Dietary Vitamin E Supplementation Does Not Inhibit *Candida albicans* Intestinal Translocation in Rats

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Summary *Candida albicans* translocation was determined in rats receiving a normal or vitamin E-supplemented and deficient diet submitted to mesenteric ischemia and reperfusion (MIR). The antioxidant effect of vitamin E on lipid peroxidation was also assessed. The animals were divided into six groups submitted to different diets for 30 d. Groups N, NI, NC and NIC were submitted to a normal diet and used as controls, and groups VITE and DEFE received a vitamin E-supplemented and vitamin E-deficient diet, respectively. Groups NIC, VITE and DEFE were submitted to MIR, inoculated with *Candida albicans* and sacrificed 24 h after the surgical procedure. The antioxidant effect of vitamin E was determined in the liver and gut mucosa using the TBARS method. *Candida albicans* translocation was assessed in lymph node, liver and kidney specimens. The results showed that lipid peroxidation was lower ($p < 0.05$) in the vitamin E-supplemented group. However, vitamin E supplementation did not protect the rats against *Candida albicans* translocation (the translocation in the Group VITE was 100% for lymph nodes).

Key Words intestinal translocation, free radical, vitamin E, *Candida albicans*, mesenteric ischemia

Some conditions are essential for the mucosal barrier to exert its protective function against microorganisms in the intestinal lumen. These functions are the maintenance of intestinal microflora, efficient host immunologic system and intact mucosal epithelium in the small intestine. When one or more factors are altered, intestinal translocation of microorganisms is favored (1). This is characterized by microorganisms passing through the intestinal wall to extraintestinal sites such as mesenteric lymph nodes and appearing in viable conditions in the circulation and various organs. Today, this mechanism is thought to be responsible for situations of sepsis without a defined primary focus that mainly occur in patients with severe impairment of general conditions, often leading to multiple organ failure (2, 3).

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When a tissue is exposed to ischemia, a sequence of chemical reactions is observed that may lead to cell dysfunction and tissue injury (4). This injury is caused by different chemical reactions that occur after oxygenated blood returns to the ischemic tissue. These reactions resulting from reperfusion involve cytotoxic oxidants derived from molecular oxygen (i.e., reactive oxygen metabolites or free radicals) (5, 6). One of the most important mechanisms and probably the initial source of free oxygen radicals during ischemia and later reperfusion in the intestine is the hypoxanthine-xanthine oxidase system, with the intestine being a site highly rich in xanthine dehydrogenase (7).

Among the lesions caused by free radicals in practically all types of organic matter, particularly important is the involvement of lipid biomembranes (8). Lipid peroxidation is generally started by the attack of several reactive species, with the occurrence of a chain reaction and of lipid peroxide accumulation on the membrane. Lipid peroxides destabilize the membranes and make them permeable to ions (9-11).

The ability of vitamin E to protect cell membrane lipids against oxidative damage has been fully elucidated (12, 13). Vitamin E acts by supplying hydrogen atoms to the cell membranes, preventing the chain reaction from propagating through lipid membranes. α -Tocopherol inhibits lipid peroxidation by reacting with lipid peroxide (6). The objective of the present study was to determine the antioxidant effect of vitamin E in the neutralization of free radicals produced by mesenteric ischemia and reperfusion and its consequent protection against *Candida albicans* translocation.

MATERIALS AND METHODS

A total of 59 adult male Wistar rats weighing on average 148 g were divided into six groups: Group 1 (control), N, rats on a normal diet not submitted to *Candida albicans* inoculation or ischemia; Group 2 (control), NI, rats on a normal diet not submitted to *Candida albicans* inoculation but submitted to mesenteric ischemia and reperfusion; Group 3 (control), NC, rats on a normal diet submitted to *Candida albicans* inoculation but not to ischemia; Group 4 (control), NIC, rats on a normal diet submitted to *Candida albicans* inoculation, mesenteric ischemia and reperfusion; Group 5 (test), VITE, rats on a diet supplemented with vitamin E submitted to *Candida albicans* inoculation, mesenteric ischemia and reperfusion; Group 6 (test), DEFE, rats on a vitamin E-deficient diet submitted to *Candida albicans* inoculation, mesenteric ischemia and reperfusion.

The diets were prepared according to the AOAC, 1995 (14). Supplementation of the diet for Group 5 was vitamin E provided in an amount 20 times higher (0.156 g/100 g of diet) than recommended for normal rat diet (0.009 g/100 g of diet). The rats received the diets described above for 30 d. On day zero of the experiment, the rats received a *Candida albicans* inoculum (the concentration of inoculum was 1.0×10^{10} colony-forming units of *Candida albicans*/ml of inoculum) by gavage, and 60 min later, were submitted to laparotomy for the induction of ischemia

Table 1. Plasma level of vitamin E.

	Groups					
	N (n=8)	NI (n=8)	NC (n=8)	NIC (n=9)	VITE (n=13)	DEFE (n=13)
Vitamin E	10.17 ^{ab} ±9.16	18.16 ^a ±11.16	17.64 ^{ab} ±14.18	9.89 ^{ab} ±8.19	28.16 ^a ±15.75	1.73 ^b ±1.62

N, group on a normal diet not submitted to ischemia or *Candida albicans* inoculation. NI, group on a normal diet submitted to ischemia but not to *Candida albicans* inoculation. NC, group on a normal diet not submitted to ischemia but submitted *Candida albicans* inoculation. NIC, group on a normal diet submitted to ischemia and *Candida albicans* inoculation. VITE, group on a diet supplemented with vitamin E and submitted to ischemia and *Candida albicans* inoculation. DEFE, group on a vitamin E-deficient diet submitted to ischemia and *Candida albicans* inoculation. Means followed by different letters are significantly different ($p < 0.05$).

Values represent mean ± SD (μmol/L).

induced by clamping the mesenteric superior artery. Reperfusion was carried out 40 min later. Twenty-four hours after surgery, the animals were anesthetised with ether and sacrificed. All mesenteric lymph nodes of the distal ileum, a liver fragment and the left kidney were removed, weighed and processed for *Candida albicans* culturing. The positives cultures were determined by counting the number of colony-forming units of *Candida albicans*/g of tissue. For the assessment of lipid peroxidation, we determined thiobarbituric acid reactive substances (TBARS) in the liver and mucosa by the method of Uchiyama and Mihara (15). Plasma α-tocopherol was determined by HPLC (16)

This experiment was done in accordance with the Recommendations of the Helsinki Declaration [*Med Clin (Barc)*, 1988; **91**(18): 702–703], the protocol was approved by the Animal Experiment Ethical Committee of the School of Medicine of the University of São Paulo.

RESULTS

Table 1 presents the plasma concentrations of vitamin E in the groups. The Kruskal-Wallis test showed a significant difference between group DEFE and groups NI and VITE.

The TBARS concentrations (mean ± SD) in the intestinal mucosa and liver are presented in Table 2. The Kruskal-Wallis test showed a significant difference in the intestinal mucosa between groups N and DEFE, NI and VITE, NIC and VITE, and VITE and DEFE. In the liver, there was a significant difference between group DEFE and the remaining groups.

Table 3 presents the percentages of positive *Candida albicans* cultures for mesenteric lymph nodes, liver and kidney. The chi-square test shows that there was

Table 2. Thiobarbituric acid reactive substances (TBARS) in the intestinal mucosa and liver.

TBARS	Groups					
	N (n=8)	NI (n=8)	NC (n=8)	NIC (n=9)	VITE (n=13)	DEFE (n=13)
Intestinal	0.355 ^{ab}	0.692 ^{bc}	0.397 ^{abc}	0.644 ^{bc}	0.294 ^a	0.776 ^c
-mucosa	±0.121	±0.233	±0.085	±0.309	±0.094	±0.319
Liver	0.546 ^a	0.819 ^a	0.787 ^a	1.329 ^a	0.599 ^a	3.029 ^b
	±0.163	±0.329	±0.487	±0.502	±0.125	±0.536

N, group on a normal diet not submitted to ischemia or *Candida albicans* inoculation. NI, group on a normal diet submitted to ischemia but not to *Candida albicans* inoculation. NC, group on a normal diet not submitted to ischemia but submitted to *Candida albicans* inoculation. NIC, group on a normal diet submitted to ischemia and *Candida albicans* inoculation. VITE, group on a diet supplemented with vitamin E and submitted to ischemia and *Candida albicans* inoculation. DEFE, group on a vitamin E-deficient diet submitted to ischemia and *Candida albicans* inoculation. Means followed by different letters are significantly different ($p < 0.05$).

Values represent mean ± SD (nmol/mg protein).

Table 3. Median and statistical variability of the minimum and maximum values (min-max) of log colony-forming units (cfu) and percentage (%) of positive *Candida albicans* cultures in mesenteric lymph nodes, liver and kidney.

Translocation	Groups					
	N (n=8)	NI (n=8)	NC (n=8)	NIC (n=9)	VITE (n=13)	DEFE (n=13)
Lymph nodes (cfu)	0	0	2.3	3.7	3.5	2.6
min-max	(0-0)	(0-0)	(0-2.7)	(0-4.4)	(2.8-4.8)	(0-4.4)
(%)	0	0	75	89	100	84.6
Liver (cfu)	0	0	0	2.7	2.4	0
min-max	(0-0)	(0-0)	(0-2.9)	(0-5.5)	(0-4.8)	(0-2.89)
(%)	0	0	25	78	69	43
Kidney (cfu)	0	0	0	1.7	0	0
min-max	(0-0)	(0-0)	(0-0)	(0-4.5)	(0-4.2)	(0-2.4)
(%)	0	0	0 ^a	55.5 ^b	30.7	38.4

N, group on a normal diet not submitted to ischemia or *Candida albicans* inoculation. NI, group on a normal diet submitted to ischemia but not to *Candida albicans* inoculation. NC, group on a normal diet not submitted to ischemia but submitted to *Candida albicans* inoculation. NIC, group on a normal diet submitted to ischemia and *Candida albicans* inoculation. VITE, group on a diet supplemented with vitamin E and submitted to ischemia and *Candida albicans* inoculation. DEFE, group on a vitamin E-deficient diet submitted to ischemia and *Candida albicans* inoculation. Means followed by different letters are significantly different ($p < 0.05$).

no significant difference between the groups submitted to inoculation ($p > 0.05$). There was a significant difference ($p < 0.05$) in the percentage of positive kidney cultures between NIC and NC.

DISCUSSION

The symptoms of vitamin E deficiency are considered to be the result of damage caused by free radicals (12). Lindley et al (17) showed that after 16 weeks of exposure to deficient and supplemented diets, rats in the deficient group had undetectable plasma α -tocopherol concentrations, whereas the supplemented group had concentrations of $34.5 \pm 1.4 \mu\text{mol/L}$. In the present study, Table 1 shows that DEF animals had significantly lower plasma vitamin E levels ($1.73 \pm 1.62 \mu\text{mol/L}$) as compared to the remaining groups that received diets containing normal or supplemented vitamin E amounts ($28.16 \pm 15.75 \mu\text{mol/L}$).

The present study also demonstrated an increase in TBARS levels in the intestinal mucosa, with a significant difference between the vitamin E-deficient DEFE group and the N group (i.e., a control group that was not submitted to surgery or *Candida albicans* inoculation) and the vitamin E-supplemented VITE group, whose TBARS concentration was lower than that in the DEFE group. There was a significant difference between group VITE (supplemented), group NI (submitted to ischemia only and receiving a normal diet) and group NIC (submitted to a normal diet, ischemia and *Candida albicans* inoculation). The TBARS concentration was lower in group VITE than in groups NI and NIC, and was similar to the concentration found in group N (Table 2). The increased TBARS production in the intestinal mucosa of rats submitted to ischemia alone or ischemia and reperfusion shows that lipid peroxidation plays an important role in the production of damage to the intestinal mucosa (18).

TBARS production in the liver was significantly higher in group DEFE as compared to groups N, NI, NC, NIC and VITE (Table 2). Other investigators have shown that rats fed normal and vitamin E-deficient diets present increased TBARS concentrations in the liver (19–21). Another report (22) showed that, after 90 min of hepatic ischemia and reperfusion from 0 to 60 min in rats injected intraperitoneally with a placebo or vitamin E, the MDA values in liver tissue remained relatively stable during the period of ischemia in both groups. After reperfusion, there was an increased production of MDA in the placebo group, whereas in the vitamin E-treated group, this MDA increase was inhibited.

In the present study, the *Candida albicans* translocation was determined by counting the log number of colony-forming units (cfu) in positive cultures. There was not a significant difference in terms of the percentage of positive cultures detected in the organs from the different groups inoculated (Table 3), however, there was a significant difference ($p < 0.05$) between the NC and NIC control groups in the kidney measurements. The mechanism of disequilibrium in the intestinal flora caused by the oral inoculation of *Candida albicans* was probably responsible for

the translocation of the microorganisms observed in the NC control group where the translocation to mesenteric lymph nodes and liver occurred at rates of 75 and 25%, respectively. No fungal colonies were detected in the kidney of this group, which was not submitted to surgery or anesthesia. The process of ischemia involves the alteration of intestinal permeability, and can provoke higher microorganism translocation to systemic circulation, as observed in groups NIC, VITE and DEFE, where translocation to all the studied tissues occurred.

The model of mesenteric ischemia and reperfusion used here reflects the hemodynamic and metabolic changes occurring in situations of hypermetabolism such as hypovolemic shock, postoperative stress and trauma. In animals submitted to ischemia and reperfusion, *Candida albicans* translocation was observed in all organs studied. For the mesenteric lymph nodes, the percentages of *Candida albicans* positive cultures were 89% for the NIC control group, 100% for the VITE experimental group and 84.6% for the DEFE experimental group. The percentages of positive cultures for the liver were 78% for the NIC control group, 69% for the VITE experimental group and 43% for the DEFE group, indicating dissemination through portal circulation. In the renal parenchyma, which reflects the translocation disseminated through systemic circulation, the percentages of positive cultures were 55.5% for group NIC, 30.7% for group VITE and 38.4% for group DEFE. When we compared the log colony-forming units of *Candida albicans* in the groups submitted to inoculation, we did not observe a significant difference in translocation, although translocation was higher in the groups submitted to ischemia (Table 3). In a previous study from our laboratory (23) using rats on a zinc-deficient diet and later submitted to mesenteric ischemia and reperfusion, we demonstrated that translocation was higher for animals submitted to ischemia than in animals that were not submitted to the procedure; demonstrating a greater magnitude of the phenomenon caused by ischemia-reperfusion.

All of these results indicate the production of free radicals during ischemia and mainly during reperfusion, probably damaging the intestinal mucosa and causing increased translocation.

Immunodeficiency plays an extremely important role in the occurrence of translocation and propagation of the microorganism to distant locations. In studies in which the immune response is not significantly altered, translocation is limited to the lymph nodes for a short period of time. These lymph nodes should normally destroy a large part of the microorganisms that reach them, but failure of the immunologic system prevents elimination of the microorganisms, permitting them to colonize the lymph nodes and to remain viable (24).

In the present study, we did not observe an effect of vitamin E on the process of *Candida albicans* translocation since no significant difference in the log colony-forming units (cfu) was observed between the groups submitted to fungal inoculation. However, there was a significant reduction of lipid peroxidation in the intestinal mucosa and in the liver of group VITE as compared to groups DEFE and NIC. It is possible that macrophage activation was altered due to vitamin E

supplementation, with the consequent inhibition of superoxide anion. It should be pointed out that the superoxide anion produced by leucocytes is an important oxygen radical for bacterial death. Since vitamin E is an antioxidant, the mechanism of bacterial killing by leucocytes can be inhibited by the administration of high doses of this vitamin. Some studies have demonstrated that very high plasma levels of vitamin E may predispose premature infants to infection, including necrotic enterocolitis and sepsis (25, 26). Similarly, there are several studies showing the longer survival of animals submitted to treatment with α -tocopherol. Some investigators have demonstrated improved survival in experimental models of endotoxemia after treatment with α -tocopherol, and others have reported a reduction of lipid peroxidation in hepatocyte membranes, with improved cell energy and increased survival of animals exposed to endotoxins (27, 28). Endogenous oxidation reactions are essential for the maintenance of the normal biochemistry of living organisms and are especially important in terms of the killing action of leucocytes against microbes (i.e., in terms of the mechanism of host defense in infectious diseases). However, reactive oxygen intermediates may damage normal tissues unless they are under the control of an antioxidant (29).

Thus, in biological systems there is an absolute necessity for the control of the reactions that release free radicals and their reactive intermediary compounds by antioxidants or scavengers. This control, however, should be balanced, since complete elimination of these free radicals from the cell may cause its destruction. It is necessary to think about oxidative stress and antioxidants as a system in which failure of either element in the balance may result in some type of disorder (29). On this basis, Krinsky (30) defines biological antioxidants as "compounds that protect biological systems against the harmful effects of processes or reactions that may cause excessive oxidation."

Antioxidants are clearly important in cell regulation but the appropriate balance between oxidative processes and antioxidants still needs to be defined. Biological antioxidants do not act separately but as part of a complex system (13, 31–33).

Thus, on the basis of the results obtained, we may conclude that vitamin E supplementation led to lower lipid peroxidation in the intestinal mucosa but did not protect the animal against *Candida albicans* translocation.

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