Ovarian carcinoma cells with low levels of β-F1-ATPase are sensitive to combined platinum and 2-deoxy-d-glucose treatment

Emma Hernlund,1 Elisabet Hjerpe,1,2 Elisabeth Åvall-Lundqvist,1,2 and Maria Shoshan1

1Department of Oncology-Pathology, Cancer Center Karolinska R8, Karolinska Institute; 2Radiumhemmet Clinic, Karolinska Hospital, Stockholm, Sweden

Abstract

We have here examined chemopotentiating effects of glycolysis inhibitor 2-deoxy-d-glucose (DG) in two epithelial ovarian carcinoma (EOC) cell lines and 17 freshly isolated ascitic EOC cell samples, and we identify low expression of the β-F1-ATPase involved in mitochondrial ATP production as a candidate marker for sensitivity to this strategy. Although in the majority of samples, DG per se did not induce apoptosis, cotreatment with DG potentiated apoptosis and total antiproliferative effects of cisplatin and, to a lesser degree, carboplatin. In the cell lines, combination treatment with DG and cisplatin or carboplatin at non-inhibitory concentrations prevented posttreatment regrowth in drug-free medium over a total of 5 days. DG per se allowed complete recuperation in drug-free medium. The more platinum-resistant a cell line was, the more sensitive it was to potentiation by DG and showed higher IC50 for cisplatin by 68% and, in the most sensitive samples, up to 90%, and DG-mediated potentiation correlated with low expression of β-F1-ATPase. By contrast, cisplatin sensitivity did not correlate with β-F1-ATPase levels. The findings validate targeting cancer cell glucose metabolism for potentiating platinum chemotherapy in EOC and indicate that reduced β-F1-ATPase/oxidative phosphorylation distinguishes cells that are amenable to this strategy. [Mol Cancer Ther 2009;8(7):1916–23]

Introduction

Ovarian cancer is a major cause of cancer death in women. Whereas standard treatment (surgery and platinum-based combination chemotherapy) may be effective initially, the 5-year survival in epithelial ovarian carcinoma is only around 45% (1). Rapid relapse to platinum-resistant disease is often seen in fast-growing malignancies, whereas slower-growing, low-grade tumors may show better survival rates despite a primarily lower responsiveness to chemotherapy (1). New strategies overcoming platinum resistance and increase platinum antitumor effects are, thus, urgently needed.

Carboplatin and cisplatin are both DNA-damaging drugs, albeit with different clinical profiles and toxicities. Tumor cell resistance to these drugs may involve reduced cellular accumulation, altered drug metabolism, altered DNA repair, and up-regulated cell survival pathways (2). In addition, the altered energy metabolism in tumor cells is now increasingly acknowledged as an important and general factor in drug resistance and, indeed, tumor progression (3–5). A major alteration in energy metabolism is the increased dependence on aerobic glycolysis rather than mitochondrial oxidative phosphorylation for ATP production. This switch allows the tumor cell to survive under hypoxia, but also confers other survival or growth advantages, such as glucose-dependent antiapototic AKT signaling, and increased purine synthesis and NADPH reducing power via the pentose phosphate pathway (3, 6, 7). Other alternative modes of energy production may also be deployed by the tumor cell, e.g., glutaminolysis and fatty acid β-oxidation. Targeting tumor-specific cell energy metabolism has, therefore, become an important new strategy for improved and specific treatment (3, 8).

The glucose analogue 2-deoxy-d-glucose (DG) inhibits glycolysis because it is phosphorylated by hexokinase, the first enzyme in glycolysis, but is not further metabolized and thus acts as a titrator of glucose. Other effects of DG are also possible; it has been shown to prevent protein glycosylation and, thereby, induce an unfolded protein response/endoplasmatic reticulum stress (9). DG has been shown to be a radiosensitizer for pancreatic cancer cells (10) and to potentiate clinical radiation effects in gliomas (11). We have previously reported that DG can potentiate the antiproliferative effects of several different classes of antineoplastic agents, thereby showing that potentiation is not necessarily linked to DNA-damaging agents (12).
However, of the 18 agents tested, cell death induced by cisplatin or its derivative oxaliplatin was particularly well potentiated by DG (12, 13).

This finding prompted us to first examine DG sensitivity and DG-mediated potentiation of cisplatin and carboplatin effects in two human ovarian carcinoma cell lines. To investigate whether a molecular and metabolic profile could be correlated with sensitivity to DG and to potentiation in the cell lines, we also examined lactate production and expression of a set of proteins involved in energy metabolism. These proteins—the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the mitochondrial β-F1-ATPase involved in the ultimate and ATP-producing step of oxidative phosphorylation, and the mitochondrial marker Hsp60—have been shown by others to provide the basis for a bioenergetic cellular (BEC) index, which strongly correlates with tumor progression in several types of carcinoma (5, 7, 14). We therefore examined the possibility that the BEC index might also be a predictive marker for DG-mediated effects. This was based on the assumption that, although the BEC index is a prognostic rather than treatment-predictive factor, it also reflects a shift away from oxidative phosphorylation toward greater glycolytic dependence.

Obviously, human cell lines provide a convenient model for the present type of study. However, because they are not necessarily representative of the actual, often multiclonal, clinical tumor, we also examined the platinum and potentiation responses, as well as expression of BEC index proteins, in freshly isolated ascitic tumor cells from 17 epithelial ovarian carcinoma patients. The results indicate that DG represents a promising strategy for improving platinum responses in epithelial ovarian carcinoma and that loss of mitochondrial β-ATPase/oxidative phosphorylation may distinguish cells that are particularly amenable to such potentiation.

Materials and Methods

Cells

All cells were cultured in RPMI 1640 supplemented with 10% FCS, l-glutamine, and penicillin streptomycin, and cells were kept at +37°C in 5% CO2. Cells plated in plastic culture dishes were treated with drugs 1 d after plating, and the drugs were present throughout the indicated incubation periods unless otherwise indicated. The ovarian carcinoma cell lines SKOV-3 and CaOv-4 were used, and human ovarian tumor cells were freshly isolated from ascites. The study was approved by the Regional Ethics Committee of Stockholm (EPN Stockholm 2006/1361-31/4) and done with patient’s informed consent.

Cells in fresh ascites were pelleted, resuspended in PBS, and then separated on a discontinuous gradient consisting of (from the bottom) Lymphoprep, Lymphoprep/Krebs HEPES Ring solution 3:1, and Lymphoprep/Krebs HEPES Ring solution 1:2. Cells were centrifuged at 1,500 × g for 20 min. Tumor cells were collected at the interphase between the top and middle layer [Lymphoprep/Krebs HEPES Ring solution: 137 mmol/L NaCl, 5.4 mmol/L KCl, 0.34 mmol/L Na2HPO4, 0.35 mmol/L KH2PO4, 8 mmol/L MgSO4, 1 mmol/L HEPES (pH 7.4)].

Viability and Apoptosis Assays

Total antiproliferative effects were studied using the sulforhodamine B–based TOX6 kit from Sigma-Aldrich Sweden according to the manufacturer’s instructions. Briefly, cells were seeded in 96-well plates, and after overnight incubation, incubation drugs were added in fresh medium. After indicated incubation times, cells were fixed in the well by the addition of trichloroacetic acid to the medium followed by incubation for 1 h at +4°C. After washing with deionized water, cells were incubated with sulforhodamine B dye for 20 min and then washed with 1% acetic acid. After dissolution of the dye in 10 mmol/L Tris Base, the total protein content of each well was assessed as absorbance at 565 nm minus background. Results are represented as the ratio of absorbance in treated to nontreated samples.

Regrowth capacity was studied using the sulforhodamine B assay. Cells were seeded at low density in three 96-well plates. Cells in the first plate were fixed at the initiation of treatment (t = 0), the second after 48 h of treatment, and the third plate after an additional 72 h of regrowth opportunity in fresh drug-free medium. Resulting absorbances (total cell protein) were compared with that at t = 0.

Apoptosis was assessed after 24 h as accumulated levels of specifically caspase-3/caspase-7 cleaved cytokeratin-30 fragments in total lysates (cells + supernatant), detected using the ELISA-type M30-Cytodeath assay (PEVIVA; ref. 15).

Western Blots

Whole-cell lysates were subjected to gel electrophoresis and blotting onto polyvinylidene difluoride membranes for protein probing using an anti-GAPDH antibody (1:8,000; Cell Signaling Technology), anti-Hsp60 antibody (1:2,000; Abcam), and anti-β-ATPase (1:10,000; generously provided by J.M. Cuezva; refs. 5, 7, 14, 16). The probes were visualized using the appropriate secondary antibody (anti-rabbit or antimouse IgG; 1:15,000; Amersham Biosciences, Uppsala, Sweden) and SuperPico developing agent (Pierce Technologies). For molecular markers, See-Blue Plus2 (Invitrogen) was used. Images were captured using Fuji LAS-1000. The same membrane filter was in each case used for all three probes, and the shown experiments were repeated with similar results. To determine BEC indices, each image was opened to the same size in Adobe Photoshop, cropped to show only the areas of interest, adjusted to 12+ brightness and 9+ contrast and copied to Adobe Illustrator to create the figure. In Photoshop, the image was then inverted. The median signal in each band, represented by equal-sized areas cropped to contain only the band, was corrected for background signal in an adjacent, empty area of equal size and then used for the calculations. Some blots were analyzed also using Aida software, and the results were virtually identical. The signals observed in SKOV-3 cells were used for normalization of signals in the ascitic tumor cell samples.
Glucose Uptake

Glucose uptake was assessed as uptake of $^{18}$F-deoxyglucose (FDG). Cells were incubated with 0.5 MBq FDG per well for 60 min at 37°C in the presence or absence of 25 mmol/L glucose. After incubation, cells were washed extensively in cold PBS and lysed. Radioactivity in the lysates was quantified using $\gamma$-counter WIZARD 3″, Wallac 1480 (PerkinElmer Sweden AB). Protein concentrations were determined using the BCA Protein Assay (Thermo Scientific) and used for normalizing radioactivity between the samples.

Lactate Production Assay

Fresh medium was added to near-confluent cells, and after 24 h, lactate levels in supernatants from test samples and controls were determined using a spectrophotometric assay kit (BioVision) according to the manufacturer’s instructions.

Statistical Analysis

All data values are given as averages ± SE, unless SD is specifically indicated. Statistical significances were determined using Mann-Whitney $U$ test or Student’s $t$ test, as indicated.

Results

Cell Line Sensitivities to DG and Platinum Drugs

Two ovarian carcinoma cell lines (CaOv-4 and SKOV-3) were cultured in 96-well plates in the presence or absence of the glucose analogue DG at 0 to 10 mmol/L. The resulting survival levels after 48 h were assessed as remaining precipitable cellular protein and expressed as percentage of protein in control samples. Concentrations of DG resulting in 50% reduction in protein were calculated (Inhibitory Concentration; IC$_{50}$; Table 1A). The values of IC$_{50}$ for CaOv-4 and SKOV-3 cells were 14 and 18 mmol/L, respectively. Sensitivities to cisplatin and carboplatin were similarly assessed (Table 1A). Compared with SKOV-3, CaOv-4 cells were more resistant to cisplatin and carboplatin.

Table 1. Summary of drug data and protein expression

(A) Inhibitory concentrations (IC$_{50}$) of DG, cisplatin, carboplatin, and combinations

<table>
<thead>
<tr>
<th></th>
<th>CaOv-4</th>
<th>SKOV-3</th>
<th>Average for 17 ascitic samples ± SE (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG (mmol/L)</td>
<td>14</td>
<td>18</td>
<td>33* ± 16 (28.5)</td>
</tr>
<tr>
<td>Cisplatin ($\mu$mol/L)</td>
<td>35</td>
<td>12</td>
<td>25 ± 7.6 (23)</td>
</tr>
<tr>
<td>Cisplatin + 5 mmol/L DG</td>
<td>14</td>
<td>10</td>
<td>13 ± 11† (8)</td>
</tr>
<tr>
<td>Cisplatin + 10 mmol/L DG</td>
<td>5</td>
<td>7</td>
<td>NC</td>
</tr>
<tr>
<td>Carboplatin ($\mu$mol/L)</td>
<td>600</td>
<td>275</td>
<td>273$^3$ ± 49 (282)</td>
</tr>
<tr>
<td>Carboplatin + 5 mmol/L DG</td>
<td>280</td>
<td>300</td>
<td>See Results</td>
</tr>
<tr>
<td>Carboplatin + 10 mmol/L DG</td>
<td>90</td>
<td>140</td>
<td>NC</td>
</tr>
</tbody>
</table>

(B) Cisplatin potentiation by DG in ascitic tumor cell samples and ratios of $\beta$-F1-ATPase: Hsp60 expression in ascitic samples in Fig. 5

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Cisplatin IC$_{50}$ ($\mu$mol/L)</th>
<th>Cisplatin IC$_{50}$ in the presence of 5 mmol/L DG</th>
<th>$\beta$:H ratio, relative to SKOV-3 cells</th>
<th>BEC index, relative to SKOV-3 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>7</td>
<td>0.582</td>
<td>0.638</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>0</td>
<td>0.310</td>
<td>0.352</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>4</td>
<td>0.448</td>
<td>0.288</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>20</td>
<td>2.90</td>
<td>1.59</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>0</td>
<td>0.478</td>
<td>0.189</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>22</td>
<td>0.650</td>
<td>0.291</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>3</td>
<td>0.383</td>
<td>0.134</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>0</td>
<td>0.458</td>
<td>0.294</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>17</td>
<td>13.3</td>
<td>5.22</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>8</td>
<td>2.12</td>
<td>0.605</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>14</td>
<td>2.66</td>
<td>0.627</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>8</td>
<td>1.38</td>
<td>0.266</td>
</tr>
<tr>
<td>13</td>
<td>23</td>
<td>9</td>
<td>0.737</td>
<td>0.144</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>9</td>
<td>1.22</td>
<td>0.340</td>
</tr>
<tr>
<td>15</td>
<td>11</td>
<td>2</td>
<td>1.69</td>
<td>0.540</td>
</tr>
</tbody>
</table>

Abbreviation: NC, not calculated.

*Excluding three outliers (>100 mmol/L).
†Significantly different from cisplatin only ($P = 0.05$; Student’s $t$ test).
‡Excluding five outliers (>1 mmol/L).
We then treated the cell lines with cisplatin at 1 to 20 μmol/L in the presence or absence of 5 and 10 mmol/L DG. After 48 hours, the resulting survival levels were assessed (Fig. 1A and B). The overall effect of the combination treatment can be expressed as a DG-mediated decrease in the IC\textsubscript{50} of cisplatin. For CaOv-4 cells, 5 and 10 mmol/L DG reduced the IC\textsubscript{50} value for cisplatin from 35 μmol/L to 14 and 5 μmol/L, respectively (Table 1A). In SKOV-3 cells, at higher cisplatin doses, DG had no or small additional effect (Fig. 1B). Thus, 5 mmol/L DG had no or small effect on the cisplatin IC\textsubscript{50}, and 10 mmol/L reduced it from 12 μmol/L to 7 μmol/L (Table 1A).

The cell lines were similarly treated for 48 h with combinations of DG and carboplatin (Fig. 1C and D). In CaOv-4 cells, the IC\textsubscript{50} of carboplatin was in the presence of 5 mmol/L DG reduced from 600 to 280 μmol/L and with 10 mmol/L DG to 90 μmol/L (Table 1). In SKOV-3 cells, 5 mmol/L DG had no effect (Fig. 1D; Table 1A), whereas 10 mmol/L reduced the IC\textsubscript{50} from 275 to 140 μmol/L (Table 1A). DG potentiation was, thus, more prominent in CaOv-4 than in SKOV-3 cells.

**Inhibition of Outgrowth by Combination Treatment with DG**

The sulforhodamine B protein assay used here to assess survival does not discriminate between proliferating and arrested, but still viable, cells. To be truly efficient, treatment must also target the tumor cell capacity for recuperation and regrowth posttreatment. To investigate regrowth capacity after low-dose platinum ± DG treatment, cells were first treated with drug(s) for 48 h and then allowed to recuperate for 72 hours in fresh medium without drugs. The resulting cell densities were then compared with those at t = 0 and t = 48 h time points. As expected, cisplatin at 2 μmol/L and carboplatin at 16 μmol/L had no effects per se in either cell line (Fig. 2). At the 48-hour time point, the outcome of combination treatment was similar to that of DG per se. After release from drugs, cells treated with DG or with platinum resumed growth. Intriguingly, the combined DG + platinum treatment inhibited regrowth after release from drugs, including after carboplatin treatment (Fig. 2). We conclude that the combination treatments have antiproliferative effects within the first 48 h and that these are irreversible.

**Metabolic Characterization of the Cell Lines**

The BEC index is a prognostic marker for several types of carcinoma, with a low BEC value indicating worse prognosis (5, 14). Because a low BEC value also reflects a shift to higher glycolytic dependence, we asked whether it could predict sensitivity to DG and/or platinum combination...
treatment. Based on digital density scanning of Western blots of CaOv-4 and SKOV-3 lysates, the BEC indices of the cell lines were calculated by dividing the ratio of β-F1-ATPase to Hsp60 (hereafter designated β:H) with the GAPDH value. Based on three samples of each lysate, the average β:H ratio was 2.69 in SKOV-3 cells and 0.89 in CaOv-4. The BEC index was 0.09 in SKOV-3 and 0.03 in CaOv-4. This suggests that CaOv-4 cells have higher glycolytic activity and that SKOV-3 by contrast have higher mitochondrial/oxidative phosphorylation activity. The lower BEC index of CaOv-4 cells is also in line with their greater drug resistance.

To verify the difference in cellular glycolytic activity, as indicated by the difference in BEC index, we assessed uptake of glucose, seen as uptake of FDG, and accumulation of the glycolytic product lactate. Both assessments were normalized for cellular protein content in each sample. In accordance with the lower BEC index of CaOv-4 cells, their FDG uptake was 1.5-fold to 2-fold higher than in SKOV-3 (Fig. 3B) and lactate production was also twice as high (Fig. 3C). The inhibitory effect of 5 mmol/L DG on lactate production showed the effect of this drug on glycolysis and was far greater in CaOv-4 (Fig. 3C), in turn suggesting that lactate production in SKOV-3 may be more due to, e.g., glutaminolysis than to glycolysis.

Together, the data show that, compared with SKOV-3 cells, CaOv-4 are more resistant to platinum treatment, but also more sensitive to the combined DG + cisplatin treatment, and that this sensitivity correlates with a lower BEC index and a more glycolytic phenotype.

Effects of DG, Cisplatin, and Carboplatin per se in Ascitic Tumor Cells

To extend the study to fresh, multiclonal clinical material for evaluation of the therapeutic potential of glycolysis inhibition, we assessed the antiproliferative effects of platinum ± DG on tumor cells isolated from ascites from 17 patients at the Karolinska University Hospital. Treatment in vitro for 48 to 72 h with DG (1–10 mmol/L) had effects ranging from 0 to 50% reduction in survival (not shown). Calculation of IC50s for DG (48 h) showed that in 6 of 17 samples, it was <20 mmol/L. Three outliers had IC50s of >100 mmol/L. After elimination of these, the average IC50 was 33 ± 16 mmol/L (median 28.5 mmol/L; Table 1A). For cisplatin, the average IC50 was 25 ± 7.6 μmol/L (median 23 μmol/L), with no outliers. After elimination of five resistant outliers for which the IC50 values could not be determined, the average IC50 for carboplatin was 273 ± 49 μmol/L (median 282 μmol/L; Table 1A).

DG-Induced Potentiation of Platinum Antiproliferative Effects in Ascitic Tumor Cells

Ascitic tumor cell samples were treated with cisplatin (10 and 20 μmol/L, i.e., 0.4 and 0.8 of the average IC50 value) in the presence or absence of 5 mmol/L DG (0.17 of the average IC50 value), and survival fractions were assessed after 48 hours. A box plot summarizing the effects of DG on growth inhibition induced by 10 μmol/L cisplatin is shown (Fig. 4A). DG-mediated potentiation was also calculated as fold reduction in cisplatin IC50. The average cisplatin IC50 was reduced by 48%, and the median by 68% (Table 1A). By contrast, DG potentiated carboplatin responses only in 7 of 17 samples. In these, DG reduced the average carboplatin IC50 from 743 to 217 μmol/L (not shown).

DG-Induced Potentiation of Platinum-Induced Apoptosis in Ascitic Tumor Cells

We have earlier shown by a number of criteria that, in different tumor cell lines, cisplatin at 10 to 20 μmol/L induces acute, “classic” apoptosis via c-Jun NH2-terminal kinase/mitogen-activated protein kinase kinase kinase 1 and the mitochondrial pathway, leading to up to 50% nuclear fragmentation within 24 h (17–20). Here, acute apoptosis was quantitated after 24 h using the M30-Cytodeath assay. This assay is based on an antibody recognizing a neoeptope formed specifically by caspase cleavage of cytokeratin 18. Unlike dying cells, the resulting fragments are stable, wherefore the assay in effect quantitates accumulated apoptosis (15, 21).
of increased apoptosis, e.g., samples 3, 10, 12, 13, and 15. We, therefore, conclude that although DG may increase platinum-induced acute apoptosis, this is not the only mode of potentiation.

**BEC Indeces of Ascitic Tumor Cells**

Fifteen ascitic tumor samples were subjected to Western blot analysis of the three BEC index proteins and tubulin (Fig. 5A). A 16th sample on the blot later turned out to be not relevant with a non–epithelial ovarian carcinoma diagnosis. To enable comparison of results on the two filters required to accommodate the samples, one and the same SKOV-3 lysate was used as an internal standard on each filter. Signal intensity values obtained by density scanning of each blotted sample could thereby be normalized to SKOV-3 results on the same filter. The resulting values were used for calculation of BEC indices, i.e., the ratio of ($\beta$:H)/(GAPDH).

To test the hypothesis that tumor cells with low BEC indices are more sensitive to treatment with DG ± cisplatin, samples were first grouped based on the percentage decrease in cisplatin IC$_{50}$ in the presence of DG. Samples showing >50% decrease were designated highly potentiated, whereas samples showing low potentiation (LP) showed 50% or less. The highly potentiated group is characterized by homogeneously low BEC indices compared with the low potentiated group (Fig. 5B). However, the $\beta$:H ratio was found to correlate better than the BEC index with potentiation ($P = 0.028$, compared with $P = 0.075$ for the BEC index; Fig. 5C). Because the $\beta$:H ratio reflects $\beta$-F1-ATPase expression levels in relation to mitochondrial mass, this result suggests that, rather than an increase in glycolysis, as represented by higher GAPDH levels (14), reduced mitochondrial ATPase/oxidative phosphorylation capacity renders cells more sensitive to DG ± cisplatin.

In contrast to DG potentiation, sensitivity to cisplatin (based on IC$_{50}$s) did not correlate with either BEC or $\beta$:H ratios (Mann-Whitney U test, $P > 0.6$). When the seven samples showing DG-mediated potentiation of carboplatin were compared with the nonpotentiated samples, there was no correlation between potentiation and BEC or $\beta$:H values ($P > 0.3$).

**Discussion**

Cisplatin and carboplatin are widely used for treatment of ovarian cancer, but the therapeutic outcome is all too often blunted by rapid relapse to resistant disease. We show here for the first time that cotreatment with DG potentiates the growth inhibitory effects of, in particular, cisplatin and also carboplatin, in ovarian carcinoma cell lines, as well as in freshly isolated ascitic tumor cell samples. We examined whether sensitivity to this combination treatment correlated with the metabolism-based BEC index, which has earlier been shown to be a metabolic prognostic marker for several types of carcinoma (5, 14, 16). More than the BEC index, however, its component ratio of $\beta$-F1-ATPase to Hsp60 ($\beta$:H) was found to correlate with sensitivity to DG-mediated potentiation of cisplatin.
There are several possible mechanisms underlying the observed potentiation: inhibition of ATP-dependent DNA repair or glycolysis-dependent macromolecule syntheses, or via an autophagic program. Inhibition of ATP-dependent expulsion of drug via avidin-biotin complex transporters or reduced levels of cisplatin-titrating glutathione (22) are less likely mechanisms, at least in the cell lines, because DG could be added as late as 24 hours after platinum and still achieve a potentiation effect at 120 hours similar to that of concomitantly added DG (not shown). Most likely, potentiation is mediated by cell-dependent combinations of these mechanisms. One response to DG (4 mmol/L) in normoxic cells is the induction of the unfolded protein response due to incorrect protein glycosylation, which in sensitive cell lines led to massive apoptosis (23). Here, we see, with three exceptions, little apoptosis induction by DG at 5 mmol/L, and in the ascitic samples, the median IC₅₀ of DG was 28.5 mmol/L. Moreover, in the same work, sensitivity to DG-induced unfolded protein response did not correlate with defective mitochondrial function (respiration; ref. 23); this is also, in some, contrast to our results.

When assessed as reduction in platinum IC₅₀, and throughout the ascitic material, carboplatin potentiation was smaller, compared with cisplatin. This difference reflects differences in the molecular events induced by the two drugs. Other findings support the existence of such differences; we have, e.g., observed a general lack of acute apoptosis induction by carboplatin and a slower development of the total antiproliferative effect compared with cisplatin. Cisplatin is a highly reactive molecule, which we have reported to induce appreciable apoptosis also in cytoplasts, i.e., cells from which nuclei were removed before treatment (18, 20). In line with involvement of nonnuclear targets, cisplatin is highly reactive toward thiolis and bionucleophiles, including proteins, whereas carboplatin is less reactive in this respect (24, 25). These observations underscore the fact that platinum compounds do not constitute a mechanistically homogenous group. Nevertheless, in experiments on posttreatment, regrowth of tumor cells treated with noninhibitory drug concentrations revealed profound, long-term antiproliferative effects of combination treatment were found, and unexpectedly, the effect was apparent also with carboplatin at only 16 μmol/L. These experiments gave encouraging indications that the effects observed at the 48-hour time point do not represent the full range of potentiation and that DG may have an early and major effect on regrowth capacity after the DNA damage that both platinum drugs do induce.

To explain the great potentiability of cisplatin treatment, which we have reported also earlier (12, 13), it is in the present context interesting that cisplatin has been shown to bind directly to mitochondrial DNA and mitochondrial proteins,
including the voltage-dependent anion channel protein (26). Both effects may have profound effects on mitochondrial function by preventing expression of mitochondrial DNA-encoded respiratory chain proteins and by affecting the voltage-dependent anion channel protein–regulated metabolic coupling between cytosol and mitochondria (27), respectively. We have also noted a transient reduction in cellular ATP and respiration levels after treatment with cisplatin (ref. 13; not shown). Impaired respiration/oxidative phosphorylation clearly represent a mitochondrial defect that the cell must compensate for, by up-regulation of glycolysis, also under normoxic conditions; this renders the cell more sensitive to DG (28). We therefore propose that one reason for the efficiency of the DG + cisplatin combination is that cisplatin can further impair the oxidative phosphorylation capacity, whereby it increases the cellular sensitivity to glycolysis inhibition.

Targeting tumor cell glycolysis or glycolytic dependence has recently attracted considerable interest as a novel cancer therapeutic strategy (8). Several small-molecule agents that inhibit glycolysis are currently used as model substances for experimental studies and/or are being tested for drug development (6). Other experimental studies often involve single-drug use of energy metabolism inhibitors, but many reports and our own observations that DG-treated cells arrest and then resume growth in the absence of drug lead us to support the prediction that the main use of energy metabolism inhibitors will be as potentiators of chemotherapeutic drugs (6, 8). Although it remains to be established whether these drugs may also increase platinum side effects, we propose that this strategy would combine the tumor-specific effects of chemotherapy with the tumor specificity of energy metabolism inhibitors, resulting in better therapeutic outcome and/or fewer side effects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Prof. J.M. Cuezva for the kind gift of antibody β-F1-ATPase and for the interesting discussions, Dr. Suzanne Egyhazi for fruitful discussions and development of the present project, Marianne Frostdvik-Stolt for expertise in isolating ascitic tumor cells from ascites fluid, and Dr. Li Lu for helping us assess 18FDGuptake.

References

Ovarian carcinoma cells with low levels of $\beta$-F1-ATPase are sensitive to combined platinum and 2-deoxy-d-glucose treatment

Emma Hernlund, Elisabet Hjerpe, Elisabeth Åvall-Lundqvist, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-09-0179

Cited articles
This article cites 28 articles, 12 of which you can access for free at:
http://mct.aacrjournals.org/content/8/7/1916.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/8/7/1916.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.