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Contrasting effects of α-tocopheryl succinate on cisplatin- and etoposide-induced apoptosis

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ABSTRACT

Targeting mitochondria is a promising strategy in tumor cell elimination. D-α-tocopheryl succinate (α-TOS), a redox-silent analog of vitamin E, is a potentially powerful tool for fighting tumors by directly affecting mitochondria. However, when used at low concentrations it can suppress apoptosis induced by the conventionally used anticancer drug cisplatin. In cells treated with cisplatin, 30 μM α-TOS prominently attenuated the manifestation of characteristic features of apoptosis — release of cytochrome *c* from mitochondria, caspase-3-like activity, and cleavage of poly(ADP-ribose) polymerase. In contrast, cell death induced by etoposide was not inhibited but rather stimulated by α-TOS. Thus, co-treatment with α-TOS and conventional antitumor drugs should be carried out with caution.

1. Introduction

Mitochondria are key participants in various cell death signaling pathways. Thus, steps leading to mitochondrial destabilization and permeabilization of the outer mitochondrial membrane (OMM) with the subsequent release of pro-apoptotic proteins might be a promising strategy in tumor cell elimination.

Recently, a range of compounds named mitocans (an abbreviation derived from MITOchondria and CANcer), were shown to cause cell death by targeting mitochondria (Neuzil et al., 2007). One of the mitocans, D-α-tocopheryl succinate (α-TOS), a redox-silent analog of vitamin E, was shown to induce multiple changes in tumor cells, ultimately leading to cell death. The effects of α-TOS are due to interaction with Complex II of the mitochondrial respiratory chain, causing the leakage of electrons and formation of reactive oxygen species (Dong et al., 2008). In addition, we found that α-TOS stimulated rapid entry of Ca^{2+} into the cytosol, compromised Ca^{2+} buffering capacity of the mitochondria and sensitized them towards mitochondrial permeability transition (Gogvadze et al., 2010; Kruspig et al., 2012). Remarkably, α-TOS was shown to selectively kill malignant cells at concentrations that are non-toxic for normal cells. In non-malignant cells α-TOS is hydrolyzed by means of esterases. As a result, α-tocopherol is gradually released to prevent membrane oxidative damage. Indeed, α-TOS was shown to rescue cells from chemical-induced toxicity (Fariss et al., 1989), or ionizing radiation (Singh et al., 2012). However, in malignant cells the hydrolysis of α-TOS is suppressed due to lower esterase activity (Neuzil et al., 2006; Ottino and Duncan, 1997). Thus, the inability of malignant cells to cleave α-TOS determines its ability to stimulate tumor cell death.

α-TOS and other vitamin E analogs, such as α-tocopheryloxyacetic acid (Dong et al., 2012), are regarded as anticancer drugs, the efficiency of which has been demonstrated in a number of studies utilizing tumor cell lines (Kruspig et al., 2012) as well as mice xenografts. Co-treatment of α-TOS and paclitaxel inhibited bladder cancer cell growth and viability in vitro and in vivo (Kanai et al., 2010). The anticancer effect of α-TOS was also demonstrated on JHU-022 solid tumor xenograft growth in immunodeficient mice (Gu et al., 2008).

However, it remains unknown whether relatively low concentrations of α-TOS that does not overcome the capacity of esterases would protect cells from conventional antitumor drugs due to the cleavage of α-TOS with the formation of α-tocopherol and succinate. In the present work we analyzed the possible consequences of combined treatment of tumor cells with various concentrations of α-TOS and drugs used in antitumor therapy — cisplatin and etoposide. We show that α-TOS should be administered with caution as a potential anticancer drug, since administered at low concentrations α-TOS together with certain drugs can rescue rather than kill cells.

Abbreviations: α-TOS, D-α-tocopheryl succinate; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; OMM, outer mitochondrial membrane; PARP, poly(ADP-ribose) polymerase; MPT, mitochondrial permeability transition.

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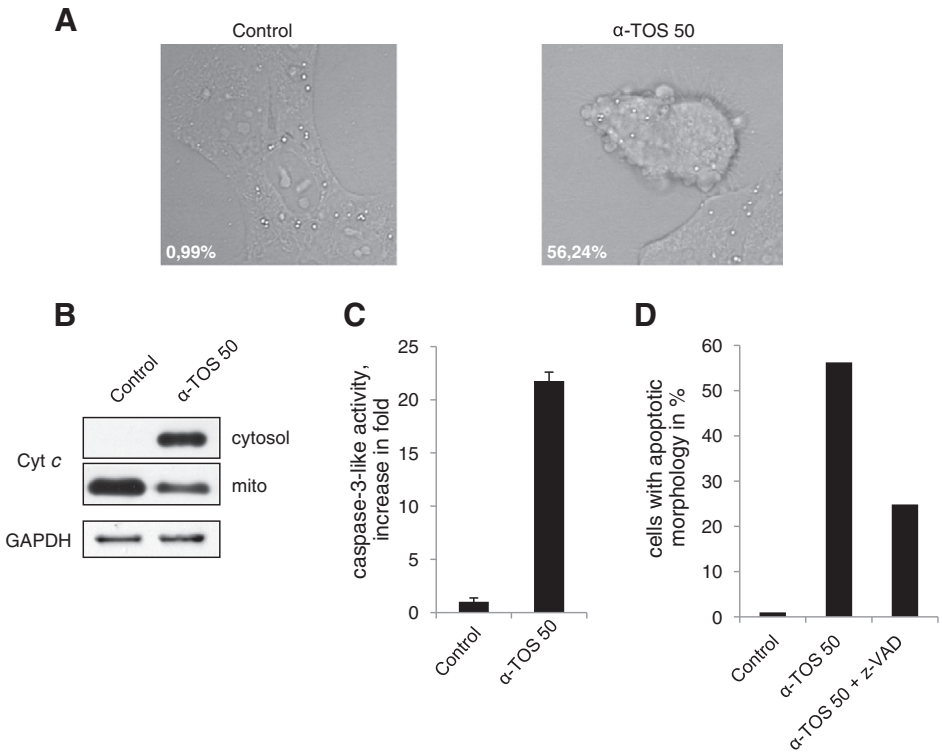

Fig. 1. α-TOS-induced apoptosis assessed by morphological changes (A), release of cytochrome *c* (B), and caspase-3-like activity (C). Pan-caspase inhibitor z-VAD-FMK (10 μM) attenuated α-TOS-induced morphological changes (D). Numbers indicate percentage of cells with apoptotic morphology. Tet21N cells were incubated with 50 μM α-TOS for 17 h, and apoptosis manifestations were assessed following harvesting, as described in the Materials and methods section.

2. Material and methods

2.1. Cells

All cells used in these experiments were cultured in RPMI 1640 complete medium supplemented with 10% (w/v) heat-inactivated fetal calf serum and penicillin/streptomycin (100 U/ml). For Tet21N cells, 100 μg/ml hygromycin and 200 μg/ml geneticine were also added to the medium. Cells were grown in a humidified air/CO_2 (5%) atmosphere at 37 °C and maintained in a logarithmic growth phase for all experiments.

2.2. Assessment of cytochrome c release

Cells were permeabilized with 0.01% digitonin for 15 min and fractionated into supernatant and pellet by centrifugation for 5 min at 16,000 × *g*. Samples were mixed with Laemmli's loading buffer, boiled for 5 min and subjected to 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at 40 mA followed by electroblotting to nitrocellulose membranes for 2 h at 120 V. Membranes were blocked for 1 h with 5% nonfat milk in phosphate-buffered saline (PBS) at room temperature and subsequently probed overnight with a mouse anti-cytochrome *c* antibody (BD Biosciences, San Jose, CA). The membranes were rinsed and incubated with a horseradish peroxidase-conjugated secondary antibody (1:10,000) and visualized by ECL™ (Amersham Biosciences, Buckinghamshire, UK) and X-ray film.

2.3. Measurement of caspase activity

Cleavage of the fluorogenic peptide substrate (Peptide Institute, Osaka, Japan) was measured using a modified version of a fluorometric assay. Cells were pelleted and washed once with PBS. After centrifugation, cells were resuspended in PBS at a concentration of 2×10^6 cells/100 μl; 25 μl of the suspension was added to a microtiter plate and mixed with the appropriate peptide substrate dissolved in a standard reaction buffer (100 mM Hepes, 10% sucrose, 5 mM DTT, 0.001% NP-40 and 0.1% CHAPS, pH 7.2). Cleavage of the fluorogenic peptide substrate was monitored by AMC liberation in a Fluoroscan II plate reader (Labsystems, Stockholm, Sweden) using 355 nm excitation and 460 nm emission wavelengths.

2.4. Analysis of oxygen consumption

Cellular respiration was monitored with an oxygen electrode (Hansatech Instruments, Norfolk, UK) and analyzed using OxygraphPlus software (Hansatech Instruments, Norfolk, UK). Briefly, cells were harvested and counted. Four million cells were spun down, resuspended in 300 μl of the medium in which the cells were grown, and transferred into the Oxygraph chamber. The chamber was closed and basal respiration was measured for 3–4 min. To assess the maximum capacity of the respiratory chain, mitochondria were uncoupled by 5 μM carbonyl cyanide 3-chlorophenylhydrazone (CCCP).

2.5. Morphological assessment of apoptosis

Cells were seeded on glass cover slips, incubated with the indicated treatment and, after staining with Hoechst (2 μg/ml) for 10 min, examined using a Zeiss LSM 510 META confocal laser scanner microscope. At least 600 cells were counted and analyzed for apoptotic morphology.