

RESEARCH COMMUNICATION

Novel Hydrophilic Taxane Analogues Inhibit Growth of Cancer Cells

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Abstract

In our era there has been several anti-cancer drugs which have undergone both experimental and clinical trials; however, due to their poor solubilities, numerous side effects, insufficient bioavailability and poor compliance, many have resulted into poor outcomes. Therefore, our aim was to investigate the effects of novel hydrophilic taxanes analogues CQMU-0517 and CQMU-0519 on growth of A549 lung, SKVO3 ovary and MCF7 breast carcinoma cell lines. Different concentrations of original paclitaxel, CQMU-0517, original docetaxel and CQMU-0519 were utilized on three cell lines, where cell growth was assessed using cell culture kit-8 and flow cytometry analysis. The results unveiled that CQMU-0517 and CQMU-0519 suppressed cell growth in the three particular cell lines, cell cycle arrest being evident in the G2/M phase. Hence, the results showed that these new taxane analogues have potential and warrant future clinical trials.

Keywords: Taxol - paclitaxel - taxotere - docetaxel - growth

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Introduction

During the past decades, innumerable experimental studies on taxol, taxotere and their homologs have been carried out all over the world so as to try to carve a pathway for clinical oncological trials. Their mechanism of actions, pharmacokinetics, activation of signal transduction pathways, side effects are few of the characteristics that have been delineated through experimental trials. Laboratory based researches have been ongoing in numerous cell lines and have yielded quite appreciable outcomes to provide a rationale for ongoing clinical investigations, in order to optimize cytotoxicity of chemotherapy. In the long run, due to some side effects, poor solubilities and outcomes, insufficient bioavailability and incompliance, many have been removed from the local market to be replaced by better ones.

In order to improve hydrophobic anti-tumor drugs, several types of vehicles have been used to provide better sustained release, specific targeting, and lower interaction with the reticuloendothelial system. On the other hand, taxanes have been amongst the most solicited chemotherapeutic drugs of our era, grouping mainly Paclitaxel (Taxol[®]) and Docetaxel (Taxotere[®]) which forms part of the second generation of Taxanes also called as mitotic poisons or mitotic inhibitors undergoing profound and meticulous laboratory and clinical investigations. Paclitaxel and docetaxel are both derived

from renewable natural sources such as *Taxus brevifolia* (bark of Pacific yew/Western yew) (Wani et al., 1971), and *Taxus baccata* (needles of European Yew) (Bissery et al., 1991) and despite being slightly more soluble in water than Taxol[®] (Hennenfent and Govindan, 2006), both need to be solubilised in polysorbate 80 for its commercial formulation, thus bearing a lot of side effects.

Moreover, both drugs have been known to decrease cell growth and induce apoptosis of various cancer cells; where several chemotherapeutic mechanisms have accounted that their cytotoxicities are related to either excessive polymerization or depolymerization of microtubules (Rao et al., 1999) or even due to some association with β -tubulin (Snyder et al., 2001) where microtubule mechanisms of action are suppressed (Derry et al., 1995; Jordan and Wilson, 1998; Yvon et al., 1999); thus resulting in aberrant mitosis and diminished cell growth during subsequent cell cycles. Somehow, taxanes have been found to be not cell specific with not all concentrations displaying similar effects on microtubules dynamics (Schiff et al., 1979; Schiff and Horwitz, 1980; Jordan et al., 1993; Derry et al., 1995; Jordan et al., 1996; Jordan and Wilson, 1998; Yvon et al., 1999) relating to unsimilar cytotoxic, anti-proliferative and apoptotic effects on various cancer cell lines namely breast, lung, ovary, prostate, hepatic, melanocyte and leukemic (Jordan et al., 1993; Jordan et al., 1996; Ferlini et al., 1998; Nehmé et al., 2001; Geng et al., 2003; Wang et al., 2005; Mahaffey et al., 2007; Mhaidat et al., 2007;

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Ichite et al., 2009; Kang et al., 2010). Though killing of malignant cells is the main aim for all researchers, scientists, cancer physicians and pharmaceutical companies; however, there are still many hedges and thorns to be overcome in order to attain a successful and irreproachable cancer therapy. Nevertheless, Taxotere® has remained amongst those anti-cancer drugs that could achieve favorable anti-proliferative and apoptotic results on a whole myriad of cancer cells both in vivo and in vitro (Liebmann et al., 1993; Ferlini et al., 1998; Nehmé et al., 2001; Geng et al., 2003; Wang et al., 2005; Mahaffey et al., 2007; Mhaidat et al., 2007; Ichite et al., 2009; Kang et al., 2010). Somehow nowadays, due to its poor oral bioavailability, solubility, drug resistance and numerous side effects; better derivatives like Abraxane® and docetaxel with several combinations like cisplatin, cyclophosphamide, capecitabine and even radiation (Calderoni and Cerny, 2001; Mäenpää, 2003; Nabell and Spencer, 2003; Pectasides et al., 2005; Wenzel and Steger, 2006; Katopodis et al., 2010) are being used in clinical trials and have yielded acceptable outcomes on patients' health conditions.

Hence, our university has synthesized two new hydrophilic anti-cancer drugs, CQMU-0517 and CQMU-0519 whose predecessors were poorly water soluble and this study was performed to highlight the main effect on growth suppression where they were found to be having better effects on lung, ovary and breast cancer cell lines compared to their siblings.

Materials and Methods

Chemical Preparation

The original docetaxel and paclitaxel were purified while the new paclitaxel (CQMU-0517) (Patent number: CN 200910104505.1; CN 201010617646.6) and docetaxel analogues (CQMU-0519) (Patent number: CN 200910104454.2; CN 201010299678.6) were synthesized by the Department of Pharmacology, Chongqing Medical University, China which were completely soluble in 0.95% normal saline (NS) compared with the old drugs. Nevertheless, due to the poor solubility of Taxol® and Taxotere®, we dissolved the two drugs into 1% methanol and 99% (0.95%) NS in order to achieve an appropriate control for comparison.

Cell Culture

Human A549 lung adenocarcinoma, SKVO3 ovarian and MCF7 breast cancer cell lines were cultured in DMEM-High Glucose medium (Hyclone) supplemented with 10% Fetal Bovine Serum (FBS), 100U/ml Penicillin and 100µg/ml Streptomycin (Hyclone) at 37°C in a 5% CO₂ incubator. Individual cell lines were treated for 24h with control (1% methanol & 99% NS); 10, 100, 1000nM of original paclitaxel and CQMU-0517, and 25, 50, 100nM of original docetaxel and CQMU-0519 in final concentrations were used to assess cell growth. However, in cell cycle analysis only one suitable and appropriate concentration was chosen to assess their effects on all three cancer cell lines. Hence, in brief our experiment consisted of these 3 major groups for each cell line.

Cell Proliferation Assay

100µl of respective cell suspensions (5×10^3 cells/well) were dispensed into triplicate in 96-well plates and incubated for 24h; then 10µl of control; 10, 100, 1000nM original paclitaxel and CQMU-0517 as well as 25, 50, 100nM original docetaxel and CQMU-0519 were added to respective individual wells and plates were further incubated for 24h. Afterwards, 10µl of Cell Counting Kit-8 (CCK-8, Dojindo Japan) solution was added to the wells and plates were incubated for 1h. Absorbance (Optical Density-OD) was read using a universal microplate reader (Bio-Tek) at 450nm and a graph of A450 (Absorbance at 450nm wavelength) against concentration was plotted. Each mark represents the mean of collected readings and the procedure was repeated at least 3 times. The percentage of inhibition of proliferation was calculated by (mean OD for control - mean OD for each drug)/mean OD for control $\times 100\%$.

Cell Cycle Analysis

A549, SKVO3 and MCF7 cells 1×10^6 cells/well were allowed to adhere for 24h in 6 well plates; then control, 1000 nM of original paclitaxel and CQMU-0517 and 50 nM of original docetaxel and CQMU-0519 were added to each well and incubated for a further 24hr. Cells were trypsinized, washed thrice with cold PBS, fixed with 70% absolute ice-cold ethanol, kept at -20 °C overnight and stained with a mixture of Propidium RNaseA (PI) and analysed with Becton Dickinson flow cytometer for cell cycle analysis (FACScan). This experiment was done thrice.

Statistical Analysis

The quantitative data were expressed as a mean of \pm standard deviation (SD). Statistical analysis was performed by one-way ANOVA or Student's t test using SPSS 13.0 software package. A '*P' value of less than 0.05 was considered to be statistically significant.

Results

New Paclitaxel and Docetaxel Analogues Inhibit Cell Growth in all Three Cell Lines

10, 100 and 1000nM of original Paclitaxel and CQMU-0517 were used, at 1000 nM after 24 hours, CQMU-0517 displayed more cell growth inhibitory effects in A549 lung, SKVO3 ovarian and MCF7 breast cell lines with IC₅₀ values of 7.36 µg/ml, 10.96 µg/ml and 10.73 µg/ml respectively calculated from concentration dependent curves. After incubation with 25, 50 and 100 nM of original docetaxel and CQMU-0519; at 50nM concentration after 24 hours, CQMU-0519 had inhibitory cell growth effects which were more evident in all three cell lines (Figure 1), with IC₅₀ values of 9.52µg/ml, 11.75µg/ml, and 12.67µg/ml.

New Drugs affect the Cell Cycle in all Three Cell Lines

Various phases of the cell cycle were affected more with the new drugs 1000nM of CQMU-0517 and 50 nM of CQMU-0519 compared with the original ones and controls (Figure 2a-f) in lung, ovary and breast cancer

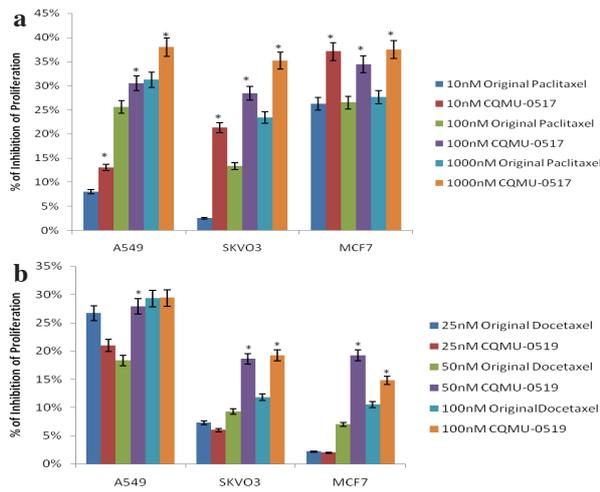


Figure 1. Growth of Viable Cells was Quantified Using CCK-8 Assay. Data points were expressed as mean of A450 ± SD from three separate experiments for each cell line for 24 hours. a) A549, SKVO3 and MCF7 cells exposed to CQMU-0517 showed an increase in inhibition of cell proliferation compared with original paclitaxel (*P < 0.05). b) A549, SKVO3 and MCF7 cells exposed to 50nM of CQMU-0519 showed an increase in inhibition of cell proliferation compared with original docetaxel (*P < 0.05)

Table 1. Effects of 1000nM Original Paclitaxel and CQMU-0517 on Cell Cycle in A549, SKVO3 and MCF7 Cell Lines

	G0/G1 (%)	S (%)	G2/M (%)
A549 Lung Control	66.38	27.15	6.48
Original Paclitaxel	50.23	30.14	19.62
CQMU-0517	32.14	32.61	34.6
SKVO3 Ovary control	72.38	21.43	6.19
Original Paclitaxel	60.66	30.15	9.19
CQMU-0517	15.79	66.36	17.89
MCF7 Breast control	61.89	30.37	7.74
Original Paclitaxel	54.94	33.71	8.35
CQMU-0517	29.38	55.1	15.53

Table 2. Effects of 50nM Original Docetaxel and CQMU-0519 on Cell Cycle in A549, SKVO3 and MCF7 Cell Lines

	G0/G1 (%)	S (%)	G2/M (%)
A549 Lung Control	62.22	33.44	4.34
Original Docetaxel	53.07	36.24	10.69
CQMU-0519	18.46	38.12	43.42
SKVO3 Ovary control	84.3	12.12	3.58
Original Docetaxel	57.21	18.68	24.11
CQMU-0519	28.71	39.13	32.13
MCF7 Breast control	65.75	26.54	7.74
Original Docetaxel	4.8	31.21	64.37
CQMU-0519	3.97	18.27	76.93

cell lines which was determined by flow cytometry using PI staining. The proportions of cells in the G2/M phases were much higher than phases G0/G1 and S, showing a mitotic cell cycle arrest in the G2/M phases which is relevant to DNA damage checkpoint. Besides, a 3.64 % of apoptotic cells (sub-G0/G1) in MCF7 cells could also be noted in cells treated with CQMU-0519 (Table 2). While in Table 1 cells treated with CQMU-0517 had cell cycle arrest evident in S and G2/M phases and both SKVO3 and MCF7 cells had sub-G0/G1 peaks.

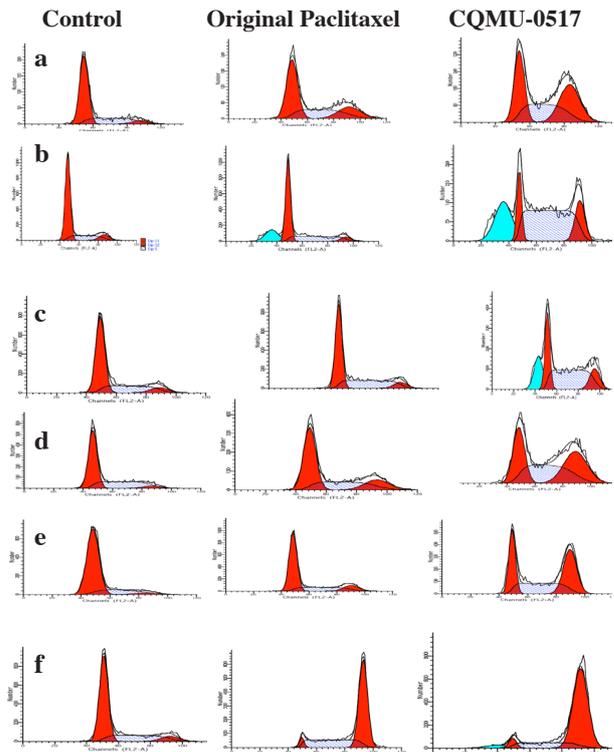


Figure 2. Cell Cycle Effects. (a & d) A549, (b & e) SKVO3 and (c & f) MCF7. The effect of CQMU-0517 and CQMU-0519 on the cell cycle of all 3 cell lines was analysed by flow cytometry analysis using channels (FL2-A) and the different phases G0/G1 (left peak), S and G2/M (right peak) were analysed

Discussion

Numerous studies involving different cancer cell lines treated with paclitaxel and docetaxel have demonstrated a whole spectrum of cell growth inhibition, induction of apoptosis and even inhibition of angiogenesis (Ferlini et al., 1998; Grant et al., 2003; Fauzee et al., 2011). Nevertheless, though both paclitaxel and docetaxel have yielded some very good outcomes in both experimental and clinical trials (Jordan et al., 1993; Ferlini et al., 1998; Grant et al., 2003; Mäenpää, 2003; Nabell and Spencer, 2003; Pectasides et al., 2005; Wenzel and Steger, 2006; Katopodis et al., 2010), their poor solubilities are associated with numerous side effects (Hennenfent and Govindan, 2006), have been a major hurdle in cancer therapy; somehow, there are numerous ongoing studies to produce better taxanes in terms of solubility, mechanism of action with fewer side effects for the benefits of patients in the long run. Nevertheless, single docetaxel concentration either low or high induces better and acceptable pro-apoptotic responses compared with taxol (Mhaidat et al., 2007) but several studies have demonstrated that docetaxel in combination with other drugs have better response rates and fewer side effects with prolongation of overall surviving time; hence representing an advantage in the optimal treatment of cancers.

In our study, 1000 nM and 50 nM concentrations of CQMU-0517 and CQMU-0519 were selected due to better outcomes in decreasing cell growth and being considered to be relatively low and safe concentrations (Chang et al., 2006; Wang et al., 2007). The original

paclitaxel and docetaxel were purified and since they are already known to be poorly soluble in water; hence, we used 1% methanol and 99% normal saline as solvent to dissolve them; however, CQMU-0517 and CQMU-0519 analogues synthesized by our pharmacy college were completely soluble in water and therefore representing an important characteristic in clinical trials for the benefit of cancer patients; in the sense that there may be fewer side effects. The control was set up so as to rule out any harmful, anti-proliferative effects on its own on A549 lung adenocarcinoma, SKVO3 ovarian and MCF7 breast epithelial cancer cell lines, in comparison with DMSO and Cremophor known to affect the cytotoxicity of taxanes. Our main aim was to define the anti-proliferative effects of CQMU-0517 and CQMU-0519. A549, SKVO3 and MCF7 cells were treated various concentrations of CQMU-0517 and CQMU-0519 respectively for 24h; it was seen that cell growth was successfully inhibited by them, where A549 treated with 1000nM of CQMU-0517 had cell growth inhibition of 38.0%, SKVO3-35.2% and MCF7-37.5%, whereas A549 treated with 50 nM of CQMU-0519 displayed higher percentage of cell growth inhibition-27.90%, SKVO3-18.6% and MCF7-19.20% (P<0.05). Besides, this was further confirmed by flow cytometry results which showed that CQMU-0517 induced cell cycle arrest both at S and G2/M phases in ovarian and breast cancer cells while in lung cancer cells, arrest was mostly appreciated in the G2/M phase similar to the effect of CQMU-0519 in all three cell lines, which may be accountable for the depolymerization of microtubules (Derry et al., 1995; Jordan and Wilson, 1998; Yvon et al., 1999; Calderoni and Cerny, 2001). Nevertheless, MCF7 had a hypodiploid sub G0/G1 peak when treated with both CQMU-0517 and CQMU-0519, which speaks for the onset of DNA fragmentation which may have occurred earlier or due to loss of PI staining (Fabbri et al., 2006). Hence, the new hydrophilic drugs had better effects on growth inhibition by affecting the S and G2/M phases compared to the original drugs.

Therefore, we could conclude that these novel hydrophilic taxane analogues do have a better effect on all the three cancer cell lines; however, further experiments for cell suicide are on the way to confirm their mechanism of action and hope is there for their introduction into clinical trials for the betterment of our patients.

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