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ORIGINAL ARTICLE

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Supraadditive effect of 2′,2′-difluorodeoxycytidine (gemcitabine) in combination with oxaliplatin in human cancer cell lines

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Abstract *Purpose*: This study assessed the cytotoxic effects of the nucleoside analog gemcitabine in combination with the diaminocyclohexane platinum compound oxaliplatin. *Methods*: Growth inhibition studies were performed using the human CEM leukemia cell line and the colon-cancer cell lines HCT 116 and Colo 320 DM. Gemcitabine-oxaliplatin combinations were compared with gemcitabine-cisplatin combinations in the same cell lines using similar experimental settings. Cells were exposed for 2 h to gemcitabine and then for 24 h to oxaliplatin or cisplatin, and vice versa. *Results*: The 50% inhibitory concentrations (IC_{50} values) in single-drug experiments using 2 h of exposure to gemcitabine and 24 h of exposure to oxaliplatin or cisplatin were, respectively, 89 p*M*, 11.1 μ*M*, and 10.3 μ*M* for CEM cells; 46 p*M*, 10.2 μ*M*, and 2.7 μ*M* for HCT 116 cells; and 102 p*M*, 4.6 μ*M*, and 8.6 μ*M* for Colo 320 DM cells. Gemcitabine-oxaliplatin combinations displayed supraadditive effects in human leukemia and colon-cancer cell lines. The sequence of gemcitabine followed by oxaliplatin was more effective than the opposite sequence in HCT 116 and Colo 320 DM colon-cancer cell lines, whereas the sequence of oxaliplatin followed by gemcitabine yielded to synergistic effects in CEM cells. The cytotoxic effects of gemcitabine-oxaliplatin combinations were better than (HCT 116 cells) or equal to (CEM and Colo 320 DM cells) those of gemcitabine-cisplatin combinations. *Conclusion*: Our data show that the combination of gemcitabine with oxaliplatin exerts potent antiproliferative effects in human leukemia and colon cancer cells, warranting further investigations in the framework of phase I–II trials as an alternative for the treatment of solid malignancies.

Key words Combination chemotherapy · Diaminocyclohexaneplatinum · Nucleoside analogs · Gemcitabine · Combination index

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Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine) is an antimetabolite that is structurally related to 1-β-D-arabinofuranosylcytosine (ara-C). Gemcitabine inhibits cell growth by interfering with several pathways of nucleic acid metabolism. The principal mechanism involves the inhibition of DNA synthesis. The triphosphate form of gemcitabine (dFdCTP) is recognized by DNA polymerases and incorporated into the growing DNA strand. This process is followed by the incorporation of one more natural nucleotide, resulting in an arrest of DNA polymerization [17]. Gemcitabine also affects deoxynucleotide pools by interfering with ribonucleotide reductase and inhibits RNA synthesis by incorporation into RNA chains [31]. Both gemcitabine and ara-C inhibit cellular proliferation in the S phase and cause cells to accumulate in the G_1–S phase of the cell cycle. Unlike ara-C, which has demonstrated antitumor activity restricted to hematologic malignancies, gemcitabine has shown a broad spectrum of activity against panels of solid murine tumors and human tumor xenografts [16]. It has been proven active and registered for the treatment of pancreatic cancer [4, 30], and its antitumor activity in various advanced malignancies such as ovarian [18], bladder [23], and lung cancers [15] is evidenced by a 20–30% response rate.

Oxaliplatin is a recent diaminocyclohexane platinum compound that causes DNA damage at the same sites of adduct formation as does cisplatin but has displayed partial, if any, cross-resistance with cisplatin in a wide range of human tumors in vitro and in vivo [21, 27, 35]. European phase II trials have reported encouraging activity and manageable toxicity in malignancies that are usually resistant to cisplatin, including colon [10, 20] and ovarian cancer [7, 22]. The main types of DNA lesions induced by oxaliplatin and cisplatin are intrastrand cross-links covalently binding the platinum compound to guanine radicals [27]. The other types of lesions include DNA interstrand cross-links, DNA protein cross-links, and DNA strand breaks [12]. Oxaliplatin has demonstrated in vitro and in vivo supraadditive effects in combination with several other antitumor agents, including 5-fluorouracil (5-FU), topoisomerase I inhibitors [37], thymidylate synthase inhibitors [26], paclitaxel [33], cisplatin, and carboplatin [29]. Moreover, whereas the sensitivity of cisplatin is decreased in cell lines and xenografts that are deficient in mismatch-repair proteins, the antiproliferative and antitumor effects of oxaliplatin are maintained regardless of the mismatch-repair status [13, 14, 36]. Interestingly, like oxaliplatin, gemcitabine displays similar or superior cytotoxicity in cell lines that are deficient in mismatch-repair proteins as compared with their proficient counterparts [28]. In vitro, gemcitabine combined with the classic compound cisplatin has demonstrated synergistic effects in the human ovarian-carcinoma cell line A2780 [3, 25]. In vivo the combination was at least additive in the head-and-neck-cancer xenograft HNX-22B [25]. Clinical trials using this combination in non-small-cell lung cancer have confirmed preclinical results and have shown high response rates ranging from 38% to 54% [9, 34]. Moreover, cisplatin in combination with gemcitabine has demonstrated significant activity in other tumor types, including mesothelioma [5] as well as urothelial [24] and ovarian cancer [19]. However, the use of this association in clinical trials is often limited by the occurrence of myelosuppression.

Both gemcitabine and oxaliplatin are active drugs in solid tumors and do not have overlapping toxicity. Moreover, on the basis of previous evidence that ara-C enhances the formation of platinum-DNA adducts, it might be expected that gemcitabine potentiate the formation of oxaliplatin-induced DNA lesions [1], which might potentially be more difficult to repair. Therefore, the association of gemcitabine with oxaliplatin might be an attractive clinical alternative for the treatment of solid tumors, including gastrointestinal malignancies.

The purpose of this study was to determine the cytotoxic activity of gemcitabine in combination with oxaliplatin in human leukemia and human colon-cancer cell lines to provide a rationale for future clinical studies. Cell lines were selected on the basis of their mismatch-repair and p53 status. Both possible sequences of gemcitabine and oxaliplatin administration were used to investigate the potential influence of the drug schedule. This growth-inhibition study was conducted in comparison with combinations of gemcitabine with cisplatin in the same cell lines.

Materials and methods

Chemicals

Gemcitabine was purchased from Eli Lilly Company (Indianapolis, Ind.), oxaliplatin was obtained from Sanofi (Great Valley, Pa.), and cisplatin was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Cells and culture conditions

Human leukemia CEM cells obtained from Dr. W.T. Beck (Chicago, Ill.) were cultured in Eagle's minimal essential medium, Joklik's modification (Sigma Chemical, St. Louis, Mo.), supplemented with 10% fetal bovine serum. Human colon cells HCT 116 provided by Dr. R. Boland (La Jolla, Calif.) were cultured in monolayers using IMEM (Gibco, Gaithersburg, Md.) supplemented with 10% fetal bovine serum. The Colo 320 DM cell line was purchased from the American Type Culture Collection (ATCC, Rockville, Md.) and cultured in monolayers using RPMI 1640 (Gibco, Gaithersburg, Md.) supplemented with 10% fetal bovine serum. Cells were regularly checked for mycoplasma infection and were grown in a humidified atmosphere containing 5% CO_2 at 37 °C. The p53 and mismatch-repair (MMR) status of the three cell lines is summarized in Table 1. CEM cells harbor two missense mutations located on separate alleles; thus, both alleles of the p53 gene may have been functionally inactivated by two different point mutations [6]. The human colon-cancer cell line HCT 116 is deficient in DNA mismatch repair (MMR) because of a genetic defect in the hMLH1 gene, which is located on chromosome 3 [11].

Cytotoxicity studies

At 1 day before drug additions, $2.5–5 \times 10^5$ cells were seeded in 35-mm 6-well plates (Becton Dickinson Labware, Franklin Lakes, N.J.) containing 2 ml of growth medium. The cells were exposed to gemcitabine for 2 h, with and without a 24-h period of exposure to oxaliplatin or cisplatin, and were then washed with phosphate-buffered saline (PBS) and cultured in drug-free media for 48 h after initial drug addition. For combination, sequential studies were performed using gemcitabine and either oxaliplatin or cisplatin in both possible sequences (Fig. 1). A range of nonconstant ratios was first investigated on the basis of the IC_{50} of single-drug experiments. The ratio leading to the best cytotoxic effect was then selected for the experiment using a constant ratio at increasing concentrations. For experiments involving exposure to a combination of gemcitabine and a platinum compound (oxaliplatin or cisplatin) the drugs were combined at gemcitabine-to-oxaliplatin/cisplatin concentration ratios of $1:10^{-4}$ for experiments using CEM cells, $1:10^{-5}$ for experiments using HCT 116 cells, and $1:10^{-5}$ for experiments using Colo 320 DM cells. Cell numbers were determined after trypsinization for monolayer cells using a ZM

Table 1 Cell line characteristics for p53 and mismatch-repair status (*ND* Not determined)

	P53 status	Mismatch-repair status
CEM	Mutated	ND
HCT 116	Wild type	Deficient[a]
Colo 320 DM	Mutated	Proficient

[a] Mismatch-repair deficiency for hMLH1 protein

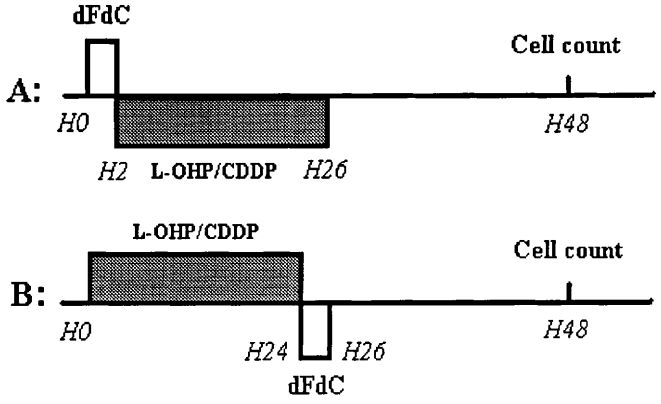


Fig. 1A,B Schedule of gemcitabine-based combinations. The cells were exposed **A** to gemcitabine for 2 h, followed by a 24-h period of exposure to oxaliplatin or cisplatin, or **B** to the opposite sequence. Cells were then washed with PBS and cultured in drug-free media for up to 48 h after initial drug addition. Cell counts were performed at 48 h after initial drug addition (*dFdC* Gemcitabine, *L-OHP* oxaliplatin, *CDDP* cisplatin)

Coulter counter (Coultronics, Luton, UK). Results were given as mean values ± SEM for three experiments performed in duplicate, except for HCT 116 (two experiments performed in triplicate).

Functional interactions between drugs

The combined drug effects were evaluated using the Chou and Talalay analysis based on the median-effect principle [8]. This method involves the plotting of dose-effect curves for each drug and for multiply diluted, fixed-ratio combinations using the equation: $f_a/f_u = (C/C_m)^m$, where C is the drug concentration, IC_{50} being the concentration required for half-maximal effect (i.e., 50% inhibition of cell growth); f_a is the cell fraction affected by drug concentration C (e.g., 0.9 if the cell growth is inhibited by 90%); f_u is the unaffected fraction; and m is the coefficient of sigmoidicity of the concentration-effect curve.

The IC_{50} used for calculation of Dm was estimated not from cytotoxicity curves but from the data recorded for each experiment. In the experiments performed, cells displayed linear growth, and there was no experiment in which fewer cells were present at the end as compared with the beginning of the experiment. The growth inhibition was calculated as the percentage of cells counted at 48 h after initial drug addition in comparison with the number of cells counted at the moment of drug addition. On the basis of the slope of the concentration-effect curves it can be determined whether the drugs have mutually nonexclusive effects (e.g., independent or interactive mode of action). The combination index (CI) is then determined by the equation:

$$CI = \{(C)_1/(C_x)_1\} + \{(C)_2/(C_x)_2\} + \{\alpha(C)_1(C)_2/(C_x)_1(C_x)_2\} \ ,$$

where $(C_x)_1$ is the concentration of drug 1 required to produce x percent of effect alone, and $(C)_1$ is the concentration of drug 1 required to produce the same x percent of effect in combination with $(C)_2$. If the mode of action of the drugs is mutually exclusive or nonexclusive, then α is 0 or 1, respectively. In this study the analysis was performed using the mutually exclusive case. We calculated CI values by solving the equation for different values of f_a (i.e., different degrees of inhibition of cell growth). CI values of < 1 indicate synergy, values equal to 1 indicate additive effects, and values of > 1 indicate antagonism.

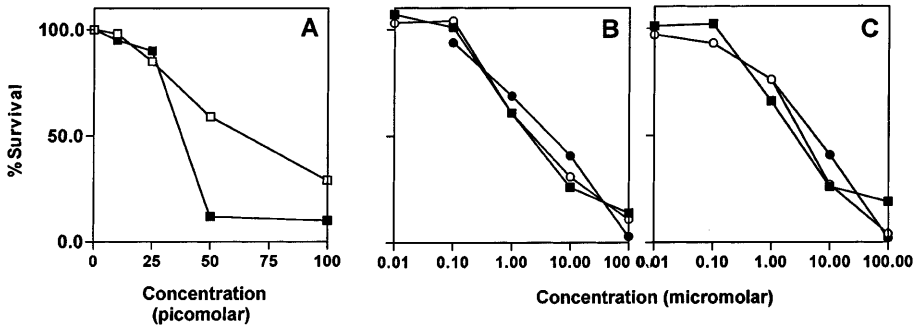
Data analysis was performed using the concentration-effect analysis for microcomputer software (Biosoft, Cambridge, UK) on an IBM PC. Statistical analysis and graphs were performed using Instat and Prism software (GraphPad, San Diego, Calif.). The dose-effect relationships for the drugs tested alone or in paired combinations were subjected to the median-effect plot for determination of their relative potency (IC_{50}), shape (m), and conformity (r) in each selected cell line. As defined previously, the IC_{50} and m values were used for the calculation of synergism or antagonism as based on the CI equation. Results were given as mean values ± SEM for three experiments performed in duplicate. In each experiment, cells were exposed to the paired combinations for 48 h. Comparisons of mean values and standard deviations used Student's t-test (two-sided P value).

Results

Single-drug experiments

For gemcitabine the cytotoxic effect of short- (2-h) versus long-term (24-h) incubation was first compared in CEM cells (Fig. 2A). The antiproliferative activity was so marked after 24 h of continuous incubation that it did not allow reproducible determinations of cytotoxicity parameters, given the shape of the curve. Moreover, the dramatic growth inhibition rates associated with this schedule would not likely permit the detection of a synergistic interaction on the use of this drug in combination. A 2-h period of exposure was therefore chosen for subsequent experiments in the leukemia and the colon-cancer cell lines.

Fig. 2A–C Cytotoxicity of drugs in single-drug experiments. **A** Cytotoxic activity of gemcitabine as determined in CEM cells using 2-h (□) and 24-h (■) drug incubation followed by 46- and 24-h incubation in drug-free medium, respectively. **B** Cytotoxic activity of oxaliplatin as determined in CEM (■), HCT 116 (○), and Colo 320 DM cells (●) using 24-h drug incubation followed by 24-h incubation in drug-free medium. **C** Cytotoxic activity of cisplatin as determined in CEM (■), HCT 116 (○), and Colo 320 DM cells (●) using 24-h drug incubation followed by 24-h incubation in drug-free medium



Using a 2-h exposure period the cytotoxic activity of gemcitabine was evaluated at 24 and 48 h after initial drug addition. The IC_{50} values are summarized in Table 2. The sensitivity to gemcitabine was higher in CEM and HCT 116 cells than in Colo 320 DM cells, the latter showing IC_{50} values above 100 p*M*, even at 48 h after initial drug addition. For all cell lines, especially Colo 320 DM, the culture in drug-free medium did not allow the cells to recover, as the IC_{50} values decreased between 24 and 48 h after initial drug addition.

For exposure to oxaliplatin and cisplatin the cells were treated by 24 h of continuous incubation. The growth inhibition rates were determined at 24 h after the end of drug removal. The dose-response curves in CEM, HCT 116, and Colo 320 DM cells are shown for oxaliplatin (Fig. 2B) and cisplatin (Fig. 2C). IC_{50} values are summarized in Table 2.

Cytotoxicity of gemcitabine-based combinations

The dose-effect interactions of the three drugs tested alone or in combination were subjected to the median-effect plot for determination of their relative potency (Dm), shape (*m*), and conformity (*r*) in each selected cell line. As defined above, the Dm and *m* values were used for the calculation of synergism or antagonism as based on the CI equation. Each experiment was repeated three times in duplicate in CEM and HCT 116 cell lines and two times in triplicate in the Colo 320 DM cell line. Sequential studies were performed using 2 h of gemcitabine exposure followed by 24 h of oxaliplatin or cisplatin exposure, and vice versa.

In the CEM cell line the combination of gemcitabine followed by oxaliplatin showed partial antagonism for concentrations corresponding to a fraction affected of above 0.50 (Fig. 3A). In contrast, the opposite sequence yielded strong synergistic effects (Fig. 3B). The combination of gemcitabine followed by cisplatin showed partial antagonism at concentrations corresponding to a fraction affected of above 0.50 (Fig. 3C), whereas the opposite sequence (cisplatin followed by gemcitabine) showed synergistic effects in the same range of concentrations (Fig. 3D).

In the HCT 116 cell line, gemcitabine combined with oxaliplatin in both possible sequences showed synergistic effects on growth inhibition, the combination of gemcitabine followed by oxaliplatin being slightly more potent than the opposite sequence (Fig. 4A, B). Gemcitabine followed by cisplatin showed additive effects, whereas the opposite sequence showed antagonistic effects (Fig. 4C, D).

In the Colo 320 DM cell line, gemcitabine followed by oxaliplatin showed synergistic effects (Fig. 5A), whereas the opposite sequence (oxaliplatin followed by gemcitabine) showed additive effects (Fig. 5B). Gemcitabine followed by cisplatin showed weak synergistic effects (Fig. 5C), whereas the opposite sequence had additive effects (Fig. 5D).

Discussion

This study was designed to assess the cytotoxic activity of the recent nucleoside analog gemcitabine in combination with the diaminocyclohexane platinum compound oxaliplatin and to compare this antiproliferative activity with the effects of gemcitabine in combination with cisplatin.

In single-drug experiments the cytotoxic effect of gemcitabine was strongly correlated with the duration of drug exposure in CEM cells. Indeed, 24 h of protracted exposure induced high rates of growth inhibition that reached 90% at 50 p*M* concentration as compared with 40% inhibition for 2 h of exposure. The IC_{50} values of platinum compounds were in the range of 2–10 μ*M* in HCT 116 and Colo 320 DM cells, cisplatin being more potent than oxaliplatin in the HCT 116 cell line. Conversely, cisplatin was slightly less cytotoxic than oxaliplatin in Colo 320 DM cells.

We observed that gemcitabine in combination with oxaliplatin resulted in supraadditive effects in one leukemia cell line and two different human colon-cancer cell lines (Table 3). Very interesting results were obtained in colon-cancer cell lines, particularly in HCT 116 cells, where gemcitabine applied in combination with oxaliplatin in both possible sequences displayed synergistic cytotoxic effects. However, in Colo 320 DM the combination of gemcitabine followed by oxaliplatin showed supraadditive effects, whereas the opposite sequence was only additive. The sequence effects of the combinations appear to be cell-line-dependent. Surprisingly, whereas the best sequence in both colon-cancer cell lines was gemcitabine followed by oxaliplatin, the most efficient combination in leukemia CEM cells was oxaliplatin followed by gemcitabine. The mechanisms underlying this sequence-dependent effect are yet unknown.

The difference in the results obtained in the individual cell lines may be explained in part by their genetic status. Both oxaliplatin [13] and gemcitabine [28] have shown potent cytotoxicity in MMR-deficient cells in comparison with MMR-proficient cells. Interestingly, in our study, oxaliplatin-gemcitabine combinations displayed better results in the MMR-deficient HCT116 cell line

Table 2 IC_{50} values recorded for single-drug experiments. Mean values are given *in boldface*; SEM values are shown *in parentheses*

	CEM	HCT 116	Colo 320 DM
Gemcitabine 2-h incubation, H24 (p*M*)	**116** (±34)	**157** (±93)	**247** (±6)
Gemcitabine 2-h incubation, H48 (p*M*)	**89** (±34)	**46** (±10)	**102** (±31)
Oxaliplatin 24-h incubation, H48 (μ*M*)	**11.1** (±0.75)	**10.2** (±4.9)	**4.6** (±1.7)
Cisplatin 24-h incubation, H48 (μ*M*)	**10.3** (±5.6)	**2.7** (±1.5)	**8.6** (±1.0)

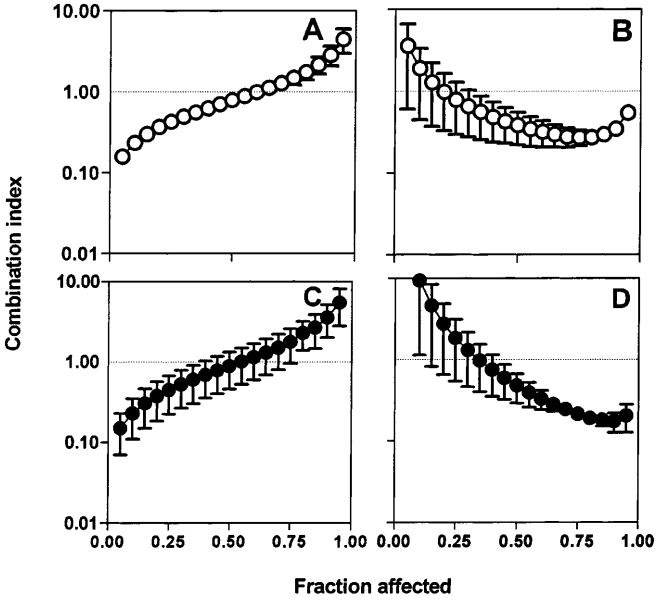


Fig. 3A–D Cytotoxicity of gemcitabine-based combinations in CEM cells as determined **A** gemcitabine (2-h incubation) followed by 24-h incubation with oxaliplatin and **B** the opposite sequence and using **C** gemcitabine (2-h incubation) followed by 24-h incubation with cisplatin and **D** the opposite sequence. Functional interactions between drugs were determined using median-effect plot analysis according to the method described by Chou and Talalay [8]. A combination index below 1 indicates synergy. *Error bars* indicate SEM values

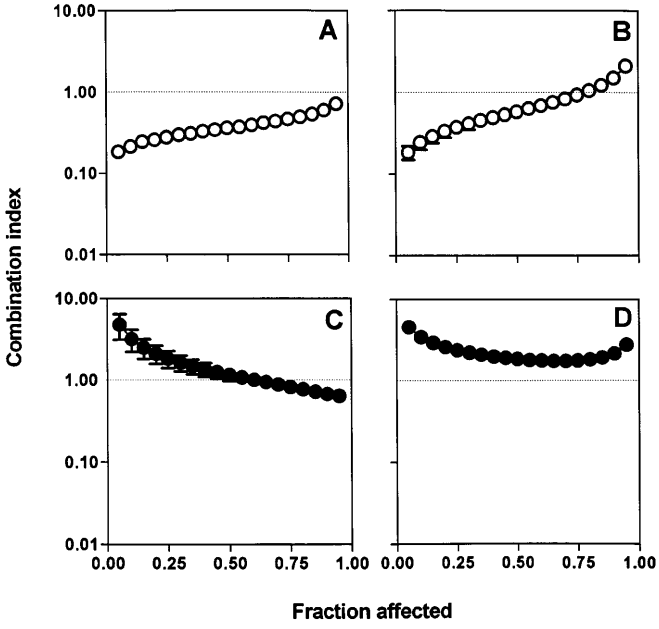


Fig. 4A–D Cytotoxicity of gemcitabine-based combinations in HCT 116 cells as determined using **A** gemcitabine (2-h incubation) followed by 24-h incubation with oxaliplatin and **B** the opposite sequence and using **C** gemcitabine (2-h incubation) followed by 24-h incubation with cisplatin and **D** the opposite sequence. *Error bars* indicate SEM values

than in its MMR-proficient counterpart Colo 320 DM. However, it is noteworthy that the combination gemcitabine-oxaliplatin remained synergistic in Colo 320 DM, in which low levels of drug sensitivity would be expected because of mutant p53 and *myc* amplification. Other factors may possibly affect the sensitivity of the cell lines, such as deoxycytidine kinase and dFdCTP accumulation and retention, incorporation into DNA and RNA, inhibition of ribonucleotide reductase, deoxyribonucleosidetriphosphate pools, and dCMP deaminase. Although the importance of p53 and MMR status needs to be confirmed in further experiments using different cell lines, these observations may have potential clinical implications since colorectal cancer is known to present a high frequency of genetic alterations, including p53 mutations [2] and MMR deficiency [32]. Our results support an increased probability of action of oxaliplatin-gemcitabine combinations in this type of cancer. Given the reproducibility of the results obtained in the two colon-cancer cell lines, the sequence of gemcitabine followed by oxaliplatin appears to be suitable for future clinical trials in solid tumors.

Another interesting aspect of our results resides in comparison of the results of oxaliplatin-gemcitabine combinations with those obtained for cisplatin-gemcitabine combinations in similar experimental settings. In all cell lines, oxaliplatin-gemcitabine combinations were at least as cytotoxic as cisplatin-gemcitabine combinations (Table 3). Moreover, in colon-cancer cell lines, especially the HCT 116 cell line, oxaliplatin was superior to cisplatin in combination with gemcitabine. Only one study has previously assessed the cytotoxicity of gemcitabine in combination with platinum compounds [25]. Peters et al. studied various combinations of gemcitabine and cisplatin using ovarian cancer cell lines. A synergistic effect was observed with gemcitabine-cisplatin combinations in the wild-type A2780 and the

Table 3 Sequence dependence of the cytotoxic effects of gemcitabine-based combinations with oxaliplatin and cisplatin. *In parentheses* are the values corresponding to a fraction affected of 0.50 ± SEM[a] (*dFdc* Gemcitabine, *L-OHP* oxaliplatin, *CDDP* cisplatin)

	dFdC → L-OHP	L-OHP → dFdC	dFdC → CDDP	CDDP → dFdC
CEM	+/– (0.797 ± 0.072)	++ (0.387 ± 0.167)	+/– (0.890 ± 0.430)	+ (0.477 ± 0.187)
HCT 116	++ (0.365 ± 0.005)	+ (0.580 ± 0.080)	+/– (1.155 ± 0.195)	– (1.865 ± 0.055)
Colo 320 DM	++ (0.517 ± 0.178)	+/– (0.807 ± 0.085)	+ (0.663 ± 0.110)	+/– (0.517 ± 0.207)

[a] Synergistic effect: +, ++; additive effect: +/–; antagonistic effect: –

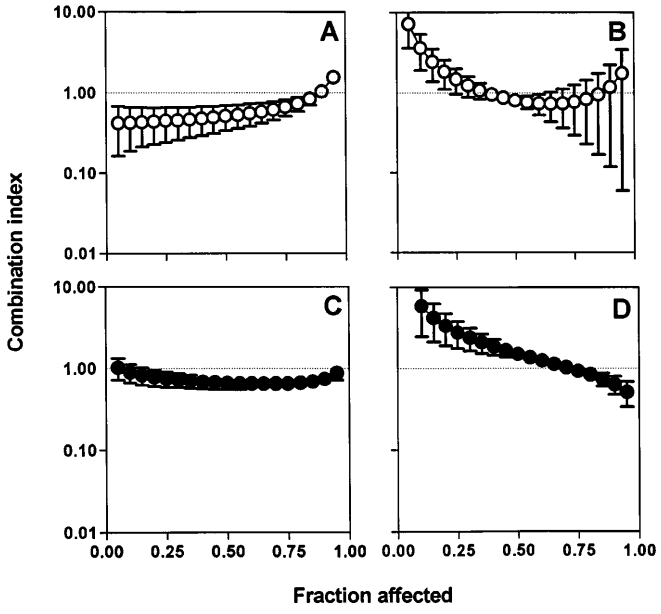


Fig. 5A–D Cytotoxicity of gemcitabine-based combinations in Colo 320 DM cells as determined using **A** gemcitabine (2-h incubation) followed by 24-h incubation with oxaliplatin and **B** the opposite sequence and using **C** gemcitabine (2-h incubation) followed by 24-h incubation with cisplatin and **D** the opposite sequence. *Error bars* indicate SEM values

cisplatin-resistant ADDP ovarian cells for periods of drug exposure longer than 4 h. In the gemcitabine-resistant AG6000 ovarian cells the effect of the combination was dependent on the scheduling of the two drugs, and the authors concluded that the optimal effects of the combinations required a certain degree of sensitivity to either of the drugs. In our study we reached a similar conclusion, as the gemcitabine-oxaliplatin combinations were more active in the HCT 116 cells than in the Colo 320 DM cells, which are characterized by a low level of sensitivity to gemcitabine. The synergistic effects of gemcitabine-cisplatin combinations described by Peters et al. [25] in ovarian cell lines were not reproducible in the colon-cancer cell lines HCT 116 and Colo 320 DM, suggesting that the effect of the combination may be dependent on the type of tumor cell line involved.

Both oxaliplatin and gemcitabine exert their cytotoxic activity at the DNA level but, interestingly, they act through different mechanisms of action. Moreover, cellular DNA is not the only target for these two drugs. Only few preclinical data are available concerning the mechanisms of interaction between platinum compounds and gemcitabine [25]. Gemcitabine-cisplatin combinations showed synergistic cytotoxic effects in ovarian cancer cells. The mechanism of this interaction was not elucidated by the authors, as this synergistic effect could not be explained by accumulation of dFdCTP or DNA damage. Because gemcitabine is structurally related to the nucleoside antimetabolite 1-β-D-arabinofuranosylcytosine (ara-C), we can draw reference from previous studies using the nucleoside analog 5-azacytidine (5-aza)-CdR in combination with cisplatin [1]. In this study the authors concluded that the synergistic interaction was likely to take place at the DNA level and that the incorporation of the antimetabolite into DNA increased cisplatin binding to DNA. Since oxaliplatin binds to DNA similarly to cisplatin, this mechanism might have been involved in the synergistic effects we observed with oxaliplatin in combination with gemcitabine.

In summary, our study showed synergistic activity between gemcitabine and oxaliplatin in leukemia and colon-cancer cell lines. The combination was as least as effective as gemcitabine-cisplatin combinations. The sequence of gemcitabine followed by oxaliplatin was slightly more effective than the opposite sequence. Given the encouraging results obtained with this combination on cell growth inhibition, further studies could be designed to determine the mechanisms underlying the synergistic effects of oxaliplatin-gemcitabine combinations. On the basis of the present results, phase I–II trials will shortly be implemented to determine the safety and efficacy of gemcitabine-oxaliplatin combinations.

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