Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters 233 (2006) 185–191
CANCER Letters
www.elsevier.com/locate/canlet

Paclitaxel suppresses the growth of primary prostate tumours (RM-1) and metastases in the lung in C57BL/6 mice

An Ling Zhang*, Pamela J. Russell

Department of Medicine, Oncology Research Centre, Level 2, Prince of Wales Hospital, Clinical Sciences Building, Barker Street, Randwick, NSW 2031, Australia

Received 8 December 2004; accepted 10 March 2005

Abstract

Paclitaxel has potent anti-cancer effects through its ability to block metaphase/anaphase transition during cell mitosis. This study shows that paclitaxel can significantly suppress both primary orthotopic murine (RM-1) prostate tumour growth (up to 60%) and the formation of pseudometastatic tumour colony formation in the lungs (by up to 46%) in C57BL/6 mice in vivo. Tumour growth suppression was associated with increased RM-1 cell apoptosis in the primary prostate tumours. In vitro studies found that the duration of exposure time to Paclitaxel was correlated with its ability to suppress cell proliferation and induce G2/M arrest.

Keywords: Paclitaxel; Prostate cancer; Mouse model; Cell apoptosis; Mitotic arrest

1. Introduction

Prostate cancer (CaP) is the second highest cause of cancer mortality in men in Western society. It may be indolent, clinically significant but localized to the prostate (>95% 5 year survival), or invasive and metastatic, with significantly increased mortality [1]. Organ confined CaP is treatable, but radical therapy can have distressing side effects. Once the cancer has spread, hormonal ablation therapy is limited by the development of androgen-independent CaP [2]. New strategies are needed to improve the quality of life and long-term survival of patients and to complement other therapies.

Paclitaxel, a new class of anti-cancer agents, has been successfully used to treat refractory cancers [3] including hormone-refractory prostate cancer [4]. It can induce apoptosis through concentration dependent cytotoxic effects on tubulin. At low concentrations, paclitaxel suppresses microtubule dynamics, but at high concentrations, it binds to beta-tubulin, inducing tubular polymerization and the formation of microtubular bundles [5,6]. This causes stabilization and dysfunction of the microtubules [7,8] that obstruct cell division, with arrest at the metaphase/anaphase (G2/M) transition in the cell cycle [9,5] leading via multiple mechanisms [10,11] to apoptosis (cell death). Whether binding of paclitaxel to microtubules leads

* Corresponding author. Tel.: +61 02 9382 2627; fax: +61 02 9382 2629.
E-mail address: anlingz@yahoo.com.au (A.L. Zhang).

0304-3835/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2005.03.051

irreversibly to cell death, or whether some cells can re-grow needs to be elucidated.

Apart from inducing microtubule stabilization and apoptosis, paclitaxel induces genes encoding tumour necrosis factor α (TNFα) and interleukins [12,13] that may have pharamacological effects [14] against metastatic tumour growth, but few studies have addressed this possibility. Our aims were (1) to see if paclitaxel treatment could suppress murine (RM-1) prostate tumour growth and inhibit the formation of metastatic tumour growth in the lung in C57BL/6 mice; (2) to determine if there were a relationship between dosage and duration of exposure to paclitaxel to G2/M phase cell arrest and suppression of cell proliferation in RM-1 cells.

2. Material and methods

2.1. Tumour cell lines

RM-1 murine prostate cancer cell line, from prostate tumours induced in the Zip ras/myc-9 infected mouse prostate reconstitution (MPR) model [15] were obtained from T. Thompson (Baylor College of Medicine, Houston, TX) and maintained in a humid atmosphere with 5% CO_2 at 37 °C in Dulbecco's modified essential medium (DMEM) plus 10% foetal bovine serum, 2 mM glutamine and 100 U/ml penicillin, 100 μg/ml streptomycin. Cells, grown in monolayers and passaged every 3 days (70–80% confluent) using 0.025% trypsin, were Mycoplasma free, and used in log phase.

2.2. Mice

C57BL/6 male mice were purchased from Biology Research Center (BRC), University of New South Wales (UNSW), maintained there and used aged 8–12 weeks. All procedures were approved by the Animal Care and Ethics Committee, UNSW.

2.3. Cell proliferation assay

RM-1 viable cells at 5×10^4/well were cultured in quadruplicate in 96 well microplates. After 24 h, cells were exposed to paclitaxel or vehicle (provided by FH Faulding Limited) at 5, 10, 20, 50, 80, 100 or 150 nM for 24, 48 and 72 h. The culture medium was replaced by fresh medium after exposure to drug. A cell proliferation assay was performed in triplicate using Cell Proliferation Reagent, WST-1 (Roche Molecular Biochemicals, Sandhofe Strasse, Mannheim, Germany) and analysed using an ELISA reader at 450 nm. Each experiment was repeated three times.

2.4. Flow cytometric analysis of G2/M cell cycle

Cultured RM-1 cells (~70% confluent) in 75 cm^2 tissue culture flasks were exposed to paclitaxel at 0, 20, 50,100 and 150 nM for 8, 24 and 48 h, then replaced in fresh medium. They were harvested using trypsin at various time points after paclitaxel treatment and fixed in 70% cold ethanol at 4 °C for at least 1 h, then washed with PBS/1% FBS and stained with 10 μg/ml propidium iodide (Sigma Chemical Co., St Louis, USA) and RNase A (50 μg/ml) for 30 min at room temperature. Cell cycle analysis was performed using a Fluorescence Activated Cell Scanner (FACScan; Becton Dickinson, Mountain View, CA) equipped with an argon-ion laser tuned to 488 nm. The sample size was 20,000 cells. Flow studies measured the number of cells versus DNA content and determined the fraction of cells in G0/G1, S, and G2/M, using ModFit software.

2.5. In vivo treatments

Cultured RM-1 cells were harvested and resuspended in Hank's balance salt solution (HBSS) for intra-prostate (iprost, 5×10^3 cells) and intravenous (iv, 5×10^5 cells) injection on days 0 and 4 to establish primary prostate tumours and pseudo-metastases in the lung in C57/BL6 mice, respectively, as previously described [16]. Mice were divided into control and paclitaxel treatment groups ($n=8$/group). Each experiment was performed three times. A single dose of paclitaxel (20 mg/kg) or paclitaxel vehicle (control) was given iv on day 6, 2 days after iv implantation of the cells. The mice were followed for general condition and body weight three times/week until sacrificed on day 13. Lungs were fixed in Bouin's fixative for 3 days and colonies were counted under a dissecting microscope. Treatment efficacy was assessed by the percentage reduction of the primary prostate tumour volume ($V(mm^3)=(d_1\times d_2)^{3/2}\times\pi/6$ [17], where d_1