Relationship of Cellular Glutathione to the Cytotoxicity and Resistance of Seven Platinum Compounds


Abstract

The role of glutathione (GSH) in the effectiveness of and resistance to 7 platinum compounds (5 Pt(II) and 2 Pt(IV) drugs) was evaluated in a 8.6-fold CDDP-resistant human small cell lung cancer cell line (GLC4/CDDP), the parent GLC4 line, a 3.7-fold CDDP-resistant human embryonal carcinoma cell line (Tera-CP), and the parent Tera line (NTera2/D1). Resistance factors for both CDDP-resistant cell lines were determined after continuous incubation (4 days) with CDDP.

Continuous incubation with the other studied platinum drugs revealed complete cross-resistance for carboplatin (CBDCA) and zeniaplatin but less for enolaplatin (ENLO) and iproplatin in both models. Tetraplatin and lobaplatin showed, respectively, partial and complete cross-resistance in GLC4/CDDP but no cross-resistance in Tera-CP.

GSH level, but not glutathione S-transferase activity, of the 4 cell lines correlated with platinum drug concentration inhibiting cell survival by 50% after continuous incubation. GSH depletion by DL-buthionine-S,R-sulfoximine (BSO) increased sensitivities, as measured after a 4-h exposure to the drugs, of GLC4/CDDP for CDDP 2.0-fold, for CBDCA 1.7-fold, for zeniaplatin 1.7-fold, and almost to the level of the sensitive GLC4 for ENLO, whereas no effect was observed for lobaplatin and the Pt(IV) compounds iproplatin and tetraplatin. BSO-modulating effect was higher in the sensitive GLC4 line for most compounds; therefore, reduction of resistance could be achieved only for CDDP and ENLO. In contrast to GLC4, no modulation occurred in Tera. In Tera-CP, BSO increased sensitivity for CDDP 1.5-fold, for CBDCA 1.9-fold, and for zeniaplatin 1.2-fold; no effect was observed for ENLO, lobaplatin, and the Pt(IV) compounds.

Reduction of CDDP resistance by BSO was known to occur with identical cellular platinum levels and higher Pt-DNA binding in GLC4/CDDP. However, pretreatment with BSO followed by a 4-h ENLO incubation increased cellular platinum levels in both GLC4 and GLC4/CDDP while Pt-DNA binding remained unchanged.

In conclusion, GSH reflected sensitivity to platinum-containing drugs. However, since the involvement of GSH differed between the models and the various platinum drugs, the effect of modulation with BSO was unpredictable.

Introduction

The development of acquired resistance to the useful anti-neoplastic agent CDDP with this drug encouraged the development of new platinum-containing drugs.

In vitro, studies have demonstrated that cellular resistance to CDDP is multifactorial. Mechanisms involved include: altered membrane transport; inactivation of the drug by cellular thiols such as GSH and metallothioneins; decreased Pt-DNA binding; and/or an increased Pt-DNA repair (1). The role of the GSH system in CDDP resistance has received much attention, because CDDP as well as other platinum-containing compounds are electrophilic agents which are most reactive towards sulfur-containing nucleophiles. A correlation between the GSH level and sensitivity to, e.g., CDDP has been reported in human tumor cell lines expressing a range of in vitro sensitivities to CDDP (2, 3). Elevated GSH levels have been found in CDDP resistant cell lines (4–13), and BSO, a specific inhibitor of the GSH synthesis, can act as an useful modulator of resistance to nucleophilic agents such as CDDP in vitro (6, 9).

The exact mechanism, however, by which GSH influences platinum-induced cytotoxicity and participates in resistance to various platinum-containing drugs is, as yet, not fully elucidated and may differ between various test models. The present study evaluated the role of GSH in the effectiveness of and the resistance to seven platinum-containing drugs in two models of CDDP resistance: a human embryonal carcinoma cell line, Tera, representing a sensitive tumor in the clinic; and a human small cell lung cancer cell line GLC4, representing a sensitive tumor type prone to resistance in the clinic, as well as their sublines with in vitro-acquired CDDP resistance, Tera-CP and GLC4/CDDP. Compared to the parent line, GSH levels were 1.4- and 2.5-fold higher in, respectively, Tera-CP and GLC4/CDDP, and GST activity was 1.5-fold higher in Tera-CP but the same in GLC4/CDDP. Platinum drug cytotoxicity was tested in the absence and presence of BSO pretreatment. Five Pt(II) drugs, namely, CDDP, the less toxic CBDCA, three platinum compounds recently introduced in clinical studies (ZENI, ENLO, and LOBA), as well as two Pt(IV) drugs, CHIP and one of the DACH compounds (TETRA) were studied (Fig. 1). For ENLO, the platinum drug most effectively modulated by BSO in this study, the role of GSH was further investigated by measuring the effect of BSO-induced GSH depletion on cellular platinum levels and Pt-DNA binding.

Materials and Methods

Chemicals. CDDP, CBDCA, and CHIP were kindly provided by Bristol Myers S.A.E. (Weesp, the Netherlands), ZENI and ENLO by Lederle Laboratories (Pearl River, NY), LOBA by ASTA (Frankfurt, Germany), and TETRA by Upjohn (Kalamazoo, MI). RPMI 1610 was obtained from Life Technologies (Paisley, United Kingdom) and FCS from Sanbio (Uden, the Netherlands). DME and Ham's F-12 medium were purchased from Flow Laboratories (Irvine, United Kingdom). GSH, BSO, and (3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were obtained from Sigma (St. Louis, MO), and dimethyl sulfoxide from Merck (Darmstadt, Germany).
were incubated continuously with the platinum drugs in a total volume of 2.5 × 10⁶ cells for 4 h with ENLO concentrations ranging from 0.45 to 2.7 mm. The cells were washed three times with PBS at 4°C. The pellets were dissolved in 0.5 ml 65% HNO₃ in an oven at 70°C for 2 h, and the amount of platinum in the samples was measured (atomic absorption spectrophotometry) as described before (6). Pt-DNA was measured after treating 5 × 10⁶ GLC₄ and GLC₄/CDDP cells with ENLO concentrations ranging from 0.45 to 2.7 mm for 4 h. The cells were washed three times with PBS at 4°C. DNA was isolated, and the DNA content (absorption at 260 nm) and the amount of platinum in the samples were measured (atomic absorption spectrophotometry) as described before (6). At least three independent experiments were performed for both cellular platinum levels and Pt-DNA binding at each concentration.

RESULTS

Survival curves of GLC₄, GLC₄/CDDP, Tera, and Tera-CP after continuous incubation with CDDP are shown in Fig. 2. Resistance factors were calculated from the drug concentrations inhibiting cell growth by 50%. IC₅₀ values were 1.2, 10.3, 0.69, and 2.6 μM for, respectively, GLC₄, GLC₄/CDDP, Tera, and Tera-CP, corresponding with a resistance factor of 8.6 for GLC₄/CDDP and 3.7 for Tera-CP.

Table 1 shows the cross-resistance for the platinum-containing drugs tested in the two CDDP resistance models. After continuous incubation complete cross-resistance for CBDCA and ZENI, but less for ENLO and the Pt(IV) compound CHIP, was observed in both resistant cell lines. TETRA showed partial cross-resistance in GLC₄/CDDP and no cross-resistance in Tera-CP. LOBA showed full cross-resistance in GLC₄/CDDP, whereas no cross-resistance could be observed in Tera-CP.

There was a significant correlation between the amount of GSH/mg cellular protein of the four cell lines and the IC₅₀ values after continuous incubation with the various platinum drugs in the cell lines (average correlation coefficient: r = 0.86; P < 0.05) (Fig. 3). All correlations between the amount of GSH/mg cellular protein of the four cell lines and the IC₅₀ values for the individual drugs were significant (Spearman rank correlation coefficient range, 0.67–0.97; P < 0.05). There was a significant correlation between the amount of GSH/mg cellular protein of the four cell lines and the IC₅₀ values after continuous incubation with CDDP.
neither a positive correlation between GST activity and IC₅₀ values for the individual platinum drugs after continuous incubation in the four cell lines nor an averaged correlation (r = -0.13, not significant).

Table 1 shows the effect of BSO-induced GSH depletion on the cytotoxicity of the tested drugs for GLC₄ and GLC₄/CDDP expressed as IC₅₀ after 4 h incubation with the various platinum drugs. BSO-induced GSH depletion increased sensitivity of GLC₄/CDDP for CDDP, CBDCA, and ZENI and did this almost to the level of the parent GLC₄ for ENLO. No effect was observed for the two Pt(IV) drugs. Except for CDDP and ENLO, dose modifying factors were higher in the sensitive GLC₄ cell line. An also increased sensitivity for the two Pt(IV) compounds, CHIP and TETRA, could be observed in this cell line. No effect of GSH depletion on the sensitivity for LOBA was found in either cell line. Reduction of resistance could be achieved for CDDP and ENLO. Table 3 shows the effect of BSO-induced GSH depletion on the cytotoxicity of the drugs for Tera and Tera-CP expressed as IC₅₀ after 4 h incubation with the various platinum drugs. BSO-induced GSH depletion increased sensitivity of Tera-CP for CDDP, CBDCA, and ZENI whereas no effect was observed for ENLO, LOBA, and TETRA. In the sensitive Tera cell line, GSH depletion had no effect on the sensitivity of any platinum drug. Reduction of resistance could be achieved for CDDP, CBDCA, and ZENI. Table 4 gives a survey of the resistance factors found in the two tested models of CDDP resistance after the various experiments.

For ENLO, the most promising BSO-modulated platinum drug in this study, the role of GSH was further evaluated by measuring the effect of BSO-induced GSH depletion on cellular platinum levels and Pt-DNA binding in GLC₄ and GLC₄/CDDP. The cellular platinum content, corrected for cellular protein or cellular volume, did not differ between GLC₄ and GLC₄/CDDP after incubation with CDDP [data published previously (5)] or ENLO. Pretreatment of cells with BSO did not alter the CDDP-induced cellular platinum content in GLC₄ and GLC₄/CDDP [data published previously (6)]. However, pretreatment with BSO increased ENLO-induced cellular platinum content in both cell lines (Fig. 4). The amount of Pt-DNA binding induced by CDDP [data published previously (5)] as well as ENLO was for both drugs significantly lower in GLC₄/CDDP than in GLC₄. Following BSO pretreatment, an increased CDDP-induced Pt-DNA binding in GLC₄/CDDP was observed whereas the Pt-DNA binding in GLC₄ remained the same. For ENLO, no effect of BSO pretreatment on Pt-DNA binding could be observed in GLC₄ and GLC₄/CDDP (Fig. 5).

**DISCUSSION**

In the present study we evaluated the role of GSH in the effectiveness of and resistance to CDDP as well as six other platinum analogues in two models of CDDP resistance in order to obtain more insight in the interaction of GSH with the different drugs and the involvement of GSH in resistance to these drugs.

In accordance with the literature (8–10, 14–18) complete cross-resistance to CBDCA was found in both CDDP-resistant cell lines. Cross-resistance to CBDCA has been described for normal (10, 14–16) and elevated cell GSH status (8–10).

**Table 2** Effect of BSO-induced GSH depletion on the cytotoxicity of the drugs for GLC₄ and GLC₄/CDDP after a 4 h incubation (n ≥ 3)

<table>
<thead>
<tr>
<th>IC₅₀ (µM)</th>
<th>GLC₄ control + BSO DMF</th>
<th>GLC₄/CDDP control + BSO DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP</td>
<td>3.0 1.9 1.6ᵃ</td>
<td>27.3 14.0 2.0ᵇ</td>
</tr>
<tr>
<td>CBDCA</td>
<td>62.1 31.8 2.0ᵃ</td>
<td>695 404 1.7ᵇ</td>
</tr>
<tr>
<td>ZENI</td>
<td>7.1 3.7 1.9ᵇ</td>
<td>41.1 23.8 1.7ᵇ</td>
</tr>
<tr>
<td>LOBA</td>
<td>3.7 2.8 1.3ᵇ</td>
<td>20.0 26.2 0.8ᵇ</td>
</tr>
<tr>
<td>ENLO</td>
<td>115 41.5 2.8ᵃ</td>
<td>472 148 3.2ᵇ</td>
</tr>
<tr>
<td>CHIP</td>
<td>41.6 14.3 2.9ᵇ</td>
<td>161 122 1.3ᵇ</td>
</tr>
<tr>
<td>TETRA</td>
<td>2.6 1.7 1.5ᵃ</td>
<td>0.15 11.7 1.3ᵇ</td>
</tr>
</tbody>
</table>

ᵃ p < 0.05 with BSO pretreatment versus control.
b Not significant with BSO pretreatment versus control.

**Table 3** Effect of BSO-induced GSH depletion on the cytotoxicity of the drugs for Tera and Tera-CP after a 4 h incubation (n ≥ 3)

<table>
<thead>
<tr>
<th>IC₅₀ (µM)</th>
<th>Tera control + BSO DMF</th>
<th>Tera-CP control + BSO DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP</td>
<td>2.3 2.2 1.0ᵃ</td>
<td>6.7 4.4 1.5ᵇ</td>
</tr>
<tr>
<td>CBDCA</td>
<td>44.9 34.5 1.3ᵇ</td>
<td>90.0 47.7 1.9ᵇ</td>
</tr>
<tr>
<td>ZENI</td>
<td>4.2 4.8 0.9ᵇ</td>
<td>5.6 4.7 1.2ᵇ</td>
</tr>
<tr>
<td>LOBA</td>
<td>3.4 2.9 1.2ᵇ</td>
<td>7.2 5.9 1.2ᵇ</td>
</tr>
<tr>
<td>ENLO</td>
<td>82.7 82.0 1.0ᵇ</td>
<td>95.6 88.6 1.1ᵇ</td>
</tr>
<tr>
<td>CHIP</td>
<td>17.0 15.5 1.1ᵇ</td>
<td>21.1 16.6 1.3ᵇ</td>
</tr>
<tr>
<td>TETRA</td>
<td>3.2 2.4 1.3ᵇ</td>
<td>3.6 3.1 1.2ᵇ</td>
</tr>
</tbody>
</table>

ᵃ Not significant with BSO pretreatment versus control.
b P < 0.05 with BSO pretreatment versus control.

**Table 4** Survey of the resistance factors for the two models of CDDP resistance after the continuous and 4 h incubation experiments

<table>
<thead>
<tr>
<th>Resistance Factor</th>
<th>Continuous incubation</th>
<th>4 h incubation</th>
<th>Continuous incubation</th>
<th>4 h incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP</td>
<td>8.6 9.1</td>
<td>3.7 2.9</td>
<td>8.6 9.1</td>
<td>3.7 2.9</td>
</tr>
<tr>
<td>CBDCA</td>
<td>8.3 11.2</td>
<td>6.1 2.0</td>
<td>8.3 11.2</td>
<td>6.1 2.0</td>
</tr>
<tr>
<td>ZENI</td>
<td>11.0 5.8</td>
<td>3.1 1.3</td>
<td>11.0 5.8</td>
<td>3.1 1.3</td>
</tr>
<tr>
<td>LOBA</td>
<td>7.2 5.3</td>
<td>1.4 2.1</td>
<td>7.2 5.3</td>
<td>1.4 2.1</td>
</tr>
<tr>
<td>ENLO</td>
<td>2.6 4.1</td>
<td>2.0 1.2</td>
<td>2.6 4.1</td>
<td>2.0 1.2</td>
</tr>
<tr>
<td>CHIP</td>
<td>2.5 3.9</td>
<td>2.1 1.2</td>
<td>2.5 3.9</td>
<td>2.1 1.2</td>
</tr>
<tr>
<td>TETRA</td>
<td>3.6 5.8</td>
<td>1.3 1.1</td>
<td>3.6 5.8</td>
<td>1.3 1.1</td>
</tr>
</tbody>
</table>

ᵃ Not significant with BSO pretreatment versus control.
b P < 0.05 with BSO pretreatment versus control.
ZENI showed complete cross-resistance in both models whereas for ENLO partial cross-resistance was found. As ZENI and ENLO differ only in their carrier ligand, with structure and charge of ZENI most similar to CBDCA, carrier ligand specificity of resistance may play a role for these compounds as it does for the DACH ligand. Interestingly, for LOBA, full cross-resistance was observed in GLC4/CDDP but none in Tera-CP. The existence of at least two platinum resistance phenotypes in human ovarian carcinoma cell lines has been reported (24). A difference in platinum resistance phenotype between GLC4/CDDP and Tera-CP may explain their differential response to LOBA, although cross-resistance patterns for the other platinum compounds hardly differ. As yet no data concerning GSH status and cross-resistance for ZENI, ENLO, and LOBA are reported.

CDDP can react with thiols such as GSH and therefore may play a role in determining cellular sensitivity to platinum-containing compounds by preventing platination of critical loci. We found a close correlation between the amount of GSH, but not between the GST activity, and sensitivity for the various platinum drugs in our cell lines.

BSO was used to investigate a possible causal role of GSH in the resistance to platinum-containing drugs. The involvement of GSH differed between the models and the various platinum drugs. BSO did not affect the CDDP sensitive Tera, whereas in Tera-CP it reduced the resistance for the Pt(II) compounds CDDP, CBDCA, and ZENI. Because almost no cross-resistance could be observed after a 4 h incubation for ENLO, CHIP, and TETRA, no modulating effect of BSO could be expected. Reduction of one-half of the CDDP resistance in Tera-CP may be ascribed to BSO-induced GSH depletion. In this model of CDDP resistance the increased GSH content, probably in combination with the increased GST activity although this cannot be proven statistically with the data from this study, seems to be a relevant mechanism, responsible for maximal 50% of the resistance, that is operational for CDDP and other platinum-containing drugs. In the GLC4/CDDP model the role of the increased GSH as a cause of resistance is less obvious. BSO-induced GSH depletion increased the sensitivity of the sensitive GLC4 line, for most platinum drugs tested, more effectively than in the GLC4/CDDP line. Reduction of resistance could be achieved only for the Pt(II) compounds CDDP and ENLO. The fact that a 7.4-fold CDDP resistance remains after BSO pretreatment underscores the limited importance of GSH as CDDP resistance mechanism in this model. An effect of differences in the potential for rebound synthesis of GSH in various cell lines, in particular between resistant and parental cell lines (6, 25), cannot be excluded in our experiments as drug treatment after BSO exposure was performed in the absence of GSH inhibition. In the future, examination of the regulation of the GSH synthesis merits further investigation.

A substantial increase in CHIP but not in CDDP or CBDCA cytotoxicity was reported in two murine cell lines after GSH depletion (26). Mistry et al. (2) found a better effect of BSO for Pt(IV) than for Pt(II) drugs. They suggested a more important role for GSH as mechanism of resistance to Pt(IV) than to Pt(II) drugs. In our study, however, dose modifying factors did not differ between Pt(II) drugs and Pt(IV) drugs.

An earlier study with GLC4 and GLC4/CDDP suggested two roles for GSH in CDDP resistance, namely, a cytosolic elimination resulting in less DNA platination and a nuclear effect on the formation and repair of Pt-DNA adducts (6). The amount of CDDP-induced Pt-DNA was lower in GLC4/CDDP than in

CHIP and TETRA showed partial to no cross-resistance in GLC4/CDDP and Tera-CP. Platinum drugs containing the DACH carrier ligand, such as TETRA, received much attention because of their potential to overcome CDDP resistance (8, 11, 15, 19–21). For the DACH carrier ligand as mechanism of resistance a carrier ligand-specific decrease in accumulation (11, 15, 20, 22), increase in ability to tolerate specific Pt-DNA lesions (20, 22), preferential DNA repair (20, 22), and an effect on the inhibition of DNA synthesis (23) has been reported. Selective activity of TETRA might also be attributed to the circumvention of detoxification systems such as GSH metabolism (12). For Pt(IV) compounds, it has been assumed that reduction to Pt(II) is required for activity. GSH can activate Pt(IV) compounds resulting in their reaction with DNA (19). If this mechanism plays a role, this cannot be in disadvantage of sensitivity to Pt(IV) drugs in resistant cells with elevated GSH levels. Most studies with CDDP-resistant cell lines report partial or complete cross-resistance, with unchanged (14) or elevated GSH levels (8, 9), to CHIP (8, 9, 14, 17).
GLC4. BSO pretreatment did not affect cellular platinum levels but increased CDDP-induced Pt-DNA binding in GLC4/CDDP but not in GLC4. For ENLO, the drug most efficiently modulated by BSO in this study, GLC4/CDDP showed also a lower DNA platination compared to GLC4. Surprisingly, BSO pretreatment increased ENLO-induced cellular platinum content almost 2-fold, in GLC4 and GLC4/CDDP, however, without an effect on Pt-DNA binding. This may occur at longer drug exposure time, explaining the increased cytotoxicity in both cell lines after BSO pretreatment. Increased cellular drug accumulation by BSO was also reported for other non-platinum drugs such as daunorubicin and the camptothecin analogue CPT-11 (27, 28). The mechanism behind this phenomenon still must be elucidated.

In conclusion, in the present study GSH seems to be a good reflector of sensitivity to platinum-containing drugs and therefore a good target for modulation in the clinic. Phase I studies using BSO with non-platinum drugs are under way (29, 30). However, the fact that the involvement of GSH differs between various drugs and models makes a general causative role of GSH in sensitivity and/or resistance to platinum-containing drugs questionable and the effect of modulation with BSO unpredictable. Despite the fact that BSO can be an excellent modulator of platinum sensitivity in vitro, care should be taken not to generalize the option of GSH as target for modulation in the clinic.

REFERENCES

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