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Vitamin E Effects on Intestinal Damages in Burned Rats

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ABSTRACT

Background: Vitamin E is a fat–soluble agent protecting cells from free radicals damages. Previous studies have shown that oxidative stress plays an important role in mucosal intestinal damages in burn trauma. This study aimed to investigate vitamin E effects on small intestinal mucosal changes in burned rats. ***Methods:*** Mature male rats (n=32) weighing 260 ± 10 g were used in this experiment. After induction of deep general anesthesia, a determined area of rats' back region (10% of body surface) was exposed to 95^{oC} water for 8 seconds to induce a second-degree wet burn. The evaluated groups in our study were: 8 rats without burning, 8 rats without burning treated with vitamin E, 300 mg/kg/day for 15 days, 8 burned rats without medication and 8 burned rats treated with vitamin E, 300 mg/kg/day for 15 days. All rats were killed on fifteenth day by ether inhalation. The samples were taken from the first part of small intestine and were stained by Hematoxylin & Eosin method. ***Results:*** Burned rats receiving vitamin E had a higher intestinal villi height and lower intestinal lumen diameter as compared to burned rats without the vitamin E treatment ($P < 0.05$ and $P < 0.01$, respectively) and those values were close to the results of unburned ones. There were no significant differences among the study groups regarding the intestinal diameter and muscular layer thickness. ***Conclusion:*** Vitamin E can improve intestinal villus height and lumen diameter and its consumption at the time of burning may protect intestine mucosa.

Introduction

Burn injury is associated with a high incidence of death and disability.[1] Fires cause 1% of the global burden of diseases[2] and 300,000 deaths per year.[3] The leading causes of death in burn patients are multiple organ failure and infection.[4] In serious burns, pathologic and physiologic changes are not limited to skin and affect the whole body systematically.[5,6] There is a significant decrease in organ blood flow, which can be complicated by sepsis and multi-organ failure.[7,8] Intestine, kidney and liver are the organs most affected by prolonged tissue hypoxia and reperfusion injury.[9,10] Shortly after thermal injuries, approximately 6 hours, a significant vasoconstriction of omental arterioles induces apoptosis of small bowel epithelium and causes increased cell death.[11-13] In addition, shortened intestinal villi and reduced DNA contents have been observed in burned patients.[14] Oxygen free radicals such as superoxide anions and hydroxyl radicals are produced by lipid peroxidation after burn.[15] It is believed that these radicals play an important role in development of burn shock and multiple organ injury.[16-18] Stopping oxidative products generation has been shown to enhance the rate of epithelialization.[19] The antioxidants can scavenge the free radicals that are produced during the burn stress.[20] This is especially true of Vitamin E.[21,22] It was reported that the burn patients have depleted stores of Vitamin E.[23] These antioxidants may reduce the lipid peroxidation[21] in intestinal cells by reducing the free radical contents. The aim of this study was the evaluation of vitamin E effects on small intestine mucosal changes in burned rats.

Methods and Materials

Animal models

Thirty-two Spraque-Dawley male rats (four months old, body weight 250-270 g) were used in this experiment. The animals were housed in metal cages in a temperature-controlled room (25 ± 2 °C) with a 12-hour light/dark cycle (lights on at 8:00 A.M.). Standard rat

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chow and water were freely available. All experiments were performed in accordance with institutional guidelines for animal care and use and also "Principles of laboratory animal care" were followed.

Study groups

The animals were randomly divided into four groups (C, CE, B and BE), each consisting eight rats (n=8). The evaluated groups of this study were: rats without burning and medication (Control: unburned rats that shaved their back and received water by gavage as vehicle) rats without burning treated with vitamin E (CE: Unburned rats that shaved their back and received Vitamin E by gavage), burned rats without medication (B: Burned rats that received water by gavage) and burned rats treated with vitamin E (BE: Burned rats that received Vitamin E by gavage). We used water - miscible vitamin E from Merck Co. with product number of 5008621000, so we used water as solvent. Rats in burn groups experienced burning trauma and were subsequently fed in the next 15 days. Animals in non-vitamin E groups were fed with standard food whilst Vitamin E groups' rats received 300 mg/kg vitamin E by gavage daily.

Anesthesia and Burning

Sixteen out of thirty-two rats of this study were burned. General anesthesia was induced by the injection of ketamine (50mg/kg) and diazepam (5mg/kg) before burning. The route of injection was intramuscular. After anesthesia, animal's back hairs were shaved and an area of 10 percent of the animal's body surface was exposed to heat. Walker's formula was used to calculate the extent of burning region according to animal's weight. An especial mold was built to precisely control the burning area. The interior surface of the mold was insulated against the heat and a 3.5×5 cm opening was made in its upper concave wall. To induce the burning stress, the mold was filled with 95$^{\circ C}$ water and animal's back region was cautiously put in the water for 8 seconds in the supine position. To prevent systemic shock, 5 ml of normal saline (18.5-20 ml/kg) was injected intraperitoneally both in control and test groups.

Sampling

The rats were initially weighted and then killed by diethyl ether inhalation on fifteenth day after the burn. After opening the peritoneal cavity, a 0.5 cm sample of the first duodenum was taken and fixed, using 10% formaldehyde in phosphate buffer (pH 7.4).

Tissue process and staining

Tissue process consists of dehydration, clearing and infiltration steps. Samples were transferred through progressively more concentrated baths of ethanol to remove water. 50%, 60%, 70%, 80%, 90% v/v and absolute alcohol were used for 30 min RT each. Xylene was then used to remove the alcohol. Finally, paraffin was used as the infiltration agent. The samples were embedded in paraffin molds. The paraffin blocks were cut into 5-micrometer-thick sections using leitz microtome. After preparing the samples, hematoxylin and eosin (H&E) method was used for staining.

Morphometric measurements

All morphometric measurements were made in tissue sections under 10x magnification of light microscope (Leica DM5000). The microscope was equipped with a reticule, especial scale, to allow measurements. Transverse circular sections were used for morphometric measurements. The measured parameters were intestinal diameter, intestinal lumen diameter, intestinal muscular layer thickness and villi height. Layers' border determination was done according to histological definitions. The greatest and the least amounts of each parameter were measured in each section and the mean value was considered as the amount of that parameter in that section. Measurements were made in ten sections for each case and the mean value of these numbers was considered as the final amount.

Statistical analysis

Statistical package for the social sciences (SPSS for Windows, version 17 Chicago, IL, USA) was used for data analysis. All data was represented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for multiple comparisons of the groups, followed by Tuckey's post-hoc test. *P value* < 0.05 was considered as statistical significance limitation.

Results

Intestinal diameter

There were no significant differences among experimental groups in intestinal diameter. Even though burn group rats had a slightly lower intestinal diameter compared to control groups, the difference was not significant (table 1).

Mean values of intestinal diameter in experimental groups are shown in figure 1-A.

Intestinal lumen diameter

Intestinal lumen diameter was significantly higher in burn group compared to control (*p value* < 0.05) and control with vitamin E (*p value* < 0.01) groups. There was not any significant difference between burn rats treated with vitamin E and control groups (table 1). Mean values of intestinal lumen diameter in experimental groups are shown in figure 1-B.

Muscular layer thickness

No significant differences were observed among study groups in muscular layer thickness. Burn group rats had a slightly higher muscular layer thickness compared to control groups, but the difference was not significant (table 1). Mean values of muscular layer thickness in experimental groups are shown in figure 1-C.

Table 1. Comparing of vitamin E effects on small intestinal mucosal changes in burned and control rats.

Groups Variables	Control (n=8)	Control with Vit E (n=8)	Burn (n=8)	Burn with Vit E (n=8)	p value
Intestinal diameter	3200 ± 167.33	3190 ± 176.36	3150 ± 187.76	3200 ± 141.42	NS
Intestinal lumen diameter	641.67 ± 80.1	621.56 ± 82.32	848.33 ± 64.93*	583.33 ± 40.82	p < 0.01
Muscular layer thickness	94.17 ± 14.28	95.13 ± 12.46	99.17 ± 10.68	93.33 ± 10.80	NS
Villi height	950 ± 34.05	951 ± 32.21	848.33 ± 58.79*	921.67 ± 27.86	p < 0.05

Villi height

Villi height was significantly lower in burn group compared to control (*p value* < 0.01) and control with vitamin E (*p value* < 0.05) groups. There was no significant difference between burn group treated with vitamin E and control groups in villi height (table 1). Mean values of villi height in different experimental groups are shown in figure 1-D.

Discussion

This study implies that vitamin E possesses inhibitory effects on morphometric changes of the small intestinal mucosa after burn injury. In other words, it reduces cell death in small bowel epithelium.

Apparently, burn injuries damage the skin more than anywhere else in the body. But in serious burns, the body is affected systematically.[6] Redistribution of blood from gastrointestinal viscera and kidneys to vital organs and the affected skin is the compensatory mechanisms. This redistribution, especially in prolonged periods, may have inevitable consequences for hypoperfused organs.[24] Gastrointestinal system is one of the most affected systems in burn patients.[25] Ischemia-reperfusion injury of the intestine is an important factor associated with high rates of morbidity and mortality.[26] One of the major functions of the gut is to prevent the absorption of toxins, antigens, proteases and microorganisms across the intestinal wall.[27]

Severe vasoconstriction occurs in gastrointestinal vasculature after the burn injury and gastrointestinal barrier dysfunction can permit the harmful agents to enter the blood circulation.[10,28] Septicemia is the leading cause of death in burn patients.[29] There are reports of as many as 30% of the burn patients who no detectable microorganisms were found in their repeated wound cultures, while their blood cultures were positive for gut flora.[30,31] It is thought that intestinal microbial flora is the major source of this group of septicemia. So it is of great importance to save this physiologic barrier after the burn injury. Based on histological examination, it was found that the small intestine is more susceptible to ischemia/reperfusion injury than the colon and most of the previous studies have focused on small intestine.[27]

Several studies have suggested that hypoperfusion and ischemia/reperfusion injury of the gut, as well as the release of proinflammatory cytokines and free radicals are associated with apoptosis of the small bowel epithelium.[28,32,33]Free radicals are very unstable molecules, which react quickly with other compounds, trying to capture the needed electron to gain stability.[34] To avoid this harmful electron transfer, intra and extra cellular antioxidants try to eliminate these unstable molecules. There is a regulated balance between oxidant and antioxidant agents in the body.[35] Oxidative stress induced by oxidant species, results in decline of antioxidant defense mechanisms in various organs of the burn patients.[36,37] If the body cannot keep the balance and free radicals overcome the defense system, they will start to attack DNA, proteins and lipids that results in cellular damage.[38,39] Free radical burst is associated with ischemia-related skin tissue injury.[40] Enhanced free radical production is accompanied by impaired antioxidant mechanisms.[41]
Vitamin E functions as a chain-breaking antioxidant in vivo and prevents the propagation of free radicals damage in biological membranes.[42,43] It is a potent peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) in cell membranes and plasma lipoproteins.[44,45] Vitamin E modulates smooth muscle cell proliferation, platelet adhesion and aggregation, and monocyte endothelial adhesion.[46-48] In addition, vitamin E has been shown to reduce intestinal cellular damages, caused by oxidative stress, in different kinds of diseases, including diabetes and infectious diarrhea.[49,50] Previous studies have shown that Vitamin E is depleted during cardiac diseases, as well as after burns, because of increased oxidative stress.[23,51]

Our results are consistent with previous studies, which reported decreased intestinal villous height in post-burn patients.[52,53] In this study, it was shown that vitamin E

Fig 1. Effects of Vitamin E on intestinal parameters in control and burn experimental groups. (A) Intestinal diameter. (B) Intestinal lumen diameter. (C) Muscular layer thickness and (D) villi height.

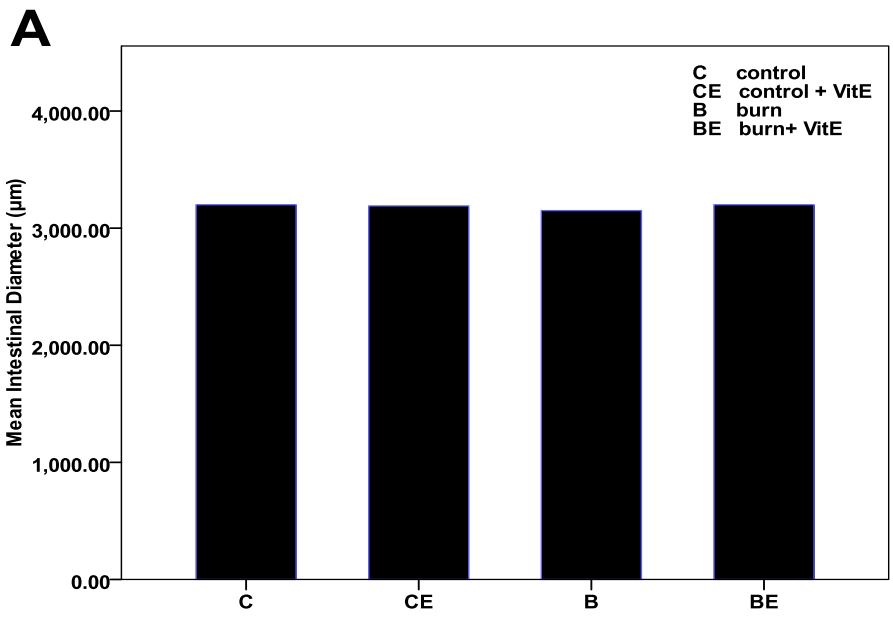

Fig 1A. Effects of vitamin E on intestinal diameter in control and burn experimental groups.
* shows the significantly different group.

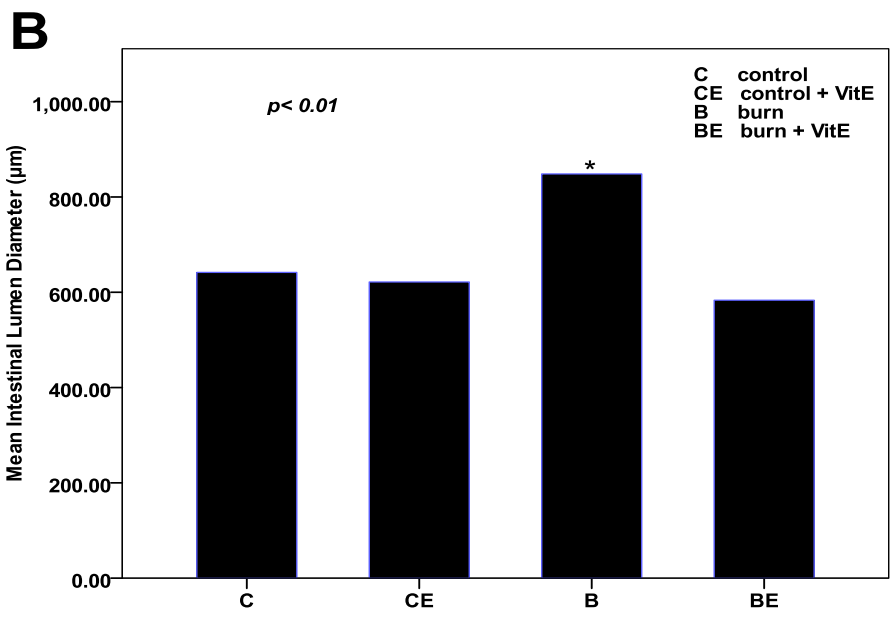

Fig 1B. Effects of vitamin E on intestinal lumen diameter in control and burn experimental groups.
* shows the significantly different group.

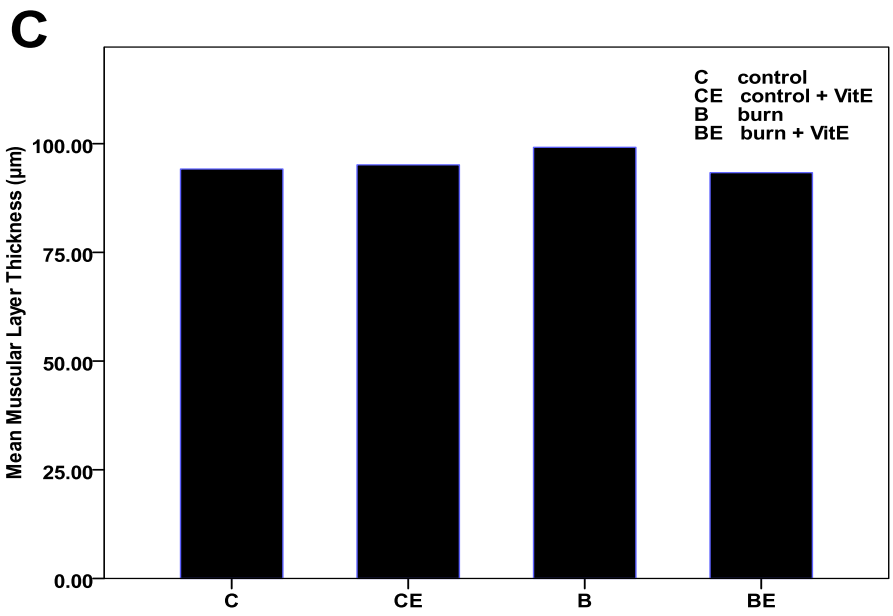

Fig 1C. Effects of vitamin E on muscular layer thickness in control and burn experimental groups. * shows the significantly different group.

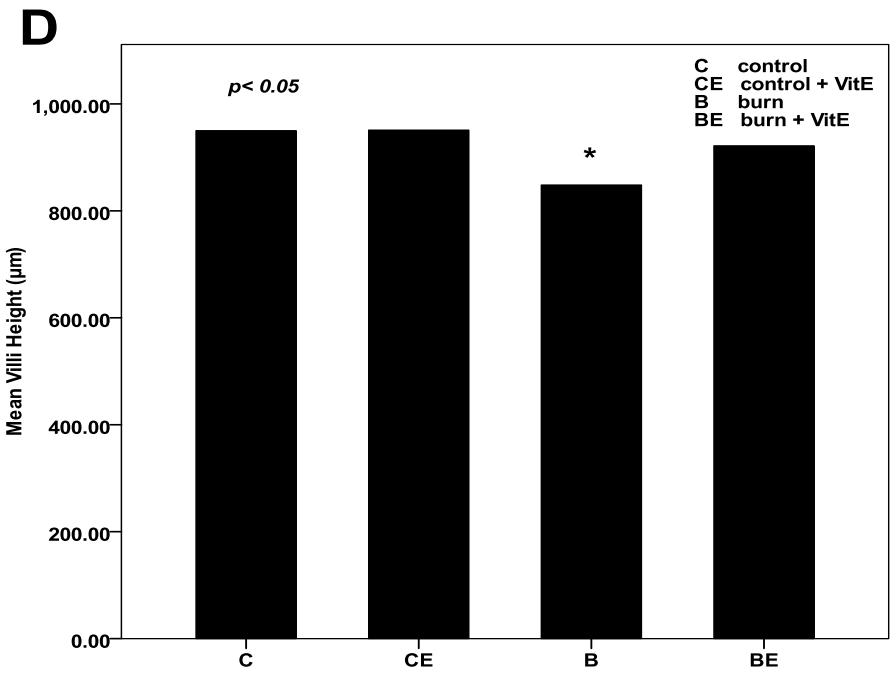

Fig 1D. Effects of vitamin E on villi height in control and burn experimental groups. villi height. * shows the significantly different group.

could reduce small intestinal mucosal injuries in burn patients. It has also helped the lumen diameter remain almost normal. Several different factors were used in previous studies to enhance the proliferation capacity of small intestinal epithelium in burn models. Growth hormone, insulin like growth factor-I and hepatocyte growth factor are among the hormonal factors that were successfully used to reduce the intestinal mucosal damage in burned rats.[13,54,55] To our knowledge, this is the first study that shows the beneficial effects of vitamin E on intestinal mucosa in burned rats. The mechanism by which vitamin E inhibits the epithelial cells apoptosis and enhances their proliferation is a matter of speculation. It is believed that vitamin E

influences the vascular diameter by increasing the production of vasoactive molecules, such as prostaglandin I2 and E2.[56] Effects of vitamin E on endothelial cells have been shown in several studies.[57] vitamin E has a regulatory effect on phospholipase A2 and increases the production of arachidonic acid in endothelial cells.[58,59] In addition, vitamin E is a well-known potent antioxidant agent, protecting the cells from oxidative stress.[60,61] There is increasing evidences that oxidative stress has an important role in the development of multiple organ failure in severe burns.[39,62] Whether the observed effect of vitamin E in this study is caused by promoting of small intestinal blood supply or by reducing destructive effects of oxidative stress is not an easy question to answer. The mechanisms of distant organs injury in severe burns are under active and continuous researches. As these mechanisms are better understood, protective effects of vitamin E are also better clarified.

In summary, the results of this research show that vitamin E can improve intestinal villuses height and lumen diameter and its consumption at the time of burning can protect intestine mucosa.

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