Nutritional Approaches to Radioprotection: Vitamin E

Guarantor: K. Sree Kumar, PhD
Contributors: K. Sree Kumar, PhD; Venkataraman Srinivasan, PhD; Raymond Toles; Msgt Lennox Jobe, MSC USAF; Thomas M. Seed, PhD

Low-level radiation injury is dependent on the radiation dose and dose rate. The major military use of any potential radioprotectant is to prevent the short-term effects of lethality and the long-term effects of cancer and other pathologies from radiation exposure that may occur in a nuclear battlefield or in a nuclear material contaminated field of operation. Therefore, a radioprotectant should not affect the ability of military personnel to perform tasks. Because exposure to ionizing radiation induces free radical species, effective antioxidants, either alone or in combination with other agents, can be used as potential radioprotectors. To test this hypothesis, we studied vitamin E for its radioprotective efficacy. Using $CD2F_1$ male mice as the model system, we observed that vitamin E at a dose of 400 IU/kg acts as a good radioprotectant against lethal doses of cobalt-60 radiation. Vitamin E was more efficacious when given subcutaneously than when given orally.

Introduction

Radiation exposure can induce an adaptive response,[1] produce a variety of cancers, as in atom bomb survivors,[2] and lead to an elevated risk of leukemia after radiotherapy.[3] In an effort to identify suitable radioprotectors, different strategies have been developed over the past several years. Prominent among these strategies are prophylactic intervention with free radical scavengers,[4] or sulfhydryl agents,[5] stimulation of a radiation-compromised hematopoietic system with immunomodulators,[6] and cytoprotection with receptor-mediated ligands like prostanoids.[7] Most of these studies were limited to the preclinical stage with rodents, but some of the preclinical studies were also performed with canines.[8] Some of the drugs studied, such as amifostine (WR-2721) and granulocyte colony-stimulating factor, were approved by the Food and Drug Administration for limited clinical applications of radio/chemical cytoprotection as well as other applications. One of the main hurdles to overcome in developing radioprotective agents for human use is that most are toxic and thus might compromise the human's ability to perform tasks; this effect has been demonstrated in animals.

Because ionizing radiation produces free radicals, we developed some approaches for testing the efficacy of nutritional antioxidants as potential radioprotective agents. In earlier reports, we showed that the antioxidant vitamin E (α-tocopherol) can alleviate radiation-induced decrement in delayed-type hypersensitivity[9] and can act as an adjuvant to another radioprotectant.[10] We also showed that a vitamin E preparation containing an emulsifier base acted as a radioprotector when given after irradiation.[10] In the present paper, we demonstrate that vitamin E given 24 hours before irradiation can be an effective radioprotector, even at superlethal radiation doses.

Radiation Casualty Management Research Team, Department of Radiation Medicine, Armed Forces Radiobiology Research Institute, 8901 Wisconsin Avenue, Bethesda, MD 20889-5603.

Presented in part at the International Conference on Low-Level Radiation Injury and Medical Countermeasures, November 8–10, 1999, Bethesda, MD.

This manuscript was received for review February 2001. The revised manuscript was accepted for publication in November 2001.

Materials and Methods

Male $CD2F_1$ mice were used throughout this study. Mice between the ages of 6 and 8 weeks, weighing approximately 22 to 26 g, were purchased from Charles River Laboratories (Wilmington, MA), held in quarantine for 10 days, tested for *Pseudomonas*, and maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility of the Armed Forces Radiobiology Research Institute before use in the experiment. Animals were kept in plastic microisolator cages (eight per cage) on hardwood-chip contact bedding with free access to food and acidified water, in an air-conditioned room, with 12 changes of air per hour. All animal procedures were done according to the protocol approved by the Armed Forces Radiobiology Research Institute Animal Care and Use Committee.

Vitamin E [(+)-α-tocopherol] was purchased from Sigma-Aldrich (St. Louis, MO); it was suspended in a mixture of an emulsifier obtained from Schering-Plough (Kenilworth, NJ), and 100% PEG-400 (polyethylene glycol). The emulsifier contained 20% ethyl alcohol and 1% benzyl alcohol. For 1,000 IU of vitamin E, we used 8.35 mL of PEG-400 and 0.55 mL of emulsifier in a total volume of 10 mL. For vehicle controls, we used the same composition of emulsifier and PEG-400 but replaced the vitamin E with olive oil.

Mice were given either 0.1 mL of vitamin E or the vehicle, by subcutaneous injection at the nape of the neck or orally, 22 to 24 hours before irradiation. Mice were held in well-ventilated Plexiglas boxes (eight per box) during bilateral irradiation in a cobalt-60 facility at the dose rate of 0.6 Gy/min. After irradiation, mice were returned to their original cages and monitored for 30 days for survival, which was used as the index of radioprotective efficacy.

Results

Table I shows the results of the protective effect of vitamin E at a dose of 400 IU/kg in mice. The vitamin E was given subcutaneously 24 hours before irradiation at 10.5 Gy at a dose rate of 0.6 Gy/min from a cobalt-60 source. The $LD_{50/30}$ radiation dose for the strain of mice used in this study was 8.14 Gy. The survival rate of irradiated mice given vitamin E, as measured by 30-day survival, was 79%. The survival rate in vehicle controls was only 4%, even at a lower radiation dose of 9.5 Gy.

To test whether protection by vitamin E is independent of the route of administration, we tested its efficacy using two different

TABLE I

RADIOPROTECTION OF MICE BY SUBCUTANEOUS INJECTION OF VITAMIN E

Treatment[a]	Radiation Dose (Gy)	Number of Mice		% Survival[b]
		Exposed	Survived	
Vitamin E	10.5	24	21	79 ± 8.9
Vehicle	9.5	24	1	4 ± 3.5

[a]Vitamin E (400 IU/kg) given 24 hours before irradiation at 10.5 Gy, at the rate of 0.6 Gy/min from a cobalt-60 source.

[b]Mean ± S.E. from three experiments with eight mice in each experiment.

routes—subcutaneous and oral. The dose of vitamin E (400 IU/kg), time of administration (24 hours), and radiation dose rate (0.6 Gy/min) were identical to those in the previous experiment, but a lower radiation dose of 9.5 Gy was used. The lower dose was used because there was almost no survival at 30 days with the vehicle. The results are shown in Figure 1.

When vitamin E was given subcutaneously, 88% of the mice were alive at the end of 30 days. There were no survivors in the group that received vitamin E orally or in the group that received the vehicle, either orally or subcutaneously.

Discussion

Vitamin E (α-tocopherol) is a major lipophyllic antioxidant present in vivo that protects critical membrane components, including phospholipids, transmembrane lipoproteins, and glycoproteins, from the free radicals produced by a variety of endogenous and exogenous sources. Vitamin E has been implicated in cancer prevention,[11] alleviation of Adriamycin cardiotoxicity,[12] immune system protection from radiation damage,[9] and other normal and pathological conditions.[13] However, in studies of the role of vitamin E in atherogenesis, a definite role for vitamin E could not be established. It was suggested that vitamin E may have an antioxidant, neutral, or pro-oxidant activity vis-à-vis atherogenesis unless other reducing agents are present along with it.[14] The results presented in this paper and our previous reports indicate that vitamin E can also protect species from free radicals produced by radiation and enhance survival after lethal irradiation.[9,10]

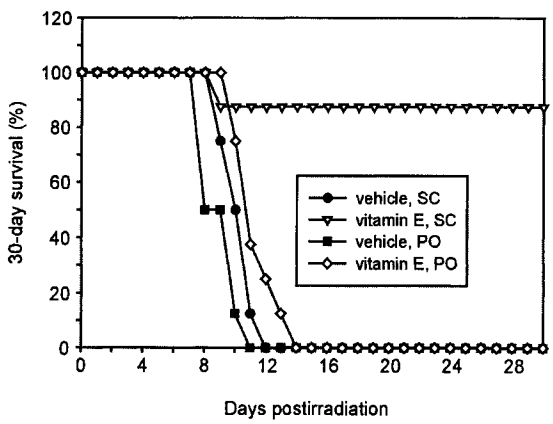

Fig. 1. Radioprotection of mice by subcutaneous injection of vitamin E (400 IU/kg) given 24 hours before irradiation at 9.5 Gy from a cobalt-60 source. SC, subcutaneously; PO, orally.

Most of the earlier studies on vitamin E were done after feeding it to the mice by gavage or incorporating it into the diet for several days[15] or months.[16] Probably the most interesting aspect of the present study is the high degree of protection obtained from a single dose of vitamin E injected subcutaneously rather than taken orally. The lack of protection with the oral administration may be attributable to the probable first-pass metabolism of vitamin E to its quinones in the liver, thereby reducing the dose available for targets that are critical for radiation damage, such as bone marrow.

Using a similar method of administration of vitamin E in our previous studies, protection was obtained when vitamin E was given 15 minutes after unilateral cobalt-60 irradiation at a dose rate of 0.2 Gy/min. In the current study, however, a greater level of protection was obtained when vitamin E was given 24 hours before radiation exposure.

The increase in protection obtained in the current study may also be attributable to the particular formulation of vitamin E used. In the previous studies, the injectable vitamin E was in a base containing 20% ethyl alcohol and 1% benzyl alcohol. This high concentration of ethyl alcohol may have caused a local denaturation at the injection site, preventing further adsorption of the vitamin. Although we used the same emulsifier, it constituted only approximately 5% of the total suspension injected. Moreover, the presence of PEG-400 may have facilitated adsorption of vitamin E. Although certain emulsifiers might complicate the results, the use of the same amount of emulsifier in the experimental and vehicle control groups obviates such a possibility.

Vitamin E can function as a radioprotectant by several mechanisms. Vitamin E acts as a sink for the free radicals formed from radiation, thus preventing them from attacking cell membrane targets; it also stops the formation of secondary radicals. The lipophilic nature and long half-life of vitamin E in vivo are very helpful in this regard. It is well known that irradiation increases the expression of interleukins, interferon-γ, tumor necrosis factor-α and -β, and intercellular adhesion molecules.[17] It is possible that vitamin E may reverse the increase of proinflammatory genes that may contribute to radiation-induced lethality.

Irrespective of the mechanisms used, vitamin E offers an excellent alternative to other known chemical radioprotectants because it is not toxic. At the dose tested in the present study, the animals did not show any gross observable toxicity like the behavioral decrement observed with other radioprotectors. Earlier studies of toxicity done at a dose range of 600 to 3,200 IU per day for 3 to 6 weeks did not show any consistent side effects or any evidence of mutagenicity, teratogenicity, or carcinogenicity.[18]

In addition to protection from lethality, vitamin E in combination with pentoxifylline is known to greatly diminish the long-term subcutaneous fibrosis induced by exposure to high-dose radiation.[19] These findings and the known anticarcinogenic potential of vitamin E[11] offer an excellent opportunity to develop vitamin E as a radioprotector for long-term low-level exposure to radiation.

References

1. Azzam EI, Raaphorst GP, Mitchel REJ: Radiation-induced adaptive response for protection against micronucleus formation and neoplastic transformation in C3H 10T1/2 mouse embryo cells. Radiat Res 1994; 138: S28–S31.
2. United Nations Scientific Committee on the Effects of Atomic Radiation (UN-

SCEAR): Effects and risks of ionizing radiation. New York, United Nations, 1988.
3. Holowaty EJ, Darlington GA, Gajalakshmi CK, Toogood PB, Levin W: Leukemia after irradiation for endometrial cancer in Ontario. Cancer 1995; 76: 644–9.
4. Kumar KS, Vaishnav YN, Smith C, Clark EP: Radiation injury and antioxidant mechanisms of protection in eicosanoids and other bioactive lipids. In Cancer, Inflammation and Radiation Injury, pp 333–9. Edited by Nigam S, Honn KV, Marnett LJ, Walden TL. Boston, Kluwer, 1993.
5. Murray D: Aminothiols. In Radioprotectors: Chemical, Biological, and Clinical Perspectives, pp 53–109. Edited by Bump EA, Malaker KM. Boca Raton, FL, CRC Press, 1998.
6. Neta RN, Duches SD, Oppenheim JJ: Interleukin-1 as a radioprotector. J Immunol 1986; 136: 2483–85.
7. Hanson WR, Houseman KA, Collins PW: Radiation protection in vivo by prostaglandins and related compounds of the arachidonic acid cascade. Pharmacol Ther 1988; 39: 347–56.
8. MacVittie TJ, Monroy RL, Patchen ML, Souza LM: Therapeutic use of recombinant human G-CSF in a canine model of sublethal and lethal whole-body irradiation. Int J Radiat Biol 1990; 57: 723–36.
9. Srinivasan V, Jacobs AJ, Simpson SA, Weiss JF: Radioprotection by vitamin E: effects on hepatic enzymes, delayed type hypersensitivity, and postirradiation survival of mice. In Modulation and Mediation of Cancer by Vitamins, pp 119–31. Edited by Meyskens FL, Prasad KN. Basel, Karger, 1983.
10. 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: 841–5.
11. Knekt P, Aromaa A, Maatela J, et al: Vitamin E and cancer prevention. Am J Clin Nutr 1991; 53: 283s–6s.
12. Myers C, McGuire W, Young R: Adriamycin amelioration of toxicity by alpha-tocopherol. Cancer Treat Rep 1976; 60: 961–2.
13. Packer L, Landvik S: Vitamin E in biological systems. Adv Exp Med Biol 1990; 264: 93–103.
14. Stocker R: The ambivalence of vitamin E in atherogenesis. Trends Biochem Sci 1999; 156: 219–23.
15. Aikawa KM, Quintanilha AT, de Lumen BO, Brooks GA, Packer L: Exercise endurance training alters vitamin E tissue levels and red-blood-cell hemolysis in rodents. Biosci Rep 1984; 4: 253–7.
16. El-Nahas SM, Mattar FE, Mohamed AA: Radioprotective effect of vitamins C and E. Mutat Res 1993; 301: 143–7.
17. Hong JH, Chiang CS, Campbell IL: Induction of acute phase gene expression by brain irradiation. Int J Radiat Oncol Biol Phys 1995; 33: 619–26.
18. Kappus H, Diplock AT: Tolerance and safety of vitamin E: a toxicological position report. Free Rad Biol Med 1992; 13: 55–74.
19. Lefaix J-L, Delanian S, Vozenin M-C, Lepalt J-J, Tricaud Y, Martin M: Striking regression of subcutaneous fibrosis induced by high doses of gamma rays using a combination of pentoxifylline and α-tocopherol: an experimental study. Int J Radiat Oncol Biol Phys 1999; 43: 839–47.