studies. These results suggest that erbstatin may exhibit an antitumor effect against several kinds of human tumors via a cytostatic action.

Imoto *et al.* [9] reported that erbstatin did not inhibit the binding of EGF to its receptor but did inhibit the internalization of EGFR complexes and that this compound did not inhibit EGF-stimulated phosphotidylinositol turnover in A431 cells. Our *in vivo* study also demonstrated the consistency of EGF binding to its receptor throughout treatment with erbstatin. However, little is known about how the inhibition of tyrosine kinase leads to inhibition of tumor cell growth *in situ*.

No side-effects such as weight loss nor warning signs in organs were observed.

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Effect of Topoisomerase Modulators on Cisplatin Cytotoxicity in Human Ovarian Carcinoma Cells

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The *in vitro* interaction of modulators of topoisomerase I and II with cisplatin in human ovarian carcinoma cells might be synergistic. The interactions were evaluated by median effect analysis of survival data derived from continuous exposure to drug combinations for 10 days in colony-forming assays. The interaction between cisplatin and the topoisomerase I inhibitor camptothecin and the topoisomerase I activator β-lapachone was additive, as was that between cisplatin and the topoisomerase II inhibitor novobiocin. Despite the clinical efficacy of the combination of etoposide (a topoisomerase II inhibitor) and cisplatin, the combination index at 50% cell kill indicated antagonism between these two drugs. Thus, biochemical synergism at the cellular level is not a prerequisite of improved therapeutic efficacy.
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INTRODUCTION

THE USE of cisplatin is frequently limited by the rapid development of resistance. Cisplatin forms intra- and interstrand crosslinks in DNA, and these adducts interfere with replication [1]. DNA topoisomerases alter the topology of DNA molecules [2, 3] and may thus influence one or both of these processes. However, repair replication induced by cisplatin can be enhanced [4], or intrastrand adducts in resistant cells removed more rapidly [5, 6]. The role of topoisomerases, particularly topoisomerase II, in DNA repair has not been fully defined [7]. These enzymes might have a role in DNA repair by making adducts accessible to repair enzymes.

Camptothecin inhibits mammalian topoisomerase I, producing single-strand breaks by blocking the rejoining step of the reaction [8, 9]. β-Lapachone inhibits the repair of potentially lethal damage by activating topoisomerase I [10]. Treatment of HEp-2 cells with β-lapachone or camptothecin produced similar sensitization to X-irradiation, indicating involvement of topoisomerase I in at least some forms of DNA repair. Novobiocin and

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etoposide both inhibit topoisomerase II [11, 12], and have been intensively studied as chemotherapeutic agents alone and in combination with other drugs. The role of topoisomerase II in DNA repair is speculative, with evidence both for [13, 14] and against [15–18]. The issue has been further clouded by the lack of specificity of these agents for the enzyme [7].

Topoisomerases are thus crucial to the normal functioning of DNA. If either topoisomerase I or II had an important role in the formation or repair of DDP intra- or interstrand DNA crosslinks, topoisomerase inhibitors might synergize with cisplatin at the cellular level. We have investigated whether topoisomerase I and II modulators can alter the cytotoxicity of cisplatin to human ovarian carcinoma cells *in vitro*.

MATERIALS AND METHODS

Cisplatin was obtained from Bristol-Myers and β-lapachone from Ciba-Geigy. Etoposide and camptothecin were obatined from the natural products branch of the National Cancer Institute. Novobiocin was obtained from Upjohn. Stock solutions of etoposide and novobiocin were made in 100% ethanol. A stock solution of camptothecin was made in dimethylsulfoxide. Stock solutions were stored at −20°C.

These experiments were performed with the human ovarian carcinoma cell line 2008 [19]. The cisplatin sensitivity and other properties of these cells have been well characterized in this laboratory [20, 21]. Cells were grown in RPMI 1640 supplemented with 5% heat-treated fetal calf serum, 2 mmol/l L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The clonogenic assay has been described [20]. Three hundred cells were plated in 5 ml medium into tissue culture dishes. Drugs were added to the desired final concentrations, and the number of colonies containing 50 or more cells counted 10 days later after staining with giemsa. The number of colonies in triplicate was averaged. The experiments with novobiocin were performed twice, those with β-lapachone and captothecin thrice, and those with etoposide seven times.

We used median effect analysis [22]. This is useful for establishing the nature of the interaction between two agents under conditions in which both are cytotoxic and the dose–response curves are non-linear. The analysis yields an esimate of the degree of interaction in the form of a combination index at a given level of effect. A combination index of 1 indicates only additivity. A value greater than 1 indicates antagonism, and a value less than 1 indicates synergy. The extent to which the combination index differs from 1 is a measure of the strength of the interaction. Data for this analysis were obtained, within every experiment, by plotting the dose–response curve for each agent alone and for both agents in a fixed concentration ratio corresponding to the ratio of the IC_{50} for each drug.

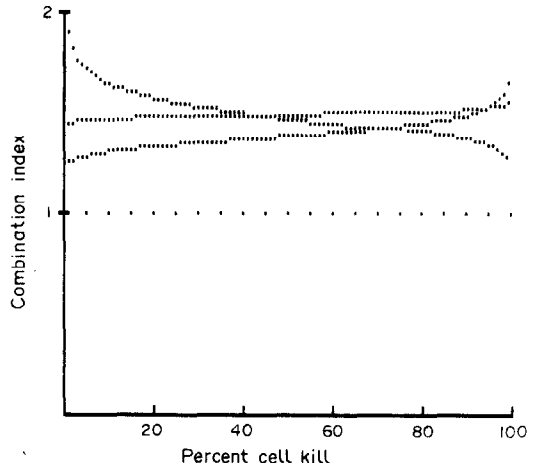

Fig. 1. Combination index plot for cisplatin and etoposide. Three experiments are shown. Concentration ratio of cisplatin:etoposide was 1:1.7.

RESULTS

The IC_{50}, the concentration ratio and the combination index at 50% level at effect are shown in Table 1. The combination index at 50% cell kill for the interaction between cisplatin and etoposide averaged 1.40 (S.D. 0.15), which was significantly different from 1 ($P < 0.05$ t test). Thus at this level of cell kill the two drugs were mildly antagonistic. The 50% combination indices for the other three agents in combination with cisplatin were all around 1, and none was significantly different from 1. Thus, under the condition of continuous exposure, there was no evidence for synergistic interaction between cisplatin and either the topoisomerase I or II modulators.

Figure 1 shows a plot of the combination index as a function of level of effect (percentage cell kill) for the interaction between cisplatin and etoposide. There was mild antagonism between the two drugs over the whole range of up to two logs of cell kill. Curves with camptothecin, β-lapachone and novobiocin (data not shown) were of the same general slope for each combination of drugs and demonstrated neither synergy nor antagonism.

DISCUSSION

We have investigated the interaction of cisplatin with drugs that affect topoisomerase. Cisplatin reacts with DNA and we expected that interfering with cellular processing of DNA might enhance the cytotoxic effects of this compound, as measured by colony forming assays, either by enhancing crosslink formation or by impeding repair. However, using a rigorous test of the nature of the interaction, we found that under conditions of continuous exposure etoposide antagonized cisplatin and that

Table 1. IC_{50}, IC_{50} *concentration ratio used for assessment of interaction, and combination index at* 50% *cell kill*

Drug	IC_{50}* (μmol/l)	Concentration ratio†	No. of experiments	Combination index at 50% cell kill
Cisplatin	0.16 (0.05)	—	—	—
Camptothecin	>0.004	100:1	3	1.01 (0.09)
β-Lapachone	0.43 (0.08)	1:6	3	1.21 (0.20)
Novobiocin	>120	1:400	2	0.98 (0.21)
Etoposide	0.24 (0.05)	1:1.7	7	1.40 (0.15)

*Mean (S.D.).
†Ratio of cisplatin to modulator concentration.

the other three topoisomerase modulators were additive. This suggests that neither topoisomerase I nor II plays a limiting role in the cell's handling of those cisplatin adducts that are cytotoxic, nor is any other effect of these drugs acting in concert with cisplatin.

Both camptothecin and β-lapachone stabilize the cleavable complex formed between topoisomerase I and DNA. Although we found no evidence of an interaction between cisplatin and β-lapachone, this drug is toxic in a 10 day exposure schedule, and the design of our experiments required the use of low concentrations. If the toxicity of β-lapachone is mediated by some other pathway, then it is possible that the concentrations used were insufficient to inhibit DNA repair. Similarly, the concentrations of camptothecin we used are lower than those reported to inhibit cellular topoisomerase I [8, 9]. In any case, no synergy was detected between DDP and whatever process was responsible for the cytotoxicity of the two topoisomerase I modulators on this schedule.

Novobiocin and etoposide both inhibit topoisomerase II. We found that the combination of cisplatin and novobiocin was additive while the combination with etoposide was antagonistic. Novobiocin synergizes with cisplatin in several *in vitro* and *in vivo* systems [23, 24] and the cisplatin novobiocin combination is currently in clinical trial. This discrepancy may be due to differences in experimental design, analysis or differences between the systems. We used continuous 10-day exposure of human ovarian cancer cells to both drugs. The IC_{50} values for a 1-h exposure indicated that the 2008 cell line is approximately 20-fold more sensitive to cisplatin than are the Chinese hamster ovary cells used in other studies. These results may indicate the importance of testing drugs in more than one model.

Previously we showed [25] cisplatin-etoposide is an effective salvage regimen when administered intraperitoneally to patients with ovarian carcinoma. Others have also reported on the value of cisplatin and etoposide in combination [26], including reports of synergy [27]. Our results may be an example of the difference between biochemical synergy at the cellular level and therapeutic synergy. Clinically, drugs are defined as acting 'synergistically' if the combination produces a better tumor response than either drug alone, with an acceptable level of toxicity. This is a less stringent criterion than that applied to median effect analysis. In patients, two drugs with different limiting toxicities may be used at maximally tolerated doses for each, producing enhanced effectiveness, even if they are antagonistic at the cellular level, i.e. 'toxicity independence' [28]. Similarly, there is no assurance that combining two drugs shown to be synergistic at the biochemical level will result in therapeutic synergism, especially if synergy *in vitro* occurs in sensitive tissues such as bone marrow as well as in the tumor cells.

The difficulties of investigating the nature of drug interactions have been considered [22, 28, 29]. We used median effect analysis [22]. As with isobologram analysis [22], this method takes into account non-linear dose–effect curves. It also permits assessment of interaction across a range of cytotoxic effect, as shown in Fig. 1. In studies at the cellular level the combination of cisplatin with etoposide was antagonistic at all levels of cell kill.

Tsai *et al.* [30] used isobologram analysis for the combination cisplatin/etoposide in an array of human lung tumor lines. Extensive testing of interaction at the cellular level demonstrated that the effect of the two drugs was no more than additive. This result supports our finding that the same combination is mildly antagonistic in a human ovarian carcinoma cell line.

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Weekly High-dose Infusion of 5-Fluorouracil in Advanced Colorectal Cancer

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18 patients with advanced colorectal cancer entered a phase I–II study of high-dose 48 h continuous infusion 5-fluorouracil (5-FU) for 6 weeks. 7 patients were included at the first dose level (3 g/m²) and only 1 had serious toxicity (grade 3 diarrhoea and mucositis). 7 patients were included at the second dose level (3.5 g/m²). 6 had a good tolerance to treatment, while the remaining patient had grade 4 leukopenia. 4 patients received 4 g/m²: 3 had severe toxicity (grade 4 diarrhoea, myelosuppression, mucositis, central nervous system), such that the entry of new patients was stopped. Anti-tumour activity was seen in 33% (95% confidence interval 13–59%) of the overall population. Only patients who had not had previous chemotherapy responded to treatment (response rate in this subgroup [43%, 18–71%]). The optimal dose of 5-FU was 3.5 g/m² weekly for six cycles.
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INTRODUCTION

5-FLUOROURACIL (5-FU) is the most widely used cytotoxic drug in advanced colorectal cancer. However, its anti-tumour activity is below 20% [1]. Several studies have suggested that continuous infusion may increase response rate and tolerance to treatment [2–5]. A weekly 48 h continuous infusion of high-dose 5-FU (60 mg/kg) had little toxicity [3, 6]. Therefore, we have done a phase I–II study of escalating doses of weekly 48 h continuous infusion of 5-FU to determine the optimal dose of this schedule and to evaluate the response rate.

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PATIENTS AND METHODS

Patients had advanced colorectal cancer, with or without previous chemotherapy. Other inclusion criteria were Karnofsky status above 70%, age under 75 years, life expectancy of at least 2 months, white cell count over 4000/μl, platelet count over 150,000/μl, bilirubin below 1.5 mg/dl and creatinine less than 1.2 mg/dl.

18 patients entered the study (Table 1). After informed consent was obtained, patients received the total dose of 5-FU administered in 2000 ml normal saline over 48 h as an inpatient. The solution was protected from the light. Chemotherapy was repeated weekly for six doses except when toxicity was severe. The initial dose we used was 3 g/m². Subsequently, we used 3.5 g/m² in other patients, and then 4 g/m² in the final group of patients.

Toxicity and response to therapy were defined with WHO criteria [7]. During treatment, the following tests and examinations were done: physical examination (twice a week), white