

Gamma tocotrienol-induced apoptosis in neoplastic +SA mammary epithelial cells is mediated through PERK/eIF2a/CHOP endoplasmic reticulum stress pathway.

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Gamma tocotrienol is a member of the vitamin E family of compounds and has been shown to display potent anticancer activity. Gamma tocotrienol induces apoptosis in neoplastic mammary epithelial cells at doses that are non-toxic to normal mammary epithelial cells. Previous studies have shown that gamma tocotrienol-induced cytotoxicity is unrelated to mitochondrial stress or death receptor-mediated apoptotic signaling in +SA mammary tumor cells. To further investigate the mechanism of gamma tocotrienol-induced cytotoxicity, studies were conducted to determine the effects of gamma tocotrienol on the endoplasmic reticulum (ER)-mediated apoptotic signaling pathway in +SA cells grown in culture. Cells were maintained in serum-free defined media containing EGF (10 ng/ml) and insulin (10 mg/ml) as co-mitogens. Treatment with 0-40 mM gamma tocotrienol significantly decreased the +SA cell viability over a 24 h-period in a dose- and time-dependent manner. Western blot analyses showed that 24 h treatment exposure to 0-30 mM gamma tocotrienol resulted in a large induction of C/EBP homologous protein (CHOP), the key component of ER stress-mediated apoptosis pathway. Similar treatment doses of gamma tocotrienol led to the cleavage of ER-resident caspase, caspase-12, and poly (ADP-ribose) polymerase (PARP). Additional studies showed that treatment with 0-30 mM gamma tocotrienol had no effect on the relative intracellular levels of ER chaperone protein Bip/GRP78, as compared with untreated control. In contrast, gamma tocotrienol treatment with similar doses resulted in a marked increase in the phosphorylation of ER transmembrane protein, PERK and its downstream effector,  $\alpha$ -subunit of eukaryotic translational initiation factor 2 (eIF2a) within 8 h of treatment exposure, followed by a decline in phosphorylation, as compared with respective controls. Furthermore, treatment of +SA cells with 0-30 mM gamma tocotrienol caused a dose-dependent increase in the expression of ATF4, a transcription factor whose expression is modulated by eIF2a, and can increase CHOP expression. Taken together, these studies demonstrate the role of ER stress-mediated apoptotic PERK/eIF2a/ATF4/CHOP pathway in mediating the cytotoxic effects of gamma tocotrienol in neoplastic +SA mammary epithelial cells. These findings strongly suggest that breast cancer cells that overexpress ER chaperones and are resistant may be rendered sensitive to ER stress-mediated apoptosis by gamma tocotrienol treatment. Additionally, gamma tocotrienol may have potential therapeutic benefit in breast cancers resistant to death receptor signaling.

Short title: Gamma tocotrienol, ER-apoptosis and cancer