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Taxol-induced polyploidy and cell death in CHO AA8 cells

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Summary

The purpose of this study was to assess whether Taxol-induced changes in microtubular dynamics are accompanied by apoptosis. CHO AA8 cells were treated with different Taxol concentrations (0.25 μM, 0.5 μM, 1 μM) for 24 h. The effects of Taxol exposure were analyzed using fluorescence microscopy and flow cytometry (TUNEL and annexin V-FITC/propidium iodide assays). 0.25 μM Taxol caused the appearance of few multinucleated giant cells exhibiting extensive arrays of fine filaments. Slight increases in the level of polyploidy, phosphatidylserine externalization and in the percentage of TUNEL positive cells were noticed. Concentrations of 0.5 and 1 μM resulted in the appearance of a large number of giant cells, which exhibited, depending on the cell, an extensive microtubular network or loose or tightly packed bundles of microtubules. Cells of reduced volume and showing chromatin condensation were also seen. Cell cycle analysis revealed that almost half of the cell population was polyploid. Except in cells exposed to 1 μM Taxol, annexin V-FITC/PI labelling did not reveal the loss of plasma membrane integrity or increase in phosphatidylserine externalization; however, TUNEL assay revealed a significant increase in the percentage of cells with DNA fragmentation. These data indicate that CHO AA8 cells treated with Taxol undergo cell death of a type which considerably differs from apoptosis.

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Introduction

The anti-mitotic, anti-cancer drug paclitaxel (Taxol) belongs to the most important class of anti-neoplastic agents, the taxanes. It is used in the treatment of patients suffering from ovarian, breast, head and neck and lung cancers (Forastiere et al., 1993; Rowinsky and Donehower, 1995; Rowinsky, 1997). Taxol binds to β-tubulin subunits in microtubules causing stabilization of spindle microtubules, an increase in microtubule polymerization, arrest of cells in prometaphase and reorganization of the microtubular cytoskeleton into asters or bundles (Checchi et al., 2003; Ganansia–Leymarie et al., 2003; Jordan, 2002). Despite extensive research demonstrating that the cytotoxicity of Taxol is mainly related to its ability to stabilize microtubules and induce block of the cell cycle at G2/M phase of mitosis (Schiff et al., 1979; Torres and Horwitz, 1998; Woods et al., 1995), many molecular and biochemical aspects of its effect remain unclear. Moreover, recent evidence indicates that depending on the drug concentration, treatment schedule and cell line, Taxol induces cell death through at least two independent pathways (Checchi et al., 2003; Ganansia-Leymarie et al., 2003). The first one results from microtubule stabilization and leads to mitotic block at G2/M phase and eventually apoptosis or necrosis (Abal et al., 2003; Fan et al., 2004; Sorger et al., 1997). In the second pathway, cell death occurs following suppression of microtubule dynamics without blocking cell cycle progression. In this case, disruption of microfilaments affects many biological processes within the cells. Consequently, the cells die regardless of the phase of the cell cycle (Fan et al., 2004; Lieu et al., 1997) or, as other results indicate, following aberrant exit from mitosis, multinucleated cells are formed (Abal et al., 2003). There are other reports indicating that Taxol may cause cell death independently of microtubule stabilization, by the modulation of specific gene expressions (Burkhart et al., 1994; Kirikae et al., 1996; Lee et al., 1997; Moos and Fitzpatrick, 1998).

Contradictory observations exist concerning the mode of cell death induced by Taxol. Some evidence indicates that this drug causes damage leading to mitotic catastrophe – the type of cell death accompanied by the appearance of the giant, multinucleated cells with non-condensed chromosomes (Castedo et al., 2002; Ricci and Zong, 2006; Roninson et al., 2001). Others claim that Taxol induces cell death with typical signs of apoptosis (Bhalla et al., 1993; Liu et al., 1994; Yeung et al., 1999), or necrosis (Michalakis et al., 2005; Yeung et al., 1999),depending on the dose of the drug.

Although several lines of evidence have indicated that p53 status may contribute to Taxol activity in some cell lines (Abal et al., 2003; Ganansia-Leymarie et al., 2003; Lanni et al., 1997; Wahl et al., 1996), it is widely believed that p53 is dispensable for Taxol-induced apoptosis (Abal et al., 2003; Bacus et al., 2001). However, after Taxol treatment, the cells with wild-type p53 have been shown to accumulate at G2/M and undergo apoptosis, whereas those with mutated or deleted p53 phenotype fail to arrest at the G2/M phase of mitosis. Instead, the cells proceed through several DNA replication cycles and eventually die through apoptosis or necrosis (Bacus et al., 2001; Bergstralh and Ting, 2006; Ganansia-Leymarie et al., 2003; Sorger et al., 1997).

In the present study, we used the CHO AA8 Chinese hamster ovary cell line that is presumed to lack functional p53, to investigate if the microtubular network reorganization induced by Taxol is accompanied by apoptosis.

Materials and methods

Cell culture

The Chinese hamster ovary cell line, CHO AA8, used in experiments was kindly provided by Prof. M. Zdzienicka (Department of Molecular Cell Genetics, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Poland). Cells were maintained in asynchronous growth as monolayers in Eagle's minimum essential medium (MEM; Sigma-Aldrich, Poznan, Poland) with the addition of 10% fetal bovine serum (Gibco) and 10 ml/l antibiotic–anti-mycotic stabilized solution (penicillin, streptomycin, amphotericin B; Sigma-Aldrich) at 37 °C and under 5% CO_2 in a humidified incubator.

Cell treatment

Exponentially growing cells in tissue culture flasks were trypsinized, transferred onto glass coverslips in individual wells (at a density of 1.5×10^4 cells/well) of 12-well plates in MEM (2 ml) with 10% fetal bovine serum and antibiotics, 24 h before treatment. For flow cytometry analysis, the cells were plated in six-well plates containing MEM (4 ml), at a density of 2×10^4 cells/well. The stock solution of paclitaxel (Taxol; Sigma-Aldrich) at a concentration of 10 mM, was prepared in 100% dimethyl sulfoxide (DMSO) and stored at −20 °C