positive and negative effects in vascular and cellular levels. NO is an important key in the pathogenesis of sepsis. Activation of iNOS will cause increased NO production and indicates that L-arginine is available at an adequate amount (Chen et al 1999).

Schorah and Downing reported that patients treated in the Intensive Care Unit showed a decrease in total Vitamin C, ascorbic acid and dehydroascorbic acid compared to gastritis patients, diabetes patients and healthy people (Schorah et al 1996). Long et al reported that parenteral administration of ascorbic acid 300 mg per day does not change the plasma level of ascorbic acid. Plasma level of ascorbic acid begins to rise at 1000 mg per day in two days but the level is still below normal. It needed three or more days after parenteral dosage of 3000 mg per day to reach normal level (Long et al 2003). Transcription factor NF-κB has a crucial role in inflammation process. NF-κB is a transcription factor responsible of inducing cytokine production. Administration of lipoplysaccharide (LPS) will activate NF-κB which increases production of inflammatory mediators such as IL-8, TNF-α, intercellular adhesion molecule (ICAM) and cyclooxygenase-2. Vitamin C as an anti-inflammatory agent is capable of suppressing nuclear factor κB (NF-κB) activity and inhibiting tumor necrosis factor α (TNF-α). NF-κB is a transcription factor responsible of the production of several inflammatory cytokines such as TNF-α, interleukin-1 (IL-1), IL-6, and IL-8 (Farris 2005).

The result of this study shows significant decrease of leukocyte level in patients treated with 3000 mg vitamin C. This is supported by the study of Farris (2005) which stated that antioxidative activity of vitamin C is related to its anti-inflammatory effect responsible of decreasing leukocyte level.

Galley et al (1996) found that total vitamin C concentration in sepsis patients is lower than healthy patients. This is consistent with the previous study that shows low levels of ascorbic acid in critically ill patients, as well as decreased ascorbic acid to dehydroascorbic acid ratio. Low concentration of vitamin C suggests that subjects were prone to oxidative stress. This research showed an insignificant decrease in serum NO level after vitamin C 3000 mg administration. This is probably caused by the short period of administration (72 hours). However there was a significant decrease in between serum NO level of control group and treatment group. Antioxidants are components of body defense system towards free radicals, endogenous and exogenous, present in normal cells. Antioxidants are capable to protect the cellular components to prevent free radical formation. High dose of vitamin C administration causes diarrhea, kidney stones and kidney disorders. In this research, we did not obtain significant increase in BUN level and serum creatinin level in the treatment group. The limitation of this research were measurement of NO level could not be conducted in Dr. Soetomo Hospital, Surabaya and difficulty of finding eligible severe burnt patients in the limited time allowed to obtain adequate sample size.

CONCLUSION

There was a statistically significant decrease in serum NO level of severe burnt injury patients given intravenous vitamin C 3000 mg/24 hours for 72 hours compared to control patients given intravenous vitamin C2x400 mg for 72 hours. We suggest further research with larger sample size, longer period and measurement of total plasma vitamin C level previous to treatment.

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