Paclitaxel- and Docetaxel-dependent Activation of CA-125 Expression in Human Ovarian Carcinoma Cells

Christian Marth,1 Alain G. Zeimet, Martin Widenschwendter, Christoph Ludescher, Janne Kærn, Claes Tropé, Günther Gastl, Günter Daxenbichler, and Otto Dopunt


ABSTRACT

Taxanes represent a new class of antineoplastic agents that are being evaluated in several malignant tumors; they have been shown to induce a high remission rate and to prolong survival in ovarian cancer patients. However, CA-125 has been suggested to be an unreliable marker for monitoring response to paclitaxel therapy. Therefore, we were interested in whether taxanes may directly modulate CA-125 expression.

Human ovarian carcinoma cell lines OVCAR-3, HOC-7, SKOV-6, 2780, 2774, and HTB-77 were treated with paclitaxel or docetaxel. Secreted, surface-associated, and cytosolic CA-125 were estimated by means of a sandwich solid-phase RIA or by immuno-flow cytometry.

In addition to in vitro antiproliferative activity, paclitaxel and docetaxel augmented the expression of the tumor marker CA-125 in the three ovarian carcinoma cell lines, OVCAR-3, HOC-7, and SKOV-6, constitutively expressing this tumor marker. The three CA-125-negative cell lines, 2780, 2774, and HTB-77, did not respond to taxane treatment by expressing this tumor marker, although their proliferation was markedly inhibited. The taxane-mediated induction of CA-125 was found to be dependent on intact protein and RNA biosynthesis. However, CA-125 concentration was increased in the supernatant medium only and not on cell surface or cytosol.

Our results demonstrate an in vitro activation of ovarian carcinoma cells in terms of CA-125 secretion by taxanes. This may explain the CA-125 fluctuations observed in vivo under paclitaxel treatment and may indicate that CA-125 is not a reliable tumor marker during taxane chemotherapy.

INTRODUCTION

The antigen CA-125, first described by Bast et al. (1), is elevated in the serum of approximately 80% of women presenting with advanced nonmucinous epithelial ovarian cancer. This tumor marker has evolved into one of the most useful diagnostic tests in the management of individuals with this malignant disease. Rising CA-125 levels may predict disease recurrence many months before a patient shows other evidence of tumor progression. The determination of CA-125 serum levels in ovarian carcinoma patients has recently gained major interest for the prediction of response to chemotherapy (2–4). Decreasing concentrations have been shown to be associated with tumor regression, whereas increasing CA-125 levels indicate resistance to the treatment and disease progression. Moreover, chemotherapy-induced rapid clearance of CA-125 from the serum is associated with good prognosis (5, 6). The close correlation with clinical and surgical findings, e.g., at second-look surgery, has evoked a discussion about using CA-125 in addition to or instead of standard criteria for defining response (4, 7). A prerequisite for this proposal is that the release of CA-125 by ovarian cancer is dependent only on the number of living tumor cells. However, it has been shown that the biosynthesis and shedding of CA-125 are not constitutively stable processes but may be modulated by several factors. It has been demonstrated that ovarian carcinoma cells reduce CA-125 production during the mitotic cycle and increase shedding in G0 (8). Moreover, IFN-γ is able to augment biosynthesis and the release of CA-125 in cultured ovarian carcinoma cells (9). However, this modulation of CA-125 production in vivo could impair the predictive value of tumor marker determination.

Taxanes represent a new class of antineoplastic agents being evaluated in several malignant tumors. Paclitaxel in combination with a platinum compound has been shown to induce a high remission rate and to prolong survival in ovarian cancer patients and is therefore currently regarded as the standard therapy (10). However, the assessment of response by measuring CA-125 has been shown to have limited value in paclitaxel-treated refractory ovarian cancer patients (11). To date, the reason for the paclitaxel-induced fluctuations in CA-125 serum levels could not be explained. One interesting possibility is an induction of CA-125 expression in ovarian carcinoma cells by paclitaxel. Although the primary action of this compound is thought to be the stabilization of microtubules (12), there is evidence for other effects in addition to this antimitotic activity. Paclitaxel was recently shown to activate the transcription of interleukin-8 in ovarian cancer cells (13). Moreover, secretion of human chorionic gonadotropin, the principal tumor marker in gestational trophoblastic disease, can be induced by paclitaxel in choriocarcinoma cells (14). Therefore, we were interested in whether paclitaxel also increases the expression of CA-125 in ovarian carcinoma cells. Because docetaxel, another taxane, has been found to act similarly to paclitaxel, we also included this substance in our studies (15).

MATERIALS AND METHODS

Substances. Paclitaxel and its solvent cremophor, as well as etoposide (VP-16) and cisplatin, were kindly provided by Dr. H. Stipsits (Bristol-Myers-Squibb, Vienna, Austria); docetaxel was donated by Dr. Haunold (Rhone-Poulenc-Rohrer, Vienna, Austria). Methotrexate, actinomycin D, and cycloheximide were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell Culture. The human ovarian carcinoma cell lines OVCAR-3, HTB-77, 2780, 2774, HOC-7, and SKOV-6 were kindly provided by Dr. C. Dittrich (University of Vienna, Vienna, Austria) and Dr L. Old (Memorial Sloan Kettering Center, New York, NY) and cultured under standard conditions in Dulbecco’s modified MEM with the addition of 10% fetal bovine serum (Biological Industries, Kibbutz Bet Haemek, Israel).

In order to determine CA-125 release, cells were seeded in 24-well tissue culture plates (Nunc, Roskilde, Denmark) and allowed to attach overnight. Culture medium was renewed, and taxanes were added in the desired concentrations (indicated in the legends to Table 1 and Figs. 1–3). In selected experiments, the paclitaxel solvent, namely cremophor, was added in concentrations equivalent to those in commercially available paclitaxel preparations. Because we could never detect any significant difference between controls and cremophor alone, we did not show these results. All substances remained in the culture medium for the entire incubation period, after which supernatant medium was collected for CA-125 determination. The medium specimens were centrifuged for 10 min at 40,000 × g to remove debris and stored at −70°C until analysis. Cells were detached from tissue culture plates with the help of 0.05% trypsin-0.02% EDTA in Dulbecco’s PBS solution (Biological Industries), and the number of cells was assessed with an electronic particle
cancer (Coulter, Dunstable, United Kingdom). To investigate whether taxane-mediated effects on CA-125 expression are dependent on intact biosynthesis of proteins or RNA, we treated ovarian carcinoma cells simultaneously with the taxanes and either 10 μg/ml cycloheximide or 10 μg/ml actinomycin D. The number of cells, as well as CA-125, was determined after 3 days of treatment.

CA-125 Determination. In culture medium specimens, CA-125 was determined by means of a sandwich solid-phase RIA (Centocor, Malvern, PA). All specimens were measured in duplicate according to the manufacturer’s instructions. The interassay and intraassay coefficients of variation were 5.3 and 4.2%, respectively. The lower limit of detection as defined by 2 SD of the zero standards was 1.2 units/ml culture medium. All CA-125 concentrations were expressed as arbitrary units related to the number of cells (microunits/cell).

CA-125 Cytosolic Levels. OVCAR-3 and HOC-7 cells were cultured in 150-cm² flasks. After treatment for 3 days with taxanes (10 nM) or without supplement (control), cells were detached using 0.05% trypsin-0.02% EDTA in PBS. Approximately 30 × 10^6 cells of each group were sonicated until more than 95% of the cells were disrupted. The homogenate was centrifuged at 40,000 × g for 30 min. In the supernatant, the concentration of CA-125 was determined using the RIA described. Results were expressed as microunits/cell.

CA-125 Flow Cytometry. Cells were cultured with or without the presence of taxanes in 25-cm² flasks. They were detached at different times, as indicated in the legend to Fig. 4, and incubated with the mouse monoclonal antibody OC-125 (CIS, GIF-Sur-Yvette, France) and FITC-labeled rabbit antimouse immunoglobulin (Dakopatts, Copenhagen, Denmark) as recently described (9). Samples with appropriate isotype-matched control antibody (Becton Dickinson, Sunnyvale, CA) were processed in parallel and taken as negative controls. Events from 10,000 cells were acquired and analyzed on a FACStar flow cytometer (Becton Dickinson).

Determination of Cell Viability. To estimate the toxic effects of taxanes, cells were treated for 3 days with paclitaxel or docetaxel, and cell viability was determined after incubation with 10 μM propidium iodide as described recently (16). Cells were analyzed by means of a FACStar flow cytometer.

Statistical Analysis. Data were analyzed with nonparametric procedures. The Mann-Whitney U test was used to analyze differences between measured values of CA-125 or counted number of cells. P < 0.05 was considered significant.

RESULTS

Paclitaxel and docetaxel inhibited the proliferation of the six ovarian carcinoma cell lines in a dose-dependent manner (Fig. 1 and Table 1). The action of the two taxanes on cell growth was statistically not distinguishable. Three of the ovarian carcinoma cell lines, namely OVCAR-3, HOC-7, and SKOV-6, constitutively released CA-125 into the culture medium. The three CA-125-negative cell lines, 2780, 2774, and HTB-77, were more sensitive to the antiproliferative activity of taxanes compared to OVCAR-3, HOC-7, and SKOV-6. At 10 nM paclitaxel, the number of cells ranged from 49–90% and from 8–31% for the CA-125-positive and -negative cell lines, respectively (P < 0.05). At 10 nM docetaxel, the number of cells was between 46 and 105% and between 10 and 21% for the CA-125-positive and -negative cell lines, respectively (P < 0.05).

Treatment with either paclitaxel or docetaxel led to a marked increase in CA-125 shedding in OVCAR-3, HOC-7, and SKOV-6 cells. Significant effects were seen at taxane concentrations sufficient to inhibit proliferation. At a concentration of 30 nM, both taxanes augmented CA-125 release and reduced growth (P < 0.05). Ten nM of paclitaxel reduced growth and induced CA-125 in OVCAR-3 and HOC-7 cells but not in SKOV-6 cells. On the other hand, docetaxel at a concentration of 10 nM was able to inhibit proliferation and augment CA-125 in OVCAR-3 and SKOV-6 cells but not in HOC-7 cells. The other three cell lines, namely HTB-77, 2780, and 2774, did not produce measurable amounts of CA-125 constitutively or after treatment with paclitaxel or docetaxel.

The taxane-induced effect on CA-125 shedding was time dependent and detectable in OVCAR-3 cells after 1 day of treatment for both paclitaxel (P < 0.05) and docetaxel (P < 0.05; Fig. 2). Treatment with taxanes for less than 24 h was not sufficient to significantly change the CA-125 release.

In contrast to taxanes, other cytostatic agents with the exception of hydroxyurea and actinomycin D did not augment the release of CA-125, although cell proliferation was markedly reduced (Table 2). Treatment of OVCAR-3 cells with the DNA synthesis inhibitor hydroxyurea resulted in an approximately 25% increase in CA-125 compared to control levels. Actinomycin D nearly doubled the CA-125 release of OVCAR-3 cells at a concentration of 10 μg/ml.

To evaluate whether taxane-induced CA-125 expression is dependent on constitutive protein biosynthesis, OVCAR-3 and HOC-7 cells were treated with 10 μg/ml cycloheximide (Fig. 3). Cycloheximide combined with either paclitaxel or docetaxel abolished the taxane-mediated CA-125 induction in HOC-7 and OVCAR-3 cells. The RNA synthesis inhibitor actinomycin D at a concentration of 10 μg/ml was also able to nullify the taxane-mediated effect, although when given alone it increased CA-125 secretion in OVCAR-3 but not HOC-7 cells. The combination of either cycloheximide or actinomycin with taxanes resulted in an increased antiproliferative activity (data not shown).

In addition to measuring CA-125 concentrations in supernatant culture medium, we were also interested in cytosolic concentrations. Therefore, we cultured OVCAR-3 and HOC-7 cells for 3 days with either 10 nM paclitaxel, 10 nM docetaxel, or without supplements (control). CA-125 measured in cytosolic preparation was 1100 ± 190, 840 ± 120, and 780 ± 180 microunits/cell for the control, paclitaxel, and docetaxel in OVCAR-3 cells, respectively (NS),2 and 250 ± 45, 210 ± 25, and 180 ± 32 μm/cell for the control, paclitaxel, and docetaxel in HOC-7 cells, respectively (NS). Ten μg/ml cycloheximide treatment of cells for 3 days reduced cytosolic CA-125 concen-

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2 The abbreviation used is: NS, not significant.
Table 1  Effects of paclitaxel and docetaxel on proliferation and CA-125 release
The ovarian carcinoma cells were treated for 3 days in the presence of the taxane as indicated. CA-125 was determined, and the cell number was assessed as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Control</th>
<th>10</th>
<th>30</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVCAR-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferation</td>
<td>100 ± 4</td>
<td>52 ± 2</td>
<td>22 ± 3</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>CA-125</td>
<td>141 ± 11</td>
<td>462 ± 31</td>
<td>1219 ± 103</td>
<td>1481 ± 122</td>
</tr>
<tr>
<td>HOC-7</td>
<td>100 ± 6</td>
<td>105 ± 11</td>
<td>83 ± 6</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Proliferation</td>
<td>238 ± 18</td>
<td>231 ± 22</td>
<td>403 ± 32</td>
<td>1326 ± 111</td>
</tr>
<tr>
<td>SKOV-6</td>
<td>100 ± 9</td>
<td>10 ± 2</td>
<td>4 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Proliferation</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>HTB-77</td>
<td>100 ± 7</td>
<td>30 ± 2</td>
<td>21 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Proliferation</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>CA-125</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docetaxel (nM)</td>
<td>30 UI</td>
<td>600</td>
<td>1200</td>
<td>2700</td>
</tr>
</tbody>
</table>

a Cell numbers are presented as the mean of four wells counted ±1 SD in percentage of untreated control.  
b CA-125 concentrations are given as the mean of four wells measured ±1 SD in microunits/cell.

discussion

Pharmaceuticals are highly active cytostatic agents in ovarian and breast carcinoma patients. A combination of paclitaxel with a platinum substitution to 270 ± 31 and 75 ± 10 microunits/cell in OVCAR-3 and HOC-7 cells, respectively.

CA-125 expression was also determined on the cell surface of OVCAR-3 and HOC-7 ovarian carcinoma cells by means of flow cytometry. In untreated OVCAR-3 and HOC-7 cells, integrity was 91 ± 1% and 98 ± 0.5%, respectively. After 3 days of treatment with either 10–100 nM paclitaxel or 10–100 nM docetaxel, estimated cell integrity was 91 ± 1.4% (NS) and 99 ± 0.6% (NS) for OVCAR-3 and HOC-7 cells, respectively.

Table 2  Effects of various cytostatic agents on proliferation and CA-125 release of OVCAR-3 cells
The ovarian carcinoma cells were treated for 3 days in the presence of the substances as indicated. CA-125 was determined, and the cell number was assessed as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Substance</th>
<th>Proliferationa</th>
<th>CA-125b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 4</td>
<td>161 ± 18</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>(0.03 µg/ml)</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>(0.1 µg/ml)</td>
<td>70 ± 5</td>
<td>178 ± 22</td>
</tr>
<tr>
<td>(0.3 µg/ml)</td>
<td>48 ± 2</td>
<td>151 ± 16</td>
</tr>
<tr>
<td>Hydroxyurea (100 µM)</td>
<td>45 ± 2</td>
<td>173 ± 13</td>
</tr>
<tr>
<td>Cycloheximide (10 µg/ml)</td>
<td>16 ± 4</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>(1 µg/ml)</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>(0.1 µg/ml)</td>
<td>18 ± 2</td>
<td>340 ± 41</td>
</tr>
<tr>
<td>(0.03 µg/ml)</td>
<td>10 µg/ml</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Hydroxyurea (100 µM)</td>
<td>57 ± 4</td>
<td>204 ± 19</td>
</tr>
</tbody>
</table>

a Cell numbers are presented as the mean of four wells counted ±1 SD in percentage of untreated control.  
b CA-125 concentration is given as the mean of four wells measured ±1 SD in microunits/cell.  
c P < 0.05 versus control.
Fig. 3. Effects of cycloheximide (CHX) or actinomycin D (ACT-D) on taxane-mediated CA-125 induction in ovarian carcinoma cells. OVCAR-3 (left) and HOC-7 (right) cells were cultured for 3 days without additives as control (○), with 30 nM paclitaxel (■), or 30 nM docetaxel (▲) alone (control) or in combination with either 10 μg/ml cycloheximide (+CHX) or 1 μg/ml actinomycin D (+ACT-D). Results are expressed as mean value of four wells measured ± 1 SD. Data shown are from one of two similar experiments.

A compound is now considered the standard first-line chemotherapy in ovarian cancer (10). High response rates in cisplatin-refractory tumors have been attributed to the unique mechanism of action. Taxanes promote the assembly of microtubules and also stabilize them, thus preventing their depolymerization (12). In addition to the antimitotic process, there is evidence that other activities may be important as well. Paclitaxel has been shown to reduce steroidogenesis in granulosa cells but to activate 17β-estradiol secretion in ovarian carcinoma cells (17, 18). The latter effect has been associated with increased aromatase activity. More recently, Lee et al. (13) observed paclitaxel-dependent transcriptional activation of the interleukin-8 gene in human ovarian carcinoma cells. Expression of human chorionic gonadotropin, the leading tumor marker in gestational trophoblastic disease, was also induced by paclitaxel in choriocarcinoma cells (14). This effect was dependent on intact protein biosynthesis but, in contrast to interleukin-8, was not regulated at the transcriptional level. In view of these various effects of taxanes, including antiproliferative and gene regulatory activities, we focused our interest on the tumor marker CA-125. Both taxanes tested, namely paclitaxel and docetaxel, showed a similar antiproliferative activity at equimolar concentrations in the six human ovarian carcinoma cell lines. In addition, in CA-125-positive cell lines, both taxanes augmented the release of this tumor marker into the culture medium. This effect was dependent on intact RNA transcription and protein biosynthesis, suggesting a direct regulation of taxanes on CA-125 gene regulation. Unfortunately, to date, the gene(s) encoding for CA-125 have not been cloned and, therefore, this hypothesis cannot be proven directly. Moreover, data obtained with actinomycin D remains controversial. Actinomycin D is known to inhibit transcription by binding to premelted DNA and immobilizing the transcriptional complex (19). However, actinomycin D is not capable of completely suppressing mRNA biosynthesis (20). Moreover, actinomycin D can also induce other cellular effects, e.g., apoptosis in tumor cells. This could explain the significant induction of CA-125 by actinomycin D in OVCAR-3 cells. Notably, the stimulatory effect of actinomycin D on CA-125 release was rather weak compared with taxanes in OVCAR-3 cells and was not observed in HOC-7 cells, whereas cell proliferation was effectively inhibited by both substances in both cell lines.

Taxanes, however, do not induce de novo biosynthesis of CA-125 but exert their action exclusively in cells with constitutive expression of CA-125. Of note, the taxane-mediated effect was not accompanied by increased cytosolic or surface concentrations of CA-125. These results suggest that taxanes may activate production and the rapid release of CA-125 from ovarian carcinoma cells. In fact, antigens recognized by the antibody OC-125 are found to be shed very rapidly, and within approximately 24 h nearly the entire cellular content must be renewed (8, 21). Because of the rapid turnover of CA-125, changes

Fig. 4. Cytofluorimetric analysis of CA-125 expression. OVCAR-3 and HOC-7 cells were cultured for 3 days with paclitaxel (30 nM, thick solid line) or without (control, thin solid line). Cells were treated with OC-125 or a nonimmune serum (dotted line), which served as the nonspecific group. Thereafter, cells were stained with a fluorescent second antibody. In each group, the fluorescence of 10,000 cells was analyzed, and the results were expressed as the number of cells per channel of fluorescence intensity on a logarithmic scale. Data shown are from one of four similar experiments.
TAXANES AND MODULATION OF CA-125 EXPRESSION

in production may also rapidly affect the rate of release so that no significant intracellular or surface accumulation can occur.

It might be argued that the increased release of CA-125 in cell culture supernatant could be due to taxane-induced cell lysis. However, using propidium iodide exclusion we were unable to show an effect on cell integrity or cell membrane permeability in taxane-treated cells. Moreover, incubation of ovarian carcinoma cells with cycloheximide augmented antiproliferative activity but eliminated taxane-mediated CA-125 release. Also, the finding that cytotoxic agents (such as cisplatin, which reduces the number of tumor cells to a similar extent as taxanes) were unable to augment CA-125 argues against a simple toxic mechanism of action. Like taxanes, hydroxyurea and actinomycin lead to a significant increase of CA-125 shedding. In summary, we can affirm that taxanes effectively induce the secretion of CA-125 in ovarian carcinoma cells by specific action and not simply by release from damaged cells.

It has been shown that CA-125 shedding varies with the cell cycle. Exponentially fast-growing cells or cells that are predominately in the S and G2-M show reduced CA-125 expression and shedding (8). Because in transformed cells taxanes lead to a block at the G2-M, the paclitaxel- or docetaxel-mediated increase in CA-125 secretion cannot be seen solely as a cell cycle-dependent phenomenon. Not only IFNs, interleukin-1β, tumor necrosis factor-α, and transforming growth factor-α but also taxanes may induce CA-125 expression (9, 22, 23). On the other hand, glucocorticoids and transforming growth factor β have been shown to suppress the release of this tumor marker in ovarian carcinoma cells (24, 25). Several endogenous, as well as exogenous factors, can modulate CA-125 expression. Therefore, CA-125 shedding is not a constitutive and stable process, but may be affected by the cell cycle and proliferation, as well as by various growth factors and chemotherapeutic drugs.

However, a major question remains whether the observed taxane-mediated induction of CA-125 in vitro will be of clinical importance. Van der Burg et al. (11) recently reported CA-125 to be an unreliable marker for response monitoring in patients with relapsed ovarian cancer. They observed marked CA-125 fluctuations, especially when the tumor marker was assessed at short intervals, i.e., weekly. However, other authors found a good correlation between clinical tumor and serological marker response in patients treated with either paclitaxel or docetaxel (15, 26, 27). One possible explanation for these discordant results could be the time schedule of tumor marker estimations. In contrast to Van der Berg et al. (11), who determined CA-125 weekly, others drew blood samples at end of chemotherapy treatment. The net effect of taxane-mediated induction of CA-125 and a fall of CA-125 by tumor regression may lead to either an elevation of serum CA-125 levels or instead show a decrease dependent on whichever effect predominates. CA-125 determination early after taxane treatment may be only minimally affected by a reduction of tumor mass and could, therefore, primarily reflect the induction effects of taxane treatment. Therefore, taxane treatment could impair the predictive value of CA-125 determination, particularly when the selected time interval between pretreatment and posttreatment checks is short, e.g., weekly, as demonstrated by Van der Burg et al. (11).

REFERENCES


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