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THE EFFECT OF MEGADOSAGE VITAMIN E CONSUMPTION ON RAT THYMOCYTE ROSETTE-FORMING CELLS AND PROLIFERATIVE RESPONSE

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Abstract

Cell-mediated immunity was studied in Wistar rats following treatment for 28 consecutive days with megadose quantities (560, 1120 and 2240 mg/kg) of vitamin E. The numbers of thymocyte rosette-forming cells were significantly increased ($p < 0.05$) only in the two groups of treated rats (560 and 1120 mg/kg). The proliferative response of thymocytes to phytohemagglutinin-P of all groups of treated rats were reduced compared to controls, but were not different between the vitamin E-supplemented groups.

Vitamin E, alpha-tocopherol, is one of the fat soluble vitamins widely used as a vitamin supplement and an ingredient in cosmetic preparations. In general, the vitamin E requirement is low with some variation with age[1]. However, it is also used as a drug in the treatment of some clinical problems such as muscular dystrophies, habitual abortion, cardiovascular disease, acne, aging, etc.[2,3] and many physicians use this vitamin for placebo like effects[4]. Vitamin consumption has increased dramatically during the last decade, both through self-prescription and from fortified vitamin-supplemented foods. Megadose vitamin consumption is known to have adverse effects. There is considerable controversy concerning the effects of megadoses of vitamin E in man and animals, in terms of both its physiological and immunological effects[5-7].

In the present study, three megadose quantities of vitamin E were subchronic administered orally to adult Wistar rats to assess the effects on cell-mediated immunity. Cells involved in cell-mediated immunity were quantitated by thymocyte rosette formation whereas cell-mediated immune function was assayed by proliferation in response to phytohemagglutinin P (PHA-P).

Groups of 10 male albino Wistar rats weighing 200-250 g were given orally with various doses of vitamin E (dl-alpha-tocopherol acetate) of 560, 1120 and 2240 mg/kg (80, 160 and 320 iu/m^2) respectively once a day for 28 consecutive days. Corn oil was used in the control group. The animals were fed with laboratory chow *(ad libitum)* throughout the experiment. On the last day of the experiment, blood was collected by cardiac puncture under light anesthesia using heparin as anticoagulant. Mononuclear cells were separated by Ficoll-Hypaque density (1.07 g/ml) centrifugation as follows. 2 ml of

heparinized blood was overlayered on 3 ml Ficoll-Hypaque solution and centrifuged at 400xg for 30 min at 25°C. Mononuclear cells were collected from the white layer, washed and adjusted to 2×10^6 cells/ml with RPMI 1640 medium. Then it was distributed into wells of flat-bottom tissue culture plates at 2×10^5 cells/100 µl. An equal volume of PHA-P solution was added to triplicate wells to give final concentrations of 0, 3, 30 and 300 µg/ml. The plate was placed in a 37°C, 5% CO_2 humidified incubator for 48 hours. Then 0.5 µCi of ^3H thymidine was added. After another 18 hours incubation, the cells were harvested using a MASH III. The radioactivity was detected by liquid scintillation spectrometry[8,9]. The stimulation index (S.I.) was calculated as:

$$\text{S.I.} = \frac{\text{cpm in cells with PHA-P}}{\text{cpm in cells without PHA-P}}$$

The proliferative response of normal rat mononuclear cells stimulated with PHA-P at final concentrations of 3, 30 and 300 µg/ml showed the highest S.I. at a concentration of 300 µg/ml. The profile seen in the normal rat could not be observed in any group of the three groups of vitamin E treated rats. There was no variation in the S.I. between the three PHA-P concentrations used, nor was there any variation between groups of rats as demonstrated in Fig. 1. The decrease in T cell proliferative response of vitamin E treated rats as compared to that of the control group has been reported by Prasad[5] and Yasunaga *et al.*[7]. However, some investigators have reported an enhancing effect of megadose vitamin E consumption[10,11]. The T cell proliferative responses in this study were decreased at all 3 concentrations of PHA-P used. This suggests that megadosage quantities of vitamin E may have induced some qualitative changes in T cells (at least in some subpopulation of T cells), possibly by increasing the T helper and T suppressor cell ratio. This is suggested by the change in the pattern of PHA response of the immunological abnormalities with the alteration of T cell subpopulations[12].

The numbers of thymocyte rosette-forming cells were assayed by mixing equal volumes of 5×10^6 thymocytes/ml and a 0.5% guinea pig erythrocyte suspension together in a tube. The mixture was incubated at 37°C for 5 min, then centrifuged at 200xg for 5 min and further incubated at 4°C for 5 min. The pellet was gently mixed and then loaded onto a hemacytometer. At least 200 thymocytes were counted and those bearing three or more attached erythrocytes were defined as rosette-forming cells[13,14]. The numbers of thymocyte rosette forming cells of the first two doses (560 and 1120 mg/kg) of vitamin E treated rats were significantly increased. The number observed in the rats treated with the highest concentration (2240 mg/kg) of vitamin E was in the same range as the control group (Table 1). The enhancing effects of 560 and 1120 mg/kg of vitamin E on the number of thymocyte rosette-forming cells were in agreement with the study of Bendich *et al.*[13]. However, the highest dosage of vitamin E (2240 mg/kg) did not show any affect as the number of thymocyte rosette-forming cells was in the same range as the control group. The increased number of mature thymocytes at the two lower megadose levels of vitamin E

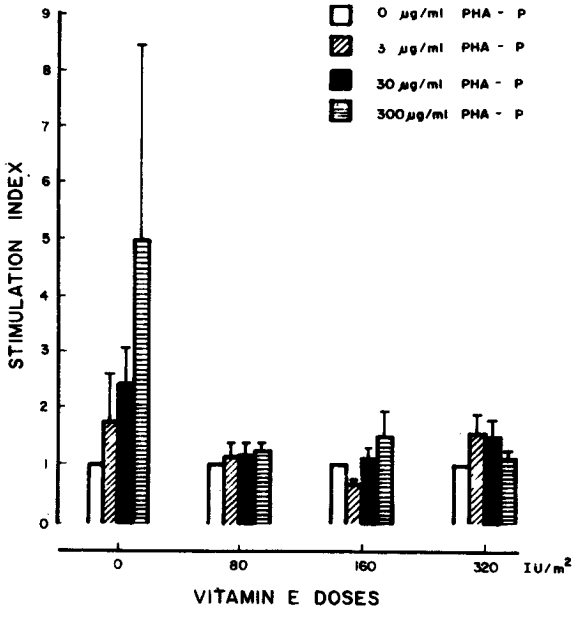

Fig. 1. Stimulation index of the proliferative response of normal and vitamin E treated rats mononuclear cells stimulated with various concentrations of phytoheamagglutinin-P (PHA-P). Vitamin E doses were 80, 160, 320 IU/m^2 (equivalent to 560, 1120, 2240 mg/kg).

Table 1. THE NUMBER OF THYMOCYTE ROSETTE-FORMING CELLS OF VITAMIN E-TREATED RATS.

Vit. E. mg/kg	Thymocyte rosette forming cells/200 thymocytes
0	4.98 $\pm 2.9^{a,}$
560	9.8 $\pm 6.2^{a,b}$
1120	13.8 $\pm 6.3^{a,b}$
2240	3.3 $\pm 3.2^{a}$

[a]Mean $\pm$ S.D., [b]Statistically significant difference from control, $p < 0.05$ (Students 't' test).

may be due to some favorable changes of the microenvironment in the thymus. At extremely high megadose level of vitamin E consumption, the microenvironment may be further altered so as to be unsuitable for the recruitment of mature thymocytes resulting in a decline in the numbers.

In conclusion, this study shows that subchronic vitamin E megadose consumption interfered with cell mediated immunity in Wistar rats. The quantitative changes observed in thymocyte rosette-forming cells were relatively vitamin E dose-dependent. However, the decreased proliferative response to PHA-P suggested some alteration in the population of T cells.

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บทคัดย่อ

ความสามารถของระบบภูมิคุ้มกันผ่านเซลล์ในหนูพันธุ์ วิสต้า ซึ่งได้รับการป้อนวิตามิน อี เป็นจำนวนมากต่าง ๆ กันคือ 560, 1120, 2240 มิลิกรัม/ กก นาน 28 วัน ติดต่อกัน ได้ถูกทำการศึกษาจำนวนของเซลล์ในระบบภูมิคุ้มกันผ่านเซลล์ โดยหาจำนวนของเซลล์ไทโมซัย ที่สามารถจับกับเซลล์เม็ดเลือดแดงของหนูตะเภา พบว่าสูงขึ้นอย่างมีนัยทางสถิติ ($P < 0.05$) ในกลุ่มของหนูวิสต้าที่ได้รับวิตามิน อี 560 และ 1220 มิลิกรัม/ กก เมื่อเปรียบเทียบกับหนูที่ไม่ได้รับวิตามิน อี ความสามารถของเซลล์ในระบบภูมิคุ้มกันผ่านเซลล์ ศึกษาโดยวิธี โพรลิเฟอเรชั่น ผลปรากฏว่าไม่มีการเปลี่ยนแปลงอย่างมีนัยทางสถิติในกลุ่มของหนูที่ได้รับวิตามิน อี จำนวนต่าง ๆ แต่อย่างไรก็ตามจะเห็นว่ามีการลดลงของความสามารถของเซลล์ในระบบภูมิคุ้มกันผ่านเซลล์