Resistance Mechanisms Following Cisplatin and Oxaliplatin Treatment of the Human Teratocarcinoma Cell Line 2102EP

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Abstract. *Background: Oxaliplatin and cisplatin are widely used in cancer chemotherapy, however, their clinical efficiency is often limited by the development of resistance. Materials and Methods: We examined different mechanisms of resistance in the human teratocarcinoma cell line 2102EP following exposure to cisplatin or oxaliplatin. Cells were exposed ten times with IC_{90}-doses of 30 μM cisplatin and 50 μM oxaliplatin, respectively. Different cell clones were tested for expression of resistance using the SRB-assay. Moreover, resistance mechanisms in terms of drug uptake, platinum-adduct formation, GSH metabolism, DNA mismatch repair and p53 protein function were investigated. Results: Three cisplatin cell clones with significant resistance factors of 2.0 to 2.6 were found. Two oxaliplatin cell clones showed only weak resistance, with resistance factors of 1.6 and 1.7, respectively. In all three cisplatin-exposed cell clones a decreased cellular uptake of cisplatin was found. Furthermore, mechanisms of DNA damage tolerance may also play a role in the development of cisplatin-resistance in these cells. However, only two cell clones showed a decreased level of platinum-DNA-adducts. An increased DNA-repair of platinum-DNA adducts was not seen. In addition, no differences in expression of mismatch-repair proteins MSH2 and MLH1, tumor suppressor protein p53, or glutathione metabolism were found. However, significant resistance mechanisms for the observed oxaliplatin resistance could not be identified, although in one oxaliplatin-exposed cell clone, there was some evidence that a decreased cellular uptake of oxaliplatin may contribute to the observed low level resistance. Conclusions: The data add weight to the hypothesis that resistance mechanisms following oxaliplatin exposure may be similar to cisplatin. The precise mechanisms of resistance in the oxaliplatin-resistant cell clones are still not fully understood and current studies are underway to further eluciate this finding.*

Cisplatin, an effective chemotherapeutic agent for the treatment of many cancers, was successfully introduced as an antitumor agent in 1970s (1). Although many patients initially respond to treatment, the development of primary and secondary resistance is a limiting factor for the clinical efficiency of this anticancer drug. This has prompted a search of new generations of platinum coordination compounds as well as a more complete understanding of the cellular mechanisms underlying resistance. Of the new generation platinum compounds those with 1,2-diaminocyclohexane (DACH) carrier ligand have been focused upon in recent years (2). Amongst the DACH-Pt-compounds, oxaliplatin (trans-L-dach (1R, 2R-diaminocyclohexane) oxalatoplatinum) is of special interest since earlier screening studies at the National Cancer Institute (USA) suggested that oxaliplatin belongs to a distinct cytotoxic family, differing from cisplatin and carboplatin (3). Several mechanisms of platinum resistance have been identified. Decreased accumulation and/or increased efflux is one of the most frequent mechanisms of cisplatin resistance and may be one of the earliest resistance mechanisms to develop in cell lines exposed to cisplatin repeatedly (5, 6). Increases in metallothionein, glutathione and/or glutathione S-transferase have been reported in many cisplatin-resistant cell lines (7). Increased nucleotide excision repair activity is also an important mechanism of platinum resistance. However, both prokaryotic and eukaryotic nucleotide excision repair complexes have very broad specificity (8). In terms of cellular DNA repair, there also appears to be little or no specificity for the repair of Pt-DNA adducts with cis-diammine, ethylenediamine(en), or DACH carrier ligands (8). Post-replication repair is best

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defined as the ability to replicate past bulky DNA adducts without introducing gaps or discontinuities into the DNA (9, 10, 11). As defined, post-replication repair is not a "true repair" process, since the damaging adduct is not actually removed from DNA (replicative bypass). This replicative bypass is enhanced in several cisplatin-resistant cell lines, but not in the replicative bypass of oxaliplatin-adducts (8). Recent experiments have suggested that defects in mismatch repair (MMR) can also lead to cisplatin resistance, which occurs frequently during the acquisition of cisplatin resistance in cell culture and has been incriminated in resistance occuring after repeated cycles of cisplatin in clinical trials (12). The defect in MMR allows resistant cells to tolerate DNA damage and replicate, instead of undergoing cell-cycle arrest or cell death. Recently, Fink *et al.* (13) have shown that colon carcinoma cell lines either defective in hMLH1 or hMSH2 MMR enzymes are 1.5 to 2-fold more resistant to cisplatin, but display little or no resistance to oxaliplatin. Moreover, several laboratories have shown that MMR complexes recognize cisplatin diadducts, but not DACH-Pt diadducts in DNA (14). Based on those results, it has been suggested that this particular resistance mechanism may account for the differential cytotoxicity profile between DACH- and cis-diammine-Pt compounds. The participation of oncogenes and tumor suppressor proteins in platinum resistance has been discussed in several reviews (15). The p53 protein acts as a tumor suppressor protein and is an important part of the stress response in cells (16, 17). Activation of this protein can induce different pathways, that culminate in cell cycle arrest or in apoptosis. The loss of p53 activity would allow damaged cells to survive following expose to platinum compounds.

DACH-Pt-complexes are effective in some, but not all, cisplatin-resistant cell lines (8). Thus, it is important to determine which resistance mechanisms affect platinum compounds with the cis-diammine carrier ligand (cisplatin and carboplatin), but not platinum compounds with the DACH carrier ligand.

In the study presented here, we investigated different resistance mechanisms following exposured of the human tumor cell line 2102EP to cisplatin and oxaliplatin in terms of drug uptake, formation and removal of platinum-DNA adducts, glutathione metabolism, DNA mismatch repair, and defects in the p53 protein function.

Materials and Methods

Cell lines. The parental cell line 2102 EP was initially derived from an untreated patient as the primary tumor and classified histologically as a teratocarcinoma with yolk sac tumor (18). The cell line and the established sublines were maintained in RPMI 1640-medium (Biochrom) supplemented with 10% fetal calf serum (FCS, Biochrom) at 37˚C with 5% CO_2. Cisplatin was purchased from Sigma Chemicals (Munich), oxaliplatin was kindly donated from Sanofi (Berlin, Germany).

Table I. *IC_{50}-dose and resistance factors of cell clones examined.*

Cell clone	IC_{50} dose (cell clone/2102EP)	Resistance factor
10xCisplatin clone A	7.2±1.3 / 3.4±0.9	2.2±0.2 ($p<0.005$)
10xCisplatin clone B	11.5±4.0 / 5.8±2.0	2.0±0.0 ($p<0.0001$)
10xCisplatin clone E	12.3±6.8 / 4.9±2.8	2.6±0.3 ($p<0.01$)
10xOxaliplatin clone A	21.3±8.4 / 13.1±4.5	1.7±0.4 ($p<0.05$)
10xOxaliplatin clone B	29.7±11.2 / 18.0±4.0	1.6±0.5 ($p>0.05$)

In terms of cisplatin resistant sublines, cells were exposed ten times to 30 μM cisplatin (IC_{90}-dose), and 50 μM (IC_{90}-dose) oxaliplatin, respectively. Cell clones were tested for their resistance and five cell clones were chosen for testing of resistance mechanisms. Routine culture conditions of the drug-resistant subclones and their properties (population doubling times, DNA and protein content), once established, were identical to those of the parental cell line.

Cytotoxicity assays. Cytotoxicity of cisplatin and oxaliplatin was measured using the sulforhodamine B (SRB) assay developed by Skehan *et al.* (19). 3,000 cells were seeded into wells of 96 well plates (Falcon) and allowed to attach overnight. Different concentration of the two platinum agents were added for two hours. Cells were then washed with RPMI-medium without FCS. The plates were incubated with normal RPMI-medium and 10% FCS for 96 h.

After incubation cells were fixed with 10% trichloracetic acid and stained with SRB. For each assay, SRB was freshly dissolved in 10 mM Tris and measured with an ELISA reader (SLT-rainbow) at 540 nm. The absorbance for each dose was expressed as a percentage of the control of untreated cells.

Cellular uptake. The parental cell line and the cisplatin resistant cell clones were treated with increased concentrations of cisplatin (20; 30; 40 and 50 μM) for 2 h. For each concentration, 5 x 10^6 cells were seeded a day before. Following drug exposure, the cells were harvested, counted and washed three times in ice-cold PBS. Cell pellets were dried and dissolved in concentrated nitric acid. Platinum uptake was measured by atomic absorption spectrophotometry (AAS; Perkin-Elmar-4100ZL). Oxaliplatin-treated cell clones and 2102EP were exposed with increasing oxaliplatin concentrations (25; 50; 75 and 100 μM) for 2 h. Drug uptake was measured as described already for the measurement of cisplatin uptake.

Quantitation of platinum-DNA adducts. For quantitation of cisplatin-DNA adducts, the immunological methods of Tilby *et al.* [20] was used. Logarithmically-growing cells were treated with 30 μM cisplatin for 2 h. At different intervals (0h; 1h; 8h; 24h and 48h) following treatment the cells were harvested and washed with PBS. Cell pellets were stored at –20˚C. The DNA was isolated using the Blood kit of Quiagen. Pt-DNA adducts were detected using the ELISA technique as described by Tilby *et al.* (20, 21). The primary antibody was a monoclonal rat IgG antibody (CP 9/19, kindly provided by M. J. Tilby) dissolved in PBS with 1% BSA and 20% Tween 20. The secondary antibody was a sheep antirat IgG
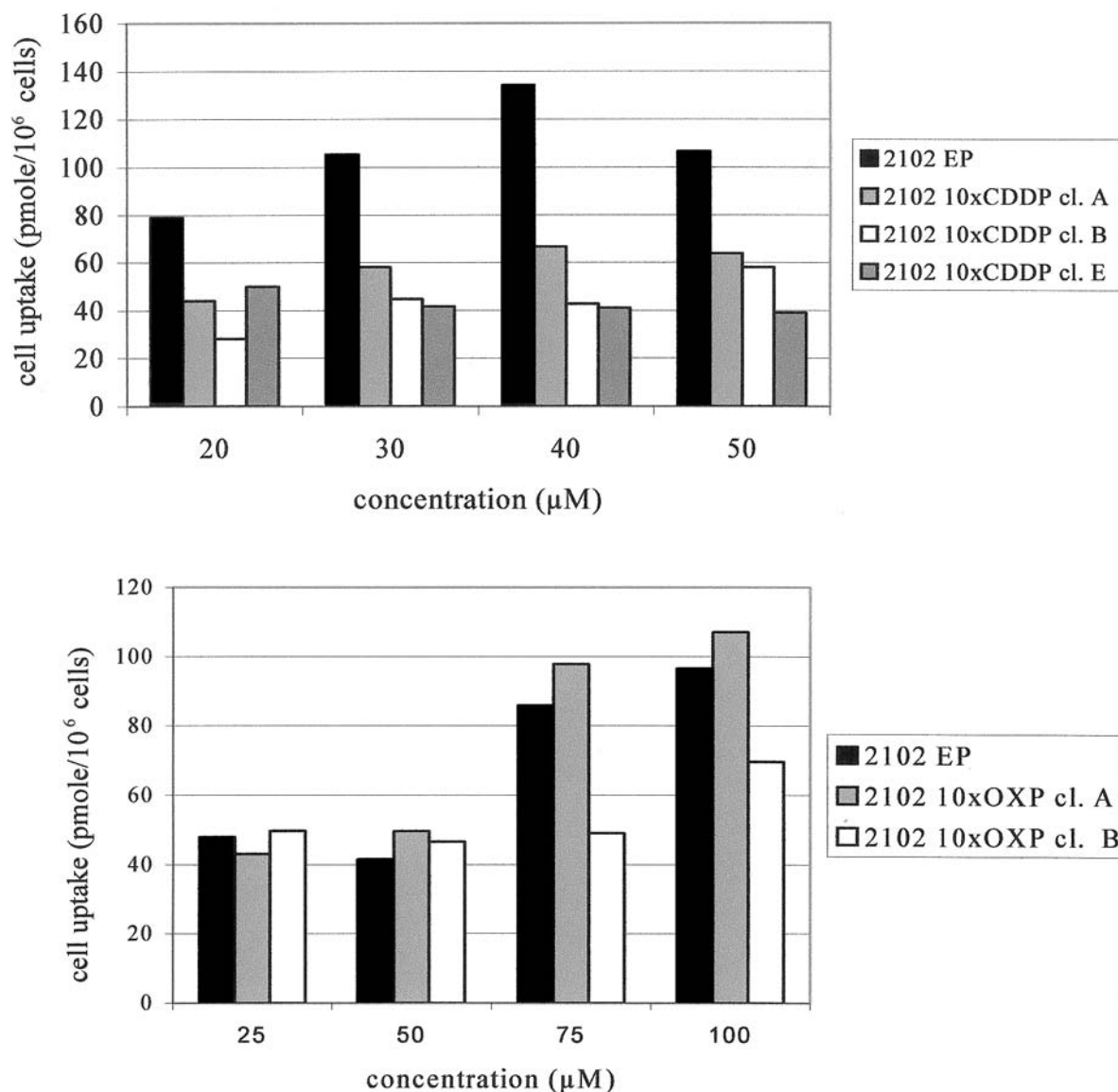
Figure 1. *Cell uptake (pmole/10⁶ cells) of cisplatin (CDDP) in cisplatin-resistant cell clones (panel A), of oxaliplatin (OXP) in oxaliplatin-resistant cell clones (panel B), and in the parental cell line 2102EP. Diagrams show the amount of drugs within the cell. Cells were exposed to cisplatin or oxaliplatin for 2 h, respectively.*

antibody with ß-galctosidase link (Sigma). After treatment with ß-galactosidase streptavidin conjugate, 4-methyl-umbelliferyl-ß-D-galactoside (Sigma; 80 μg/ml in PBS plus 10 mM MgCl$_2$) was added and probes were then analysed with a fluorescence plate reader (Fluoroscan, 355/460 nm). Platinum-DNA adduct levels were corrected for dilution by DNA synthesis as described earlier (40).

GSH and related enzymes. Logarithmically-growing cells were harvested, cell pellets washed and approximately 1 x 10⁷ cells were lysed with 990 μl Aqua dest.. 0.3% sulfosalicyl acid (10 μl) was used for protein precipitation, and the supernatant was used for measurement. GSH content was assayed using the methods of Meister (22). GST-activity was measured using the method of Habig

and Jakoby (23). Protein was assayed by the method of Bradford (24). *Western Blotting.* The cell lines were exposed with 30 μM cisplatin and 50 μM oxaliplatin for 2 h. After different times (0h, 24h, 48h) cells were harvested. A total of 10⁷ of treated and untreated cells were resuspended in 1 ml RIPA lysis buffer (100 mM NaCl, 0,5% Igepal, 0.5% SDS, sodium deoxycholate, 50 mM Tris [pH 8] containing 1 mM PMSF, 0.1 mM DTT and 50 μl protease inhibitor [Sigma]). After centrifugation at 13,000 rpm for 15 minutes the protein content of the supernatant was estimated by the Bradford protein-assay. Equal amounts of protein (60 μg) were separated in 8% (for MSH2) or 12% (for MLH1 and p53) SDS-PAGE (45 minutes, 200 V), and were transferred to nitrocellulose membrane (Bio-Rad) by electroblotting. Blots were stained with Ponceau S. Blots were pre-blocked in PBS
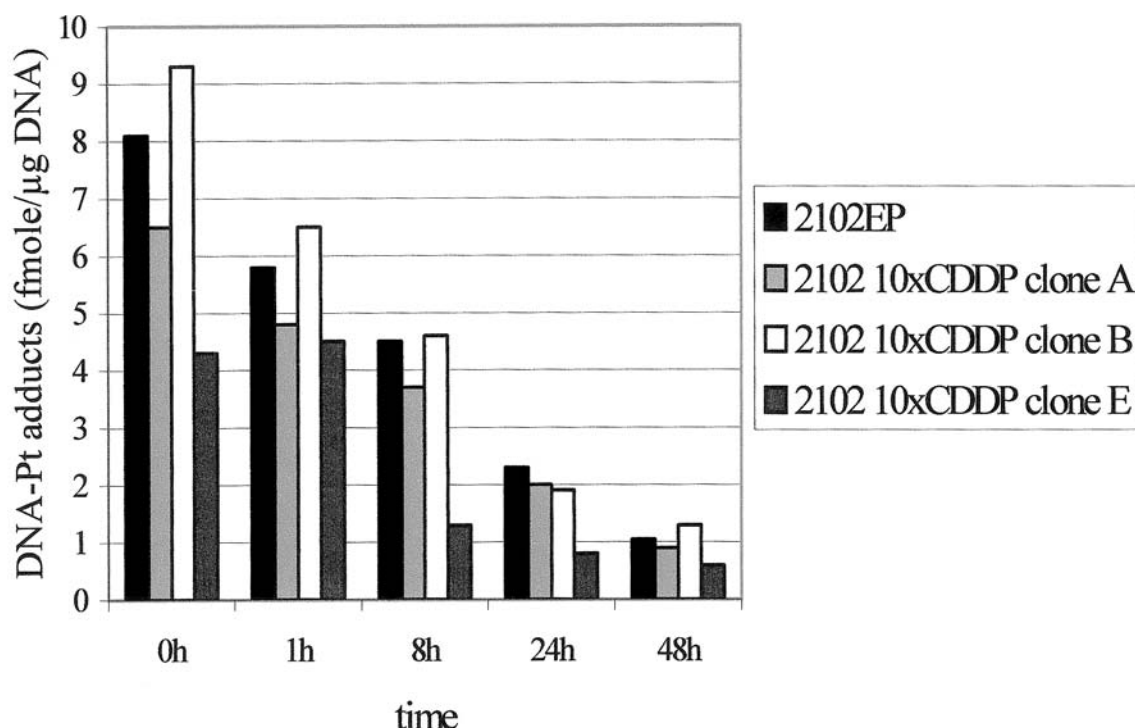
Figure 2. *Platinum-DNA adducts (fmole/μg DNA) following cisplatin exposure of cisplatin-resistant cell clones and the parental cell line. Cells were exposed to cisplatin (CDDP) for 2 h, and zero levels represent the time immediately thereafter.*

Table II. *Induction and removal of platinum-DNA adducts (fmole/μg DNA) following cisplatin exposure of 2102EP cells and drug-resistant subclones A, B, and E at different posttreatment incubation periods.*

Cell line	0 h	1 h	8 h	24 h	48 h	Repair rate after 24 h
2102EP	8.1	5.8	4.6	1.6	1.1	19.8%
Clone A	6.5	4.8	4.1	2.0	0.9	30.1%
Clone B	9.3	6.5	4.6	1.9	1.3	20.4%
Clone E	4.3	4.6	1.3	0.8	0.5	18.6%

with 0.1% Tween 20 (PBST) and 5% dried nonfat milk for 1 h. The following primary monoclonal mouse IgG antibodies were used for detection: MSH2: Ab-1 (Oncogene research products); MLH1: G168-15 (PharMingen), p53: Do-7 (Santa Cruz). The blots were treated with primary antibodies for 2 h in PBST with 5% dried nonfat milk. Blots were washed three times with PBST and membranes were then incubated with the secondary polyclonal peroxidase-conjugated anti-mouse IgG antibody (Santa Cruz) in PBST with 1% dried nonfat milk for 1 h and washed again. For detection the enhanced chemiluminescence (ECL) procedure according to manifacture's protocol (Amersham) on Hyper-ECL-Film (Amersham) was used.

Results

Cisplatin and oxaliplatin cytotoxicity assays. As comparative value for resistance we used IC_{50}-dose of cisplatin and oxaliplatin from full dose response curves of SRB-assays. The IC_{50}-doses and the resistance factors are shown in Table I. A total of nine cell clones after cisplatin exposure and three cell clones of oxaliplatin-treated cells were tested. These three cisplatin-treated cell clones A, B and E showed a significant degree of resistance, with resistance factors of 2.0 to 2.6. Two oxaliplatin-treated cell lines (cell clone A and B) expressed only a weak level of resistance to oxaliplatin when compared with the parental cell line 2102EP.

Cellular uptake of cisplatin and oxaliplatin. Results of the uptake experiments with cisplatin and oxaliplatin are shown in Figure 1. For each measurement, cells from two different exposures were pooled. These three cisplatin-resistant cell clones showed an decreased cellular uptake (Figure 1A). With increased levels of cisplatin concentrations, a linear increase between 20 μM and 40 μM in cell lines 2102EP, cisplatin-resistant clone A and B, but not cell clone E was found. Drug uptake following oxaliplatin treatment did not showed a clear difference between the two cell clones and the parental cell line at lower doses (Figure 1B). However,

Table III. *Levels of glutathione (nmole/10^6 cells), and activity of glutathione S-transferase (GST) (U/mg protein).*

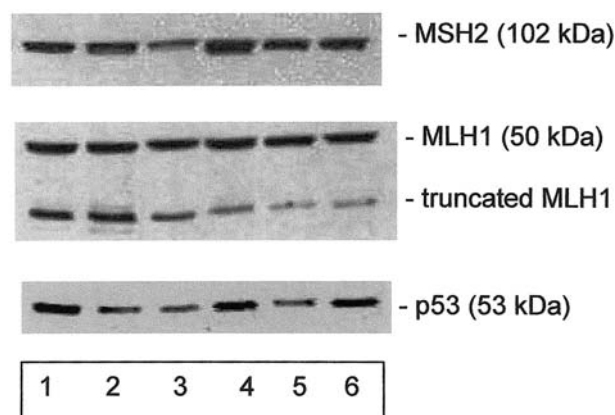| Cell line	Gluthatione	Activity of GST
2102 EP	4.5±1.1	76.1±30.4
2102 10xCisplatin clone A	6.7±0.8	66.2±11.9
2103 10xCisplatin clone B	5.5±1.0	82.1±16.9
2104 10xCisplatin clone E	6.3±1.2	79.1±8.8
2102 10xOxaliplatin clone A	5.4±0.2	77.1±22.1
2103 10xOxaliplatin clone B	5.7±0.3	67.5±12.4

Figure 3. *Expression of the proteins MSH2, MLH1 and p53 without drug exposure of the cell line 2102EP, cisplatin-resistant clones A, B, E, and oxaliplatin-resistant clones A and B. Equal amounts of protein (60 μg) were loaded on each lane. Lane 1: 2102EP 10xCisplatin clone A; lane 2: 2102EP 10xCisplatin clone B; lane 3: 2102EP 10xCisplatin clone E; lane 4: 2102EP; lane 5: 2102EP 10xOxaliplatin clone A; lane 6: 2102EP 10xOxaliplatin clone B.*

in the resistant clone B a reduced uptake was found at 75 μM and 100 μM oxaliplatin.

Measurement of cisplatin-DNA adducts. The level of cisplatin-DNA adducts could only be measured following cisplatin exposure, since the level of oxaliplatin-DNA adducts was below the detection limit of AAS (2 pmole) and the antibody for cisplatin-DNA adducts did not detect the oxaliplatin adducts. The level of cisplatin-DNA-adducts and the repair is shown in Figure 2. DNA of two experiments was pooled and measured in duplicate. In all cell lines removal of DNA lesions was found. However, levels of cisplatin-induced DNA-adducts immediately after cisplatin exposure (0 h) showed a great difference with adduct levels ranging from 9.3 fmole/μg DNA in clone B to 4.3 fmole/μg DNA in clone E (Table II). Moreover, the repair rate following 24 h posttreatment incubation was comparable between the different cell lines. Only in the resistant clone A a decreased repair rate was found (30.1%), however, values did not reach the level of statistical significance (Student's *t*-test).

Glutathione and related enzymes. Levels of total glutathione and GSH activity are shown in Table III. Experiments were performed in triplicates. No significant differences between the cell lines examined were found.

Measurement of MSH2, MLH1 and p53. In our study we examined the expression of MSH2, MLH1, and p53 by Western blot analysis in 2102EP parental cells and in the drug-resistant subclones (Figure 3). Both, the parental cells and the subclones expressed the two major mismatch repair proteins MSH2 and MLH1 and the p53 protein. However, there were no differences between the parental cell line and the cisplatin- and oxaliplatin-resistant subclones.

Following exposure of drug-resistant cells to cisplatin or oxaliplatin, an increase of p53 expression in all cell lines was found. In the more resistant oxaliplatin subclone A p53 appeared to become strongly activated already 2 h after addition of oxaliplatin to the cells. Levels of MSH2 and MLH1 where unaltered in all cell lines (Figures 4 and 5).

Discussion

Oxaliplatin is a third generation platinum complex that has shown activity in several tumors including metastatic colorectal cancer (25) and ovarian cancer (26). Oxaliplatin shows activity in a number of cell lines which exhibit intrinsic or acquired resistance to cisplatin (8). Studies carried out in cell lines of the NCI anticancer drugs screening panel comparing oxaliplatin and other platinum agents have shown that cisplatin and oxaliplatin have different sensitivity profiles, suggesting that the two complexes may have different mechanism(s) of action and/or resistance (27). In terms of oxaliplatin resistance, there are only a few experimental studies published so far and the results are controversial (Table IV). In the present study, therefore, we have attempted to gain further understanding of the oxaliplatin resistance phenotype. Thus, we have developed oxaliplatin-resistant cell clones in our laboratory by repeated exposures to oxaliplatin. This procedure was adopted in an attempt to mimic the manner of administration oxaliplatin (or cisplatin) to patients with testicular cancer. Two of these oxaliplatin-treated clones expressed resistance to oxaliplatin whereas three cisplatin-exposed clones expressed cisplatin resistance. The level of cisplatin and oxaliplatin resistance shown under these experimental conditions was relatively modest, but the effect was reproducible. Similar low levels of platinum resistance have been reported in other human tumor cell lines and are considered to be characteristic of platinum coordination complexes and possibly other alkylating agents (28) and may reflect the clinical situation more realistically.
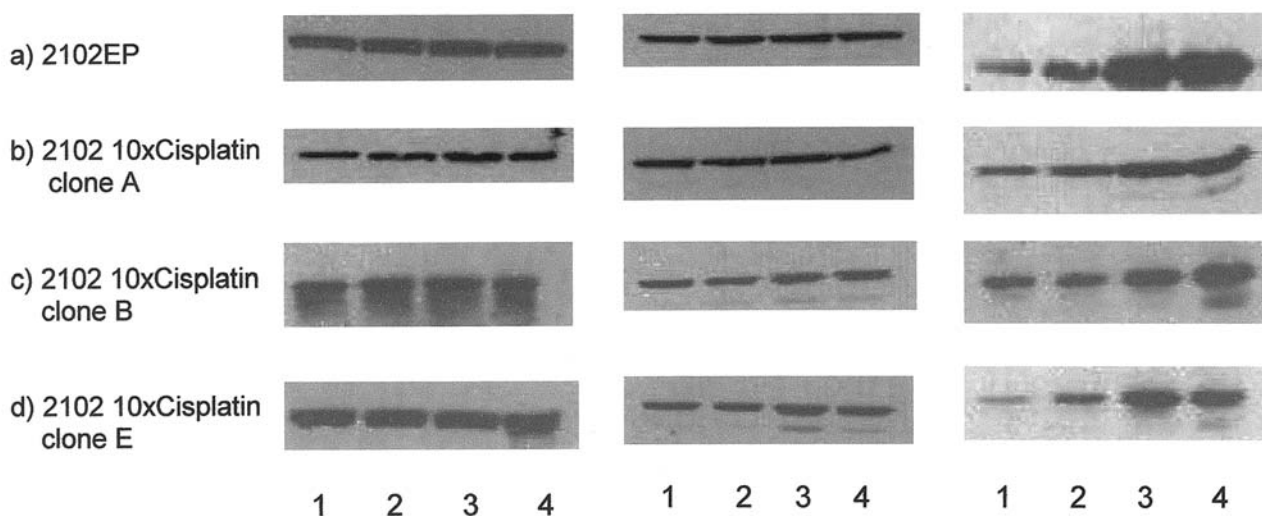
Figure 4. *Expression of MSH2 (left), MLH1 (middle) and p53 (right) after treatment of cisplatin-resistant clones A, B, E and the parental cell line with 30 μM cisplatin for 2 h. Lane 1: control without treatment; lane 2: 0 h after treatment; lane 3: 24 h after treatment; lane 4: 48 h after treatment.*
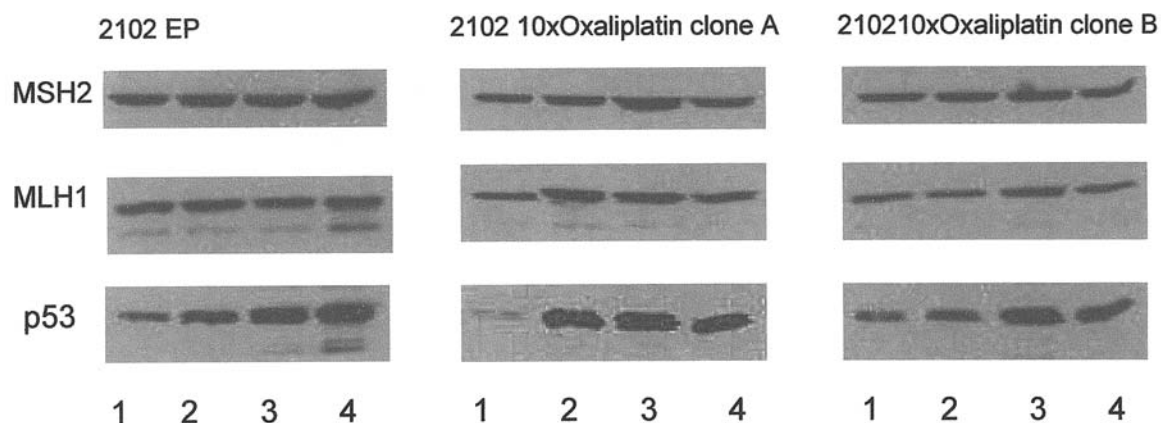

Figure 5. *Expression of proteins MSH2, MLH1 and p53 following 2 h treatment with 50 μM oxaliplatin of oxaliplatin-resistant clone A, B and the parental cell line 2102EP. Lane 1: control without treatment; lane 2: 0 h after treatment; lane 3: 24 h after treatment; lane 4: 48 h after treatment.*

The data obtained in this study indicate that at equimolar concentrations of treatment there was more drug accumulation after cisplatin exposure that after oxaliplatin exposure in the resistant clones. This is in agreement with results recently published by Hector *et al.* (29) who also found significantly more cisplatin accumulation than oxaliplatin accumulation in the human ovarian carcinoma cell lines A2780. However, the molecular mechanisms underlying cisplatin or oxaliplatin uptake remain unclear. Although some studies reported altered expression of cisplatin-binding proteins to be associated with cisplatin resistance (38), this was not seen in the majority of cisplatin-resistant cell lines or in oxaliplatin-resistant cells. To date, there is no evidence that both platinum derivatives can be brought out of the cell by active efflux mechanisms. *In vitro* studies have shown that oxaliplatin produces qualitatively similar DNA-Pt adducts as cisplatin with predominantly intrastrand crosslinks (GG > AG). These studies have also shown that at equimolar concentrations, oxaliplatin produces fewer DNA lesions than cisplatin (30, 31). These observations suggest that, relative to cisplatin, oxaliplatin can exert its cytotoxicity through a lower intracellular drug concentration. The bulky oxaliplatin-DNA adducts presumably result in more interference with the replication and/or transcription process affecting cell survival. Although cisplatin is known to react with DNA with a delayed kinetics (maximum platination levels 4-6 h after pulse-exposure), an increase of DNA platination 1 h after removal of the drug

Table IV. *Comparison of resistance mechanisms in published oxaliplatin resistant cell lines (MEDLINE serach from 1994-2004).*

Cell line	Resistance factor	Mechanism(s)	Reference
Ovary (A2780)	8-12-fold	increased γ-glutamyl-transpeptidase (→ GSH↑)	El-akawi *et al.* [33]
Colon (HCT116)	28-68-fold	reduced apoptosis (bax expression ↓)	Gourdier *et al.* [34]
Ovary (A2780)	25-fold	decreased oxaliplatin uptake	Hector *et al.* [29]
Colon (HCT116, HT29) Ovary (A2780, CH1)	3-15.8-fold	p53 mutation (phe172) loss of hMLH1	Sharp *et al.* [35]
Colon (various cell lines)	3.1-7.6-fold	decreased oxaliplatin uptake decreased adduct formation	Mishima *et al.* [36]
Testis (2102EP)	1.6-1.7-fold	decreased oxaliplatin uptake	this study

was not seen in our study suggesting that kinetics mechanisms may also be dependent on the cell lines tested.

A reduction in the interaction between cisplatin or oxaliplatin and DNA could be caused by an elevation of GSH and/or the activities of the related enzymes (*e.g.* GST). Elevated GSH levels have been associated with platinum resistance in several cell lines, but not in others. Furthermore, the modified GSH levels have been associated with either no change or significant elevations in the activity of certain GSH-associated enzymes (reviewed in (32)). Intracellular inactivation of cisplatin or oxaliplatin *via* these mechanisms, however, would not appear to be implicated in our drug-resistant cell clones since neither GSH levels nor GST activities were found to be altered. Similar results have been detailed by Arnould *et al.* (37) who also found no correlation between oxaliplatin resistance and GSH metabolism. Resistance to cisplatin in all three subclones was associated with significantly reduced drug uptake, whereas only in the oxaliplatin-resistant clone B a reduced drug uptake was found at higher oxaliplatin concentrations. Although reduced drug uptake has been implicated in some platinum-resistant cell lines (29, 38, 39, 40), the frequent lack of correlation between the extent of uptake and the degree of cisplatin or oxaliplatin resistance in many other reports (reviewed in (32)) suggests that there may be no simple relationship between these two parameters.

Enhanced DNA repair as a mechanism of platinum resistance has been detected in certain cisplatin-resistant cell lines, but not in others (reviewed in (32)). However, these studies also provided evidence that the extent of repair did not correlate directly with the degree of drug resistance. Using *in vitro* repair assays, Reardon *et al.* (41) reported similar extents of repair for cisplatin, oxaliplatin, and JM216 lesions in several cell lines with different cisplatin sensitivities suggesting that Pt-DNA adducts induced by these drugs are removed from DNA with similar *in vitro* efficiencies by the nucleotide excision repair pathway. In the three cisplatin-resistant cell clones reported here, there were no significant differences in the repair of cisplatin-DNA adducts. Only the resistant subclone appeared to remove cisplatin-DNA adducts less efficiently. Although this was statistically not significant it is conceivable that these cells may tolerate cisplatin-induced DNA damage. Due to the fact that the antibody used to detect Pt-DNA adducts cannot detect oxaliplatin-DNA adducts, it is still unclear whether repair of oxaliplatin-DNA lesions may contribute to the observed drug resistance. In a recently published study, Hector *et al.* (29) have examined DNA-repair following oxaliplatin exposure in the human ovarian carcinoma cell line A2780 and its oxaliplatin-resistant subline A2780/25. They found no significant difference in terms of removel of oxaliplatin-induced DNA adducts between these two cells lines suggesting that enhanced DNA repair may not contribute to the observed oxaliplatin-resistant phenotype. In contrast, in human ovarian carcinoma cell lines oxaliplatin resistance was accompanied by defects in drug uptake, DNA adduct formation and DNA damage tolerance. However, these changes were not correlated with the degree of resistance. Thus, it is possible that the accumulation of cisplatin- or oxaliplatin-induced DNA-damage in certain genomic regions, caused by localized repair heterogeneity, may be more important than overall levels of DNA damage or repair. It has been postulated that a functional MMR complex recognizes cisplatin-DNA adducts, but not oxaliplatin-DNA adducts and that MMR proteins are involved in mediating apoptotic responses to DNA damage (42, 43, 44). Molecular modeling studies have revealed distinct differences between the structures of cisplatin and oxaliplatin-DNA-adducts supporting this hypothesis (45). In MMR-proficient and -deficient cells, a differential activation of damage response pathways has been shown for cisplatin but not for oxaliplatin (45). Other studies have indicated that MMR defects may contribute to increased replicative bypass of cisplatin adducts (8). Loss of mismatch repair leads to tumor cell resistance by desensitizing cells to specific DNA-damaging agents. In our study we did not observe any differences in terms of MSH2 and MLH1 protein expression in the drug-resistant clones when compared to the parental line suggesting that altered MMR mechanisms may not contribute to the observed drug resistance. This is in line with a recently

published paper where also no influence of MMR mechanisms on the cisplatin resistance was demonstrated (46). In contrast, however, Sharp *et al.* (35) observed a loss of hMLH1 and a p53phe172 mutation in an oxaliplatin-resistant A2780 subline (1.7-fold resistant). This was not seen in other more resistant sublines, and re-introduction of hMLH1 caused no significant change in the sensitivity to oxaliplatin. Most recently, Zdraveski *et al.* (47) have demonstrated that the E. coli MMR protein MutS recognized cisplatin-modified DNA with twofold higher affinity in comparison to the oxaliplatin-modified DNA suggesting that the differential affinity of MutS for DNA modified with different platinum compounds could provide the molecular basis for the distinctive cellular responses to cisplatin and oxaliplatin. An important protein for triggering apoptosis following cisplatin exposure of cells is p53. Furthermore, there is some evidence that p53 mutations are associated with an abnormal expression of the MMR protein MSH2 (42) and other MMR proteins (48). In addition, p53 is thought to be involved in MMR and thereby in mechanisms associated with tolerance of DNA damage (42). In contrast, Brown and Wouters (49) stated in a recently published review that neither p53 status nor the ability of cells to undergo apoptosis appear to play a significant role in the sensitivity or resistance of these cells to DNA-damaging agents, a conclusion, which is contrary to the widely held tenet that tumor cells with mutations in p53 and/or that are resistant to apoptosis are also resistant to cancer treatment. In our study we did not observe any difference of p53 expression in the drug-resistant sublones when compared to the parental cell lines. Assuming that p53 upregulation is related to the level of DNA strand breaks, it is unclear why p53 became strongly activated 2 h after oxaliplatin exposure in the oxaliplatin-resistant subclone A. However, the anti-p53 monoclonal antibody Do-7 used in our experiments, cannot discriminate between wild-type and mutated p53 proteins. Thus, it is conveivable that a mutated (inactive) p53 protein may modulate DNA repair processes and thereby confer drug resistance. Recently, the contribution of apoptotic mechanisms to the resistance to oxaliplatin has been investigated (34). Using different oxaliplatin-resistant sublines of the human colon carcinoma cell lines HCT116 a reduction in apoptosis was found to be associated with oxaliplatin resistance (loss of bax expression). However, this was only observed in the most resistant subline (68-fold) and not in the sublines with lower resistance levels. Examination of the impact of loss of p53, bax, caspase-3 and/or MMR on the platinum-induced cell cycle checkpoint activation, p53 induction, ability of the cell to tolerate adducts in its DNA, and the rate of repair of platinum from genomic DNA indicated the effects of the loss of p53 and/or MMR on all these parameters, suggesting a multifactorial etiology for the changes in sensitivity to the cytotoxic and mutagenic effects of platinum compounds.

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