Cisplatin, doxorubicin and paclitaxel induce mdr1 gene transcription in ovarian cancer cell lines

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Abstract

The clinical observation of the multidrug resistance (MDR) phenotype is often associated with overexpression of the mdr1 gene, in particular with respect to ovarian cancer. However, until now the mdr1-inducing potential of commonly used antineoplastics has been only incompletely explored. We performed short-term cultures of six ovarian cancer cell lines (MZOV4, EFO27, SKOV3, OAW42, OTN14, MZOV20) exposed to either blank medium or cisplatin, doxorubicin or paclitaxel at concentrations related to the clinically achievable plasma peak concentration. A highly specific quantitative real-time RT-PCR was used to detect the Mdr1 transcripts. Mdr1 mRNA contents were calibrated in relation to coamplified GAPDH mRNA. Mdr1 mRNA was detectable in each cell line. In 13 out of 18 assays (72%) the specific anticancer drug being tested induced mdr1 transcription. No decrease in mdr1 mRNA concentration was observed. Our data suggest that mdr1 induction by antineoplastics is one of the reasons for failure of ovarian cancer therapy but may vary individually.

Introduction

Ovarian cancer is one of the main causes of death related to gynecological malignancy: Nearly 65% of ovarian cancer patients will die from their disease within 5 years [8]. Although ovarian carcinomas are considered highly responsive to cytotoxic treatment, they rapidly develop chemoresistance [6]. Thus the multidrug resistance (MDR) phenotype of ovarian tumor cells is one of the major obstacles to the therapy of ovarian cancer [12].

On the molecular level, increased expression of the mdr1 gene is the best-studied mechanism for the MDR phenotype [6]. The mdr1 gene encodes the p170 glycoprotein, a transmembrane protein that eliminates toxic agents from
the intracellular compartment and thus confers resistance to a wide variety of natural products. However, insufficient information is available concerning the regulation of the mdr1 gene during the clinical course of a cancer patient undergoing antineoplastic chemotherapy. In ovarian cancer as well as in other neoplasms, p170 overexpression leads to the MDR phenotype and indicates a worse prognosis [8, 18].

Consequently, intensified research efforts are needed to obtain more basic data with respect to mdr1 gene regulation in ovarian cancer [17]. The design of these studies should take into account techniques with increased sensitivity. Recently, mdr1 gene amplification was excluded as a cause for mdr1 overexpression in ovarian cancer [19]. Thus, in this tumor entity, p170 overexpression is more likely a result of increased transcription/translation of the mdr1 gene. The study presented here was designed to explore whether antineoplastics are capable of inducing the mdr1 gene. We therefore employed a mdr1 mRNA detection assay using real-time PCR, which is more sensitive than in any other study performed so far. With this assay system the mdr1-inducing potency of commonly applied anticancer drugs was investigated.

Materials and Methods

Tumor Cell Culture

The ovarian cancer cell lines were kind gifts from L.G. Poels (Nijmegen, The Netherlands) (OTN14) and V. Möbus (Ulm, Germany) (MZOV4, MZOV20) or were obtained from DSMZ (Braunschweig, Germany) and DFKZ (Heidelberg, Germany). Tumor cells were grown in AIM V medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, 100 IE/ml penicillin and 100 µg/ml streptomycin at 100 cells/µl (37°C, humidified 95% air-5% CO₂ atmosphere). Cells were exposed for 3 days to either blank medium (control) or the different antineoplastic agents: doxorubicin (DOX) 0.5 µg/ml, cis-diamino-dichloroplatinum(II) (CDDP) 3.8 µg/ml, and paclitaxel (PCT) 13.6 µg/ml. The cytostatics assayed referred to either the clinical peak plasma concentration (PPC) after administration of an intravenous standard dose (DOX, CDDP) or the equivalent of the area under the plasma elimination curve (PCT). Each assay was performed in triplicate.

Quantitative Real-Time RT-PCR

Cells were harvested by centrifugation (5 min, 8,000 g), washed in phosphate-buffered saline (PBS), and resuspended in lysis buffer. Total RNA was extracted with the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and quantified with the RiboGreen RNA quantification kit (MoBiTec, Göttingen, Germany).

A mdr1-specific 411-bp sequence was amplified in the presence of an intrinsic fluorescein-labeled mdr1 probe. The TaqMan EZ RT-PCR Kit (GAPDH mRNA, Applied Biosystems, Weiterstadt, Germany) was used as an internal control. The mdr/GAPDH biplex-qu-RT-PCR contained 5 ng of RNA, 300 µM dATP, dCTP, and dGTP, 600 µM dUTP, 60 nM reference dye, each primer at 200 nM, each probe at 100 nM, 0.01 U/µl AmpErase UNG, and 0.1 U/µl Tth DNA polymerase in TaqMan EZ buffer (50 mM bicine, 115 mM K-acetate, 0.01 mM EDTA, 40% glycerol, 3 mM Mn-aceate, pH 8.2) in a final volume of 50 µl. Quantitative real-time RT-PCR was performed by reverse transcription for 30 min at 50°C, denaturation for 10 min at 95°C and 40 cycles of 15 s at 95°C, and annealing and elongation for 1 min at 60°C. Resulting fluorescence was detected at each PCR cycle by the ABI 7700 Sequence Detection System (Applied Biosystems) automatically. Each mdr1 or GAPDH signal, respectively, was quantified by the specific threshold cycle number (CT).

Results

All ovarian cancer cell lines investigated were successfully analyzed. For GAPDH and mdr1 expression, both single quRT-PCR and biplex-quRT-PCR revealed similar absolute results, indicating reproducibility and reliability of the assay. The expression of the mdr1 gene was indicated as a quotient of mdr1 C₉/GAPDH C₉. The quantitative real-time RT-PCR was performed in triplicate to validate the test. For each RT-PCR the assay system produced comparable results with acceptable variation: The quotients ranged from 0.60 to 0.88 and the intra-assay deviation did not exceed 0.08.

Summarizing all control experiments of the six cell lines, the mdr1 C₉/ GAPDH C₉ quotient was 0.66±0.04. Therefore, quotients of 0.62–0.70 represent a “normal” distribution and are equivalent to mdr1 expression rates ranging between 94% and 106% in relation to the control. For further analyses, each blank medium control was set at 100%.

As summarized in Table 1, all cell lines displayed increased mdr1 gene expression in response to some of the cytostatics. In none of the assays was downregulation of mdr1 gene expression detected. In four out of six (66%) ovarian cancer cell lines, treatment with CDDP or DOX increased mdr1 expression to an average of 118% of the control, ranging from 109% to 133% (CDDP) or 107% to 130% (DOX), respectively. PCT-induced mdr1 expression was detected in five out of six (83%) ovarian cancer cell lines. The degree of PCT-induced mdr1 mRNA augmentation ranged between 109% and 119% with a mean of 114%.

Discussion

The unsatisfactory clinical results in refractory ovarian cancer are likely traced back to an increase in expression of the mdr1 gene [8, 11]. Further-
more, an increase of mdr1 mRNA is a highly predictive determinant of patients’ survival [16]. Accordingly, the impact of mdr1 expression is a major concern in basic research of the molecular biology of ovarian cancer. We thus designed a study concerning the mdr1 mRNA increase induced by a particular anticancer drug itself, thereby investigating the role of the mdr1 gene in the development of drug resistance.

It is reported that anticancer drugs are able to induce mdr1 transcription [4]. Resistance against doxorubicin or paclitaxel is mediated by the mdr1 gene in OAW42 cells [13]. Accordingly, chemoresistant ovarian cancers display high mdr1 mRNA levels [14]. Exposure to DOX results in a fast and dramatic increase of mdr1 gene expression in human sarcoma in vivo [1]. Consequently, and in contrast to earlier data [5, 9, 10, 22], the predictive value of mdr1 expression has recently been shown [2]. A study performed with both primary and recurrent native ovarian carcinomas revealed that the increased mdr1 mRNA levels may not be maintained for a longer interval [20].

In 1999, Robert hypothesized that the mdr1 gene might be expressed at very low levels in all tumors, including ovarian cancer [17]. New basic research involving new strategies with increased sensitivity is required to investigate the role of the mdr1 gene in development of the MDR phenotype of ovarian cancer precisely and thus to unravel the aforementioned controversial studies. Here, an assay is presented to determine the mdr1 expression rate with a high specificity and sensitivity, which is easily incorporated into clinical routine. This study shows a detectable mdr1 mRNA presence in all cancer cell lines, confirming the hypothesis of low but persistent mdr1 mRNA levels in ovarian cancer [17]. Furthermore, a drug-induced augmentation of the mdr1 transcription rate was observed in 72% of the experiments and no decrease occurred. This also holds true for CDDP exposure, although this drug is known not to be a mdr target but capable of selecting multidrug-resistant ovarian cancer cells exhibiting high mdr1 levels [21]. Our data and those of the earlier studies suggest that a particular drug induces mdr1 transcription and, consequently, supports its own extrusion out of the tumor cell. However, the response rates indicate that the extent of this phenomenon is variable and should be investigated for each single tumor separately.

Nevertheless, the assay presented here performed with native cancer cells may be useful in identifying patients who will definitely benefit from a regimen of a common chemotherapy combined with a mdr1-inhibiting drug. Clinical trials have been performed recently with encouraging results [3, 7, 15], but they lack a molecular definition of the tumors.

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References


Table 1. Relative increase of mdr1 gene expression induced by cytostatics in ovarian cancer cell lines. Tumor cells were grown overnight with either blank medium (control) or commonly applied anticancer therapeutics. For better comparison, each control was set as 100%. Increased mdr1 expression values exceeding the normal distribution are indicated in bold numbers. For each value, the standard deviation is indicated.

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<tr>
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<th>OTN14</th>
<th>EPO27</th>
<th>MZO1V</th>
<th>MZO20V</th>
<th>SKO3</th>
<th>OAW42</th>
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<tr>
<td>Cisplatin</td>
<td>120±4.3</td>
<td>105±1.6</td>
<td>109±0.5</td>
<td>109±2.9</td>
<td>101±5.7</td>
<td>133±4.6</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>130±7.2</td>
<td>106±4.5</td>
<td>107±0.5</td>
<td>101±0.5</td>
<td>108±0.8</td>
<td>128±5.2</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>115±4.0</td>
<td>113±2.8</td>
<td>119±2.2</td>
<td>119±1.3</td>
<td>99±1.2</td>
<td>119±3.4</td>
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