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JM216-, JM118-, and cisplatin-induced cytotoxicity in relation to platinum-DNA adduct formation, glutathione levels and p53 status in human tumour cell lines with different sensitivities to cisplatin

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Abstract

The aim of this study is to establish anti-tumour potency of the new oral platinum drug JM216 and its metabolite JM118 in relation to the platinum (Pt)-DNA adduct formation, glutathione (GSH)-levels, and p53 status in human cancer cell lines with different sensitivities to cisplatin (CDDP). These parameters were studied in the CDDP sensitive human germ cell cancer cell line Tera and the small-cell lung cancer cell line GLC_4 and their sublines with *in vitro* acquired CDDP resistance, Tera-CP and GLC_4-CDDP, in a human ovarian cancer cell line transfected with mutant p53 (A2780/mt273) and with an empty vector as control (A2780/cmv), and in the intrinsic CDDP resistant human non-small-cell lung cancer cell line SW1573/S1 and colon carcinoma cell line Caco-2. Cytotoxicity was tested with the microculture tetrazolium (MTT)-assay. Pt-DNA adduct levels were assessed immunocytochemically. Quantitative analysis was performed by double fluorescence video microscopy. Results were correlated with GSH levels and p53 status of the cell lines. This study showed that both JM216 and JM118 can partially circumvent intrinsic and acquired resistance to CDDP. Drug-induced cytotoxicity only correlated negatively with GSH levels for JM216 and CDDP in the tested unselected cell lines. At equimolar basis, JM216 induced lower levels of Pt-DNA adducts in the various cell lines than JM118 and CDDP, whereas the JM118-induced amount and pattern of Pt-DNA adducts was comparable to CDDP. No difference in initial Pt-DNA adducts levels was observed between cell lines sensitive, acquired or intrinsic resistant to CDDP suggesting a Pt-resistance mechanism based on tolerance or increased repair, rather than decreased initial Pt-DNA adduct formation.

Keywords: JM216; JM118; Cisplatin; Pt-DNA adducts; Glutathione; p53

1. Introduction

In the past two decades, CDDP has proven its activity against several solid tumours such as testicular, ovarian, head and neck and small-cell lung cancer and became a widely used anticancer drug. However, several tumours are intrinsic resistant to CDDP or acquire resistance to CDDP during chemotherapy. Resistance can be caused by several cellular mechanisms. *In vitro* studies have demonstrated that these mechanisms include: altered membrane transport, inactivation of the drug by cellular thiols such as GSH and metallothioneins, decreased Pt-DNA binding, tolerance for Pt-DNA adducts, increased DNA repair or combinations of these mechanisms [1–5]. More recently, activation of the programmed cell death route (apoptosis) is recognised as an event of major importance in drug cytotoxicity with p53 as one of the key proteins involved [6,7]. In order to circumvent CDDP resistance and to reduce side effects, several new Pt-based drugs have been developed. Recently, JM216 (bis-acetato-ammine-dichloro-cyclohexylamine-platinum(IV)) has been developed (Fig. 1) as an oral Pt drug. After oral administration, a

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Abbreviations: AAS, atomic absorption spectrophotometry; BSA, bovine serum albumin; CDDP, cisplatin; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GSH, glutathione; IC_{50}, concentration inhibiting 50% of cell growth; MTT-assay, microculture tetrazolium assay; PBS, phosphate buffered saline (0.15 M NaCl, 7.7 mM $Na_2HPO_4{\cdot}2H_2O$, 1.6 mM KH_2PO_4, pH 7.35); Pt, platinum; RF, resistance factor.

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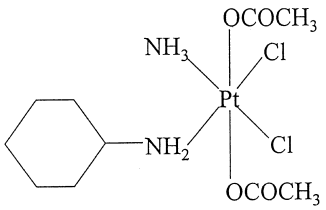
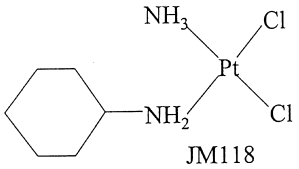
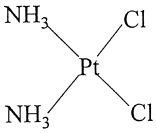


JM216



JM118



CDDP

Fig. 1. Chemical structures of JM216, JM118 and CDDP.

rapid and complete conversion of JM216 into at least seven different Pt-containing metabolites is observed [8,9]. JM118 (Fig. 1) and to a lesser account JM383 are the two major metabolites in human plasma. Human phase I studies revealed leucopenia and thrombocytopenia as dose limiting toxicities and mild non-haematological toxicities [10,11]. A phase II study with JM216 in small-cell lung cancer showed a tumour response rate of 38% [12]. *In vitro*, JM216 has shown activity against both Pt-sensitive and -resistant human tumour cell lines, obtained from small-cell lung cancer [13], cervical squamous cell carcinoma [14], and ovarian carcinoma [15]. The present study evaluated whether cytotoxicity and circumvention of Pt-resistance by JM216 or its metabolite JM118 is related to formation of different amounts of Pt-DNA adducts in comparison to CDDP or whether GSH levels or p53 status are of importance in explaining different Pt sensitivities. This was investigated in a panel of human cancer cell lines consisting of Pt-sensitive and -resistant cell lines with either acquired or intrinsic resistance to CDDP.

2. Materials and methods

2.1. Chemicals

CDDP, JM216 and JM118 were purchased from Bristol–Myers Squibb. RPMI 1640 medium, DME medium, Nutrient HAM F12 medium, fetal calf serum (FCS), and trypsin were obtained from Invitrogen-Life Technologies. Phosphate buffered saline (PBS) was made fresh in our own laboratory. GSH, glutathione reductase, 5,5 dithiobis(2-nitrobenzoic acid) (Ellman reagents), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) and protease were obtained from Sigma. Dimethyl sulfoxide and NADPH were purchased from Merck, fluorescein isothiocyanate (FITC) conjugated swine anti-rabbit antibody from Dakopatts, Hoechst 33258 from Calbiochem, and immunofluor mounting medium from ICN Biomedicals. Bovine serum albumin (BSA) was provided by CLB (Amsterdam, The Netherlands) and human AB serum by the Blood Bank Groningen (The Netherlands).

2.2. Cell lines and culture conditions

The origin and characteristics of the cell line panel used in this study are described in Table 1. All cell lines, except SW1573/S1, were cultured in RPMI 1640 medium with 10% heat-inactivated FCS. SW1573/S1 was cultured in DME medium with 10% heat-inactivated FCS. Tera, Tera-CP, SW1573/S1 and Caco-2 were grown as monolayers, GLC_4 and GLC_4-CDDP, A2780/cmv and A2780/mt273 were grown loosely attached to the flask. For experiments, cells from the monolayer cultures were harvested after a short incubation with trypsin or protease. All cell lines were cultured at 37° in a humidified atmosphere with 5% CO_2 [16–20].

2.3. Cytotoxicity

Cytotoxicity of continuous exposure of the different cell lines to JM216, JM118 and CDDP was determined with the MTT-assay as described before [20]. Each cell line was seeded at optimal density in order to test cell survival after at least two or three cell divisions had taken place under

Table 1
Characteristics of cell lines

Cell line	Tumour of origin	Type of resistance	Total GSH level ($\mu g/10^6$ cells ± SD)	p53 status	References
Tera	Germ cell	–	0.44 ± 0.04	Wild-type	[16]
Tera-CP		Acquired	0.62 ± 0.09	Wild-type	
GLC_4	Small-cell lung cancer	–	0.83 ± 0.09	Mutant	[17]
GLC_4-CDDP		Acquired	1.5 ± 0.32	Mutant	
A2780/cmv	Ovarian carcinoma	–	3.4 ± 0.89	Wild-type	[18]
A2780/mt273		–	3.3 ± 0.78	Mutant	
SW1573/S1	Non-small-cell lung cancer	Intrinsic	4.6 ± 0.22	Wild-type	[19]
Caco-2	Colon adenocarcinoma	Intrinsic	4.1 ± 0.59	Deletion mutant (no p53)	[20]

controlled conditions. For Tera, Tera-CP, A2780/cmv, A2780/mt273 and Caco-2, 1×10^4, 1×10^4, 1.25×10^3, 1.25×10^3 and 3.75×10^3 cells, respectively, were incubated with the drugs in a total volume of 0.2 mL culture medium for 4 days. For GLC_4, GLC_4-CDDP and SW1573/S1, 3.75×10^3, 1×10^4 and 5×10^3 cells were incubated with the drugs in a total volume of 0.1 mL culture medium for 4 days. Drug-induced cytotoxicity was expressed as concentration inhibiting 50% of cell growth (IC_{50}) compared to control conditions for each cell line. All experiments were performed at least three times.

2.4. GPt, a polyclonal antibody against platinated DNA

Pt-DNA adducts were measured immunocytochemically using GPt, a polyclonal antibody raised against cisplatin-platinated DNA. GPt detects the main Pt-containing intrastrand crosslinks, the Pt-GG adducts, and the interstrand crosslinks [21].

2.5. Validation of the immunoreactivity of GPt

Validation of the immunoreactivity of GPt for JM216- and JM118-induced Pt-DNA adducts compared to CDDP-induced Pt-DNA adducts was performed by atomic absorption spectrophotometry (AAS). GLC_4 cells were incubated with 0, 33 μM CDDP, 33 μM JM216 and 16.5 μM JM118 for 4 hr. Thereafter, cells were immediately washed twice in PBS at 0°, DNA was isolated and the DNA content (absorption at 260 nm) and the amount of Pt in the samples (AAS) were measured as described before [4]. Three independent experiments were performed at each concentration for each drug. Results were compared to the arbitrary units median immunosignal as determined by immunocytochemistry and analysed by double fluorescence video microscopy (see next subsection).

2.6. Immunocytochemical detection of Pt-DNA adducts

Tumour cells were incubated with either 0, 3.3, 16.5 or 33 μM JM216 or CDDP, or 1, 3.3 or 16.5 μM JM118 for 4 hr. Tumour cells incubated without any Pt compound served as background control for the GPt antibody binding. Immediately after drug incubation, cells were washed twice with PBS at 0° followed by preparation of cytospin slides. Subsequently, slides were air dried, fixed in cold (−20°) methanol for 10 min followed by cold (−20°) acetone for 2 min, air dried again and stored at −20° until staining. Upon staining, slides were washed in PBS and incubated for 30 min with 1% human AB serum and 1% BSA in PBS to block non-specific antibody binding, followed by an overnight incubation at room temperature with the GPt antibody (1:6 dilution) or with 1% BSA in PBS, as negative control for the staining procedure. After washing with PBS, slides were incubated with a FITC-labelled swine anti-rabbit antibody for 1 hr at room temperature and counterstained with Hoechst 33258 (10 min) for DNA detection. An antifade immunomounting medium was applied and slides were stored at 4° in the dark until analysis.

Double fluorescence microscopy image analysis with Hoechst fluorescence to locate the nuclei, and FITC fluorescence to measure GPt binding to Pt-DNA adducts, was used to quantify the level of Pt-DNA adducts [21]. The amount of Pt-DNA adducts was expressed as median immunosignal of at least 50–100 nuclei per slide. The results obtained from the immunocytochemical detection of Pt-DNA adducts were corrected for differences in immunoreactivity of the GPt-antibody for Pt-DNA adducts formed by the different drugs. Results are expressed as mean (SD) of these experiments. At least three independent experiments were performed.

2.7. GSH level and p53 status

The data for the GSH levels and p53 status of the cell lines used were obtained from previous investigations or measured in this study. Total GSH was determined in the A2780/cmv and the A2780/mt273 cell lines by the enzyme recycling method according to Tietze under conditions similar to those described earlier [4]. For SW1573/S1 p53 status was determined by RT-PCR followed by sequence analysis of the exons 3–9 (hot spot mutation sequence) of the *p53 gene*.

2.8. Statistics

Differences in cytotoxicity and the amount of Pt-DNA adducts between cell lines per drug concentration and between drug concentrations per cell line were tested with the Student's *t*-test. Differences in cytotoxicity, the amount of Pt-DNA adducts and GSH levels between cell lines with different p53 status were also tested by the Student's *t*-test. Correlations were tested with the Spearman's rank test. Differences or correlations were considered statistically significant if $P < 0.05$.

3. Results

Table 2 shows drug-induced cytotoxicity after continuous incubation (4 days) with JM216, JM118 and CDDP in the used cell line panel, indicated by IC_{50} and resistance factor (RF). JM216 and moreover JM118 can at least partly overcome CDDP resistance in the tested cell lines. The RF for the drugs decreased from CDDP to JM216 to JM118 for all resistant cell lines compared to their parent or control cell line. JM216-induced cytotoxicity correlated with both JM118- and CDDP-induced cytotoxicity ($r = 0.71$, $n = 8$, $P < 0.05$ and $r = 0.93$, $n = 8$, $P < 0.001$, respectively). JM216 and CDDP showed the same sensitivity ranking for the cell line panel. JM118 was the most potent of the three

Table 2
Drug-induced cytotoxicity in the different cell lines as measured by the MTT-assay

Cell line	CDDP		JM216		JM118	
	IC_{50} ± SD (μM)[a]	RF[b]	IC_{50} ± SD (μM)[a]	RF[b]	IC_{50} ± SD (μM)[a]	RF[b]
Tera	0.69 ± 0.10		0.20 ± 0.12[c]		0.23 ± 0.06[c]	
Tera-CP	2.57 ± 0.74	3.7	0.55 ± 0.16[c]	2.7	0.49 ± 0.07[c]	2.1
GLC_4	0.77 ± 0.13		0.46 ± 0.15[c]		0.17 ± 0.04[c,d]	
GLC_4-CDDP	16.6 ± 4.44	22	4.77 ± 0.29[c]	10	1.27 ± 0.31[c,d]	7.4
A2780/cmv	1.43 ± 0.35		0.98 ± 0.15[c]		0.23 ± 0.03[c,d]	
A2780/mt273	3.10 ± 0.46	2.2	1.73 ± 0.38[c]	1.8	0.26 ± 0.03[c,d]	1.1
SW1573/S1	3.43 ± 0.81		1.23 ± 0.93[c]		3.10 ± 0.36[d]	
Caco-2	9.93 ± 1.29		5.84 ± 0.97[c]		2.62 ± 0.94[c,d]	

[a] $n = 3$–7.
[b] RF: the resistance factor compared to parent cell line.
[c] Significantly different from CDDP ($P < 0.05$).
[d] Significantly different from JM216 ($P < 0.05$).

drugs tested, except for the non-small-cell lung cancer cell line SW1573/S1, where JM216 showed more cytotoxicity than its metabolite JM118. JM118 was more cytotoxic in cell lines with acquired resistance to CDDP (Tera-CP and GLC_4-CDDP) than in the cell lines with intrinsic CDDP resistance (SW1573/S1 and Caco-2).

Pt-DNA adducts were measured immunocytochemically using the polyclonal antibody GPt. First validation of the immunoreactivity of GPt to the drug-induced Pt-DNA adducts was performed. Table 3 shows levels of total Pt-DNA adducts measured by AAS as compared to the arbitrary units median immunosignal determined by immunocytochemistry and analysed by double fluorescence video microscopy in GLC_4 cells after 4 hr incubation with the drugs. Correction factors for immunoreactivity of the GPt antibody to Pt-DNA adducts induced by CDDP, JM216 and JM118 were calculated (9.08, 2.19, and 4.05 for CDDP, JM216, and JM118, respectively) and used to correct the measured median immunosignal for differences in antibody recognition for the drug-induced Pt-DNA adducts. Staining intensity observed was rather heterogeneous in all cell lines for all the tested drugs and the immunosignal of at least 50–100 nuclei per drug concentration, per cell line was analysed to assure a reliable measurement of the amount of Pt-DNA adducts.

Fig. 2 shows the levels of immunoreactive Pt-DNA adducts induced by JM216 (Fig. 2A), JM118 (Fig. 2B) and CDDP (Fig. 2C) in the various cell lines. The corrected median immunosignal increased with the drug concentration for all tested cell lines. JM216 induced lower levels of Pt-DNA adducts than CDDP and JM118. JM118 induced Pt-DNA adduct formation comparable to CDDP, except for GLC_4-CDDP, A2780/cmv and A2780/mt273 where 16.5 μM JM118 yielded more Pt-DNA adducts than equimolar CDDP ($P < 0.05$). JM118 yielded higher Pt-DNA adduct formation than JM216 for all cell lines ($P < 0.05$). In general, no differences in initial Pt-DNA adduct levels were observed between cell lines sensitive, acquired resistant or intrinsic resistant to CDDP. Fig. 3 shows representative pictures of a staining experiment as observed with fluorescence microscopy.

Table 1 shows the GSH levels and p53 status of the various cell lines. GSH levels increased in the unselected cell lines from the CDDP sensitive Tera, to GLC_4, to A2780/cmv and were equally high in the intrinsic CDDP resistant SW1573/S1 and Caco-2 cell lines. Both *in vitro* acquired CDDP resistant sublines, Tera-CP and GLC_4-CDDP, showed an increased GSH level compared to their parental cell line [16,17]. Transfection with mt273 in A2780 did not result in an altered GSH level in A2780/mt273 as compared to A2780/cmv. Tera, Tera-CP, A2780/cmv and the SW1573/S1 cell lines expressed a wild-type p53 status. GLC_4, GLC_4-CDDP and the A2780/mt273 cell lines showed a mutated p53 status whereas in Caco-2 a deletion mutation (resulting in no p53 protein) was observed.

In the unselected cell lines (Tera, GLC_4, A2780/cmv, SW1573/S1 and Caco-2), drug-induced cytotoxicity correlated with GSH levels for both JM216 and CDDP ($r = -0.90$, $n = 5$, $P < 0.037$ for both drugs), whereas

Table 3
Validation of the immunoreactivity of GPt to the drug-induced Pt-DNA adducts in GLC_4 cells after 4 hr incubation with the drugs

	Pt-DNA (μg GPt/mg DNA) (AAS)	Pt-DNA arbitrary units (immunocytochemistry)	Correction factor
CDDP 33 μM	78.5 ± 12.2 (7.0)[a]	8.65 ± 5.10 (2.94)	9.08
JM216 33 μM	19.9 ± 9.2 (5.3)	9.10 ± 7.64 (4.41)	2.19
JM118 16.5 μM	88.2 ± 23.6 (13.6)	21.8 ± 5.48 (3.16)	4.05

[a] Mean ± SD (SEM).

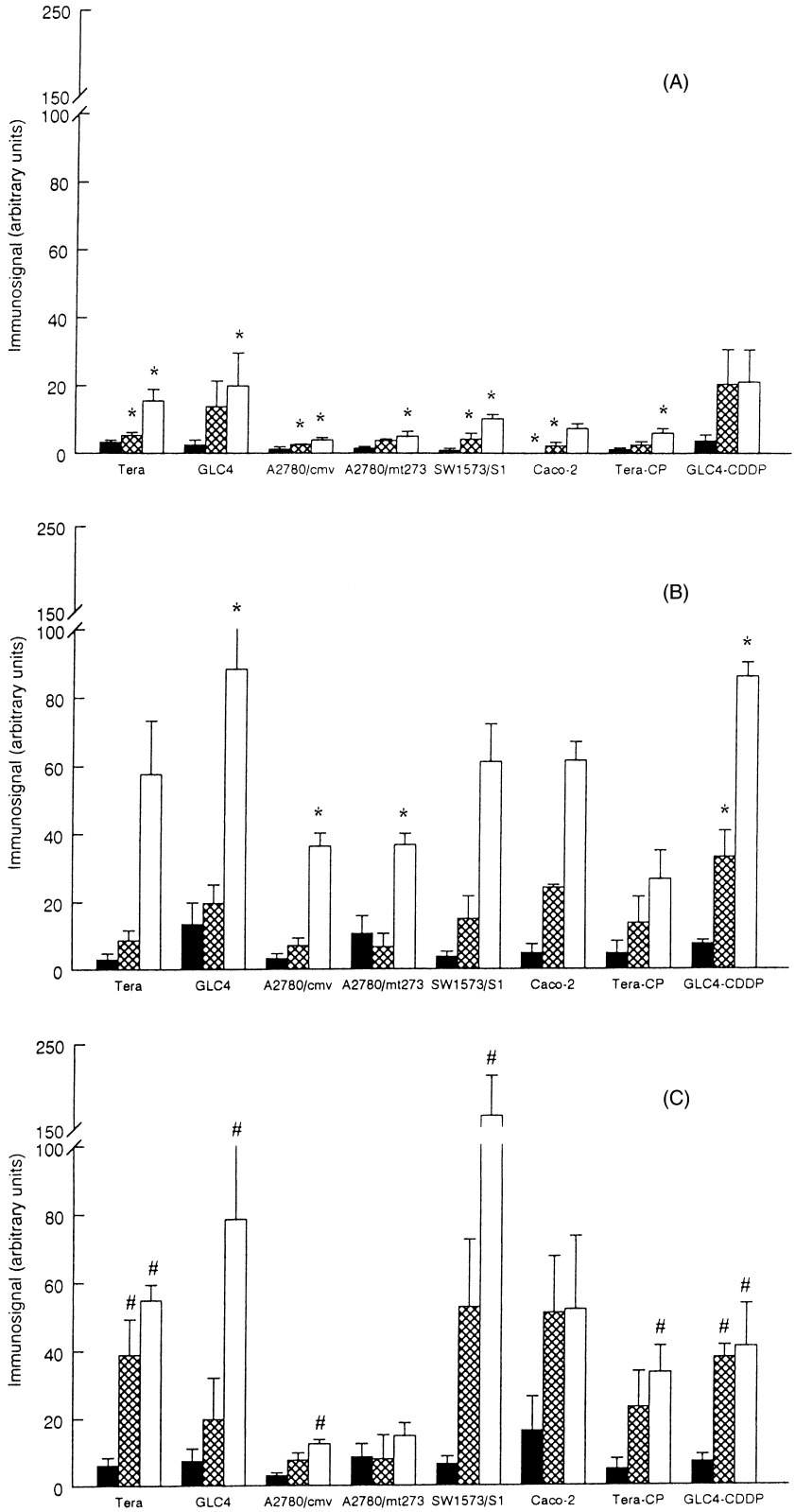


Fig. 2. Levels of drug-induced immunoreactive Pt-DNA adducts expressed as (corrected) median immunosignal after 4 hr incubation of the various cell lines with JM216 (A), JM118 (B) and CDDP (C); mean ± SEM, $n \geq 3$. (A) (■) 3.3 μM JM216, (⊠) 16.5 μM JM216, (□) 33 μM JM216; (B) (■) 1 μM JM118, (⊠) 3.3 μM JM118, (□) 16.5 μM JM118, (C) (■) 3.3 μM CDDP, (⊠) 16.5 μM CDDP, (□) 33 μM CDDP; (∗) significant different vs. the comparable CDDP concentration (A, B), (#) significant different vs. the 3.3 μM CDDP concentration (C).

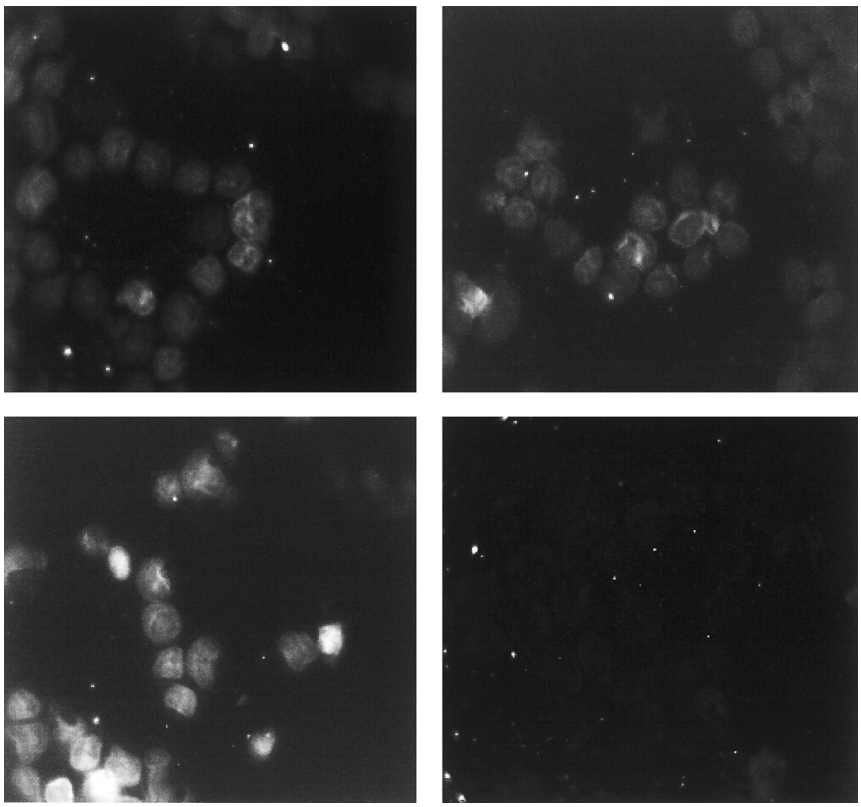


Fig. 3. Immunostaining of GLC_4 cells shown as pictures of the (uncorrected) FITC signal. Upper left, GLC_4 cells after 4 hr incubation with 33 μM CDDP; upper right, GLC_4 cells after 4 hr incubation with 33 μM JM216; lower left, GLC_4 cells after 4 hr incubation with 16.5 μM JM118; lower right, control GLC_4 cells (background staining).

for JM118 a trend was observed ($r = -0.82$, $n = 5$, $P < 0.089$). No correlations could be found between (1) drug-induced cytotoxicity and initial Pt-DNA adduct levels or p53 status, (2) initial Pt-DNA adduct levels and GSH levels or p53 status, (3) GSH levels and p53 status.

4. Discussion

In the present study cytotoxicity of JM216 and its active metabolite JM18 was evaluated in relation to initial Pt-DNA adduct formation, GSH levels and p53 status in a panel of human cancer cell lines with different sensitivities to CDDP. JM216 and especially JM118 were both capable of circumventing CDDP resistance in the tested cell lines. JM118 was the most potent drug and showed to be more cytotoxic in cell lines with acquired resistance to CDDP than in cell lines with intrinsic resistance to CDDP. JM216 is a third generation Pt drug that is highly lipophilic which facilitates cellular uptake and has shown to be capable of circumventing resistance to CDDP caused by decreased intracellular accumulation of CDDP [15]. Kelland and coworkers published several studies showing the accumulation related circumvention of acquired resistance to CDDP by ammine/amine-platinum IV dicarboxylates (e.g. JM216) in several human tumour cell line models [15,22–24]. However, in none of the two models of acquired CDDP resistance (Tera/Tera-CP and GLC_4/GLC_4-CDDP) used in the present study, an accumulation defect was proven which could explain the observed circumvention of CDDP resistance by JM216 and JM118 [16,17].

Many *in vitro* studies have shown a correlation between intracellular GSH levels and sensitivity to CDDP [2,5]. GSH can bind directly to reactive Pt resulting in a decrease in DNA-platination and has been postulated to play a role in the formation and repair of Pt-DNA adducts. However, results obtained from *in vitro* studies with buthionine sulfoximine, a specific inhibitor of the GSH synthesis leading to GSH depletion, vary from complete restoration to partial reversal or no effect at all on sensitivity to CDDP [5]. Raynaud *et al.* [25] studied the influence of GSH on intracellular metabolism of JM216 in a CDDP-sensitive (CH1) and -resistant (SKOV-3) human ovarian carcinoma cell line and suggested that GSH represents a major deactivation pathway for JM216. Mellish *et al.* [14] however found no correlation between GSH and JM216-induced cytotoxicity. In agreement with earlier studies from our laboratory performed in panels of both drug-induced and non-induced (unselected) cell lines [3–5,16,17,20], also in the presented study GSH levels of the unselected cell lines correlated negatively with sensitivity to JM216, JM118 and CDDP. Therefore, GSH conjugation seems of importance in Pt-drug detoxification.

Until now, two studies evaluated JM216- and JM118-induced cytotoxicity in relation to Pt-DNA levels. No correlation was observed between cytotoxicity of JM216 or JM118 and total DNA-platination levels in the CDDP-sensitive human ovarian carcinoma cell line CH1, the acquired CDDP-resistant cell line CH1cisR, and the intrinsic CDDP-resistant SKOV-3 cell line. Differences in gene-specific repair could at least partly explain differences in sensitivity in this model [24,26]. We studied initial JM216-, JM118- and CDDP-induced Pt-DNA adduct formation by immunocytochemistry in a pharmacologically relevant dose range in a panel of 8 cell lines from different origin including CDDP-sensitive, acquired CDDP resistant and intrinsic CDDP resistant cell lines. The used polyclonal antibody GPt was shown to detect the main Pt-containing intrastrand crosslinks (the Pt-GG adducts) and the interstrand crosslinks [21]. Validation of the immunoreactivity of GPt against JM216– and JM118– compared to CDDP-induced Pt-DNA adducts showed an increase in immunoreactivity of GPt from CDDP to JM118 to JM216 suggesting a carrier ligand involvement. It also shows that caution should be taken in measuring and comparing various drug-induced adducts in different cells by indirect immunocytochemistry. Differences between cell lines and differences in the nature of the formed adducts may influence the accessibility of the antibody and thereby the measured immunosignal (the presented study validated for different drug-induced adducts within one cell line). We also found no correlation between initial Pt-DNA adduct formation and cytotoxicity to JM216, JM118 or CDDP. Our data indicate that the amount of Pt-DNA formed by JM118 and CDDP are comparable whereas for JM216 even lower levels of Pt-DNA adducts were found. Nevertheless, both JM216 and JM118 can overcome resistance to CDDP suggesting that repair mechanisms and/or tolerance of DNA-damage must be of importance for drug-induced cytotoxicity. JM216 (and JM118) belong to the Pt compounds that form the same type of DNA-adducts as CDDP but differ from CDDP primarily in the nature of the carrier ligand attached to the Pt atom (*cis*-diammine vs. *cis*-amminecyclohexylamine for CDDP and JM216, respectively). Recently the group of Chaney and coworkers have made major advances in characterising the carrier-ligand specificitiy of the repair mechanisms used by the cell to remove and/or tolerate the presence of Pt-DNA adducts. These processes include nucleotide excision repair, post-replication repair, mismatch repair and damage-recognition proteins. Available data from their studies suggest that the DNA polymerases that catalyse translesion synthesis, the mismatch repair status of the cell and Pt-damage recognition proteins all interact to influence the overall carrier-ligand specificity for replicative bypass of Pt-DNA adducts [27–29]. The carrier ligand specificity of DNA repair seems to be of great importance in revealing the mechanism by which JM216 and JM118 can overcome CDDP resistance.

Current information shows that apart from the occurrence of adducts and perhaps more important the degree of tolerance to such damage, the potential to activate apoptotic cell death is another important factor in determining the fate of the cell [30]. We investigated the relationship between the status of one of the key proteins involved in the activation of apoptosis, p53, of the cell lines and sensitivity to the tested drugs, GSH levels, or initial Pt-DNA adduct formation. None of these parameters appeared to be directly related to p53 status.

In conclusion, this study confirms that both JM216 and JM118 are capable of at least partially circumventing CDDP resistance. This applies for both acquired and intrinsic CDDP-resistance. In the unselected cell lines, drug-induced cytotoxicity correlated negatively with GSH levels for JM216 and CDDP. No difference in initial Pt-DNA adduct levels was observed between cell lines sensitive, acquired or intrinsic resistant to CDDP, suggesting a role for a Pt-resistance mechanisms based on tolerance or increased DNA-repair.

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