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α-Tocopheryl succinate alters cell cycle distribution sensitising human osteosarcoma cells to methotrexate-induced apoptosis

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Abstract

α-Tocopheryl succinate (α-TOS) exerts pleiotrophic responses in malignant cells leading to cell cycle arrest, differentiation and apoptosis. We tested the ability of α-TOS to induce apoptosis or cell cycle perturbation in three human osteosarcoma (OS) cell lines which differ in their pRB and p53 status. We found high levels of apoptosis in OS cells carrying wild-type p53 gene when exposed to α-TOS, while the mutant p53 cells were resistant. A S/G2 transition arrest was observed in two OS cell lines exposed to α-TOS, which sensitised them to methotrexate, an agent whose activity is cell cycle-dependent. We propose that α-TOS may be used as a drug or an adjuvant for treatment of osteosarcomas.

Keywords: Osteosarcoma cells; Cell cycle; Apoptosis; α-Tocopheryl succinate; Methotrexate

1. Introduction

Osteosarcoma (OS) is one of the most common bone tumours occurring mainly in adolescents or young adults [1]. Although surgery combined with chemotherapy has markedly improved patient survival during recent years [2], the use of anti-cancer drugs is associated with serious problems, such as the frequent acquisition of drug-resistant phenotypes and the occurrence of 'second malignancies' [3]. In addition, cytotoxic effects of chemotherapy on normal tissues remain a major drawback in the treatment of OS patients [4]. High dose of methotrexate (MTX), a potent inhibitor of dihydrofolate reductase (DHFR), is a major component of current protocols for OS treatment [2,5,6], while the 'conventional' MTX dose therapy is ineffective. Intrinsic resistance to MTX can occur through a variety of mechanisms, including impaired transport of drugs into the cells via the reduced folate carrier, an increase in DHFR due to gene amplification or increased transcription [7,8], and circumvention of the inhibition of de novo nucleotide biosynthesis via the salvage of extracellular nucleosides and bases [8].

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There has been considerable effort to identify alternative approaches to the current chemotherapy. A promising treatment involves combination of anti-cancer agents with different modes of action that could improve their effects. In this context, it has been shown that α-tocopheryl succinate (α-TOS), a redox-silent analogue of α-tocopherol, is a highly selective cytotoxic agent, both reducing proliferation and inducing apoptosis in many cancer cells [9–11] and synergising with other apoptogens in vitro and in vivo [10,12].

Proliferation and apoptosis are intimately coupled, and modulators of the cell cycle can influence both cell division and apoptosis [13,14]. The cell cycle is coordinately controlled by cyclin-dependent kinases (CDKs) and their cyclin partners, whose levels fluctuate throughout the cell cycle [15]. The pRB-pathway plays a central role in cell proliferation by modulating the activity of the transcription factor E2F [16]. E2F1 can signal p53 phosphorylation that is coincident with p53 accumulation and apoptosis [17]. p53 is a key component of the cellular 'emergency-response' mechanism [18,19]. A variety of stress-associated signals activate p53 that induces growth arrest or apoptosis, thereby eliminating damaged and potentially dangerous cells [20]. The p53 gene is frequently lost or mutated in many cancers [21,22]. Lack of functional p53 is accompanied by elevated rates of genomic instability, rapid tumour progression, and resistance to anticancer drugs and radiation [23,24].

Here, we investigated the effect of α-TOS in three OS cell lines which differ in their RB and p53 phenotype. We tested the ability of α-TOS to induce apoptosis in the OS cells and sensitise them to MTX, an agent whose action is cell cycle-dependent.

2. Materials and methods

2.1. Reagents

α-TOS, annexin V-FITC, RNAase A, propidium iodide (PI), methotrexate (MTX), and Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma (St Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Roche Diagnostic (Basel, Switzerland). Anti-caspase-8, anti-caspase-9 and anti-caspase-3 monoclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-cyclin A and anti-cyclin E polyclonal antibodies were purchased from Oncogene Research (Cambridge, MA, USA).

2.2. Cell culture and treatment

The human osteosarcoma cell lines, SAOS ($pRB^{-/-}$, $p53^{+/+}$); U2OS ($pRB^{+/+}$, $p53^{+/+}$) and MG63 ($pRB^{+/+}$, $p53^{-/-}$) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 in a humidified incubator. Cells were treated with α-TOS and MTX in the range of concentrations up to 100 μM and 1 mM, respectively. MTX was added directly to the medium, whereas α-TOS was dissolved in ethanol and diluted in complete DMEM to the final concentration, and was then added to cells at 0.1% (v/v) of ethanol. Control cells were incubated with 0.1% (v/v) of ethanol alone.

2.3. Proliferation and cytotoxicity assay

OS cells were seeded in 96-well flat-bottom plates at 10^4 cells per well. The cells were allowed to attach overnight and treated with α-TOS at 30 μM. Proliferation curves were evaluated for up to 4 d by MTT analysis. Cytotoxicity was evaluated at 24 h using increasing concentrations of α-TOS or MTX. The drug combination was studied by incubating OS cells with α-TOS (30 μM) or MTX (1 μM) alone or in combination for up to 72 h. Cell viability was determined using the MTT assay [25]. Briefly, following exposure to the drug(s), 10 μl of MTT (5 mg/ml in PBS) was added to the cells, and after incubation for 4 h at 37 °C, the medium was removed and combined with 200 μl of 1% SDS. Absorbance was read at 550 nm using an ELISA reader.

2.4. Annexin V-FITC assay

Apoptosis was quantified using the annexin V-FITC method, which detects phosphatidyl serine (PS) externalised in the early phases of apoptosis [26]. Briefly, cells were plated at 10^5 cells per well in