Cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide may affect drug sequence choices in metastatic castration-resistant prostate cancer

R.J. van Soest\textsuperscript{a,*}, M.E. van Royen\textsuperscript{b}, E.S. de Morrée\textsuperscript{a}, J.M. Moll\textsuperscript{a}, W. Teubel\textsuperscript{a}, E.A.C. Wiemer\textsuperscript{c}, R.H.J. Mathijssen\textsuperscript{c}, R. de Wit\textsuperscript{c}, W.M. van Weerden\textsuperscript{a}

\textsuperscript{a} Department of Urology, Erasmus University Medical Center, Erasmus MC Cancer Institute, Rotterdam, The Netherlands
\textsuperscript{b} Department of Pathology, Erasmus University Medical Center, Erasmus MC Cancer Institute, Rotterdam, The Netherlands
\textsuperscript{c} Department of Medical Oncology, Erasmus University Medical Center, Erasmus MC Cancer Institute, Rotterdam, The Netherlands

Available online 24 October 2013

Abstract Introduction: Treatment options for patients with metastatic castration-resistant prostate cancer (mCRPC) have expanded in recent years with the introduction of cabazitaxel, abiraterone and enzalutamide. With new systemic therapies available, the optimal treatment sequence of these drugs in mCRPC becomes increasingly important. As shown recently, patients who had previously been treated with abiraterone showed impaired responses to docetaxel, suggesting clinical cross-resistance \cite{1}. In the present study, we aimed to identify cross-resistance between taxanes (docetaxel and cabazitaxel) and the new hormonal agents abiraterone and enzalutamide. As a potential mechanism for cross-resistance, we investigated the effects on androgen receptor (AR) nuclear translocation of these compounds.

Methods: To identify cross-resistance, we determined the effects of docetaxel, cabazitaxel, abiraterone and enzalutamide on cell viability in prostate cancer cell lines with acquired resistance to abiraterone and enzalutamide. Time-lapse confocal microscopy was used to study the dynamics of AR nuclear translocation.

Results: We observed impaired efficacy of docetaxel, cabazitaxel and enzalutamide in the abiraterone-resistant cell line, compared to the non-resistant cell line, providing evidence for \textit{in vitro} cross-resistance. Impaired efficacy of docetaxel, cabazitaxel and abiraterone was also observed in the enzalutamide-resistant cell line. Furthermore, docetaxel and cabazitaxel inhibited AR nuclear translocation, which was also observed for abiraterone and enzalutamide.
Conclusions: In conclusion we found substantial preclinical evidence for cross-resistance between the taxanes docetaxel and cabazitaxel, and AR targeting agents abiraterone and enzalutamide. Since these compounds all interfere with AR-signalling, this strongly suggests a common mechanism of action, and thus a potential mechanism for cross-resistance in mCRPC.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Prostate cancer cells are dependent on androgen receptor (AR) signalling for growth and survival. Therefore, patients with metastatic prostate cancer initially respond well to luteinising hormone releasing hormone (LHRH) analogues or surgical castration, with or without anti-androgens. However, eventually all patients develop castration-resistant prostate cancer. Docetaxel is the standard first line chemotherapy for metastatic castration-resistant prostate cancer (mCRPC) and has shown survival benefit as well as palliative benefit in phase III clinical trials [2,3]. For patients who progress after docetaxel chemotherapy several new treatment options have become available recently. Cabazitaxel and AR targeting agents abiraterone and enzalutamide all demonstrated improved overall survival (OS) in patients with mCRPC who progressed after docetaxel-based chemotherapy [4–6]. Taxanes (i.e. paclitaxel, docetaxel, and cabazitaxel) act through microtubule interaction and polymerisation inducing mitotic arrest and apoptosis. Recent reports demonstrated that paclitaxel and docetaxel also impair AR-signalling, which in the setting of mCRPC might in fact be responsible for part of the therapeutic efficacy [7,8]. AR-signalling remains an important target for therapy in mCRPC, which has been demonstrated by the survival benefit obtained by abiraterone and enzalutamide. Enzalutamide exerts its effect by inhibiting AR nuclear translocation, DNA-binding and co-activator recruitment [9]. Abiraterone inhibits androgen biosynthesis by irreversibly blocking CYP17A1, a crucial enzyme in steriodogenesis [10,11].

Recently abiraterone has shown improved radiographic progression-free survival (PFS) and a trend towards improved OS in chemotherapy-naive mCRPC patients [12]. Based on this trial, the US Federal Food and Drug Administration (FDA) and the European Medicines Agency (EMA) lent approval to the use of abiraterone in patients with mCRPC prior to docetaxel chemotherapy. With new therapies available in the pre-docetaxel setting, the challenge has become to determine the treatment sequence which yields the greatest survival benefit for patients with mCRPC. In this light, it was reported that the activity of docetaxel post-abiraterone appeared lower than anticipated, with a median OS of only 12.5 months, which was less than the 19 months observed in the TAX327 trial [1,2]. Moreover, fewer patients had a \( \geq 50\% \) prostate-specific antigen (PSA) response (26%) as compared to a similar abiraterone-naive patient cohort (54%), and compared to TAX327 (48%). No PSA responses to docetaxel were observed in patients who did not have a PSA response on abiraterone either.

Likewise, the activity of abiraterone appears to be higher when used before chemotherapy than in patients who have been previously exposed to docetaxel. In two phase II trials with abiraterone, a \( \geq 50\% \) PSA decline was observed in 67% and 79% of chemotherapy-naive patients, respectively, compared to 29% in the post-chemotherapy COU-AA-301 phase III trial [6,13,14]. In addition, a \( \geq 50\% \) PSA decline was observed in 62% of patients in the randomised phase III trial of abiraterone pre-chemotherapy [12].

Taken together, these data may be explained by cross-resistance, a condition in which sensitivity to one compound is impaired by another compound with a similar or overlapping mechanism of action. In this report, we describe preclinical evidence for cross-resistance between the taxanes docetaxel and cabazitaxel and new hormonal agents abiraterone and enzalutamide, all four drugs currently registered for the use in mCRPC. Furthermore, as a potential mechanism for cross-resistance, we investigated the effects of these compounds on AR nuclear translocation.

2. Materials and methods

2.1. Cell lines

The PC346C human prostate cancer cell line was derived and maintained as described previously [15–17]. Briefly, cells were cultured in special Prostate Growth Medium (PGM) based on Dulbecco’s Modified Eagle’s Medium (DMEM)-F12 medium with several prostate cancer growth factors [15], 100 U/ml Penicillin, and 100 \( \mu \)g/ml Streptomycin (Cambrex BioWhittaker, Verviers, Belgium), supplemented with 2% foetal calf serum (FCS) (PAN Biotech, Aidenbach, Germany) and 0.1 nM of the synthetic androgen R1881 (NEN, Boston, MA). The PC346Abi101 and PC346Enza cell lines were generated by continuous culturing of PC346C cells in PGM medium supplemented with 2% dextran-coated charcoal stripped serum (DCC), with
the addition of 1 μM abiraterone for PC346Abi101 and 1 μM enzalutamide for PC346Enza. After initial cell death, resistant cells started to grow out under the selection conditions used. PC346C cells stably expressing green fluorescent protein (GFP) labelled AR (GFP-AR) were generated using lentiviral transduction. For the experiments, cells were cultured in the same DCC-containing PGM medium [15].

The Hep3B cell lines stably expressing GFP-AR and yellow fluorescence protein (YFP)-β-tubulin were generated and maintained as described previously [18,19]. The YFP-β-tubulin expression construct was kindly provided by Dr. Galjart (Erasmus University Medical Center).

2.2. Confocal microscopy

For confocal microscopy, Hep3B GFP-AR, Hep3B YFP-β-tubulin and PC346C GFP-AR cells were seeded on a glass cover slip and cultured in DCC-containing medium. After overnight attachment, cells were treated with docetaxel (1 μM) [8,20], cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM) [21,22] and enzalutamide (1 μM). Incubation times were 48 and 4 h for Hep3B GFP-AR and PC346C GFP-AR cells, respectively. Docetaxel and cabazitaxel were kindly provided by Sanofi (Paris, France). Abiraterone and enzalutamide were obtained from Sequoia Research Products (Pangbourne, United Kingdom). Mitoxantrone was obtained from EMD Serono (Rockland, MA). Confocal microscopy was performed on a Zeiss LSM510 microscope (Carl Zeiss, Jena, Germany) equipped with a 63×/1.3 NA oil immersion objective using the 488 nm (GFP) and 514 nm (YFP) laser line of a 200 mW Ar laser. Cells were transferred to a live-cell chamber and maintained at 37 °C and 5% CO2. For time-lapse imaging, images of Hep3B GFP-AR cells were acquired every 5 min during 130 min at multiple locations of the same sample. After 10 min of imaging, 1 nM of the synthetic androgen R1881 was added to the medium to induce AR nuclear translocation. Average fluorescence intensities in the nucleus and cytoplasm were measured at each time point using Image J software (RSB, NIH, Bethesda, MD). The percentage of AR nuclear localisation was expressed as: nuclear signal intensity/(nuclear signal intensity + cytoplasmatic signal intensity) × 100, after background subtraction. The mean percentage of AR nuclear localisation of 18–28 cells in three independent experiments ± standard error of the mean (SEM) was plotted for the treatment conditions at every time point.

2.3. Cell proliferation assays

To determine the effects of docetaxel, cabazitaxel, abiraterone, enzalutamide and mitoxantrone on cell viability, we used an assay based on the enzymatic reduction of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St.Louis, MO) by metabolically active cells as described previously [23]. Briefly, cells were seeded in 96-well dishes at 5000 cells per well in DCC medium. After overnight attachment, PC346C, PC346Abi101 and PC346Enza cells were incubated for 10 days with docetaxel, cabazitaxel, abiraterone, enzalutamide, mitoxantrone or vehicle at indicated concentrations, with the addition of 0.1 nM R1881. Hep3B GFP-AR cells were incubated for 48 h with the same compounds. Four replicates per condition were used. Data are expressed as mean ± SEM of three independent experiments. IC50 values were calculated in Prism GraphPad 5.0 using the following formula: Y = 100/(1 + 10^(X-LogIC50)). To statistically test differences in IC50 values between cell lines we used the extra sum-of-squares F test with a boundary for significance of p < 0.01.

3. Results

3.1. Docetaxel and cabazitaxel efficacy is impaired in PC346Abi101 and PC346Enza cells

To identify cross-resistance between docetaxel and cabazitaxel, and the hormonal agents abiraterone and enzalutamide, we investigated the effects of docetaxel and cabazitaxel on cell viability in PC346Abi101 and PC346Enza cells, in which acquired resistance to abiraterone (PC346Abi101) and enzalutamide (PC346Enza) was developed in vitro (Figs. 1A and 2A). Protein expression of AR and PSA for PC346C, PC346Abi101 and PC346Enza was determined using Western blotting (Supplementary Methods) and is shown in Supplementary Fig. S1.

We observed that docetaxel and cabazitaxel efficacy was significantly impaired in both PC346Abi101 and PC346Enza cells, as compared to the parental PC346C cells (Figs. 1B, C and 2B, C), suggesting cross-resistance between both taxanes and abiraterone, as well as both taxanes and enzalutamide. To determine whether the observed cross-resistance was specific for the microtubule-targeting agents docetaxel and cabazitaxel, we used mitoxantrone as a control cytotoxic agent that does not target microtubules. Mitoxantrone efficacy was not significantly impaired in PC346Abi101 and PC346Enza cells, showing similar efficacy as in PC346C cells (Figs. 1D and 2D). IC50 values for the various compounds in PC346Abi101 and PC346Enza versus PC346C are shown in Table 1.

3.2. Abiraterone and enzalutamide efficacy is impaired in PC346Enza and PC346Abi101 cells

To investigate cross-resistance between the AR targeting agents abiraterone and enzalutamide, we determined
the efficacy of abiraterone in the enzalutamide-resistant cell line PC346Enza, and the efficacy of enzalutamide in the abiraterone-resistant cell line PC346Abi101. We observed impaired efficacy of enzalutamide in PC346Abi101 cells as compared to PC346C (Fig. 1). Likewise, the efficacy of abiraterone was diminished in PC346Enza as compared to PC346C, which suggests cross-resistance between these two hormonal agents (Fig. 2).

3.3. Docetaxel, cabazitaxel, abiraterone and enzalutamide inhibit R1881-induced AR nuclear translocation

To investigate the dynamics of AR nuclear translocation, time-lapse microscopy was used to determine AR nuclear localisation at regular time intervals after addition of R1881 in Hep3B GFP-AR cells pre-treated for 48 h with docetaxel, cabazitaxel, abiraterone, enzalutamide and mitoxantrone (Fig. 3A and B). Pretreatment
of cells with docetaxel and cabazitaxel inhibited AR nuclear translocation with 21% and 34%, respectively, compared to vehicle control. We investigated mitoxantrone as a control to determine whether this effect could be linked to the interference of microtubule dynamics by docetaxel and cabazitaxel. As expected, mitoxantrone pretreatment did not cause an impairment of AR translocation to the nucleus. Together with the observation that docetaxel and cabazitaxel clearly affected microtubules after 48 h of treatment this strengthens the hypothesis that microtubules may at least partly facilitate AR transport (Fig. 3C). The reduced AR translocation after pretreatment with docetaxel and cabazitaxel for 48 h could not be explained by cytotoxic effects, since neither of these compounds showed evidence of cellular toxicity under these conditions (Supplementary Fig. S2A) and overall cell viability had even increased compared to day 0 (Supplementary Fig. S2B).
Table 1
IC50 values for abiraterone, docetaxel, cabazitaxel, mitoxantrone and enzalutamide in PC346C versus PC346Abi101 and PC346Enza.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell line</th>
<th>PC346C</th>
<th>PC346Abi101</th>
<th>PC346Enza</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 (95% CI)</td>
<td>IC50 (95% CI)</td>
<td>IC50 (95% CI)</td>
<td>IC50 (95% CI)</td>
</tr>
<tr>
<td>Abiraterone (µM)</td>
<td>0.6 (0.5–0.8)</td>
<td>19.3 (15.1–24.8)</td>
<td>12.2 (9.4–15.9)</td>
<td></td>
</tr>
<tr>
<td>Docetaxel (nM)</td>
<td>0.3 (0.2–0.5)</td>
<td>9.3 (4.5–19.4)</td>
<td>23.9 (13.6–42.0)</td>
<td></td>
</tr>
<tr>
<td>Cabazitaxel (nM)</td>
<td>0.2 (0.1–0.5)</td>
<td>24.1 (11.4–51.0)</td>
<td>7.2 (3.6–14.4)</td>
<td></td>
</tr>
<tr>
<td>Mitoxantrone (µM)</td>
<td>0.2 (0.1–0.5)</td>
<td>0.5 (0.2–1.1)</td>
<td>0.05 (0.02–0.1)</td>
<td></td>
</tr>
<tr>
<td>Enzalutamide (µM)</td>
<td>3.3 (2.4–4.6)</td>
<td>26.6 (12.3–57.4)</td>
<td>102.7 (35.7–295.8)</td>
<td></td>
</tr>
</tbody>
</table>

* IC50 significantly higher as compared to IC50 PC346C (p < 0.01).

Pretreatment of Hep3B GFP-AR cells with abiraterone inhibited AR nuclear translocation with 58% as compared to control. This observation demonstrates that besides inhibiting CYP17A1, abiraterone can act as an anti-androgen in the presence of R1881. As expected, no AR nuclear import was observed in enzalutamide treated cells after the addition of R1881. Cell viability of the cells was not affected by abiraterone and enzalutamide (Supplementary Fig. S2C and D).

We confirmed our observations from the Hep3B GFP-AR cells in a prostate cancer specific model using PC346C cells stably expressing GFP-AR. Fig. 4 demonstrates that docetaxel and cabazitaxel, as well as abiraterone and enzalutamide inhibited R1881-induced AR nuclear transport in these cells as compared to vehicle control and mitoxantrone.

4. Discussion

In this study we present in vitro evidence for cross-resistance between taxanes (docetaxel and cabazitaxel) and AR targeting compounds abiraterone and enzalutamide in mCRPC. Furthermore, our data demonstrate that docetaxel, cabazitaxel, abiraterone and enzalutamide all act on AR nuclear transport, which is a crucial step in AR-signalling, and provide a mechanistical explanation for potential cross-resistance between the two taxanes that are currently registered for treatment in mCRPC and the novel AR targeting agents abiraterone and enzalutamide. The observation that mitoxantrone did not affect AR transport and did not show impaired efficacy in the abiraterone- and enzalutamide-resistant cells, strengthened our hypothesis that cross-resistance between both taxanes and the hormonal agents might be caused by the effects on AR nuclear import of these compounds.

Darshan et al. recently reported that paclitaxel inhibits AR nuclear import [20]. Paclitaxel, however, is not approved for use in mCRPC. Zhu et al. showed that also docetaxel impairs AR-signalling [8]. Thus far no data have been reported on cabazitaxel, which was approved in 2010 by the FDA and in 2011 by the European Medicines Agency (EMA) for the use in mCRPC after prior treatment with docetaxel. To our knowledge, we are the first to describe preclinical evidence for cross-resistance and the effects on AR translocation dynamics by docetaxel, cabazitaxel, and abiraterone, drugs that are all three approved for the treatment of mCRPC.

Interestingly abiraterone is able to block AR nuclear import in the presence of R1881. Like testosterone or dihydrotestosterone, R1881 does not require steroidogenic conversion to bind and activate the AR. Consequently, our observed inhibition of AR nuclear transport cannot be related to CYP17A1 inhibition, as therefore must be an effect of abiraterone directly acting on the AR. This finding is supported by Richards et al., who found that abiraterone binds and inhibits AR at high but clinically relevant concentrations (≥ 5 µM) [21,22]. Our observations of abiraterone and enzalutamide both directly inhibiting AR nuclear translocation, and cross-resistance between these compounds in vitro are concordant with recent clinical observations demonstrating modest efficacy of abiraterone in patients with mCRPC progressing after enzalutamide [24,25], as well as modest efficacy of enzalutamide in patients progressing after abiraterone [26,27].

The inhibiting effects on AR nuclear import by abiraterone and docetaxel strongly suggest a common mechanism of action in mCRPC. Such an interaction is further augmented by our observed cross-resistance between these compounds in vitro. Although the exact mechanism needs to be further elucidated, this data may explain recent clinical observations of cross-resistance between abiraterone and docetaxel in mCRPC reported by Mezynski et al. [1].

The effects of docetaxel and cabazitaxel on AR transport could be explained by a mechanism proposed by Thadani-Mulero et al. in which AR transport is facilitated by microtubules and the motor protein dynein [7]. This model could help to better understand the effect of taxanes on AR and the molecular basis of taxane resistance. In our study the effects of the taxanes on AR transport were more pronounced in PC346C as compared to the Hep3B model system, suggesting that the ability of taxanes to suppress microtubule dynamics may be cell specific, exerting optimal effects in prostate cancer cells.
Fig. 3. Docetaxel, cabazitaxel, abiraterone and enzalutamide inhibit androgen receptor (AR) nuclear import. Hep3B cells expressing green fluorescent protein (GFP)-AR were pre-treated with docetaxel (1 μM), cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM), enzalutamide (1 μM) or vehicle control for 48 h. Subsequently the synthetic androgen R1881 (1 nM) was added at $t = 0$ to induce AR nuclear translocation. Time-lapse images were acquired every 5 min at multiple locations per sample. (A) Dynamics and quantification of AR nuclear localisation. (B) Representative high resolution confocal images of AR localisation were acquired after 130 min of incubation with R1881. Bar represents 10 μm. (C) Docetaxel and cabazitaxel cause microtubule rearrangement. High resolution confocal images of Hep3B cells expressing yellow fluorescence protein (YFP)-tubulin were acquired after treatment with docetaxel (1 μM), cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM), enzalutamide (1 μM) and vehicle control for 48 h. Bar represents 10 μm.
Docetaxel, cabazitaxel, abiraterone and enzalutamide inhibit androgen receptor (AR) nuclear import in prostate cancer cells. PC346C cells stably expressing green fluorescent protein (GFP)-AR were pre-treated with docetaxel (1 μM), cabazitaxel (1 μM), mitoxantrone (100 nM), abiraterone (6 μM), enzalutamide (1 μM) or vehicle control for 4 h. High resolution confocal images were acquired after 2 h of incubation with 1 nM R1881 to induce AR nuclear translocation.
With new compounds for the treatment of mCRPC becoming available for clinical use, it is warranted, especially in light of our current findings, to determine the optimal treatment sequence of these compounds in the management of patients with mCRPC. Following the recent approval by FDA and EMA of abiraterone for the use prior to docetaxel chemotherapy, prospective clinical research aiming to define the treatment sequence that provides the maximum survival benefit has become of paramount importance. The ultimate proof of clinical cross-resistance between these compounds and the magnitude of the impact of drug sequencing can only be answered in a prospective clinical trial of abiraterone or enzalutamide followed by taxane chemotherapy, versus chemotherapy followed by abiraterone or enzalutamide.

In conclusion we found substantial evidence for cross-resistance between the taxanes docetaxel and cabazitaxel, and the new hormonal agents abiraterone and enzalutamide in vitro. These preclinical observations are concordant with clinical reports of cross-resistance between docetaxel and abiraterone, as well as abiraterone and enzalutamide in mCRPC [1,24–27]. Since docetaxel, cabazitaxel, abiraterone and enzalutamide all interfere with AR-signalling, this strongly suggests a common mechanism of action, and thus a potential mechanism for cross-resistance in mCRPC. Prospective clinical studies should further define if this cross-resistance impacts the treatment sequence of these treatment options in patients with mCRPC. Survival benefit of abiraterone has been shown post-docetaxel, but since the efficacy of the taxanes may be impaired in this setting, it is critically important to demonstrate overall survival benefit when testing these agents prior to chemotherapy.

Conflict of interest statement

R. de Wit; consultancy and speaker honoraria from Sanofi, Janssen, and Millenium, research grants from Sanofi.

Acknowledgements

This work was supported by a research grant from Sanofi. The sponsor had no role in the design, execution or interpretation of the study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejca.2013.09.026.

References


学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页　文献云下载　图书馆入口　外文数据库大全　疑难文献辅助工具