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Antitumor activity of oxaliplatin in combination with 5-fluorouracil and the thymidylate synthase inhibitor AG337 in human colon, breast and ovarian cancers

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Oxaliplatin, classical [5-fluorouracil (5-FU)] and non-classical (AG337) thymidylate synthase inhibitors have shown promising activity in the treatment of cancer. This study investigates the cytotoxic effects of oxaliplatin in combination with 5-FU and AG337 in cultured human colon (HT29, CaCo2), breast (MCF-7, MDA-MB-231) and ovarian (2008) cancer cell lines, and their derived counterparts selected for their resistance to 5-FU (HT29-5-FU), doxorubicin (MCF-7mdr) or cisplatin (2008C13). Therapeutic experiments were conducted in mice bearing colon-HT29 xenografts and in the GR hormone-independent mammary carcinoma model. *In vitro*, oxaliplatin shows potent cytotoxic activity in colon (IC_{50} from 2.1 ± 1.1 to 5.9 ± 1.7 μM), ovarian (IC_{50} = 10 ± 1.6 μM) and breast cancer cells (IC_{50} from 7.4 ± 2.7 to 17.9 ± 7.1 μM). Oxaliplatin was a potent inhibitor of DNA synthesis and bound to cellular DNA. Surprisingly, the overall amount of oxaliplatin DNA binding was significantly inferior to that induced by isocytotoxic concentrations of cisplatin in HT29 (p=0.026). *In vitro*, synergistic antiproliferative effects were observed when oxaliplatin was added to 5-FU and AG337. Those synergistic effects of combinations were maintained in colon HT29-5-FU cancer cells. *In vivo*, 5-FU increased significantly the antitumor activity of oxaliplatin in HT29 xenografts (p=0.0036), and similarly 5-FU and AG337 increased the activity of oxaliplatin in the GR tumor model (p=0.0012). These data may encourage further clinical investigation of oxaliplatin in combination with classical and non-classical thymidylate synthase inhibitors in the treatment of human cancers.

Key words: Combination chemotherapy, platinum, quinazoline antifolate.

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Introduction

Diaminocyclohexane platinum compounds have received considerable attention in recent years because of their activity against cisplatin-resistant tumors and their safe toxicity profile as compared to cisplatin.[1] The substitution of the amine radical in cisplatin by a 1,2-diaminocyclohexane motif yielded a family of molecules of which oxaliplatin [(1*R*,2*R*-diaminocyclohexane)oxalatoplatinum] was selected for clinical development.[2,3] Oxaliplatin has demonstrated original cytotoxic effects against several human cancer cell lines,[4,5] antitumor activity in patients with cisplatin-refractory ovarian and colon cancer,[6,7] no nephrotoxicity, and reduced myelotoxicity in clinical trials.[7,8] *In vivo* data have suggested synergy between oxaliplatin and 5-fluorouracil (5-FU)/folinic acid combinations in a leukemia model.[3] Since resistance to chemotherapy is a common feature in colon,[9] breast[10] and ovarian cancer,[6] oxaliplatin and oxaliplatin-based combinations with other active drugs deserve further preclinical investigations.

Thymidylate synthase inhibition is thought to be the primary mechanism of 5-FU cytotoxicity, leading to inhibition of DNA synthesis through alterations of the pyrimidine pathway.[11,12] Recent progress in protein crystallography allowed the design and synthesis of new compounds which bind thymidylate synthase with high specificity.[13] Classical folinic acid analogs that contain a glutamate residue require facilitated transport across the cell membrane and are frequently converted to polyglutamate derivatives inside the cells. Conversely, non-classical inhibitors that lack the terminal glutamate moiety are more lipophilic rapidly cross the membranes by passive diffusion and are not subject to polyglutamation. Those criteria may allow

the inhibition of thymidylate synthase in cells resistant to classical antifolate. Among these drugs, the non-classical thymidylate synthase inhibitor AG337 [3,4-dihydro-2-amino-6-methyl-4-oxo-5-(4-pyridylthio)-quinazolone dihydrochloride] has demonstrated activity against human cancers with acceptable toxicity and adverse effects in phase I trials.[14] Both classical and non-classical thymidylate synthase inhibitors have demonstrated additive or synergistic effects with cisplatin.[12] Therefore, the potentially favorable cytotoxicity of oxaliplatin in cells resistant to cisplatin and its non-overlapping undesirable effects with the thymidylate synthase inhibitors in clinical trials make oxaliplatin and thymidylate synthase inhibitors suitable pairs for future clinical combination chemotherapy.

The purpose of our study was to evaluate the cytotoxic and antitumor effects of oxaliplatin in combinations with 5-FU and the non-classical thymidylate synthase inhibitor AG337 in human cancer cells. To parallel clinical situations, we selected 5-FU-resistant colon cancer, cisplatin-resistant ovarian cancer, and doxorubicin-resistant and hormone-independent breast carcinomas. Oxaliplatin-based combinations were studied in human colon HT29 tumor xenografts and in the GR mouse mammary tumor model. The transplantable GR mouse mammary carcinoma model mimics human breast cancer in its hormone dependency at early passages, with progression to a hormone-independent phenotype.[16] Since protracted or repeated infusions of 5-FU and continuous oral treatment with AG337 seem to induce superior antitumor effects in clinical trials we decided to expose cells to drug combination for 48 h. We applied the combination index–isobologram method to analyze experimental data and to determine synergism or antagonism between drugs.[17]

Materials and methods

Chemicals

Oxaliplatin was obtained from Debiopharm (Lausanne, Switzerland). Cisplatin and 5-FU were purchased from Sigma (St Louis, MO). AG337 was provided by Agouron (La Jolla, CA).

Cell lines

Colon cancer cell lines HT29 and CaCo2 were obtained from Dr J Fogh (Sloan Kettering Institute for Cancer Research, NY). The HT29-5-FU cell line, obtained from Dr T Lesuffleur (INSERM U178, Villejuif, France), was selected from parental HT29 cells after progressive growth adaptation to 5-FU.[18] Breast cancer cell line MCF-7 and the derived subline MCF-7mdr selected for growth in the presence of doxorubicin were obtained from Dr F Calvo (Hôpital Saint-Louis, Paris, France). MDA-MB-231 is a hormone-independent mammary cancer cell line obtained from Dr J Foekens (Daniel Den Hoed Clinic, Rotterdam, The Netherlands). The A2008 ovarian cancer cell line and the platinum-resistant subline 2008C13, selected *in vitro* by stepwise increases of cisplatin concentrations in the medium,[19] were obtained from Dr P Canal (Centre Claudius Regaud, Toulouse, France).

Culture conditions

Colon and breast cancer cells were cultured at 37°C in a humidified atmosphere of 95% air–5% CO_2 in Dulbecco's modified Eagle's minimum essential medium (DMEM; Eurobio, Paris, France). Culture medium contained 10% (v/v) heat-inactivated fetal calf serum (FCS; Boehringer, Mannheim, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin and 8 mM glutamine. Ovarian cancer cells were maintained in RPMI 1640 medium containing 10% FCS and antibiotics. The cisplatin-resistant 2008C13 cells were challenged weekly with 50 μM cisplatin for 1 h. The MCF-7mdr and the HT29-5-FU cells were maintained in media containing doxorubicin (1 μg/ml) and 5-FU (10 μM), respectively. The medium was renewed every 2 days and the cells were passaged once or twice a week by trypsin/EDTA to maintain the cultures in exponential growth.

Cytotoxicity studies

Two days before drug additions, $1.5–2 \times 10^5$ cells were seeded in 35 mm six-well flasks (Corning, New York, NY) containing 2 ml of growth medium. Growth rates were determined from days 0 to 4. One six-well flask was used for each drug concentration. Cell numbers were determined after trypsinization using a Coulter counter ZM (Coultronics, Luton, UK). Results were given as mean ± SD of three experiments performed in duplicate.

Functional interactions between drugs

The combined drug effects were evaluated by using the Chou and Talalay analysis based on the median-effect principle.[17] This method involves plotting of

dose-effect curves for each drug and for multiply diluted, fixed-ratio combinations by using the equation: $f_a/f_u = (C/C_m)^m$, where C is the drug concentration, IC_{50} is 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. Based on the slope of the concentration-effect curves, it can be determined whether the drugs have mutually non-exclusive 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 effect alone and $(C)_1$ is the concentration of drug 1 required to produce the same x percent effect in combination with $(C)_2$. If the mode of action of the drugs is mutually exclusive or non-exclusive, then α is 0 or 1, respectively. CI values were calculated by solving the equation for different values of f_a (i.e. different degrees of inhibition of cell growth). CI values of below 1 indicate synergy, values equal to 1 indicate additive effects and values above 1 indicate antagonism.

Data analysis was performed using the concentration-effect analysis for microcomputer software (Biosoft, Cambridge, UK) on an IBM-PC computer. Statistical analysis and graphs were performed using Instat and Prism software (GraphPad, San Diego, CA). The dose-effect relationships for the drugs tested alone or in paired combinations were subjected to the median-effect plot in order to determine 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 calculating synergism or antagonism based on the CI equation. Results were given as mean ± SD of three experiments performed in duplicate. In each experiment, cells were exposed to the paired combinations for 48 h.

DNA synthesis and cell cycle analysis

For thymidine incorporation, cells were seeded in 24-well plates ($10\text{-}50 \times 10^3$ cells/well). The inhibition of DNA synthesis induced by the platinum salts was measured by the relative incorporation of [^{3}H]thymidine (2 μCi/ml) after 30 min pulses. For flow cytometry analysis, $1\text{-}2 \times 10^6$ cells were harvested from 100 mm Petri dishes by trypsin, fixed in ethanol (70%) and stored at 4°C. Cells were centrifuged, suspended in 0.1% Triton X-100, treated with RNase (0.2 g/ml) and propidium iodide (20 g/ml) for 30 min at room temperature, and analyzed in an Orthocyto-fluorograph 50H (Ortho Diagnostic Systems, Westwood, MN).

Determination of platinum–DNA binding

Cell DNA was extracted after sodium dodecyl sulfate lysis and proteinase K digestion. The plasma-mass spectrometry method has been described.[20] Platinum-DNA adducts were determined by an inductively coupled argon plasma-mass spectroscopy method in an Elan 5000 Perking Elmer IPC Mass spectrometer. The method yields a linear response for a platinum concentration range between 0 and 3.3 μmol. In water, the limit of platinum detection is 0.16 fmol and the limit of quantification is 0.26 fmol platinum.[21] Platinum binding to DNA has been evaluated after different incubation times with isomolar concentrations of oxaliplatin, cisplatin and in the presence or absence of 5-FU. Experiments were repeated three times.

Antitumor activity

The antitumor effect of drug combinations was investigated in the athymic nude mouse xenograft and the GR mouse mammary tumor models. Mice care was in accord with institution guidelines. At least six mice were used per experimental group. Treatment was given by i.p. injection. The maximum tolerated dose (MTD) of oxaliplatin alone and in combination with 5-FU and AG337 was determined. The MTD was defined as the dose leading to reversible 25–30% body weight loss, without toxic deaths. The HT29 human colorectal xenografts ($1\text{-}2 \times 10^6$ cells) were transplanted s.c. into the hind limb of nude mice. The treatment was started after the tumor became palpable by day 13. The GR mice, obtained from M Sluyser (NKI Amsterdam, The Netherlands), were transplanted s.c. with a late-passage steroid-independent suspension of tumor fragments.[16] Treatments were performed on days 4, 6 or 10 after transplantation. The animals were weighed concurrently with tumor measurements. In all cases, mice were sacrificed before the tumor load or general condition of the animals reduced their mobility. At the time of sacrifice, the tumor was excised, weighed, and when required paraffin-embedded and microscopically analyzed. Antitumor

activity was defined following the recommendation of Schabel.[22] Four separate experiments were conducted using 28 animals for each experimental group (control and paired combinations).

Statistical analysis

Data were expressed as means ± SD. Statistical analyses were performed using the χ^2 test, the *t*-test and the two-way ANOVA for comparisons. The *p* values reported represent two-sided tests of statistical significance.

Results

Effects of single drugs in cultured cells

In the HT29 colon cancer cell line, the antiproliferative activity of oxaliplatin increased with the time of exposure, from the inhibitory potency (IC_{50}) of 14 μM after 8 h incubation to 2.1 μM after 48 h. Subsequent cytotoxic experiment with 48 h exposure of oxaliplatin in other cancer cell lines are shown in Table 1. Cell cycle analysis showed that after 24 or 48 h of oxaliplatin exposure, the proportion of HT29 cells in the G_1 phase increased, with a concomitant decrease of cells in S and G_2 phases (data not shown). After 24 and 48 h incubation with oxaliplatin, we observed a potent inhibition of DNA synthesis in HT29 cells (IC_{50} = 1.8–2 μM concentration range). A time-related increase of total adduct formation in cellular DNA was observed with oxaliplatin (Figure 1).

In subsequent experiments, the cytotoxic effects of oxaliplatin and thymidylate synthase inhibitors have been evaluated in colon, breast and ovarian cancer cell lines (Table 1). In the HT29 cells, the most potent cytotoxic compound was AG337 (IC_{50} = 0.17 ±

Table 1. Cytotoxic activity of oxaliplatin, cisplatin, 5-FU and AG337 in human colon, breast and ovarian cancer cell lines

Cancer cell lines	IC_{50} values (μM)[a]		
	Oxaliplatin	5-FU	AG337
Colon			
HT29	2.1 ± 1.1	2.7 ± 1.2	0.17 ± 0.1
HT29-5-FU	1.7 ± 0.8	12.6 ± 2.8	0.27 ± 0.07
CaCo2	5.9 ± 1.7	12.5 ± 3.0	nd
Breast			
MCF-7	7.4 ± 2.7	23 ± 3.2	nd
MCF-7mdr	12.2 ± 9.1	43.2 ± 16.8	nd
MDA-MB-231	17.9 ± 7.1	57.6 ± 7.1	nd
Ovarian			
2008	10 ± 1.6	11.7 ± 1.9	0.7 ± 0.1
2008 C13	13.5 ± 4.6	8.4 ± 1	7.5 ± 2.8

[a]The cytotoxic activity is expressed in terms of inhibitory potency IC_{50} (drug concentration giving half-maximal inhibition of cell growth) determined after 48 h of incubation. Results are given as IC_{50} values (means ± SD) from three separate experiments performed in duplicate. ND: not determined.

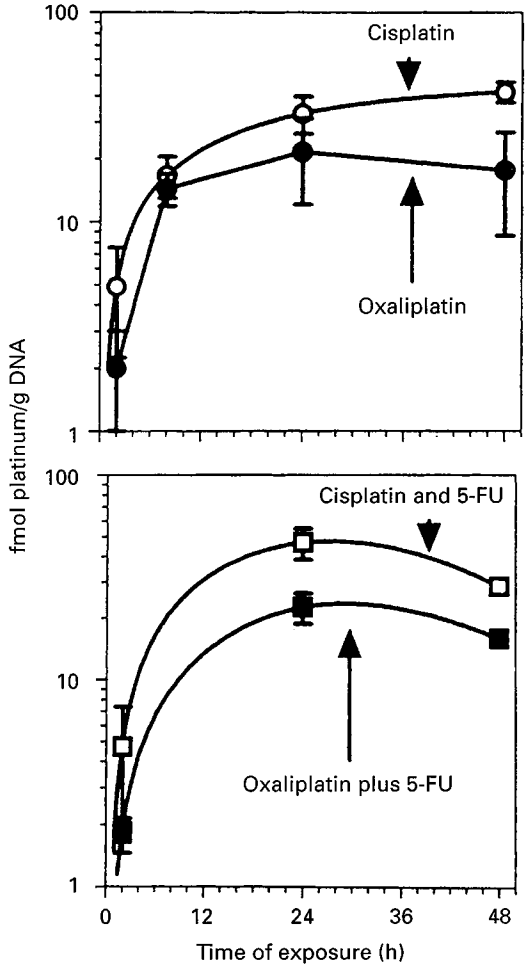

Figure 1. Quantitative platinum DNA binding in human HT29 colon cancer cells. Cells were exposed to oxaliplatin (●, 1 μM), cisplatin (○, 1 μM) or platinum–5-FU combinations (1 μM platinum compounds plus 1 μM 5-FU; oxaliplatin: ■, cisplatin: □). The μM concentration of oxaliplatin (IC_{50} = 2.1 ± 1.1 μM) induced an equitoxic effect, as compared to cisplatin (IC_{50} = 2.8 ± 0.5 μM) in HT29 cells. Cellular DNA was extracted after sodium dodecyl sulfate lysis and proteinase K digestion. Platinum was measured by plasma-mass spectrometry.[21] Experiments were performed in triplicate. Results are given as means ± SD. Total platinum–DNA binding was significantly higher with cisplatin than with oxaliplatin (p = 0.0025), the difference being maximal between 24 and 48 h. 5-FU did not significantly modify platinum–DNA binding of oxaliplatin (p = 0.88) and cisplatin (p = 0.99).

0.1 μM), while 5-FU ($IC_{50} = 2.7 \pm 1.2$ μM) demonstrated antiproliferative potency similar to oxaliplatin ($IC_{50} = 2.1 \pm 1.1$ μM). HT29-5-FU cells were 4.6-fold more resistant to 5-FU ($IC_{50} = 12.6 \pm 2.8$ μM) than the parental HT29 cells ($p = 0.043$). The cytotoxicity of oxaliplatin was not significantly modified in colon cancer cells resistant to 5-FU, as compared to parental cells (Table 1, $p = 0.44$). In the human breast cancer MCF7 cell line, oxaliplatin ($IC_{50} = 7.4 \pm 2.7$ μM) had superior antiproliferative activity than 5-FU ($IC_{50} = 23 \pm 3.2$ μM, $p < 0.01$). In doxorubicin-resistant MCF-7mdr cells, the sensitivity to oxaliplatin was not significantly altered ($p = 0.24$). The 2008 ovarian cancer cells showed an intrinsic resistance to oxaliplatin.

Effects of drug combinations *in vitro*

In HT29 and CaCo2 colon cancer cells, combinations of oxaliplatin and 5-FU yielded a potent cytotoxic synergy (Figure 2). In our study, 5-FU did not modify significantly the total platinum DNA binding of oxaliplatin in HT29 cells ($p = 0.88$). Surprisingly, at equitoxic concentrations, adduct formation induced by oxaliplatin was 1.3–2.5 times lower than that induced by oxaliplatin, the maximal difference being observed at 48 h ($p = 0.025$). In the MCF-7 and MCF-7mdr cell lines, combinations between oxaliplatin and 5-FU showed additive effects. In the breast MDA-MB-231 and ovarian 2008 cancer cells, there was a synergy between oxaliplatin and 5-FU. Synergy was not observed in 2008C13 cisplatin-resistant cells.

In the colon HT29 and ovarian 2008 cancer cells, combinations of oxaliplatin with AG337 showed synergistic effects (Figure 3). The synergy between oxaliplatin and AG337 was significantly reduced in the 2008C13 cisplatin-resistant cells ($p = 0.045$).

Effects of drug combinations *in vivo*

In athymic nude mice, we established that the MTD dose of oxaliplatin alone was 20 mg/kg in a single i.p. injection and the MTD of 5-FU was 100 mg/kg in two separate i.p. injections at a 3 day interval. In the human HT29 colon cancer xenografts, the MTD of oxaliplatin had antitumor activity similar to that of the MTD of 5-FU ($p = 0.8$). At the non-toxic doses of 10 mg/kg oxaliplatin or 100 mg/kg 5-FU, the two drugs do not exert a significant therapeutic effect when administered alone (Figure 4A), whereas the combination of 10 mg/kg oxaliplatin plus 100 mg/kg 5-FU given at the same time in two injection sites exerted significant higher antitumor activity ($p = 0.0036$) with minimal adverse effects (less than 15% weight loss).

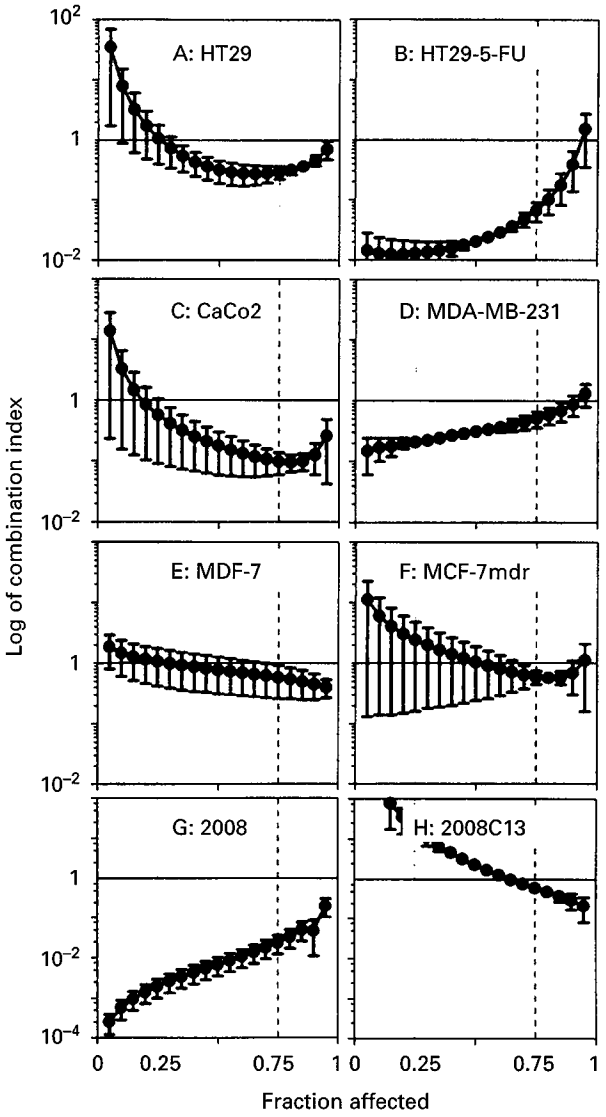

Figure 2. Cytotoxicity of oxaliplatin in combination with 5-FU. The cytotoxic activity of oxaliplatin-based combinations was studied using the quantitative method based on the median-effect principle.[17] Drugs were given simultaneously at a 1:1 molar ratio. A combination index below 1, 1 and above 1 indicates synergy, additivity and non-functional interaction, respectively. The fraction-affected values below 0.25 and above 0.75 are usually subject to important variations. Thus, we analyzed drug interactions between 0.25 and 0.75 (dashed lines). Cytotoxicity of oxaliplatin–5-FU combinations was evaluated in the colon cancer cells HT29 (A), the 5-FU-resistant HT29-5-FU (B) and CaCo2 (C); in the breast cancer cells MDA-MB-231 (D), MCF-7 (E), and the doxorubicin-resistant MCF-7mdr (F); and in the ovarian cancer cells 2008 (G) and the cisplatin-resistant 2008C13 (H).

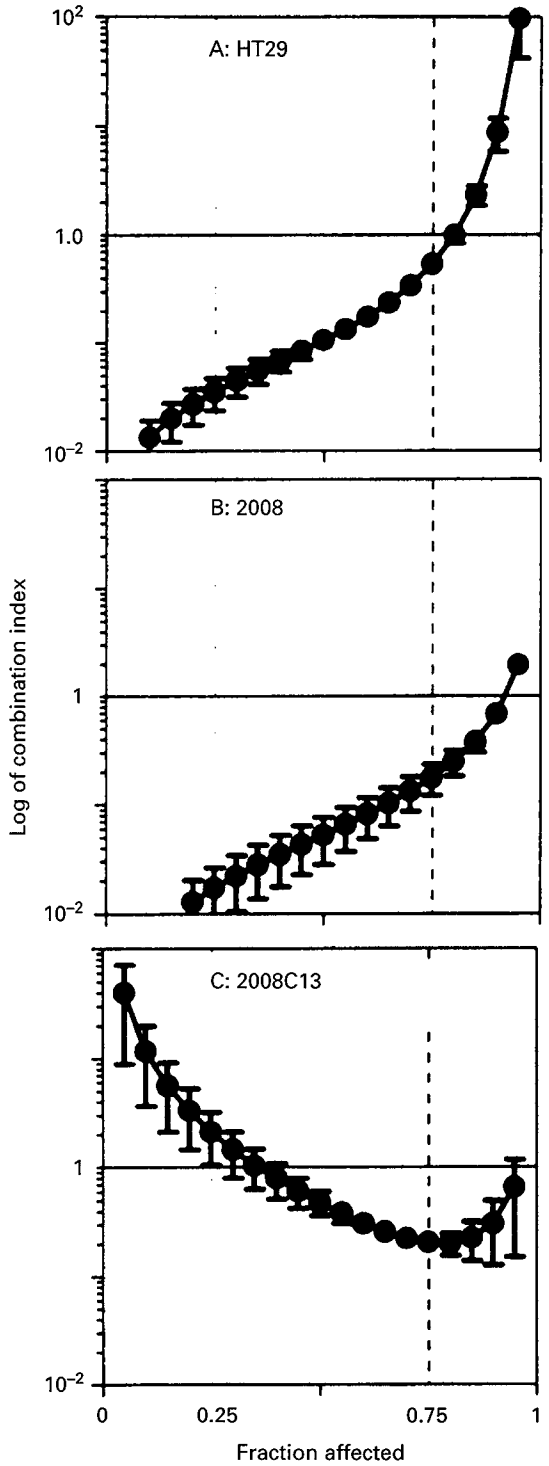

Figure 3. Cytotoxicity of oxaliplatin in combination with the non-classical thymidylate synthase inhibitor AG337. Cytotoxic effects of oxaliplatin–AG337 combinations were evaluated in colon HT29 (A), ovarian 2008 (B) and cisplatin-resistant 2008C13 cancer cells (C). Combinations were studied using a method based on the median-effect principle.[17] Drugs were given simultaneously at a molar ratio 1:1. A combination index below 1 indicates synergy. The effect was analyzed for a fraction affected range from 0.25 to 0.75 (dashed lines). Resistance to cisplatin significantly reduces the synergy between oxaliplatin and AG337 cytotoxicity in 2008 cancer cells ($p=0.045$).

In late-passage hormone-independent GR mouse mammary tumors, oxaliplatin alone given at a dose of 10 mg/kg showed no adverse effects and demonstrated antitumor activity against the GR mouse mammary tumors (oxaliplatin versus control, $p=0.0006$). In GR mice, 50 mg/kg 5-FU enhanced significantly the antitumor activity of 10 mg/kg oxaliplatin ($p=0.0012$), the combination being about twice as active as single oxaliplatin or 5-FU (Figure 4B). We subsequently investigated the effects of AG337 in combination with oxaliplatin.

We established that 10 mg/kg of oxaliplatin can be combined safely with 60 mg/kg AG337 given by six injections of 10 mg/kg over 24 h. In this model, AG337 alone showed potent antitumor activity (Figure 5 and Table 2) and increased significantly the antitumor activity of oxaliplatin ($p<0.05$). There were no morphologically different features in the histopathology of control and oxaliplatin-treated tumors (data not shown).

Discussion

The data presented in this study show that oxaliplatin alone and in combination with both classical and non-classical thymidylate synthase inhibitors exerts potent cytotoxic effects and antitumor activity in human colon, breast and ovarian cancer.

Cytotoxicity of platinum compounds is believed to result from the formation of platinum-DNA adducts.[23,24] DNA adducts formed by oxaliplatin include single-strand DNA binding to repeating deoxyguanosine, intrastrand cross-links at adenine-guanine sites and interstrand cross-links with guanosines.[25,26] Thus, oxaliplatin-induced DNA complexes seem to be similar to those of cisplatin. In our study, we have measured total platinum-DNA binding using a mass spectrometry method.[21] Comparing oxaliplatin-DNA and cisplatin-DNA binding at isomolar and isocytotoxic doses, we observed that the amount of oxaliplatin-DNA binding is about half of the cisplatin-induced DNA binding. This feature has been reported by other authors who have estimated that oxaliplatin forms fewer DNA adducts than cisplatin at equicytotoxic concentrations.[27,28] Moreover, previous reports have shown that the kinetics of interaction with DNA

appears to be slower with cisplatin than with oxaliplatin.[1,20] Nevertheless, there is no clear correlation between inhibition of DNA synthesis induced by oxaliplatin, its antiproliferative effects and the amount of platinum bound to DNA, suggesting that other factors, beside quantitative DNA binding, may contribute to the cytotoxicity of oxaliplatin. Recent data have shown that the patterns of lesions induced by

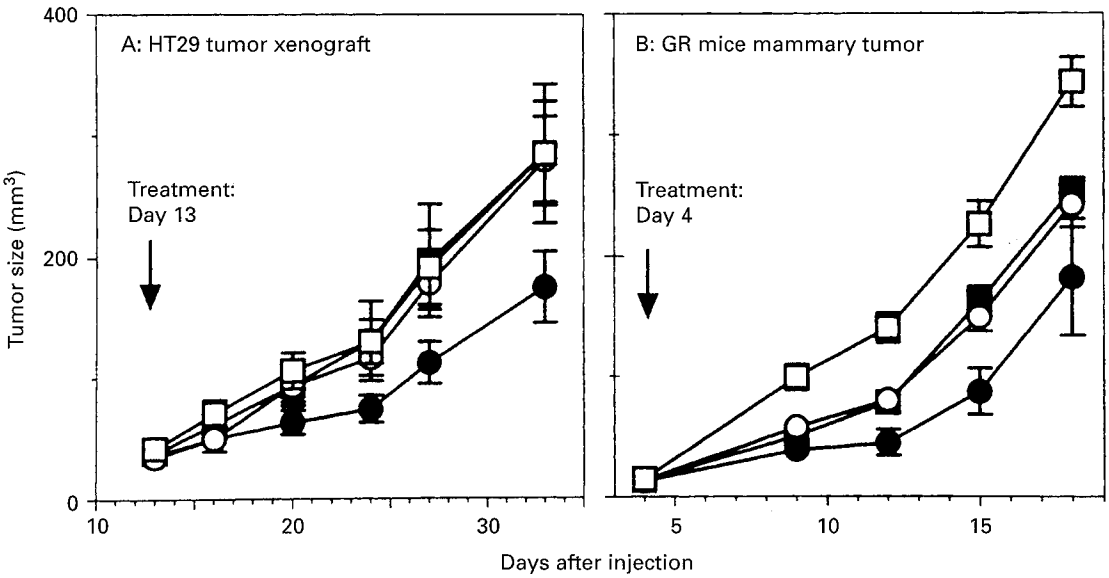

Figure 4. Antitumor activity of oxaliplatin in combination with 5-FU in human HT29 colon cancer xenografts and the GR mouse mammary tumors. Antitumor activity of oxaliplatin and oxaliplatin–5-FU in athymic mice (A) or the GR mouse mammary tumor model (B). Control (□), 10 mg/kg oxaliplatin (○), 50 mg/kg 5-FU (■) and 10 mg/kg oxaliplatin plus 50 mg/kg 5-FU (●). 5-FU increases significantly the antitumor activity of oxaliplatin in HT29 xenograft and GR mouse mammary tumor models ($p=0.0036$ and $p=0.0012$, respectively). Three separate experiments were performed using six or seven mice for each group.

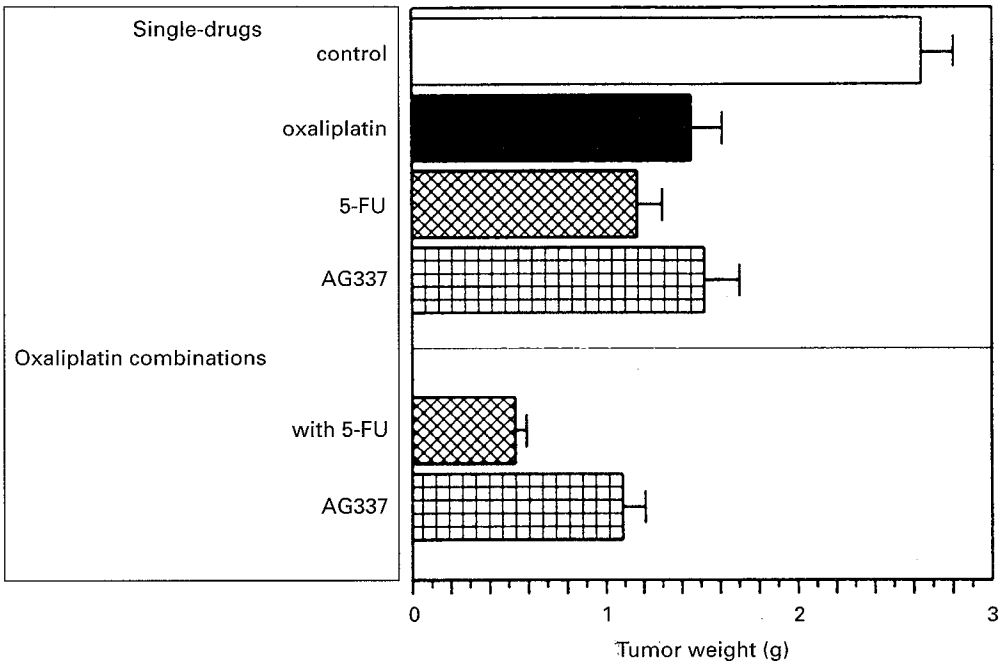

Figure 5. See Table 2 for details.

Table 2. Antitumor activity of oxaliplatin alone or in combinations with 5-FU and AG337 in GR mouse hormone-independent mammary tumors

Comparisons	Mean difference	*t*-test	95% CI		*p* value
			Lower	Upper	
Oxaliplatin versus oxaliplatin–5-FU	0.92	7.36	0.57	1.26	<0.001
Oxaliplatin versus oxaliplatin–AG337	0.36	2.89	0.01	0.70	<0.05
Oxaliplatin–5-FU versus oxaliplatin–AG337	−0.55	4.46	−0.90	−0.21	<0.001

The antitumor activity of single drugs and oxaliplatin-based combinations was evaluated by the measurement of tumor weight 10 days after treatment. 5-FU and AG337 induce a significant increase of oxaliplatin antitumor effects. Experiments were performed three times using six or seven mice in each group.

oxaliplatin and cisplatin in nuclear DNA were markedly different. Oxaliplatin induces relative higher levels of DNA strand breaks but lower levels of DNA interstrand breaks.[29] The nature of the platinum drug and consequently the resultant DNA damage have been shown to correlate with the induction of DNA single-strand breaks with novel amine (cyclohexylamine)-platinum compounds.[30] Moreover, a recent study showed that loss of mismatch repair in colon cancer cells does not confer resistance to oxaliplatin and cyclohexamine platinum derivatives but does to cisplatin.[31] The proposed mechanism to explain the failure to recognize the adducts produced by oxaliplatin may be the result of a difference in the distortion produced by oxaliplatin adducts in DNA or may be due to the steric hindrance of the binding of hMSH2/GBP by the diaminocyclohexane ring of oxaliplatin-induced DNA adducts. Taken together, those differences in the mechanism of action and repair between oxaliplatin and cisplatin may account for different spectra of cytotoxicity that have been reported in several human cancer cell lines.[5]

In our study, we have observed a significant synergy in the cytotoxicity and antitumor activity of oxaliplatin plus 5-FU in human colon cancers. Despite the observation of interesting potentiation between cisplatin and 5-FU or AG337 in the human tumor system, little is known about the mechanism of synergy between platinum salts and thymidylate synthase inhibitors.[32] The proposed mechanism of interaction between cisplatin and 5-FU implies a rise in the folate pool. This classical mechanism of action may account for the interaction between platinum salts and 5-FU but may not be relevant in the case of new thymidylate synthase inhibitors, such as AG337, that do not require folate to stabilize their interaction with thymidylate synthase. Our data showed that the amount of oxaliplatin bound to DNA was identical in the cells exposed to oxaliplatin in the presence or absence of 5-FU. Therefore, other factors than quantitative platinum-DNA binding appear to determine the synergy between these drugs; interaction of 5-FU and cisplatin in colon cancer cells may be associated with a greater degree of DNA fragmentation.[33] Interestingly, a recent report suggests that the pyrimidine antimetabolite 5-FU has multiple mechanisms of action, including incorporation into RNA and p53-dependent induction of apoptosis.[34] Since DNA fragmentation seems to be more frequent with oxaliplatin than cisplatin, further mechanistic study will be required to determine whether the synergy of oxaliplatin with 5-FU and AG337 is related to increased DNA strand breaks.

In conclusion, our data show that oxaliplatin exerts potent cytotoxic and antitumor activity in human colon, breast and ovarian cancer. We observed a synergy between oxaliplatin in combination with 5-FU and AG337 in parental and 5-FU-resistant colon cancer cells. Those data correlate with clinical trials that showed an overall response rate of 10% with oxaliplatin alone[35,36] and about 40% in combination with 5-FU in patients with 5-FU-refractory colorectal cancers.[37] Since AG337 showed remarkable antitumor activity, our preclinical data strongly encourage further clinical trials with oxaliplatin in combination with new thymidylate synthase inhibitors in human cancers.

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