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Modulation of melphalan and cisplatin cytotoxicity in human ovarian cancer cells resistant to alkylating drugs

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We investigated the effect of pharmacological modulators on the cytotoxic activity of melphalan and cisplatin in human ovarian cystadenocarcinoma cells sensitive (OAW42) or resistant (OAW42MER) to bifunctional alkylating agents. By filter elution experiments we observed a reduced accumulation and a faster repair of melphalan-induced DNA interstrand cross-links in the OAW42MER resistant cells than in the OAW42 parental, sensitive cells. Moreover, resistant cells were characterized by an increased level of mRNA encoding enzymes involved in the nucleotide excision repair pathway, such as ERCC (excision repair cross complementing)1 and ERCC2. Among the modulators used, the topoisomerase I inhibitor topotecan was able to increase melphalan cytotoxic activity in sensitive and resistant cell lines. Topotecan also positively modulated cisplatin activity, although to a variable extent in the two cell lines, as a function of treatment schedule. The energolytic compound lonidamine markedly enhanced the cytotoxicity of melphalan and cisplatin, with a potentiating effect in the OAW42MER resistant cells almost 2-fold that of in the OAW42 sensitive cells. No significant potentiation was observed by using calcium channel blockers, such as verapamil and nimodipine. Conversely, an increase in melphalan cytotoxic activity was determined by flunarizine in OAW42MER resistant cells and, to a lesser extent, in OAW42 sensitive cells. However, the calcium blocker failed to modulate cisplatin activity in both cell lines.

Key words: Alkylating drugs, cisplatin, melphalan, OAW42, OAW42MER.

Introduction

Alkylating agents, such as mephalan, constitute a major class of anticancer drugs with well-established clinical activity against a broad spectrum of human malignancies including ovarian cancer.[1] Unfortunately, the occurrence of *de novo* or acquired resistance to such agents is a frequent cause of treatment failure.

Previous investigations have demonstrated that resistance to alkylating agents is multifactorial, and that the mechanisms involved include drug transport alterations[2], increased drug detoxification,[3,4] and, mainly, enhanced DNA damage repair.[5,6] Clinical strategies to treat alkylator-resistant neoplasms are still limited and there is the need to preclinically identify effective therapies. In particular, attempts to modulate the function of specific cellular targets involved in cellular response to alkylating agent treatment are expected to provide a rational basis for pharmacological intervention.

We evaluated the efficacy of possible modulators of melphalan and cisplatin activity in two human ovarian cystadenocarcinoma cell lines, one sensitive (OAW42) and one with experimentally induced resistance to melphalan (OAW42MER) and cross-resistant to other alkylators. In particular, we analyzed the activity of chemical agents supposed to interfere with DNA repair processes, such as topotecan, an inhibitor of the enzyme DNA topoisomerase I,[7,8] and the energolytic compound lonidamine, for which an inhibiting effect on potentially lethal damage repair has already been demonstrated.[9,10]

Moreover, based on recent evidence indicating a potentiating effect by calcium channel blockers on melphalan cytotoxicity consequent to modifications in drug transport,[11,12] we also assessed the ability of verapamil, flunarizine and nimodipine to modulate melphalan and cisplatin activity.

Materials and methods

Cell lines

OAW42 is a cell line derived from the ascites of a patient with a papillary serous cystadenocarcinoma

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of the ovary.[13] The melphalan-resistant OAW42MER subline was obtained by exposure of the OAW42 line to increasing stepwise concentrations of melphalan.[14] Both cell lines were kindly supplied by Dr RA Britten (Department of Radiation Oncology, University of Liverpool, UK). Cell lines were maintained as monolayers at 37°C in a 5% CO_2 humidified atmosphere in air, using DME/F12 medium supplemented with 10% fetal calf serum, 2 μM L-glutamine, 0.25 U/ml insulin and 0.1% gentamicin. The melphalan-resistant subline was used for all the experiments described below, within the first four passages of growth in medium without melphalan. Both cell lines are characterized by a wild-type p53 gene, as detected by single-strand conformation polymorphism and direct DNA sequencing analysis (data not shown).

Drugs

Melphalan (Sigma, St Louis, MO) was dissolved at 50 mg/ml, just before use, in perchloric acid and ethanol solution (1:20), and diluted in 0.9% NaCl solution. Cisplatin (Bristol-Myers, Evansville, IL), mitomycin C (Kyowa Hakko, Tokyo, Japan), doxorubicin (Pharmacia, Milan, Italy) and verapamil (Knoll Farmaceutici, Milan, Italy) were dissolved in 0.9% NaCl solution. Taxol (Bristol-Myers) and nimodipine (Bayer, Garbagnate Milanese, Italy) were dissolved in absolute ethanol, fludarabine (Schering, Berlin, Germany) in phosphate-buffered saline (PBS), lonidamine (F. Angelini Research Institute, Rome, Italy) in *N*-methyl-D-glucamine, flunarizine (Jansen Research Fundation, Beerse, Belgium) in dimethylsulfoxide, and topotecan (SmithKline Beecham Pharmaceuticals, King of Prussia, PA) in H_2O and then diluted in saline solution. Immediately before each experiment, the drugs were diluted with fresh medium. Control samples were always run with the specific solvent of each drug.

Cell survival assay

After harvesting in logarithmic phase, cells were seeded in 6-well plates (1.2×10^5 cells/well for OAW42 and 1×10^5 cells/well for OAW42MER) in 2 ml of fresh medium for 24 h and then exposed for 1 h to melphalan (0.1–20 μg/ml), cisplatin (0.1–50 μg/ml), mitomycin C (0.05–5 μg/ml) or doxorubicin (0.05–1 μg/ml), for 1 or 24 h to topotecan (0.001–1 μg/ml) and for 24 h to taxol (0.001–1.0 μg/ml) or fludarabine (0.05–1 μg/ml).

In the combination experiments, cells were exposed to melphalan or cisplatin for 1 h, washed and incubated with lonidamine (25 μg/ml) for 24 h. In the combined experiments with topotecan, cells were simultaneously exposed to melphalan (or cisplatin) and topotecan (0.01 μg/ml) for 1 h, or alternatively, cells were pretreated with melphalan or cisplatin for 1 h and then washed and incubated with topotecan (0.001 μg/ml) for 24 h. Finally, in experiments with calcium channel modulators, cells were simultaneously exposed to melphalan (or cisplatin) and flunarizine (2 μg/ml), or nimodipine (4 μg/ml) or verapamil (5 μg/ml) for 1 h.

At the end of different treatments, cells were washed and incubated at 37°C until the sixth day after seeding. At that time, detached cells were removed with PBS and monolayers were trypsinized with a trypsin–EDTA solution. Single-cell suspensions obtained by repeated pipetting in an isoton solution (azide-free balanced electrolyte solution; Coulter Scientific, Luton, UK) were checked at the microscope and counted in a particle counter (Coulter Counter, Coulter Electronics, Luton, UK). Cell viability, determined by the Trypan blue dye exclusion test, always exceeded 95%. Each experimental sample was run in triplicate. The results were expressed as the number of treated cells compared with control cells.

Alkaline filter elution

The method developed by Kohn[15] was used. The OAW42 and OAW42MER cells were grown in medium containing [^{14}C]thymidine (0.1 μCi/ml; specific activity 56 mCi/mmol) for 24 h. The medium was discarded and cultures were treated with melphalan for 1 h, and then incubated in fresh medium for 6, 24 and 48 h. Cultures were then trypsinized, washed, gamma-irradiated (600 rad; 1000 rad/min^{-1}) on ice and deposited on a 2.0 μm pore polycarbonate filter (25 mm diameter, Nucleopore, Pleasant, CA). Cells were lysed with 2% sodium dodecylsulfate (SDS), 0.02 M disodium EDTA (pH 10.0) and 0.5 mg/ml proteinase K. The DNA was then eluted at a flow rate of 0.035 ml/min with a 0.02 M EDTA–0.1% SDS solution adjusted to pH 12.15 with tetrapropylammonium hydroxide. The lysate from a 15 h elution was collected in fractions and counted by liquid scintillation. Data were plotted as the relative amount of [^{14}C]DNA retained on the filter versus the elution time. DNA interstrand cross-link (DNA-ISC) frequency was calculated in rad equivalents by the formula:[16]

$$ISC = \left[\left(\frac{1 - r_0}{1 - r}\right)^{1/2} - 1\right] \times 600 \text{ rad}$$

where r_0 is the fraction of [^{14}C]DNA remaining on the filter in irradiated control cells and r is the fraction of [^{14}C]DNA remaining on the filter in drug-exposed irradiated cells, after 12 h of elution.

RNA extraction and Northern blot hybridization

Total cellular RNA was extracted according to the method of Chomczynski and Sacchi.[17] RNA (20 μg) was denatured with formaldehyde and formamide and electrophoresed through a 1% agarose gel containing formaldehyde. The RNA was transferred overnight to Hybond nylon membrane (Amersham, Buckinghamshire, UK) by capillary blotting in 20 × SSC (SSC, 3 M sodium chloride, 0.3 M sodium citrate, pH 7). After baking for 30 min at 80°C and UV cross-linking for 30 s, the filter was prehybridized for 2 h at 42°C in 5 × SSC, 5 × Denhart's solution (0.1% bovine serum albumin, 0.1% Ficoll and 0.1% polyvinyl pyrrolidone), 0.5% w/v SDS, 50% formamide and 100 μg/ml denatured salmon sperm DNA, and then hybridized in the same solution at 42°C for 18–20 h. DNA probes used for hybridization were [α-^{32}P]deoxycytidine labeled with a random primer kit (specific activity 2–3 × 10^8 c.p.m./μg of DNA) (Amersham) and denatured before addition to the membrane. After hybridization, the filter was rapidly washed with 2 × SSC followed by 1 × SSC and 0.1% SDS at 55°C and then with 0.1 × SSC and 0.1% SDS at 65°C. The filter was exposed at −80°C to Hyperfilm MP (Amersham). To reprobe with alternative probes, the filter was immersed twice in a stripping solution (0.1% SDS) for 10 min at 95°C and then reprobed. Gene transcript levels were calculated by optical densitometry of autoradiograms using an LKB ultrascan XL laser densitometer equipped with a Gelscan software package and normalized to human β-actin expression.

Probes

The pE12-12 plasmid containing the human ERCC1 cDNA fragment was a gift from Professor JHJ Hoeijmakers. Human ERCC2 fragment cDNA subcloned into pKSV10, human glutathione S-transferase (GST) π cDNA subcloned into pGem-4 and human MT-IIA (metallothionein IIA) fragment cDNA subcloned into pBR322 were supplied by American Type Culture Collection (Rockville, MD).

Results

Initial experiments were performed to define the sensitivity/resistance profiles of OAW42 and OAW42-MER cell lines to the anticancer agents with different action mechanisms (Table 1). OAW42MER cells showed a resistance to melphalan more than 2-fold that of OAW42 parental cells and were cross-resistant to the other alkylating agents, cisplatin and mitomycin, and to the antimetabolite fludarabine, whereas no cross-resistance to doxorubicin or the topoisomerase I inhibitor topotecan was observed. Conversely, OAW42MER cells were found to be almost four times as sensitive to taxol as the OAW42 parental cells.

Table 1. Cross-resistance profile of OAW42MER cells

Drug	Exposure time (h)	IC_{50} (μg/ml)		Relative factor of resistance[a]
		OAW42	OAW42MER	
Melphalan	1	4.50 ± 0.41	10.30 ± 1.10	2.29
Cisplatin	1	5.31 ± 0.60	32.00 ± 2.81	6.03
Mitomycin C	1	0.51 ± 0.04	2.71 ± 0.24	5.4
Doxorubicin	1	0.50 ± 0.05	0.45 ± 0.03	0.9
Topotecan	1	0.10 ± 0.01	0.11 ± 0.01	1.1
	24	0.0075 ± 0.0008	0.0075 ± 0.0004	1.0
Taxol	24	0.1 ± 0.008	0.026 ± 0.003	0.26
Fludarabine	24	0.14 ± 0.02	0.30 ± 0.04	2.1

The IC_{50} value was determined graphically from the survival curve as the concentration inhibiting by 50% cell growth (average of four independent experiments ± SE).
[a]Ratio of IC_{50} OAW42MER/IC_{50} OAW42.

Table 2. Accumulation and repair of melphalan-induced DNA-ISC

Time[a] (h)	DNA-ISC (rad-equivalents)			
	OAW42		OAW42MER	
	5 μg/ml	10 μg/ml	5 μg/ml	10 μg/ml
6	112 ± 15	187 ± 23	70 ± 12	143 ± 21
24	88 ± 11 (21%)	146 ± 18 (22%)	40 ± 10 (43%)	78 ± 19 (45%)
48	46 ± 10 (59%)	69 ± 15 (63%)	12 ± 8 (83%)	20 ± 6 (86%)

Data represent the average of three independent experiments ± SE.
In parenthesis, the percentage of DNA-ISC repaired.
[a]Incubation time in drug-free medium after a 1 h exposure to melphalan.

Analysis of the accumulation and removal of induced DNA-ISC, performed by alkaline filter elution (Table 2) 6 h after a 1 h exposure of cells to 5–10 μg/ml of melphalan, showed a reduced formation of DNA lesions (−24 to −37%) in OAW42MER resistant cells compared to the parental sensitive cells. Moreover, a faster repair of DNA-ISC was observed in OAW42MER than in OAW42 cells. Specifically, 24 h after drug treatment, about 40% of DNA lesions were repaired by resistant cells, whereas in sensitive cells the repair rate was only around 20%. After an additional 24 h, the removed DNA-ISC further increased to 80% in resistant cells and to only about 60% in sensitive cells.

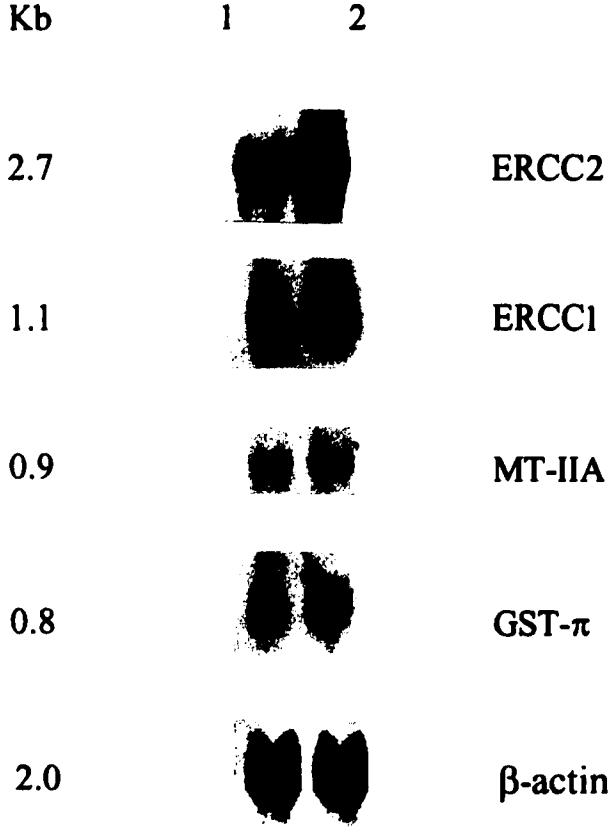

Figure 1. Expression of putative resistance factors (GST-π, MT-IIA, ERCC1 and ERCC2) in OAW42 and OAW42MER cells (lanes 1 and 2, respectively). Total (20 μg) RNA was fractionated in a 1% agarose–formamide gel, transferred to a nylon membrane and hybridized with the indicated probes.

In an attempt to explain the different patterns of drug sensitivity and DNA repair ability in the two cell lines, we examined the expression, in terms of mRNA level, of proteins involved in cellular drug detoxifications systems (GST-π and MT-IIA) and in DNA repair pathways (ERCC1 and ERCC2) (Figure 1). Densitometric analysis showed a slightly higher GST-π mRNA level in resistant than in sensitive cells, whereas no differences in MT-IIA mRNA expression were observed between the two cell lines. Moreover, a markedly higher ERCC1 and ERCC2 mRNA expression was recorded in resistant than in parental, sensitive cells (Table 3).

In a further step of the study, we assessed the ability of different modulators to increase the activity of melphalan and cisplatin in OAW42 and OAW42-MER cell lines. Preliminary experiments were performed to define a non-toxic or moderately toxic concentration of each modulator to use in combination experiments. Simultaneous exposure to melphalan and 0.01 μg/ml topotecan for 1 h significantly increased the alkylator cytotoxic activity in both cell

Table 3. Densitometric analysis of mRNA levels

	OAW42	OAW42MER
ERCC1	0.45	0.96
ERCC2	0.22	0.92
GST-π	0.24	0.34
MT-IIA	0.13	0.19

The levels of the transcripts have been normalized to β-actin gene expression.

lines (Table 4). Similar results were obtained when OAW42 and OAW42MER cells were first exposed to melphalan and then incubated with 0.001 μg/ml topotecan for 24 h. The extent of potentiation was comparable following the two treatment schemes and it was consistent in the two cell lines. Combined treatment with topotecan also produced an increase in cisplatin cytotoxic activity. Specifically, the potentiating effect was higher in sensitive than in resistant cells following the short-term, simultaneous exposure to the two agents. Conversely, a greater effect of the combination was recorded in OAW42MER than in OAW42 cells when cells were sequentially exposed to cisplatin and topotecan.

The potential of lonidamine as a modulator of melphalan and cisplatin was also assessed in the two cell lines. Sequential exposure to melphalan for 1 h and lonidamine (25 μg/ml) for 24 h induced a 4-fold increase in the cytotoxic activity of the alkylator in the OAW42MER resistant cells (Table 4). Although to a lesser degree, a potentiating effect was also recorded in OAW42 sensitive cells. Very similar results were observed when the two cell lines were sequentially exposed to cisplatin and lonidamine.

Finally, we evaluated the ability of calcium channel blockers to modulate the cytotoxic activity of the alkylating agent (Table 5) by exposing cells simultaneously to melphalan and flunarizine (2 μg/ml),

Table 4. Effect of DNA repair modulators on the cytotoxic activity of melphalan and cisplatin

	OAW42		OAW42MER	
	IC_{50} (μg/ml)	DMF	IC_{50} (μg/ml)	DMF
Melphalan alone	4.50 ± 0.41		10.30 ± 1.10	
+ topotecan[a] (1 h)	1.80 ± 0.21[d]	2.50	4.21 ± 0.35[d]	2.45
→ topotecan[b] (24 h)	2.21 ± 0.32[d]	2.04	3.80 ± 0.29[d]	2.71
→ lonidamine[c] (24 h)	1.61 ± 0.24[d]	2.80	2.52 ± 0.31[d]	4.09
Cisplatin alone	5.31 ± 0.42		32.00 ± 2.81	
+ topotecan[a] (1 h)	2.10 ± 0.31[d]	2.53	22.05 ± 2.10[d]	1.45
→ topotecan[b] (24 h)	3.81 ± 0.53	1.39	10.04 ± 1.12[d]	3.19
→ lonidamine[c] (24 h)	1.83 ± 0.24[d]	2.90	6.84 ± 0.71[d]	4.68

Data represent the average of four independent experiments ± SE.
DMF, dose-modifying factor: IC_{50} in the absence of the modulator/IC_{50} in the presence of the modulator.
[a]0.01 μg/ml.
[b]0.001 μg/ml.
[c]25 μg/ml.
[d]$p < 0.05$, Student's *t*-test.

Table 5. Effect of Ca^{2+} channel blockers on the cytotoxic activity of melphalan and cisplatin

	OAW42		OAW42MER	
	IC_{50} (μg/ml)	DMF	IC_{50} (μg/ml)	DMF
Melphalan alone	4.50 ± 0.41		10.30 ± 1.10	
+ flunarizine[a] (1 h)	3.01 ± 0.35	1.50	4.21 ± 0.61[d]	2.45
+ nimodipine[b] (1 h)	4.52 ± 0.39	1.00	8.35 ± 0.94	1.23
+ verapamil[c] (1 h)	4.66 ± 0.43	0.97	9.61 ± 0.73	1.07
Cisplatin alone	5.31 ± 0.40		32.00 ± 2.81	
+ flunarizine (1 h)	5.12 ± 0.61	1.04	33.40 ± 3.12	0.96
+ nimodipine (1 h)	5.03 ± 0.58	1.06	34.07 ± 2.85	0.94
+ verapamil (1 h)	4.91 ± 0.24	1.08	33.61 ± 2.19	0.95

Data represent the average of four independent experiments ± SE.
DMF, dose-modifying factor.
[a]2 μg/ml.
[b]4 μg/ml.
[c]5 μg/ml.
[d]$p < 0.05$, Student's *t*-test.

nimodipine (4 μg/ml) or verapamil (5 μg/ml) for 1 h. No potentiation of melphalan activity was consistently observed in OAW42 cells, whereas a significant increase in the cytotoxic effect of the alkylator was observed only after combined exposure with flunarizine in OAW42 cells. None of the modulators was able to increase cisplatin activity in sensitive or resistant cell lines.

Discussion

The present study was designed to evaluate the efficacy of pharmacological modulators to increase the cytotoxic activity of melphalan and cisplatin in two ovarian cancer cell lines, sensitive or with experimentally induced resistance to melphalan. The melphalan-resistant cell line was also refractory to cisplatin and mitomycin C, thus confirming that the development of resistance to a single alkylating agent can confer cross-resistance to other alkylators, as previously observed in other experimental systems.[18]

A decreased accumulation and a faster repair of melphalan-induced DNA-ISC was observed in resistant than in sensitive cells, suggesting that the differential effect of the alkylating agent in the two cell lines is at least partially dependent on the different balance between DNA lesions and DNA repair. A role of enhanced DNA repair in alkylating drug resistance of OAW42MER cells was also suggested by increased ERCC1 and ERCC2 mRNA expression. The product of the ERCC1 gene is an enzyme involved in the incision step of excision repair of bulky adducts in DNA induced by cisplatin and bifunctional alkylators.[19] The implication of ERCC1 in resistance to cisplatin is supported by the demonstration that cisplatin-resistant ovarian and bladder cancer cell lines have higher levels of ERCC1 mRNA than cisplatin-sensitive tumor cell lines.[20] In addition, increased expression of ERCC1 mRNA has been shown to be associated with clinical resistance to platinum compounds and alkylating agents in ovarian[21] cancer and chronic lymphocytic leukemia[22] patients, respectively. ERCC2 is a helicase, which is supposed to cooperatively work with ERCC3 to unwind the duplex DNA on both sides of the lesion.[23] Previous observations on DNA-repair-deficient mutant CHO cell lines suggest only a marginal role of the ERCC2 gene product in the cellular response to cytotoxic effects of cisplatin and melphalan.[24]

Based on the evidence of a role of topoisomerase I in DNA repair,[25] in our study we used the enzyme-specific inhibitor topotecan as a potential modulator of melphalan and cisplatin cytotoxicity. Topotecan alone caused a comparable cytotoxic effect in sensitive and resistant cell lines. In combination experiments with melphalan, topotecan significantly increased the alkylator's cytotoxic activity, independently of the treatment schedule used. Moreover, the degree of the potentiating effect was similar in the two cell lines. Even though it can be hypothesized that the enhancement of melphalan cytotoxicity by topotecan is due to inhibition of repair of melphalan-induced DNA lesions, it does not seem that such an inhibition was more effective in resistant cells, which are characterized, under basal conditions, by a more efficient DNA repair.

The cisplatin and topotecan combination differentially affected cisplatin cytotoxicity in the two cell lines as a function of treatment scheme. In particular, the best potentiating effect obtained in resistant cells after sequential exposure to cisplatin and topotecan is still consistent with an inhibitory effect of topotecan on the repair of cisplatin-induced DNA lesions. However, the differential effect of topotecan on the activity of cisplatin and melphalan could be related to the different main genotoxic lesions induced by the two alkylators (i.e. DNA *intra*strand cross-links for cisplatin and DNA *inter*strand cross-links for melphalan) and to what degree topoisomerase I is a determinant in the repair of such lesions.

Lonidamine markedly enhanced the cytotoxic activity of melphalan and cisplatin in OAW42 and OAW42MER cells. The potentiation in the resistant cell line was almost 2-fold that of the sensitive cell line. A potentiating effect by the energolytic compound on the activity of alkylating agents has been observed in other experimental models[9,10,26–28] and has been tentatively explained by an inhibitory effect of lonidamine on DNA repair processes.[9] Moreover, in a previous study performed in ovarian cancer cell lines, we observed a significant enhancement of DNA lesions induced by cisplatin after sequential exposure to cisplatin and lonidamine in the cisplatin-resistant cell line but not in the sensitive cell line.[10] Such findings suggest the specific ability of lonidamine to interfere with cisplatin adducts by preventing the repair of those potentially cross-linkable in resistant cells.

Based on recent evidence suggesting a positive modulating effect by calcium channel blockers on melphalan activity, we assessed the effect of flunarizine, verapamil and nimodipine in combination with melphalan or cisplatin. Flunarizine markedly increased melphalan cytotoxicity in the OAW42MER resistant cell line. Although to a lesser degree, a

potentiating effect was also observed in OAW42 parental cells. Such results are in agreement with previous observations by Castellino *et al.*,[12] who described a flunarizine enhancement of melphalan activity in melphalan-sensitive and -resistant rhabdomyosarcoma xenografts. Such an enhancement appeared to be consequent to specific modifications induced by the calcium entry blocker on the cellular transport of melphalan and not of other alkylators. In fact, in the rhabomyosarcoma experimental models, flunarizine failed to potentiate cyclophosphamide activity.[12] Similarly, no enhancement of cisplatin cytotoxicity was observed after concomitant exposure to flunarizine in our ovarian cancer cell lines.

Nimodipine, a calcium entry blocker demonstrated to enhance doxorubicin accumulation as a consequence of increased electric transmembrane potential in doxorubicin-resistant colon cancer cells,[29] failed to affect melphalan or cisplatin cytotoxicity in OAW42 and OAW42MER cell lines. Again, we did not observe any significant effect by verapamil on melphalan and cisplatin activity in the two ovarian cancer cells, which is consistent with the observation of Rogan *et al.*[30] who showed that resistance to melphalan was unaffected by the calcium entry blocker in a human ovarian cancer cell line with primary resistance to the alkylator. Conversely, Robinson *et al.*[31] reported potentiation by verapamil on melphalan cytotoxicity and cellular uptake in murine fibrosarcomas and murine bone marrow, but not in human bone marow. More recently, Averill-Bates and Courtemanche[11] showed that verapamil selectively increased melphalan cytotoxicity in a pleiotropic drug-resistant CHO cell line but not in parental, sensitive CHO cells, and that such an increase was accompanied by alterations in membrane permeability to melphalan. Such findings indicate a different effect of verapamil on melphalan cytotoxicity in the different cellular systems, although there is no clear explanation for the variable sensitization of cells.

Overall, results from our study indicate that, among the potential modulators studied, topotecan, lonidamine and flunarizine are the most promising for combination therapies with alkylators. However, it should be stressed that the main effect of such compounds is not overcoming alkylating drug resistance, but, more generally, increasing drug efficacy also in cells already sensitive to alkylators.

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