Biomedicine & Pharmacotherapy 69 (2015) 186–190

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Altered expression of platelet factor 4 and basic fibroblast growth factor correlates with the inhibition of tumor growth in mice

Peace Mabeta [a,*], Michael S. Pepper [b]

[a] Department of Physiology, Faculty of Health Sciences, University of Pretoria, Tshwane, South Africa
[b] Immunology, Faculty of Health Sciences, University of Pretoria, Tshwane, South Africa

ARTICLE INFO

Article history:
Received 28 October 2014
Accepted 12 November 2014

Keywords:
Angiogenesis
Vascular tumor
Cell migration
Taxol
bFGF
PF4

ABSTRACT

Herein, we describe the effects of Taxol on endothelioma cell growth and migration in vitro and on vascular tumor growth in vivo. The effects of Taxol on endothelioma cell growth were determined using the crystal violet assay, while cell migration was measured using the xCELLIgence Real-Time Cell Analysis system. To study the effects of Taxol on tumor growth, mice were inoculated with endothelioma cells to induce vascular tumor development and were treated with the drug. At termination, tissue samples from Taxol-treated and control mice were stained with hematoxylin and eosin for histological examination, while blood samples were collected for hematological analysis, as well as for the analysis of the expression of angiogenic markers. In vitro, Taxol inhibited cell growth and migration. The drug also inhibited vascular tumor growth in mice, and this correlated with a recovery of mice from thrombocytopenia. Array analysis of blood samples from mice revealed that there was an increase in the expression of platelet factor 4 and a suppression of the proangiogenic molecule basic fibroblast growth factor in Taxol-treated animals. Our findings suggest that Taxol may have potential in the treatment of vascular tumors.

1. Introduction

Paclitaxel, commonly known as Taxol, is a member of the taxane class of drugs, which are derived from the American Pacific yew tree (*Taxus brevifolia*) [1,2]. It inhibits microtubule dynamics by binding to the N-terminal region of β-tubulin, which in turn results in the formation of stable microtubules [3]. Paclitaxel was the first taxane to enter clinical trials and to receive food and drug association (FDA) approval for cancer treatment [2]. It is currently used as first-line therapy for many common malignancies, including lung, breast, ovarian and head and neck cancers [1,4]. In addition, it has high antitumor activity against some uncommon malignancies such as the endothelial cell tumors angiosarcoma and Kaposis sarcoma [3].

Besides its known anticancer properties, another characteristic of paclitaxel, namely, its antiangiogenic activity, started a second life for this compound and has led to the investigation of new therapeutic approaches using the drug [5].

Dordunoo et al. reported the first published evidence about a possible antiangiogenic activity of paclitaxel more than 15 years ago [6]. Their study showed that Taxol inhibits angiogenesis in the chick chorioallantoic membrane (CAM) model [6]. Since then, a number of studies have reported on the antiangiogenic effects of Taxol in vitro and in vivo [7,8]. Recent studies further showed that Taxol inhibits the growth and migration of endothelial cells [8].

Endothelial cell neoplasms such as hemangioendotheliomas and hemangiomas have no definitive treatment. Given the potent direct effects of Taxol on endothelial cells, we sought to investigate the effects of Taxol on the development of vascular tumors in a mouse model previously employed as a model of for these endothelial cell neoplasms. The effects of the drug on endothelioma cell growth and migration, and on the expression of angiogenic markers were also investigated.

2. Materials and methods

2.1. Cell culture

The endothelioma cell line, sEnd.2, derived from Pym T-induced vascular tumors in the skin of the thorax of C57BL6 mice was grown in Dulbecco's Minimum Essential Medium (Sigma-Aldrich, Germany) supplemented with 10% heat inactivated fetal bovine

* Corresponding author. P.O. Box 2034, Department of Physiology, Faculty of Health Sciences, University of Pretoria, Pretoria 0001, South Africa.
Tel.: +27 12 529 8354; fax: +27 12 529 8305.
E-mail address: peace.mabeta@up.ac.za (P. Mabeta).

http://dx.doi.org/10.1016/j.biopha.2014.11.018
0753-3322/

serum (FBS) (Invitrogen, USA), 20 mM glutamine (Invitrogen, USA), and 1% penicillin-streptomycin (Whitehead Scientific, South Africa). Bovine microvascular endothelial (BME) cells were maintained in α-modified Eagle's Medium, supplemented with 15% donor calf serum (Invitrogen, USA), and 1% penicillin-streptomycin (Whitehead Scientific, South Africa). The cell lines were maintained in a 37 °C incubator in a humidified atmosphere containing 5% CO_2.

2.2. Cell growth assay

Cell viability was assessed using the crystal violet nuclear staining assay. Endothelioma cells were seeded in 24-well culture plates at a density of 10,000 cells per well for 24 hours, and then treated with Taxol (0–10 μM) or dimethylsulfoxide (DMSO) for 48 hours. The time and dose were selected following initial screening over a period of 12 to 72 hours. At termination, cells were fixed with 1% glutaraldehyde in phosphate buffered saline (PBS) for 15 minutes, followed by staining with a 0.1% crystal violet solution (Sigma-Aldrich, Germany) for 30 minutes. The dye was extracted with 0.1% Triton X-100 (Sigma-Aldrich, Germany) per well. The absorbance was read at 570 nm on an ELx 800 Universal Microplate Reader (Bio-Tek instruments Inc., SA). Three wells were analyzed for each concentration.

2.3. Cell migration assay

Cell migration experiments were performed using 16-well cell invasion and migration (CIM) plates (Roche Applied Science, South Africa). Prior to each experiment, BME and sEnd.2 cells were deprived of FBS for 24 hours. For the migration assay, 160 μL serum free medium with or without a chemo-attractant, namely, 10 ng/mL basic fibroblast growth factor (bFGF), as was added to each well of the lower chamber of a CIM plate. The cells were added to the upper chamber of the CIM plate at a density of 6×10^3 cells/well and treated with Taxol (0–10 μM) or dimethylsulfoxide (DMSO). The migration of cells through the filter into the bottom chamber was monitored over 20 hours with the xCELLigence RTCA DP instrument (Roche Applied Science, South Africa). The cell index (CI) was recorded by the instrument analyzer and analysis was performed with the supplied RTCA software (vs. 1.2.1). Each condition was analyzed in quadruplicate. Results are based on raw data without CI-normalization.

2.4. Effects of Taxol on tumor development

Female C57BL6 mice (8–12 weeks old) were housed at the University of Pretoria Biomedical Research Center (UPBRC). The mice were given standard commercial food (EPOL) and water ad libitum. Animal experiments were conducted according to a protocol approved by the University of Pretoria Animal Use and Care Committee (project 16/2005).

For tumor induction, 2×10^6 endothelioma cells were inoculated s.c. in the flanks of the mice as previously described. Mice were preloaded with Taxol on day one. The animals were injected with Taxol at doses of 0–6 mg/kg i.p. The mice were monitored every second day for tumor formation. The mice were sacrificed 10 days after inoculation due to the size of the tumors in the control group, and the tumors were isolated.

2.5. Histological analysis

The isolated tumors were fixed in buffered 4% paraformaldehyde and embedded in paraffin using standard procedures. Five μm sections were cut and stained with hematoxylin and eosin. Sections were viewed under a light microscope and photos were taken using a digital camera.

2.6. Protein array studies

The screening of angiogenic proteins in plasma samples of control and Taxol-treated mice was performed using a mouse angiogenic protein array kit (RayBiotech Inc., GA, USA). Membranes spotted in duplicate with antibodies against angiogenic factors were blocked with blocking buffer. This was followed by the incubation of the membranes with biotin-conjugated antibodies against various angiogenic factors (Fig. 4). The membranes were subsequently washed with wash buffer and then incubated with horseradish peroxidase–conjugated streptavidin. The intensity of the protein signal (two spots for each protein) was compared with the relative positive signals using the Flourchem Imaging System Software (ProteinSimple, USA).

2.7. Hematological analysis

For hematological analysis, blood samples were collected in heparinized tubes. The red cell count, hematocrit percentage, hemoglobin concentration, white cell and platelet counts, were determined using a hemocounter, Cell-Dyne 3700 (Abbott, IL, USA).

2.8. Data analysis

Statistical analyses were performed using either the Student's *t* test or analysis of variance with the Bonferroni method. Values are expressed as the mean ± SEM from triplicate studies unless stated otherwise. Differences were considered significant when the calculated P value was < 0.05.

3. Results

3.1. Cell growth

The effects of Taxol on endothelioma (sEnd.2) cell growth were evaluated over 48 hours. Taxol induced a decrease in sEnd.2 cell numbers in a dose-dependent manner, with an IC_{50} of about 0.102 μM (Fig. 1).

3.2. Cell migration

The migration of host endothelial cells and endothelioma cells appear to contribute to vascular tumor development in the Pym T

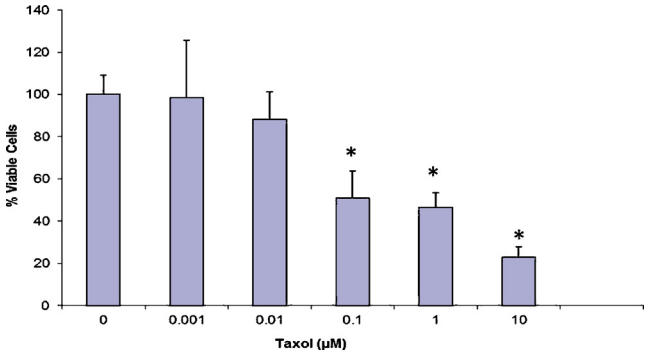

Fig. 1. Taxol inhibits endothelioma cell growth. Taxol reduced the percentage of viable endothelioma cells in a dose-dependent manner. Data represents mean values ± SD of three wells from triplicate studies. $^*P < 0.05$ compared to control.