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Utilization of Vitamin E Analogs to Protect Normal Tissues While Enhancing Antitumor Effects

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Despite advances in radiation delivery techniques, side effects of radiation therapy due to radiation exposure of normal tissues are common and can limit the deliverable dose to tumors. Significant interests lie in pharmacologic modifiers that may protect against normal tissue toxicity from cancer treatment while simultaneously enhancing the tumor response to therapy. While no such treatments are available in the clinic, this is an area of active preclinical and clinical research. This review summarizes research studies that provide evidence to indicate that tocotrienols, natural forms of vitamin E, are potent radiation protectors and may also have antitumor effects. Hence, several current clinical trials test tocotrienols as concomitant treatment in cancer therapies.
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Introduction

Approximately half of all cancer patients receive radiation therapy as part of their treatment regimen. Technical improvements in radiation therapy and the combination with novel chemotherapeutic therapies have led to an increase in cancer cure rates. Despite advances in radiation delivery techniques, radiation exposure of the surrounding normal tissues still occurs and can limit the deliverable dose to tumors. Acute and long-term side effects associated with exposure of normal tissues to radiation can affect the quality of life of cancer patients and survivors. Significant interests lie in pharmacologic modifiers that may protect against normal tissue toxicity from cancer treatment while simultaneously enhancing the tumor response to therapy. While no such modifiers have been approved for clinical use, preclinical and clinical studies have identified potential safe and effective treatments that may be administered during cancer therapy. This review focuses on tocotrienols, natural forms of vitamin E, as one class of molecules that is an active area of research with much potential.

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Molecular and Cellular Properties of Tocotrienols

Since its discovery in 1922, vitamin E attracted attention in the biomedical community at an increasing pace.[1] Vitamin E is not a single compound, but a general term, which includes the family of lipid soluble tocochromanols. These molecules possess structural similarities, yet distinct features and biological properties. All members of the vitamin E family have an aromatic chromanol head determining the nomenclature of the isomers (α-, β-, γ-, and δ-) based on the number of methyl groups located on the chromanol head. Saturation of the 16-carbon hydrocarbon tail also defines the analogs' names as tocopherol (saturated) or tocotrienol (unsaturated).[2]

The putative antioxidant activity of the vitamin E family is believed to arise from the chromanol head. By donating its hydrogen atom and scavenging free radicals in the biological membranes, the phenolic hydroxyl group prevents the propagation of lipid peroxidation.[3,4] The accessibility and mobility of the vitamin E analogs also significantly contribute to their antioxidant strength.[5-8] Compared to tocopherols,

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tocotrienols have higher protective potential against lipid peroxidation due to their increased mobility and better distribution in the lipid bilayer, enhancing their ability to interact with lipid radicals in the membranes.[9-14] Studies in cell culture indicate that γ-tocotrienol is superior to α-tocopherol in protecting against oxidant injury and subsequent mitochondrial dysfunction.[15] Moreover, tocotrienols induce the expression of endogenous antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and NADPH quinoneoxidoreductase.[16-18]

In addition to antioxidant properties, tocotrienols have many "pleiotropic" effects, as described below, which make tocotrienols attractive molecules in the development of radiation countermeasures.

Tocotrienols are Potent Radiation Protectors

Tocotrienols Improve Survival in Animal Models of the Acute Radiation Syndrome

Tocotrienols were discovered as strong radiation protectors in animal models of the acute radiation syndrome from whole-body exposure to γ- or X-rays.[19-26] The optimal time of administration appears to be 24 hours before irradiation. Of all natural compounds tested to date, tocotrienols are among the strongest radiation protectors.[27,28] As a result, efforts are underway to develop γ-tocotrienol as a countermeasure against the acute radiation syndrome caused by accidental radiation exposure in human subjects.[29]

Although the mechanisms by which tocotrienols protect against the acute radiation syndrome are not yet fully known, preclinical studies have provided evidence that tocotrienols may act at least in part via the induction of granulocyte colony-stimulating factor.[30,31] On the other hand, induction of granulocyte colony-stimulating factor by γ-tocotrienol is not always associated with potent radiation protection,[32] indicating that additional mechanisms of action must be involved. For instance, tocotrienols have anti-inflammatory properties, by suppressing expression of tumor necrosis factor α, inducible nitric oxide (NO) synthase, and interleukins 6 and 8, as well as inhibiting NF-κB signaling.[33-36] Other mechanisms may be discerned from studies on spleen microRNA expression in response to γ-tocotrienol in a mouse model of whole-body irradiation[37] and studies on the metabolic response to γ-tocotrienol administration in nonhuman primates.[38]

Tocotrienols Protect From Radiation Injury in Part by Modifying Endothelial Cell Function

Damage to the vascular endothelial cells plays a critical role in radiation toxicity in various tissues. Therefore, modulation of endothelial cell damage is a promising strategy to use to try to interfere with radiation toxicity. Irrespective of cell type, an early adverse event of radiation exposure is the formation of DNA double strand breaks, which are considered some of the most critical cellular lesions in radiation injury. γ- and δ-Tocotrienol are the only natural forms of vitamin E known to inhibit the cholesterol biosynthesis pathway. They induce the intracellular degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis.[39,40] Moreover, γ-tocotrienol accumulates in 30-fold higher levels than α-tocopherol in endothelial cells.[41] As demonstrated in cultured human primary endothelial cells, γ-tocotrienol suppression of radiation-induced DNA double strand break formation and cytogenetic alterations may indicate its therapeutic potential in limiting radiation-induced genetic damage.[42]

Inhibition of the cholesterol biosynthesis pathway is associated with increased endothelial expression of thrombomodulin (TM), an endothelial cell surface receptor that has intrinsic anti-inflammatory, antioxidant, anticoagulant, and antiapoptotic properties.[43-46] By forming a complex with the coagulation factor thrombin, TM can exert beneficial effects via generation of activated protein C.[47] Ionizing radiation negatively affects TM expression,[48,49] while activation of the activated protein C pathway and administration of exogenous recombinant TM protect against radiation toxicity.[50,51] γ-Tocotrienol induces the expression of TM in primary and immortalized human endothelial cells.[45] Moreover, γ-tocotrienol-mediated protection against radiation lethality is dependent on TM, indicating the importance of TM in γ-tocotrienol's mechanisms of action.[52]

In vitro studies revealed that γ- and δ-tocotrienols are potent inducers of endothelial NO synthase (eNOS),[53] an enzyme involved in prevention of atherosclerosis, inflammation, oxidative stress, and platelet aggregation. Ionizing radiation negatively affects eNOS-mediated NO generation, in part due to radiation-induced oxidation of tetrahydrobiopterin, an essential co-factor of eNOS.[54,55] As a result, production of superoxide is promoted, a phenomenon popularly known as "eNOS uncoupling." Uncoupled eNOS enhances generation of peroxynitrite and induces vascular nitrosative stress.[56] We have previously shown that γ-tocotrienol suppresses radiation-induced vascular nitrosative stress.[57] Coadministration of the 3-hydroxy-3-methylglutaryl-CoA reductase product mevalonate reversed the effects of γ-tocotrienol, indicating that γ-tocotrienol acted through inhibition of the cholesterol biosynthesis pathway.

Both eNOS and TM are under positive transcriptional control by Kruppel-like factor 2 (KLF2). KLF2 has been associated with endothelial anti-inflammatory, anticoagulant, antioxidant, antiadhesive, and antipermeability properties.[58] γ-Tocotrienol and statins synergistically enhance endothelial KLF2 expression as well as the expression and functional activity of TM.[45] TM's transcription can also be positively regulated by the transcription factor, heat shock factor 1 (HSF1). Statins cause NO-dependent dissociation of HSF1 from heat shock protein 90, nuclear translocation of HSF1, and binding to heat shock elements in the TM promoter to induce TM expression.[44]

Endothelial cells activated through ionizing radiation or other insults express an array of adhesion molecules, including but not limited to intercellular adhesion molecule 1,

vascular cell adhesion molecule 1, and E-selectin, which allow attachment of hematopoietic immune and nonimmune cells to the endothelial surface,[59] thereby contributing to inflammation and activation of the coagulation cascade. Tocotrienols suppress lipopolysaccharide-mediated upregulation of intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin in endothelial cells, potentially by inhibiting NF-κB pathway.[53] Moreover, δ-tocotrienol reduces monocyte-endothelial cell interaction by inhibiting the surface expression of adhesion molecules in oxysterols- or tumor necrosis factor α-activated human endothelial cells.[60,61]

Do Tocotrienols Reduce Adverse Tissue Remodeling After Radiation Therapy When Administered in Combination With Pentoxifylline?

Pentoxifylline (PTX) is a phosphodiesterase inhibitor that was first developed as a rheological agent and is currently approved for the treatment of certain vascular diseases.[62-64] Because it also has antioxidant and anti-inflammatory properties, it has received interest as a potential treatment of fibrosis.[65] In fibrosis due to radiation, it has been tested mostly in combination with α-tocopherol. Efficacy of PTX and α-tocopherol against radiation fibrosis has been shown in animal models[66,67] and clinical studies.[68,69] In many of these studies, treatment with PTX and α-tocopherol is started after radiation-induced fibrosis has become clinically manifest. On the other hand, not all studies show a beneficial effect of PTX and α-tocopherol on radiation fibrosis.[70]

ClinicalTrials.gov lists 3 clinical trials that are recruiting patients to test the impact and safety of PTX and vitamin E against treatment-related toxicity in radiation therapy of head and neck cancer (NCT02397486), nonsmall cell lung cancer (NCT01871454), and metastatic brain cancer (NCT01508221).

Although some studies suggest that PTX enhances the radiation protective properties of γ-tocotrienol,[71,72] additional studies are required to determine whether PTX combined with a tocotrienol may be a better radiation protector or mitigator than combined with α-tocopherol.

Tocotrienols Have Anticancer Properties

In addition to the protection against normal tissue radiation injury as described above, several studies show that tocotrienols have cancer preventive properties.[73-76] Stable and unstable structural changes in chromosomes are considered the "hallmark" features of cancer cells. Administration of γ-tocotrienol 24 hours before total body irradiation in a mouse model suppresses cytogenetic alterations in bone marrow cells.[42] Other suggested mechanisms of cancer prevention by γ-tocotrienol include anti-inflammatory activities and direct effects on cancer cell proliferation and death.[77,78]

In addition to cancer prevention, researchers are exploring the therapeutic role of tocotrienols in cancers.[79-82] Here we present a selection of studies published in this area. Tocotrienols have cytotoxic properties against cancer cells in culture.[83-87] Many molecular mechanisms have been proposed, including induction of the mitochondrial apoptosis pathway,[88,89] altered sphingolipid metabolism,[90] endoplasmic reticulum stress,[91] inhibition of telomerase,[92] inhibition of signaling pathways involved in cytoskeletal organization[93] induction of cyclin dependent kinase inhibitors p21 and p27,[94] inhibition of canonical Wnt signaling,[95] and modification of the Ras-ERK pathway.[96]

Tocotrienols have shown efficacy against tumor growth in in vivo models. For instance, γ-tocotrienol reduced tumor growth in mouse models of human colorectal cancer.[97] Similarly, a tocotrienol-rich fraction inhibited the growth of human colon cancer xenografts in a mouse model.[98] δ-Tocotrienol inhibited tumor growth and number of metastases in a mouse model of orthotopic human pancreatic ductal adenocarcinoma.[99] Oxazine derivatives of γ- and δ-tocotrienols showed enhanced antitumor activities compared to regular γ- and δ-tocotrienol in a syngeneic mouse model of breast cancer.[100] While mechanisms by which oxazine derivation enhances antitumor activity are not yet exactly known, mechanisms may involve attenuation of mammary tumor cell compensatory response to hypoxia.[101]

Angiogenesis is an important pathologic process during tumor development. Vascular endothelial growth factor is one of the critical proangiogenic factors, which is upregulated by hypoxia-inducible factor-1, a transcription factor induced under hypoxic conditions but also by ionizing radiation.[102,103] Tocotrienols have antiangiogenic effects via down-regulating vascular endothelial growth factor expression, indicating that tocotrienols have the potential to restrict tumor growth by exerting antiangiogenic effects.[104-107] For instance, γ-tocotrienol reduced tumor growth in a mouse model of human hepatocellular carcinoma, together with indications of antiangiogenesis effects.[108]

In Vivo Bioavailability of Tocotrienols

Despite the advantages in the cellular effects of tocotrienols over tocopherols, lower in vivo bioavailability of tocotrienols as compared to that of tocopherols may decrease its effectiveness.[109,110] Both tocopherols and tocotrienols are cleaved by esterases and absorbed in the small intestine. Following absorption, both tocopherols and tocotrienols undergo a similar pattern for transportation to the lymphatic system via chylomicrons.[111] The major obstacle for tocotrienols' bioavailability following oral administration arises from their initial tissue distribution via α-tocopherol transfer protein. While this hepatic protein has an excellent affinity for α-tocopherol, its affinity for tocotrienols is less because the unsaturated isoprenoid chain of tocotrienols obstructs the α-tocopherol transfer protein binding pocket.[112-114]

Concerns related to bioavailability of tocotrienols after oral administration have led to the development of self-emulsifying formulations for enhanced intestinal absorption.[115,116] In addition, tocotrienol absorption is enhanced when taken together with food.[110,117] Other recent developments in the advancement of novel anticancer approaches with tocotrienols include the PEGylation of γ- and δ-tocotrienol for enhanced bioavailability,[118] or synthesis of nanoemulsions or new tumor-targeted nanocapsules loaded with anticancer medications and tocotrienols.[119-124]

Clinical Development of Tocotrienols

Tocotrienols are safe, nontoxic, and well-tolerated dietary supplements that exhibit no known interactions with other medications.[125,126] Based on the broad evidence of antiradiation toxicity and anticancer properties of tocotrienols in preclinical and clinical studies, current clinical trials test tocotrienols as concomitant treatment in cancer therapies. Several clinical trials currently listed in ClinicalTrials.gov are recruiting patients to test tocotrienols in the treatment of cancer. δ-Tocotrienol is tested in combination with standard chemotherapy in non-small cell lung cancer (NCT02644252). Also, efficacy and safety of tocotrienol (isomer not specified) is tested in patients who have developed chemoresistant ovarian cancer (NCT02560337), and tocotrienol (isomer not specified) is tested in combination with bevacizumab in advanced ovarian cancer (NCT02399592). Tocotrienol (isomer not specified) is used to improve efficacy of neoadjuvant chemotherapy in breast cancer (NCT02909751). Lastly, a trial is testing whether tocotrienol (isomer not specified) can reduce side effects of 5-fluorouracil, oxaliplatin, irinotecan in metastatic colorectal cancer (NCT02705300).

Conclusions

In conclusion, tocotrienols have a high safety profile and do not interfere with other medications. Preclinical and clinical evidence support the use of tocotrienols to reduce tumor growth and protect normal tissues from radiation injury, making tocotrienols attractive compounds that may increase both efficacy and safety of cancer treatment.

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