ABCC4 Decreases Docetaxel and Not Cabazitaxel Efficacy in Prostate Cancer Cells In Vitro

DANIELA E. OPREA-LAGER1, IRENE V. BIENSDORP2, REINDERT J.A. VAN MOORSELAAR2, ALFONS J.M. VAN DEN EERTWEGH3, OTTO S. HOEKSTRA1 and ALBERT A. GELDOF1,2

Departments of 1Radiology and Nuclear Medicine, 2Urology and 3Oncology, VU University Medical Center, Amsterdam, the Netherlands

Abstract. Background: This study aimed to investigate cabazitaxel efficacy in a model for docetaxel-resistant prostate cancer cells and to evaluate the involvement of ATP-cassette binding protein 4 (ABCC4) with regard to multidrug resistance. Materials and Methods: Docetaxel and cabazitaxel sensitivity was measured in PC3 and R3327-MATLyLu (MLL) cell lines, using the sulforhodamine B (SRB) assay. ABCC4 expression was examined by western blotting and its functional involvement in drug sensitivity by blocking with MK571 inhibitor. Results: The docetaxel-resistant MLL cells (4.5-fold compared to cabazitaxel; p<0.001) were shown to express high levels of ABCC4, while non-resistant PC3 cells had no detectable ABCC4 expression. Functional inhibition of ABCC4 in MLL cells resulted in a two-fold decrease in effective concentration of docetaxel and had no effect on toxicity of cabazitaxel. Conclusion: Cabazitaxel showed an improved therapeutic efficacy over docetaxel in ABCC4-expressing prostate cancer cells. ABCC4 appears to be an important determinant of docetaxel resistance, since its inhibition almost completely reversed resistance.

Correspondence to: A.A. Geldof, Ph.D., Department of Radiology and Nuclear Medicine, Department of Urology, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands. Tel: +31 20 4445104, Fax: +31 20 44446031, e-mail: aa.geldof@vumc.nl

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4, also known as ABCC4, as compared to benign prostate tissue (10, 11). Therefore we investigated whether ABCC4 influences docetaxel and cabazitaxel resistance in two cell lines with different ABCC4 expression levels.

Materials and Methods

Cell lines. PC3 human prostate cancer cell line (derived from a metastasis in a Caucasian patient with prostate cancer) was originally obtained from the American Type Culture Collection, Rockville, MD, USA (ATCC# CRL 1435).

The R3327-MATLyLu (MLL) prostate tumor variant was maintained in castrated male Copenhagen rats by trocar transplantation, and has been used as a source for in vitro culture. The establishment of cell culture for this cell line has been described elsewhere (12).

In vitro, both cell lines were cultured in RPMI-1640 culture medium (Gibco BRL, Life Technologies Europe BV, Bleiswijk, the Netherlands), supplemented with 10% fetal calf serum (Cambrex Fetal Calf Serum EU Standard, #14-801F; Lonza Verviers, Belgium), 100 U/ml penicillin/streptomycin (Gibco BRL), 1 mM sodium pyruvate and insulin/transferrin/ selenite medium supplement (Sigma-Aldrich Chemicals, St. Louis MO, USA) at 37˚C in a humidified atmosphere of 5% CO2/95% air. Semi-annual screening demonstrated the cultures to be mycoplasma-free.

Drugs. Docetaxel was obtained from Sigma-Aldrich Chemicals (Zwijndrecht, the Netherlands) and was dissolved in dimethylsulfoxide to prepare stock concentrations of 10 nM. Cabazitaxel was obtained from Sanofi-Aventis (Aventis Pharma, Antony, France) and was dissolved in phosphate-buffered salt solution (10 nM). The MRP inhibitor MK571 (Enzo Life Sciences BVBA, Antwerp, Belgium) was kindly provided (courtesy of Dr. G. Jansen, Department of Rheumatology, VU University Medical Center, Amsterdam, the Netherlands). All stock solutions were stored in aliquots at -20˚C.

Sulforhodamine B (SRB) assay. Evaluation of drug cytotoxic effects was performed using the Sulforhodamine B (SRB) assay, as described elsewhere (13). In short: 1500 MLL cells or 3500 PC3 cells were seeded in each well of 96-well plates (Cellstar #655180; Greiner BioOne, Frickenhausen, Germany). After 24 h drugs were added in appropriate concentrations and cells were allowed to proliferate for an additional three days. After this incubation time wells were treated with trichloroacetic acid (1 h at 4˚C) and stained using SRB solution (0.4% SRB in 1% acetic acid). The optical density was measured at 492 nm after reconstitution of the dye in 150 μl 10 mM Tris buffer. Extinction values at termination of the incubation period served to measure the resulting cell density and were compared with values for day 0 plates (start of drug incubation) to compare the cellular proliferation in the absence and in the presence of increasing drug concentrations. Values were normalized to the cell density of control cultures (100%). Values for the concentration inhibiting 50% of cells (IC50) were subsequently derived graphically and are given as the mean ±SEM (n=3).

To study the effect of ABCC4 on drug sensitivity, MK571 was used. MK571 was added 30 min before incubation with Cabazitaxel and Docetaxel (with and without inhibitor) to the cultures in a series of SRB assays.

Western blot. Western blotting of untreated cells was performed as described previously (14). Cells grown in log-phase were scraped in lysis buffer (Cell signalling, Denver, Co, USA) and centrifuged at 11,000 g at 4˚C for 10 min. The protein concentration in the supernatant was determined using a Bio-Rad protein assay according to the manufacturer’s instructions (Bio-Rad Laboratories, Venenendaal, the Netherlands). From each condition, 30 μg of protein were separated on sodium dodecyl sulfate 10% polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto a polyvinylidenedifluoride (PVDF) membrane. Blots were blocked for 1 h at room temperature in 5% milk in TBST (0.15 M NaCl, 0.05% Tween-20, 10 mM Tris-HCl, pH 8.0)

Figure 1. Effect of cabazitaxel and docetaxel concentrations on cellular proliferation (SRB assay) after three days of drug incubation on PC3 (a) and R3327-MATLyLu (b) prostate cancer cells in vitro. Data are mean values ±SEM (n=3).
and subsequently incubated at 4˚C overnight with the anti-ABCC4 antibody (courtesy of Georg Scheffer, Ph.D., Department of Pathology, VU University Medical Center, Amsterdam), diluted at 1: 500 in TBST with 5% milk. After washing, the secondary antibody was added and signal was detected using Enhanced Chemo-luminescence (ECL) or ECL-plus on Hyperfilms (Amersham International, Chalfont St. Giles, UK).

Analysis and statistics. Potential differences between treatment effects of cabazitaxel and docetaxel and differences between effects with and without the ABCC4 inhibitor were evaluated using the two-tailed Student’s $t$-test. Treatment effects were considered to be significantly different when $p<0.05$.

Results

Effects of cabazitaxel and docetaxel on prostate cancer cell proliferation. Effects of docetaxel and cabazitaxel on cancer cell proliferation were investigated using colorimetric SRB assays after incubation of drugs with logarithmically growing cultures of PC3 and MLL PCa cell lines. PC3 cells exhibited comparable sensitivity towards cabazitaxel and docetaxel, with IC$_{50}$ values of 0.9 and 0.8 nM, respectively (Figure 1). MLL cells were less sensitive to the drugs compared to PC3 cells, especially to docetaxel, with an IC$_{50}$ value of 8.6 nM (Figure 1 and Table I). Remarkably, MLL cells were ±4.5-fold less susceptible to docetaxel compared to cabazitaxel.

Expression of the multidrug resistance pump protein ABCC4. To determine whether the expression level of ABCC4 is related to docetaxel or cabazitaxel sensitivity, its protein expression was determined by western blotting. In MLL cells, a clear band identifying ABCC4 was observed, while no ABCC4 was detectable in PC3 cells (Figure 2).

Inhibition studies using MK571. To study whether ABCC4 contributed to the docetaxel resistance in MLL cells, ABCC4 was inhibited by MK571 before treatment with the drugs. The resulting IC$_{50}$ values clearly show a significant ($p<0.05$) increase in sensitivity to docetaxel incubation of MLL cells (about two-fold, to a value of 4.2 nM). The ABCC4 inhibitor did not affect docetaxel sensitivity of PC3 cells and did not affect sensitivity to cabazitaxel in either cell line (Table I).

Discussion

In the present study an ABCC4-negative cell line was used next to a ABCC4-positive PCa cell line. This permits direct comparison of the effects of cabazitaxel and docetaxel in the two models and relates the cytotoxic effects to ABCC4 status. Docetaxel was found to be far less effective in the ABCC4-positive cell line, while cabazitaxel was equally effective in both models. This finding is in line with our hypothesis that ABCC4 plays a functional role in the docetaxel resistance of PCa cells. The subsequent abolishment of such a difference in treatment effect by a specific ABCC4 inhibitor underscores the functional significance of this phenotype. The current experimental data therefore do confirm the expression and functionality of ABCC4 in PCa cells. Moreover, the differential effects on cell proliferation and viability by two chemically related taxanes point to differences in the roles of drug transporters in these cells in general and of ABCC4 in specific. However, in addition to ABCC4, there might be a functional role for other drug transporters in docetaxel resistance, since MK571 did not completely restore efficacy of docetaxel in the ABCC4-positive cell line.

In the current article, we describe the differential effects of cabazitaxel compared with docetaxel in two PCa cell lines with different ABCC4 phenotypes. Cabazitaxel was developed as an approach for therapy of docetaxel-resistant PCa. In this resistance phenomenon, drug transporter pumps are purported to play an important role by eliminating intracellular cytotoxic drugs from the cancer cells (15-17).
The ABC transporter superfamily of membrane proteins is best known for its contribution to chemoresistance through efflux of cancer drugs from cancer cells (for overview see 15, 18). One of these MRPs, ABCC4, has been described to be expressed in normal prostate and PCa (10-11, 19). Elevated ABCC4 expression has been found in malignant, compared to benign prostate tissue (11, 15), and is highly expressed in both the cytoplasm and cell membrane (www.proteinatlas.org). Additionally, Cai et al. (10) described ABCC4 as being regulated by the androgen receptor both in androgen-dependent and androgen-independent PCa cells. The exact molecular role of ABCC4 in the origin of docetaxel-resistant phenotype in PCa remains to be elucidated.

The lack of ABCC4 expression in PC3 cells is in line with earlier findings by Cai et al. (10). The expression of ABCC4 in MLL cells has not been described before, to the best of our knowledge. The poor therapeutic effect of docetaxel on these latter cells is in accordance with the observed poor therapeutic effect on this cell line in vivo, when grown in syngeneic Copenhagen rats (studies from this laboratory, Jacobs et al., unpublished data).

Since all patients with CRPC with a good clinical status may benefit from chemotherapy, independently of their age, new treatment options that prolong survival and improve the quality of life are needed (20). Therefore it is essential to define new biomarkers that can predict docetaxel resistance and, consequently, to early identify the category of patients in whom recurrence on docetaxel therapy may arise. This could result in improved patient selection strategies (20). For example, patients that have a high ABCC4 expression in PCa tissue can be selected for first-line cabazitaxel therapy. Moreover, ABCC4 as a potential predictive marker for docetaxel-based therapy responsiveness should be studied in future trials.

Conclusion

In conclusion, our data indicate that ABCC4 may be an important regulator for docetaxel sensitivity in PCa cells, while cabazitaxel has therapeutic efficacy independently of ABCC4 expression. Since many prostate carcinomas have a relatively high expression of ABCC4 protein (10), this underlines the potential advantage of using cabazitaxel over docetaxel. The utility of assessing ABCC4 expression in PCa tissues, as a patient selection criterion for first-line chemotherapy, should be explored.

Disclosure

The Authors report they have no conflicts of interest in regard to this work.

References


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