

Intestinal Radioprotection by Vitamin E (Alpha-Tocopherol)

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Objectives

The major objective of this study was to test vitamin E as a potential radioprotectant for the small bowel of the rat.

Summary Background Data

Vitamin E has previously been shown to provide radioprotection in animal models: increased survival after whole-body irradiation, diminished absorptive malfunction, and modest diminution in postirradiation hemolysis. The luminal route for intestinal radioprotection has not been tested.

Methods

Rat mid-small bowel was surgically exteriorized and segmented by ties into compartments, each of which was filled with a test solution 30 minutes before 1100 cGy of x-irradiation was administered. After the rats were killed 5 days later, the various segments were evaluated for surviving crypts, mucosal height, and goblet cell preservation. Luminal agents included alpha-tocopherol phosphate and alpha-tocopherol acetate. In a separate study, dietary supplements of alpha-tocopherol were given for 10 days before irradiation, and the same irradiation sequence was carried out.

Results

Small bowel crypt cell numbers, mucosal height, and goblet cell numbers were significantly protected from radiation effects by dietary alpha tocopherol pretreatment and by luminal application of the vitamin.

Conclusions

These studies indicate that vitamin E can serve as a partial protectant against acute irradiation enteritis, whether given as chronic oral systemic pretreatment or as a brief topical application.

Therapeutic irradiation regimens are designed to maximize tumoricidal effects while causing minimal damage to normal organs. Radiation to abdominal or pelvic malignancies unavoidably injures the intestine. Largely because of rapid cell turnover, the intestine is highly sensitive to radiation injury and is therefore the limiting factor in the permissible dosage for abdominal and pelvic irradiation. An early clinical consequence of intestinal

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injury is an acute enteritis syndrome with tenesmus, diarrhea, and sometimes bleeding. These side effects may result in modification or cancellation of planned therapeutic regimens.

The effects of ionizing radiation are mediated mainly by oxygen free radicals that are generated by its action on water. These highly reactive oxidants remove hydrogen atoms from fatty acids, causing lipid peroxidation with resultant changes in membrane permeability and fluidity and ultimately in cell death. The oxyradicals also induce DNA strand breaks and protein oxidation.

Vitamin E is a natural component of mammalian cell membranes and is considered the main line of defense against lipid membrane peroxidation.^{1,2} The propagating free radical chain reaction leading to lipid peroxidation is interrupted by formation of a vitamin E-free radical complex.³ In essence, the vitamin contributes a hydrogen atom and is itself oxidized. The oxidized vitamin E is rapidly reduced by various systems, particularly by vitamin C. Vitamin E is a mixture of tocopherols, the major and most active form being alpha-tocopherol. The molecule has a hydrophilic side chain that favors localization in lipid bilayers. Antioxidant properties reside in a hydroxyl phenolic group.

More than adequate vitamin E is provided in the normal diet. Deficiency has been reported only with disease states involving severe fat malabsorption. The literature is replete with suggestions of prophylactic or therapeutic benefits from pharmacologic doses of vitamin E. A partial list includes prevention of cancer,⁴ atherosclerosis,⁵ aging,⁶ and cataract development⁷ as well as reduction of platelet aggregation,⁸ amelioration of ischemia reperfusion injury,⁹ avoidance of hemolysis,¹⁰ and enhancement of the immune response.¹¹

Because of its free radical scavenging capabilities, supraphysiologic doses of vitamin E have been proposed as a potential radioprotectant.

Our laboratory and others have demonstrated the general principal that circumstances prevailing in the intestinal lumen during radiation delivery play a significant role in the severity of acute mucosal damage.¹²

The current investigation was designed to test the possibility that vitamin E in the small-bowel lumen at the time of irradiation could provide mucosal protection against acute damage. A second aim was to compare alpha-tocopherol acetate, which is fat soluble, to water-soluble alpha-tocopherol phosphate. A third goal was to investigate if a vitamin E-enriched diet would diminish acute mucosal radiation damage.

MATERIALS AND METHODS

Detailed descriptions of the experimental protocol have been published elsewhere.¹²⁻¹⁷

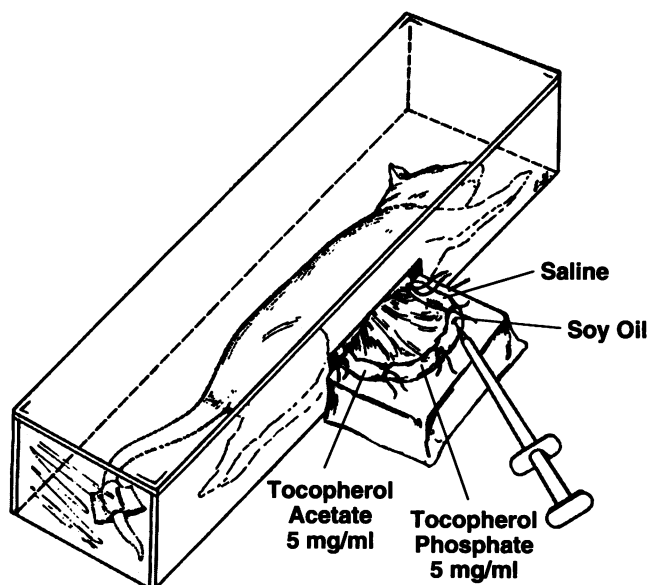


Figure 1. The anesthetized rat is shielded in a lead box while the exteriorized small bowel is irradiated. The loop is exposed to 1100 cGy of X irradiation. Each segment was filled with 0.2 mL; 30 minutes' each exposure.

Briefly, adult female Sprague-Dawley rats weighing 300–400 g were used. Animals were housed in temperature- and humidity-controlled environments with 12-hour light and dark cycles and spent at least a week in the laboratory environment before use. They were cared for by Research Animal Resources of the University of Minnesota according to the principles stated in the current “Guide For Care and Use of Laboratory Animals” from the National Research Council.

Radiation

After a 20-hour fast, the rats were injected with intraperitoneal pentobarbital (40 mg/kg) to induce anesthesia. The mid-small intestine was surgically exteriorized and 4-cm segments developed by loosely occluding sutures. The rat was placed in a 3-mm lead box. The segment of intestine to be irradiated was passed through an aperture, kept moist with saline-soaked gauze, and maintained at 36–37 C. by means of a heating lamp. Intestinal and body temperatures were monitored by thermistors. One intestinal segment was injected by means of a 27-gauge needle with 0.2 mL of normal saline, the second with a soybean oil vehicle, the third with alpha-tocopherol phosphate in saline (5 mg/mL), and the fourth with alpha-tocopherol acetate in soybean oil (5 mg/mL). This volume fills a 4-cm segment of rat small bowel without causing distention. The remainder of the intestine and of the animal were shielded from radiation. The experimental arrangement is shown in Figure 1. Previous

dose-response studies have shown that concentrations above 5 mg/mL of alpha-tocopherol did not amplify the radioprotective effect. Similarly, mucosal exposure times exceeding 30 minutes led to no further enhancement of radioprotection. Therefore, we used this dosage and exposure time for the luminal studies.

A second group of rats was fed a vitamin E-enriched diet containing 10 IU/g rat chow for 10 days before irradiation. The latter group was subjected to the same surgical procedure and the same irradiation, but the intestinal segments were filled with normal saline.

Thirty minutes after drug or saline injection into the lumen, irradiation was delivered by a 250 KV machine at 15 milliamps with a 1.0 mm aluminum and a 0.20 mm copper filter at a dose rate of 108.7 cGy/minute. The total dosage was 1100 cGy. Source-to-target distance was 55.2 cm.

When irradiation was completed, the occluding sutures were removed and additional sutures placed in the mesentery next to the bowel to mark the sites of occlusion. The intestine was replaced and the abdominal incision closed. Animals were returned to their cages after recovery from anesthesia and were allowed food and water *ad libitum* until they were killed 5 days later.

Drugs

Alpha-tocopherol acetate was solubilized in soybean oil for intraluminal administration and alpha-tocopherol phosphate in saline. The vitamin E-fortified diet contained 10 IU vitamin E per gram of chow. The control diet contained 0.04 IU vitamin E per gram. In both groups, the rats consumed approximately 25 g of chow per day, which provided 250 IU vitamin E in the enriched-diet group and 1 IU vitamin E in the standard-diet group.

Histologic Findings

Animals were killed by an anesthetic overdose 5 days later. The abdomen was opened and the irradiated segments located. The intestine was divided at the points defined by the mesenteric marking sutures. Samples of shielded, nonirradiated intestine were taken 10-cm proximal and 10-cm distal to the irradiated bowel to serve as controls. The intestinal specimens were opened along the mesenteric border, rinsed with saline, and pinned flat on a corkboard, mucosal side up. The tissues were fixed in 10% neutral buffered formalin for 48 hours, then dehydrated, cleared in xylene, and infiltrated with paraffin. Five-micron sections were cut and stained with hematoxylin and eosin or with periodic acid-Schiff.

Tissues were evaluated by one microscopist, who was unaware of their origin, using an eyepiece micrometer at

Table 1. HISTOLOGY SCORES FOR RATS RECEIVING α -TOCOPHEROL INTRALUMENALLY BEFORE 1100 cGy X-IRRADIATION

	CPTS	MUHT	GCEL	n
Saline	35 (3.4)	306 (23)	3.1	11
Soy oil	56* (4.5)	380 (20)	2.2	14
Tocopherol phosphate	70* (5.6)	400* (14)	2.3	11
Tocopherol acetate	83* (5.0)	467* (15)	1.7	14
Control	126 (1.0)	584 (7)	0.0	14

Values in parentheses are standard errors.

CPTS = crypts per circumference; MUHT = mucosal height; GCEL = goblet cell score.

* $p < 0.01$ vs. saline.

a magnification of $\times 100$. Mucosal thickness was measured at five representative sites where villi could be followed for their full length. Using the method of Withers and Elkind,¹⁸ viable crypts were counted and expressed as the number of crypts per circumference. Significance was determined with the Student's *t* test. Goblet cell loss was scored on a scale of 0 to 4. Total absence was designated 4, and normal or no irradiation was designated 0.

RESULTS

The results are summarized in Table 1 and illustrated in Figure 2. Protection was achieved with intraluminal administration of alpha-tocopherol acetate or phosphate given 30 minutes before radiation exposure. Crypts per circumference and mucosal height were increased significantly compared with the saline controls, as was goblet cell preservation. Protection by luminal alpha-tocopherol acetate in oil was not significantly different from that of the phosphate form dispersed in saline. Luminal soybean oil provided a small but significant improvement in surviving crypt numbers and in mucosal thickness compared with saline. One should note that soybean oil contains large concentrations of vitamin E, approximately 1.1 mg tocopherol per milliliter.¹⁹

Significant crypt preservation was observed in the animals fed a vitamin E-enriched diet compared with those receiving standard rat chow (Table 2). Daily consumption and weight gain were identical with the control and vitamin E-enriched diets. Figure 3 shows the percentage of protection in terms of crypts, with saline designated as 0% and nonirradiated mucosa as 100% protection.

Neither dietary systemic nor intraluminal administration of alpha-tocopherol altered the crypt numbers or mucosal height of the nonirradiated control mucosa.

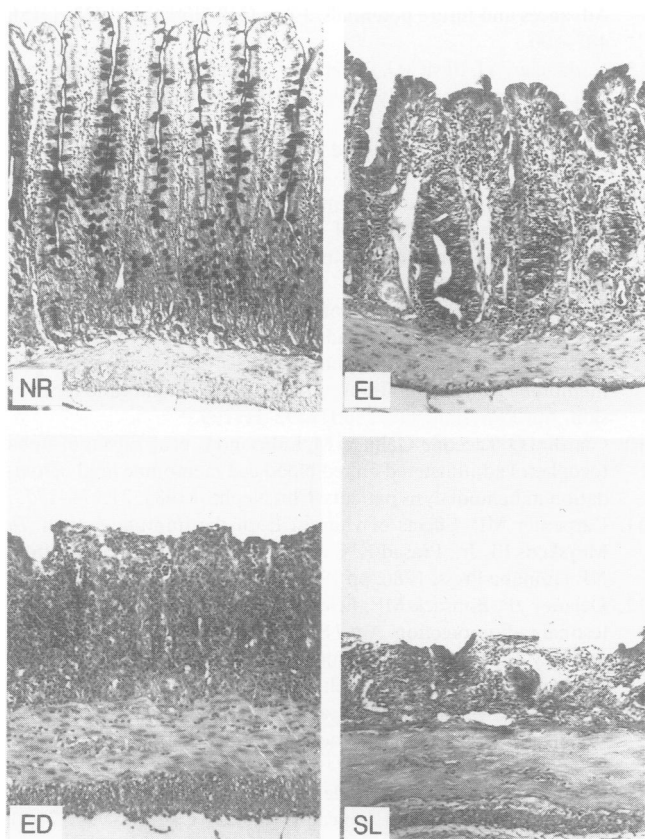


Figure 2. Photomicrographs of mucosa harvested 5 days after irradiation. X120 NR: control, no radiation; EL: luminal alpha-tocopherol acetate; ED: dietary vitamin E; SL: luminal saline. Note the denudation of the surface epithelium and the inflammatory infiltrate in the saline group.

DISCUSSION

In an earlier study in which we used the experimental model described here, we found that luminal neutralization of bile salts or pancreatic enzymes led to reduced mucosal radiation damage.¹⁵ We also showed that the intraluminal presence of alkaline buffer, WR-2721, lazaroids, or misoprostol can diminish acute injury without systemic effects.¹⁴⁻¹⁷

Srinivasan and Weiss²⁰ observed significantly increased postirradiation survival in mice pretreated with systemic vitamin E. Alpha-tocopherol (100 IU/kg body weight) administered subcutaneously either 1 hour before or 15 minutes after irradiation (10 Gy) had similar effectiveness in reducing mortality.

Empey et al.²¹ observed that systemic pretreatment with vitamin E protected the mucosa of the intestine against radiation-induced absorptive malfunction. L-alpha-tocopherol (20 mg/kg) was administered intraperitoneally once daily for 6 consecutive days before 10 Gy whole-body gamma irradiation was administered.

Oral administration of vitamin E has also been re-

Table 2. HISTOLOGY SCORES FOR RATS RECEIVING α -TOCOPHEROL RICH DIET OR REGULAR DIET BEFORE 1100 cGy X-IRRADIATION

	CPTS	MUHT	GCEL	n
Regular diet	29 (1.9)	273 (14)	3.0	8
Vitamin E diet	62* (5.5)	389* (19)	2.1	8
Control	127 (1.4)	579 (8)	0.0	8

Values in parentheses are standard errors.

CPTS = crypts per circumference; MUHT = mucosal height; GCEL = goblet cell score.

* $p < 0.001$ vs. regular diet.

ported to have radioprotective properties. Shaheen and Hassan²² observed that enteral vitamin E alone or combined with cysteine ameliorated radiation damage to red blood cells. Alpha-tocopherol acetate (400 IU/kg) was given orally once a day for 5 consecutive days. Animals were then exposed to 7.5 Gy whole-body gamma irradiation. Hemolysis was diminished in the group of animals pretreated with vitamin E. The combined administration of vitamin E and cysteine provided better erythrocyte protection than did either of the agents alone.

The luminal route has proven to be an effective method for achievement of radioprotection of intestinal mucosa.¹² The agent is delivered in high concentrations directly to the mucosal surface. Compared with systemic administration, a small total dosage can provide therapeutic tissue concentrations. Potential systemic toxicity is avoided. Target tumor protection is less likely with limited absorption of small amounts of the agent.

This study demonstrated marked radioprotection of the intestinal mucosa by topical vitamin E. Long-term

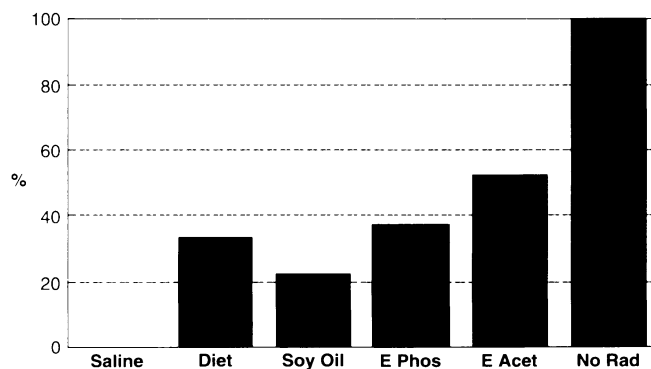


Figure 3. Percentage of crypt preservation for rats receiving luminal saline, dietary vitamin E, luminal tocopherol phosphate (E PHOS), or luminal tocopherol acetate (E ACET) before 1100 cGy x-irradiation. Saline is designated 0%—no protection. No radiation is designated 100%—no damage.

feeding with a vitamin E-enriched diet also yielded significant protection of the intestinal mucosa against acute radiation damage.

In the context of luminal effects, interpretation of the results of oral pretreatment is ambiguous. Vitamin E is absorbed from the gut and circulates systemically. It concentrates in lipids and lipid bilayer membranes. Studies with radiolabeled alpha-tocopherol have indicated that absorption is incomplete.²³ It is unknown whether the unabsorbed vitamin remains physiologically active throughout the full length of the gut lumen. If so, vitamin E would reach the enterocytes directly from the lumen as well as through the systemic circulation.

Acute radiation enteritis is common and causes severe but generally self-limiting symptoms. Late complications in the form of stricture, ulceration, or malabsorption are relatively infrequent but cause severe morbidity and significant mortality. These delayed injuries are progressive and are merely palliated by operative interventions. The relationship between acute injury and late complications is uncertain. Studies are underway to address the question of whether amelioration of acute damage eventuates in diminished late effects.

Most of the experimental studies regarding radioprotectors and the gastrointestinal tract have involved systemic administration of drugs. These agents generally cause substantial toxicity and have the inherent potential to protect target tumor cells.

In clinical trials that have involved marginally effective doses of systemic WR-2721, the most thoroughly studied radioprotectant, multiple side effects, including hypotension, somnolence, nausea, and vomiting, were observed. These were severe enough to make the drug impractical for clinical use.²⁴

The experimental procedure of injecting high concentrations of vitamin E directly into the lumen cannot be duplicated clinically. Use of oral vitamin E as a radioprotectant could solve some of the problems encountered previously in the development of a practical pharmacologic regimen. High doses of this agent have essentially no adverse side effects, acute or chronic. Whether vitamin E can reduce damage to target malignancies will require extensive study.

References

- Oski FA. Vitamin E: A radical defense. *N Engl J Med* 1980; 303:454-455.
- Jacobson HN. Dietary standards and future development. *Free Rad Biol Med* 1987; 3:203-213.
- Burton GW, Cheeseman KH, Doba T, et al. Vitamin E as an antioxidant in vitro and in vivo. *In Biology of Vitamin E*. London: Pitman, 1983, pp 4-18.
- Prasad KN, Edwards-Prasad J. Vitamin E and cancer prevention: Advances and future potentials. *J Am Coll Nutrition* 1922; 11(5):487-500.
- Verlangieri AJ, Bush MJ. Effects of d-alpha tocopherol supplementation on experimentally induced primate atherosclerosis. *J Am Coll Nutr* 1992; 11:130-137.
- Cutler RG. Antioxidants and aging. *Am J Clin Nutr* 1991; 53:373S-379S.
- Minkova M, Drenska D, Pantev T, Ovcharov R. Antiradiation properties of alpha tocopherol, anthocyanins and pyracetam administered combined as a pretreatment course. *Acta Physiol Pharmacol* 1990; 16(4):31-36.
- Jandak J, Steiner M, Richardson PD. Alpha tocopherol, an effective inhibitor of platelet adhesion. *Blood* 1989; 73:141-149.
- Massey KD, Burton KP. Alpha tocopherol attenuates myocardial membrane related alterations resulting from ischemia and reperfusion. *Am J Physiol* 1989; 256:H1192-H1199.
- Giardini O, Taccone-Gallucci M, Lubriano L, et al. Effects of alpha tocopherol administered on red blood cell membrane lipid peroxidation in hemodialysis patients. *Clin Nephrol* 1984; 21:174-177.
- Carpenter MP. Effects of vitamin E on the immune system. *In Meyskens FL Jr., Prasad KN, eds. Vitamins and Cancer*. Clifton, NJ: Humana Press, 1986, pp 199-211.
- Delaney JP, Bonsack ME, Felemovicius I. Luminal route for intestinal radioprotection. *Am J Surg* 1993; 166:492-501.
- Delaney JP, Bonsack ME. Acute radiation enteritis in rats: Bile salts and trypsin. *Surgery* 1992; 112:587-592.
- Delaney JP, Kimm GE, Bonsack ME. The influence of luminal pH on the severity of acute radiation enteritis. *Int J Radiat Biol* 1992; 61:381-386.
- Delaney JP, Bonsack ME, Felemovicius I. Radioprotection of the rat small intestine with topical WR-2721. *Cancer* 1994; 74(8):2379-2384.
- Delaney JP, Bonsack ME, Hall P. Intestinal radioprotection by two new agents applied topically. *Ann Surg* 1992; 216:417-422.
- Delaney JP, Bonsack ME, Felemovicius I. Misoprostol in the intestinal lumen protects against radiation induced injury of the intestinal mucosa of the small bowel. *Radiat Res* 1994; 137:405-409.
- Withers HE, Elkind MM. Microcolony survival assay for cells of the mouse intestinal mucosa exposed to radiation. *Int J Radiat Biol* 1970; 17:261-267.
- United States Department of Agriculture, Science and Education Administration. Composition of foods: Fats and oils, raw, processed, prepared. Agriculture handbook no. 8-4. Washington, DC: U.S. Government Printing Office, 1978, p 51.
- Srinivasan V, Weiss JF. Radioprotection by vitamin E: Injectable vitamin E administered alone or with WR-3689 enhances survival of irradiated mice. *Int J Radiat Oncol Biol Phys* 1992; 23(4):841-845.
- Empey LR, Papp JD, Jewell LD, Fedorak RN. Mucosal protective effect of vitamin E and misoprostol during acute radiation-induced enteritis in rats. *Dig Dis Sci* 1992; 37(2):205-214.
- Shaheen AA, Hassan SM. Radioprotection of whole body gamma-irradiation-induced alteration in some haematological parameters by cysteine, vitamin E, and their combination in rats. *Strahlenther Onkol* 1991; 167:498-501.
- Bieri JG. Kinetics of tissue alpha-tocopherol depletion and repletion. *Ann NY Acad Sci* 1972; 203:181-91.
- Kligerman MM, Glover DJ, Turrisi AT, et al. Toxicity of WR-2721 administered in single and multiple doses. *Int J Radiat Oncol Biol Phys* 1984; 10(9):1773-1776.

Discussion

DR. WILEY W. SOUBA (Boston, Massachusetts): I rise to congratulate Dr. Delaney on a fine study. This is an area that he