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Metabolic and Immune Effect of Vitamin E Supplementation After Burn

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ABSTRACT. The effect of dietary vitamin E supplementation was studied in burned guinea pigs. Forty-four guinea pigs bearing a catheter gastrostomy received a 30% total body surface area full thickness flame burn and were given identical enteral diets (175 kcal/kg/day) except for the amount of vitamin E. Groups 1, 2, 3, and 4 received 0, 4 mg/kg/day (approximately equivalent to guinea pig's RDA) 20 or 100 mg/kg/day of vitamin E respectively. After 14 days of enteral feeding, there were no significant differences between groups in the body weights and the weights of carcass, gastrocnemius muscle, liver, and spleen. Resting metabolic expenditure on PBD 3, 6, 9, and 12 was similar in all groups. No statistical differences were seen in ear-thickness response to 2,4-dinitrofluorobenzene and lymphocytic proliferative responses to phytohemagglutinin. However, mucosal weight and protein content in group 1 were significantly less compared to groups 2 and 4 ($p < 0.05$). Anemia was also significantly greater in group 1. Histologic examination of the intestinal wall, however, did not yield any physical differences associated with the addition of vitamin E to the diet. This study suggests that vitamin E supplementation in diets of burned animals may have a beneficial effect on maintenance of intestinal mucosa and erythrocyte counts over a wide-dose range. (*Journal of Parenteral and Enteral Nutrition* 15:22–26, 1991)

Recent studies have shown that vitamin E is an effective antioxidant *in vivo* and has the ability to enhance both humoral and cell mediated immunity.[1–3] It is also well known that host defense mechanisms are suppressed following thermal injury. However, adequate nutritional support has a beneficial effect on immune functions as well as on metabolic responses.[4,5] There have been few reports on the effect of short-term dietary vitamin E supplementation and immune functions *in vivo* following thermal injury. Vitamin E has been suggested to have some relationship to maintenance of the gut mucosal cell,[6] which is thought to be important as a barrier against translocation of bacteria. This study was designed to evaluate the effect of short-term enteral vitamin E supplementation on selected immune responses and nutritional status after burn.

MATERIALS AND METHODS

Experiment I

Preparation of animals. Forty-four female Hartley guinea pigs (body weight, 414 ± 8.1 g) underwent catheter gastrostomies using a Silastic tube (size, 0.062 inch ID by 0.095 inch OD, Dow Corning Co, Midland, MI) under general anesthesia by intramuscular injection of 50 mg/kg of ketamine hydrochloride, 0.2 mg/kg of acepromazine maleate and 0.04 mg/kg of atropine sulfate. After 7 days of recovery from the operation, all animals were given a 30% total body surface area full thickness flame burn and were resuscitated with an intraperitoneal injection of 20 ml of lactated Ringer's solution. After burn the guinea pigs were placed into individual metabolic cages and the gastrostomy tubes were connected to a pump-controlled continuous infusion system.

Diets and enteral feeding. Animals were randomized into four groups and except for vitamin E content, received identical diets (175 kcal/kg/day) through the gastrostomy tubes (Table I). For the first 24 h following burn all the animals received 80 ml of lactated Ringer's solution through the tubes. During the second 24 h, the animals were given one-third of the planned caloric intake in order to allow them to adapt to the diet. For the next 24 h they received two-thirds of the planned intake, and then full-strength diets (175 kcal/kg/day) on the following day. Water was added to the diet so that the total volume was 100 ml for each day, which provided the animals with sufficient fluid. The composition of the diet is shown in Table II. Twenty percent of total calories were given as whey protein, 68% as glucose polymer, and 12% as lipid from safflower oil. The animals in groups 1, 2, 3, and 4 received 0, 4 mg/kg/day (approximately equivalent to guinea pig's RDA),[7] 20 mg/kg/day (5 × the RDA), or 100 mg/kg/day (25 × the RDA) of vitamin E (dl-alpha tocopheryl acetate: Aquasol E, Armour Pharmaceutical Co., Kankakee, IL), respectively. The animals in groups 2, 3, and 4 were given vitamin E followed by 2 ml of water by bolus administration through the gastrostomy tubes once a day from postburn day (PBD) 0 to 14, whereas those in group 1 were given the same volume of water.

Nutrition and immunological evaluation. The animals were weighed at the same time daily. Resting metabolic expenditure (RME) was measured by indirect calorimetry (Respiratory gas monitor, Webb Associates, Inc. Yel-

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TABLE I
Groupings of the animals and body weight, weights of carcass, gastrocnemius muscle, liver, and spleen on PBD 14

Group	1	2	3	4
Number	10	12	10	12
Vitamin E (mg/kg/day)	0	4	20	100
Body weight* (%)	**87.2 ± 1.5**	**87.8 ± 1.4**	**93.0 ± 2.4**	**92.8 ± 1.5**
Carcass weight* (%)	28.9 ± 0.7	29.7 ± 0.8	31.0 ± 1.1	30.6 ± 0.9
Gastrocnemius muscle (g)	0.97 ± 0.05	0.97 ± 0.08	1.09 ± 0.13	1.08 ± 0.09
Liver (g)	20.7 ± 1.6	19.7 ± 1.9	20.8 ± 1.0	18.6 ± 1.4
Spleen (g)	0.90 ± 0.08	0.90 ± 0.08	0.94 ± 0.08	0.96 ± 0.08

* Percent of preburn body weight.

TABLE II
Composition of diets (1 kcal/ml)

Component	Amount
Protein (ProMix)*	62.5 g
Carbohydrate (Nutrisource)†	212.0 ml
Fat (Microlipid)‡	26.7 ml
Arginine hydrochloride	2.5 g
Sodium chloride (2.5 m Eq/ml)	24.0 ml
Potassium chloride	10.0 ml
Potassium acetate (2 m Eq/ml)	24.0 ml
Potassium phosphate (4.4 m Eq/ml)	10.0 ml
Calcium gluconate (0.46 m Eq/ml)	50.0 ml
Magnesium sulfate (4 m Eq/ml)	24.0 ml
Mixture of trace elements (M.T.E.-5)§	6.0 ml
Vitamin C	500.0 mg
Choline chloride	1,000 mg
Folic acid	2.0 mg
Thiamine HCl	50.0 mg
Riboflavin	10.0 mg
Pyridoxine HCl	15.0 mg
Niacinamide	100.0 mg
Dexpanthenol	25.0 mg
Vitamin A	10,000.0 IU
Vitamin D	1,000.0 IU
Water added to make total volume	1,000.0 ml

* Navaco Laboratories, McAllen, TX.
† Sandoz Nutrition, Minneapolis, MN.
‡ Cheesebrough Ponds, Inc., Greenwich, CT.
§ Lympho-Med, Inc., Melrose Park, IL.

low Springs, OH)[8] before burn (day 0) and on PBD 3, 6, 9, and 12. After 14 days of continuous enteral feeding all animals were killed and body weight, weights of carcass, gastrocnemius muscle and liver were measured. Blood was also drawn by cardiac puncture to count blood cells and measure serum levels of vitamin E, C_3, and IgA. A 10-cm segment of small intestine was excised midway between the duodenum and cecum. The mucosa was scraped according to the method of Levine et al[9] and the wet and dry weights of mucosa were measured. Thereafter, dried samples were homogenized with 5 ml of normal saline and protein content was measured by the method of Lowry et al.[10] Cell-mediated immunity was evaluated by ear-thickness response to 2,4-dinitrofluorobenzene (DNFB) on PBD 13, using a previously described method.[11] Briefly, the animals were sensitized by cutaneous application of 1.0 ml of 0.5% DNFB solution on PBD 5. On PBD 12 ear thickness was determined, and 0.25 ml of the 0.5% DNFB solution was applied to each ear. Ear thickness was measured again on PBD 13 and percent increase was calculated.

Serum levels of C_3 and IgA were measured by nephelometry, using anti-guinea pig C_3 antiserum (Cappel, Westchester, PA) and anti-guinea pig IgA antiserum (Bethyl Laboratories, Montgomery, TX). Serum vitamin E levels were measured by spectrofluorometry.[12]

At the time of death, the spleens were removed aseptically and splenic lymphocytes were separated to evaluate the mitogenic response to phytohemagglutinin (PHA). Lymphocyte proliferation was measured by the following method. The spleens were placed in Hanks' balanced salt solution, without calcium or magnesium. With the use of an ice bath, the spleens were minced with forceps and a scalpel and filtered through gauze to remove debris. Splenic cells were centrifuged and resuspended in a tissue culture medium containing 95 ml of RPMI-1640 with 1-glutamine (Gibco Co., Columbus, IN), 5 ml of heat-inactivated fetal bovine serum, and 1 ml of penicillin-streptomycin-gentamicin mixture (Gibco Co., Columbus, IN) to achieve a final concentration of 1×10^6 lymphocytes/ml. A 0.1-ml aliquot of each cellular suspension was plated into wells of a Falcon 3847 Culture Plate (Becton Dickinson Labware, Oxnard, CA) and incubated at 37°C in 5% CO_2. A 0.1-ml aliquot of a solution of PHA (5 μg/ml) was added to the wells,

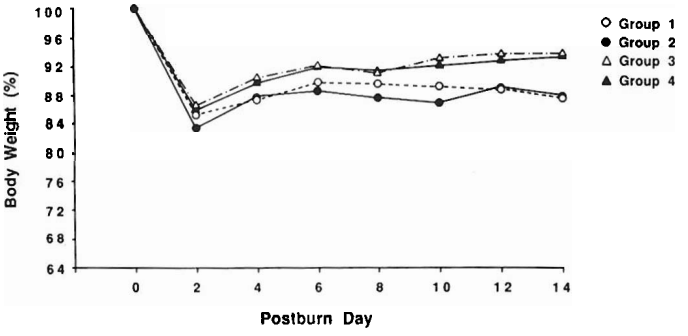

FIG. 1. Changes in body weight following thermal injury (expressed as percentage of preburn body weight). No statistical difference was seen between groups throughout the experiment.

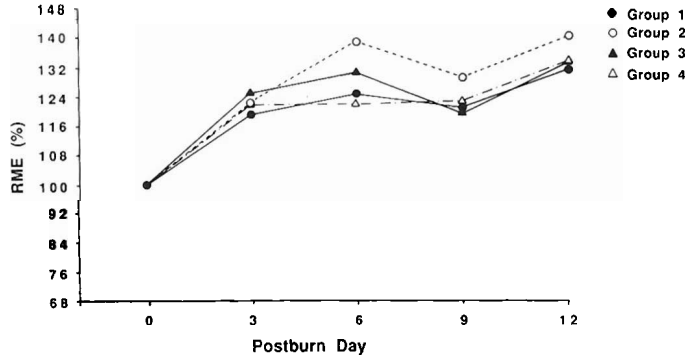

FIG. 2. Changes in resting metabolic expenditure (RME) (expressed as percentage of preburn).

TABLE III
Intestinal mucosal weight on PBD 14

Group	1	2	3	4
Wet weight of mucosa (mg/10-cm gut)	333 ± 14	394 ± 25*	384 ± 9	402 ± 26*
Ratio of mucosa† (%)	64.8 ± 1.8	74.5 ± 1.7*	73.8 ± 2.2*	71.3 ± 2.5
Ratio of dry weight to wet weight (%)	23.8 ± 0.6	23.5 ± 0.3	22.9 ± 0.3	23.5 ± 0.4
Protein content of mucosa (mg/10-cm gut)	57.3 ± 2.4	69.6 ± 3.8*	69.7 ± 3.1*	67.4 ± 5.3

* $p < 0.05$ when compared to group 1.
† Ratio of mucosa was calculated by weight of mucosa/weight of whole gut × 100.

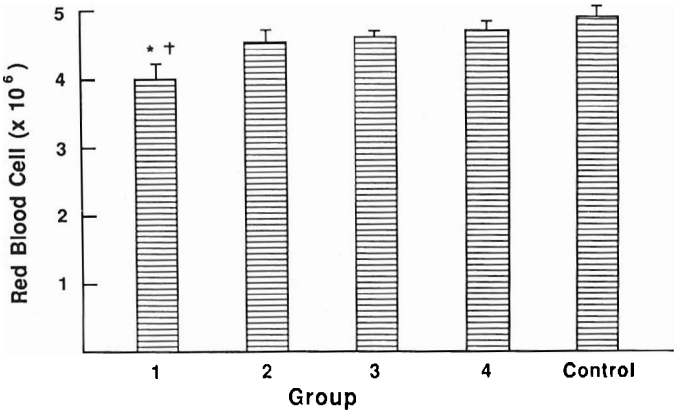

FIG. 3. Red blood cell counts on PBD 14. Group 1 showed significantly lower value, when compared to groups 3, 4, and unburned control. The control animals were not burned and fed guinea pig chow *ad libitum*. * $p < 0.05$ when compared to groups 3 and 4; † $p < 0.01$ when compared to control.

including a control without mitogen. The tissue culture plates were then incubated for 24 hr. Each tissue culture well then had 0.5 μCi of tritiated thymidine (New England Nuclear, Boston, MA) in 10 μl of tissue culture medium added and the incubation was continued for an additional 18 hr. All cultures were then harvested on a Mash II and counted on a Beckman scintillation counter.

Statistics. All data are expressed as mean ± SEM. Student's *t*-test and one-way analysis of variance (ANOVA) were used for comparisons where appropriate.

Results. After an abrupt loss of body weight immediately following burn, all groups maintained their weights at approximately 90% of preburn levels, showing no significant differences among the groups (Fig. 1). Similarly, no statistical differences were seen among the groups in the weights of carcass, gastrocnemius muscle and liver (Table I). RME, expressed as percentage of preburn level, is shown in Figure 2. RME increased gradually after burn in all groups and reached 130 to 140% of preburn level on PBD 12, which demonstrated a similar pattern. The wet weight of mucosa of 10-cm ileal segment was highest in group 4 and lowest in group 1. There were significant differences between group 1 and groups 2 and 4 ($p < 0.05$). The ratio of mucosal weight to whole gut weight was also lowest in group 1 ($p < 0.05$, group 1 *vs* group 2). However, the ratio of dry weight to wet weight of mucosa was very similar in all groups. Protein contents of mucosa samples in group 1 were significantly lower than those in groups 2 and 3 (Table III). All the burned animals had slightly lower red blood cell counts than the control animals who were unburned and fed guinea pig pellet (Wayne Feeds Research Division, Libertyville, IL) and water *ad libitum*. Significant differences were observed when group 1 was compared to the control animals ($p < 0.01$), and groups 3 and 4 ($p < 0.05$) (Fig. 3). The hemoglobin level in group 1 was also significantly lower than that of the control animals (11.7 ± 0.3 *vs* 13.6 ± 0.2 g/dl).

Serum levels of vitamin E in group 1 animals were significantly lower than those in the control animals (3.55 ± 0.33 *vs* 5.90 ± 0.62 mg/ml). Although the animals in group 4 were given 25× the RDA of vitamin E, their vitamin E levels were only twice as high as those of group 2 (Table IV). There were no significant differences in serum levels of C_3 and IgA between groups. Similarly, neither ear-thickness response to DNFB nor mitogenic responses of splenic lymphocytes in PHA showed any differences between groups (Table IV).

Experiment II

Experiment II was designed to histologically investigate the effect of enteral vitamin E supplementation on the intestinal wall of burned guinea pigs.

Methods. Ten female Hartley guinea pigs (350–400 g) were burned and fed enterally as described in experiment

TABLE IV
Serum levels of vitamin E, C_3, and IgA, and immunological response

Group	1	2	3	4
Serum level of vitamin E (μg/ml)	3.55 ± 0.33	6.80 ± 0.50*	10.83 ± 1.03*	11.11 ± 1.24†
Serum level of C_3 (%)‡	122.3 ± 6.7	130.6 ± 5.7	130.8 ± 4.6	120.8 ± 5.2
Serum level of IgA (%)‡	132 ± 11	104 ± 8	139 ± 12	125 ± 10
Ear-thickness response to DNFB (%)§	39.1 ± 2.8	34.7 ± 2.9	32.1 ± 4.1	32.1 ± 4.0
Mitogenic response to PHA (cpm)	2472 ± 390	2712 ± 843	2026 ± 572	2445 ± 568

* $p < 0.05$ when compared to group 1.
† $p < 0.05$ when compared to group 2.
‡ Expressed as % of serum level of normally fed animals.
§ Expressed as % increase of ear thickness.

I. Following burn, the animals were randomly divided into two groups (n = 5/group): 0 vitamin E or 25× RDA (100 mg/kg/day) vitamin E supplementation.

Light microscopy. At the time of death (day 14 postburn) samples of guinea pig small intestine (ie, jejunum and ileum) were excised and cut into 0.5 to 1.0 cm lengths, washed with normal saline, and placed into a fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffered saline (PBS). After fixation, samples were cut into 1- to 2-mm segments, washed three times with PBS, postfixed in 1% osmium tetroxide, washed three times with PBS, dehydrated through a graded series of alcohols, and embedded in Spurr's resin. Embedded samples were then sectioned at a thickness of 1.0 μm, and transferred onto glass slides coated with 0.5% gelatin. Tissue sections were stained with 0.1% toluidine blue, destained with 50% ethanol, dried, and coverslips were attached with Permount. Tissue sections were then photographed on a Zeiss Axiophot microscope.

Results. Examination of the tissue sections did not show any physical differences associated with the addition of vitamin E to the diet.

DISCUSSION

High amounts of vitamin E supplementation in the diet have been found to stimulate the immunoglobulin production to antigen[1,13] and to enhance the mitogenic responses of lymphocytes.[2,3] Conversely, it has been reported that vitamin E deficiency suppresses the lymphocytic responses to mitogen,[14] suggesting an adverse effect of vitamin E deficiency on cell-mediated immunity. However, in this experiment no differences between groups were observed in ear-thickness response to DNFB or in lymphocytic proliferative responses to PHA, both of which are known to be dependent on T cell function.[15,16] This result may be partly because the duration of vitamin E supplementation was too short to enhance the immunological activity, or that serum levels of vitamin E did not become high enough. Serum levels of vitamin E in group 4 animals were only twice those of group 2 animals despite the fact that they were given 25 times as much vitamin E. Machlin and Gabriel[17] showed that vitamin E levels in plasma and tissue depend not only on the amount ingested but also the duration of supplementation in unstressed animals. It may be possible that mucosal damage following thermal injury has affected the absorption of vitamin E, because hydrolysis by mucosal esterase has been reported to be essential before absorption of tocopheryl acetate.[18] Furthermore, Nockels[1] reported that in guinea pigs, 300 IU of vitamin E administered orally for 2 weeks had no effect on antibody response to vaccination. In contrast, 33 IU of vitamin E injected intraperitoneally elevated the antibody levels. The reason why immune response was not depressed in group 1 animals is not clear, but it may be due to the high arginine content which is known to enhance immune response.[19,20]

The lack of significant differences in RME and body and muscle weights among the groups demonstrated that vitamin E supplementation failed to show any beneficial effect on postburn hypermetabolism. However, the mucosal wet weights of group 1 were significantly lower than those of groups 2 and 3 on PBD 14. As the ratios of mucosal dry weight to wet weight were almost the same among the groups, the difference could not be attributed to water content. It is well known that the gastrointestinal mucosa is impaired following thermal injury. Previous studies reported that impairment of mucosa allows the translocation of pathogenic organisms.[21,22] Several mechanisms have been reported to be responsible for this mucosal damage following burn injury.[23] Although we did not find any significant physical differences associated with the addition of vitamin E to the diet, Parks et al[24] reported that superoxide radicals play an important role in ischemic injury of the small intestine. Vitamin E, an antioxidant, has been reported to protect the cell membranes of various organs[25–27] against ischemic injury. In the small bowel, it has been suggested[28] that vitamin E is absorbed through the mucosa by a passive diffusion process. Therefore, it may be possible that mucosal cells in vitamin E-supplemented groups were exposed to a high concentration of vitamin E, which lessened the mucosal damage compared to those in group 1, who were not given any vitamin E. It has been also reported that vitamin E supplementation normalizes the ultrastructure of mucosal cells.[6]

This study also demonstrated that vitamin E supplementation has a beneficial effect on the prevention of postburn anemia, even though it could not explain the mechanism whereby vitamin E affects the red blood cells. One possible reason may be that vitamin E protects the red blood cell membrane from lipid peroxidation. It has been reported that the lipid peroxide level in serum or plasma increases following thermal injury,[29,30] and in red blood cells polyunsaturated fatty acid levels decrease significantly.[31] A high level of lipid peroxidation products in red blood cells is reported to have a relationship to decreased cellular deformability and a reduction of osmotic resistance.[32,33] This results in an increased susceptibility to hemolysis. Another possibility is that vitamin E may affect the synthesis of heme proteins, which has been reported in the rat.[34]

In conclusion, dietary vitamin E supplementation failed to produce any beneficial effect on either nutritional state or immune response following burn. Yet, in terms of the maintenance of intestinal mucosa and recovery from postburn anemia, addition of vitamin E was effective over a wide-dose range.

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