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Research paper

Cytotoxic effect of interferon-α2a in combination with *all-trans* retinoic acid or cisplatin in human ovarian carcinoma cell lines

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Ovarian cancer has a poor prognosis due to the frequent appearance of a drug-resistant state. An alternative therapeutic approach may lie in combinations of conventional chemotherapeutic agents with new classes of drug, such as interferons (IFN) and differentiation-inducing agents. There is clinical evidence that both IFN-α2a–*all-trans* retinoic acid (ATRA) and IFN-α2a–cisplatin have significant activities on growth of malignant cells, cell differentiation or programmed cell death in solid tumors. In order to throw more light on the cellular basis of these findings and to optimize a schedule of such drug combinations, we examined the cytotoxic effects of various combinations on five human ovarian carcinoma cell lines. The experiments were based on a clonogenic assay on plastic. The different cell lines exhibited different sensitivities to the three drugs tested. Using the cell line most sensitive to these drugs, we then examined the effect of different sequences of two drug combinations. We observed a potentiation after pretreatment with ATRA followed by IFN-α2a and ATRA or after pretreatment with IFN-α2a followed by IFN-α2a and cisplatin. Using this schedule of administration, cytotoxic interactions between the two drugs were investigated by median effect analysis. Synergism or antagonism were observed depending on the intrinsic sensitivity of the cell line to the first drug and the concentrations used. The magnitude of these interactions was found to be influenced by the cellular sensitivity to the second drug. These results show that schedules of drug combinations are not easy to design and may help account for the various failures and the discrepant effects observed in clinical trials. [© 1998 Rapid Science Ltd.]

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Introduction

Ovarian carcinoma is one of the leading causes of cancer mortality in women. Its dismal prognosis is due to the fact that the clinical efficiency of cisplatin, a valuable cytotoxic agent in this disease,[1] is often attenuated by progression of the tumor to a cisplatin-resistant state.[2]

An alternative therapeutic approach might be to use a combination of standard chemotherapeutic agents along with biological response modifiers (BRM) such as interferon (IFN) or differentiating agents such as *all-trans* retinoic acid (ATRA). Another possibility would be to associate BRM with differentiation-inducing drugs.

IFNs are a family of naturally occurring glycoproteins with anti-viral and immunomodulating actions. IFNs exert their effects by binding to cell surface receptors through which they transmit a signal which triggers the transcription of at least 30 genes.[3] Additionally, IFNs have been shown to possess antitumor activity against a wide variety of cancers both *in vitro* and *in vivo*.[4] In view of their relatively weak cytotoxic action, IFNs have been proposed in combination with cytotoxic agents or other BRM.

Retinoids are a family of molecules, structurally related to vitamin A, which have been shown to be involved in cell growth and differentiation.[5]

They act intracellularly through specific nuclear receptors, retinoic acid receptors (RARs and RXRs), belonging to the superfamily of steroid receptors, and which have been shown to control gene transcription.[6] In ovarian cancer, ATRA has been shown to exert a reversible inhibitory effect.[7] This finding suggests that it might find a place in combination with differentiation inducers and cytotoxic agents.[8]

Many of the studies on combinations of retinoids and IFNs have utilized myeloid malignant cell lines. The most frequently studied is HL60, a myelocytic leukemia cell line. In this cell line,[9] a combination of these two agents has been reported to result in enhanced antiproliferative and differentiating effects over those observed with either agent alone.

The IFN-ATRA combination on human solid tumors has also been examined, while the action of ATRA in combination with either IFN-α2a or IFN-γ has been studied in human mammary cell lines.[10] The antiproliferative effects of ATRA and other retinoids, either alone or with IFN-α, on various histological types of cancer cell lines have also been described.[11] Some neoplasms with single agent sensitivity to IFN-α and retinoids were found to exhibit a synergistic sensitivity to combinations of the two drugs.[11] Moreover, in a neuroblastoma cell line (NVB6), a differentiating effect has also been observed.[12]

A large number of studies have been devoted to the combination of IFNs with conventional cytotoxic agents.[13] IFN-α was shown to enhance the activity of cisplatin, both *in vitro* and *in vivo*, against non-small cell lung cancer.[14] Several clinical trials combining IFN-α and carboplatin in the treatment of ovarian cancer have been conducted.[15,16] Beneficial results appeared to depend on the tumorigenicity of the primary tumor. However, to our knowledge, there are no reports on different schedules or the time courses of action of such drug combinations.

The present study was designed to evaluate the cytotoxicity of combinations of IFN-α-ATRA or IFN-α-cisplatin on five human ovarian carcinoma cell lines. The rationale was to design schedules of delivery of these agents in the treatment of ovarian cancer. After optimization of a schedule of incubation in the most sensitive cell line to these three drugs, we have analyzed interactions between the agents in these human ovarian cell lines.

Our results are the first demonstration of the marked influence of schedule of administration of such combinations, and they may throw more light on the failures observed with certain combinations in clinical trials.

Materials and methods

Cell lines

The human ovarian carcinoma cell lines used for the present study included four serous cell lines, NIHOVCAR$_3$ (ATCC; HTB161), OVCCR$_1$,[17] 2008 and its cisplatin-resistant subline 2008/C13*, a generous gift from Dr Stephen Howell (University of California, San Diego, La Jolla, CA), and an endometroid cell line, IGROV$_1$ cells, a generous gift from Dr J. Bénard (Villejuif, France).

The cells were grown in RPMI 1640 medium supplemented with 5% FCS, 2 mM glutamine (Seromed, Polylabo, Strasbourg, France), 2 ng/ml epidermal growth factor (Boehringer, Mannheim, Germany) and 5 μg/ml insulin, in humidified 5% CO_2/95% air at 37°C.

Drugs and chemicals

Cisplatin was obtained from Bellon Rhone-Poulenc Rorrer (Montrouge, France). The stock solution was stored at 4°C and working solutions were prepared immediately before use, by dilution in the culture medium. IFN-α2a was obtained from Produits Roche (Neuilly/Seine, France) at 3×10^6 IU. Reconstituted stock solution was stored at −80°C and the working solutions were prepared before use by dilution in the medium. ATRA was supplied from Sigma (St Quentin-Fallavier, France), the stock solution was prepared in DMSO at 10^{-2} M each week, stored at −20°C, and ATRA preparations and incubations were performed in the dark.

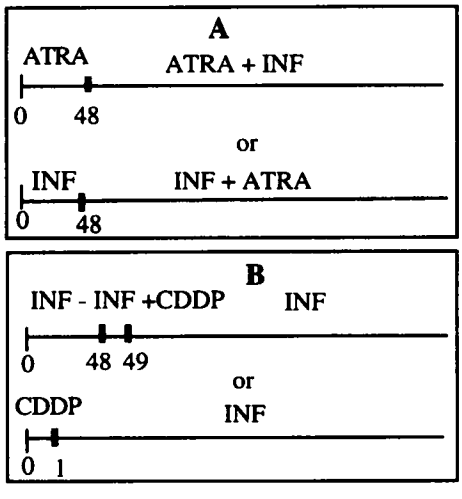
Cytotoxicity assays

IFN-α, ATRA or cisplatin cytotoxicity was measured by clonogenic assay on plastic. The cell lines were plated at various densities ranging from 200 cells/cm^2 for IGROV$_1$, 2008, 2008/C13* to 500 cells/cm^2 for NIHOVCAR$_3$ cells and to 750 cells/cm^2 for OVCCR$_1$ cells, in order to obtain, for each cell line, the same number of plated cells. After plating, the cells were exposed to various concentrations of ATRA or IFN-α throughout the experiments. For cisplatin, the cells were incubated for 1 h in the presence of various concentrations of this agent,

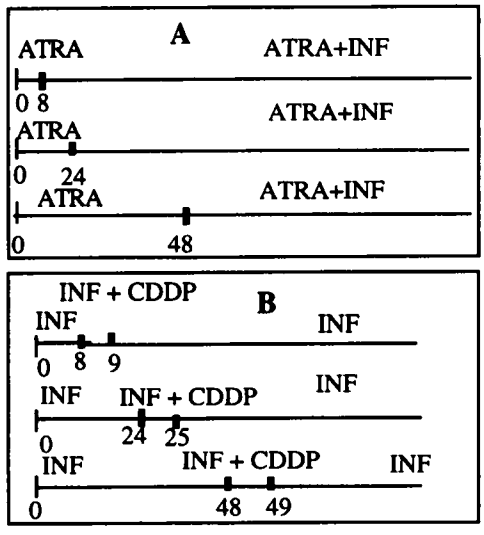
then washed and re-fed with a new medium without cisplatin and left to form colonies. The colonies were stained and those having more than 50 cells were counted.

The $NIHOVCAR_3$ cell line was employed in the following experiments.

Investigation of administration schedules for combinations of the two drugs: IFN-α2a-ATRA (combination A) or IFN-α2a-cisplatin (combination B) was conducted as follows: (A) the cultures were exposed for 48 h to either IFN-α2a or ATRA and then to both agents until the end of the experiment; (B) to either IFN-α2a for 48 h followed by cisplatin and IFN-α-2a for 1 h and then IFN-α2a until the end of the experiment or cisplatin for 1 h followed by IFN-αa2a until the end of the experiment. The two combinations were delivered at the individual 20% inhibitory (IC_{20}) and 50% inhibitory concentrations (IC_{50}) of each drug for $NIHOVCAR_3$ cells. The following diagrams summarize the different schedules of ATRA and IFN-α2a administration. The time is expressed in hours.



After determination of the best order of drug delivery, the duration of pre-incubation with the first drug was optimized for maximum sensitivity to the second drug. After plating, the cells were treated with either ATRA (combination A) or IFN-α (combination B) for different durations (0, 8, 24 and 48 h) before simultaneous incubation with IC_{50} IFN-α for 3 weeks or with IC_{50} cisplatin for 1 h. The cells were exposed to the drugs until the formation of colonies. The following diagrams summarize the different schedules of ATRA, IFN-α2 and cisplatin administration. The time is expressed in hours.



Median effect analysis

The nature of the interactions between either IFN-α and ATRA, or between IFN-α and cisplatin was evaluated in the five ovarian carcinoma cell lines by median effect analysis according to Chou and Talalay.[18] The combination index (CbI) was determined from a clonogenic assay on plastic at increasing levels of cell kill according to the best administration schedule obtained from the above experiments. For this investigation, the drugs were combined in a fixed concentration ratio corresponding to the ratio of the individual IC_{50} for each cell line. For the ATRA-IFN-α combination the ratio was 1:200 for $OVCCR_1$, 1:4000 for $NIHOVCAR_3$ cells, 1:500 for 2008/C13*, whereas for $IGROV_1$ and 2008 cells it was fixed at 1:10 000. ATRA and IFN-α2a were delivered at 10^{-6} M and 3000 IU/ml, respectively, to the cell lines which were insensitive to these agents. For the IFN-α-cisplatin combination: the ratio was 30:1 for $OVCCR_1$, 500:1 for $NIHOVCAR_3$, 30 000:7 for 2008 and 150:1 for 2008/C13*. For $IGROV_1$ cells it was fixed at 7500:1.The cytotoxicity was compared to the cytotoxicity of each drug administered separately in all the experiments and each experiment was performed in triplicate using three different cultures for each data point.

Results

Cytotoxicity of IFN-α, ATRA and cisplatin on human ovarian carcinoma cell lines

Figure 1(A) represents the cisplatin cytotoxicity of human ovarian carcinoma cell lines. As described

elsewhere,[8] the different cell lines exhibit different sensitivities to cisplatin with an IC_{50} for cisplatin ranging from 0.2 to 5 μg/ml (Table 1).

A similar heterogeneity[7,8] was observed (Figure 1B and Table 1) in the sensitivity of these ovarian carcinoma cell lines to ATRA: two cell lines were insensitive to ATRA ($IGROV_1$ and 2008 cells), one cell line was slightly sensitive (2008/C13*), and only $OVCCR_1$ and $NIHOVCAR_3$ cells had an $IC_{50} < 10^{-6}$ M.

A range of cytotoxic responses was also observed for IFN-α2a (Figure 1C and Table 1): two cell lines ($OVCCR_1$ and $NIHOVCAR_3$) were sensitive ($IC_{50} < 100$ IU/ml), two others (2008 and 2008/C13* cells) responded to higher IFN-α concentrations, whereas no IC_{50} could be determined for $IGROV_1$ cells.

Table 1 contains the exact values of $IC_{20,50,80}$ (20, 50 and 80% inhibitory concentrations, respectively) for the three drugs in these ovarian cell lines.

Schedule of IFN-α2a administration in combination with either ATRA or cisplatin

The schedule of administration of either IFN-α2a-ATRA or IFN-α2a-cisplatin and their respective time course were analyzed in the most sensitive cell line to the three agents: $NIHOVCAR_3$ cells (Figure 2A and B).

For the first combination (IFN-α2a-ATRA), simultaneous addition of drugs followed by a 3 week incubation of both gave rise to an additive effect (data

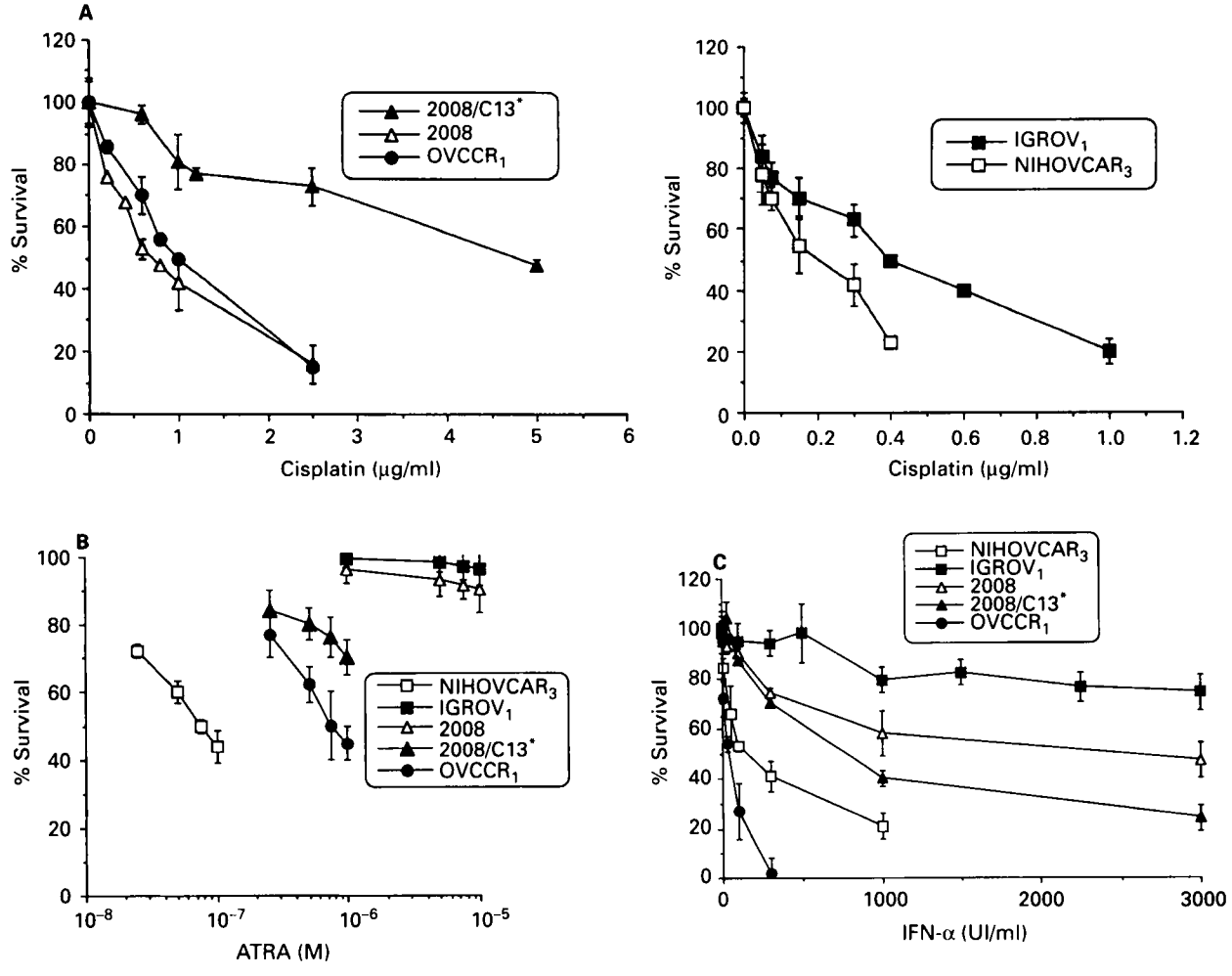


Figure 1. Cytotoxicity of cisplatin (A), ATRA (B) and IFN-α2a (C) on five human ovarian carcinoma cell lines. The sensitivity to the three drugs was evaluated by clonogenic assay on plastic as described in Materials and methods. ATRA and IFN-α were incubated in the presence of the cells for the whole experiments whereas cisplatin was left for 1 h. The results are expressed as mean ± SD of the percentage of control survival from four separate experiments.

not shown). The same result was obtained when IFN-α2a was added before ATRA with subsequent exposure to both agents for 3 weeks (Figure 2A). Only a pre-incubation for a doubling time (48 h) with ATRA, followed by a simultaneous incubation for 3 weeks with IFN-α, potentiated the inhibitory effect of IFN-α. This potentiation was observed at low ('1', IC_{20}) and high ('2', IC_{50}) concentrations of the two drugs. For the second combination (IFN-α-2a-cisplatin, Figure 2B), a similar result was observed: only a 48 h pre-incubation with IFN-α2a prior to 1 h of exposure to cisplatin potentiated cisplatin cytotoxicity at either drug concentration ('1', IC_{20}; or '2', IC_{50}).

In the second part of this study, we investigated the optimal pre-incubation time of the first drug before addition of the second in order to obtain potentiation. $NIHOVCAR_3$ cells were pre-treated with ATRA IC_{50} (Figure 3A) or IFN-α IC_{50} (Figure 3B) for different times (0, 8, 24 or 48 h) before the simultaneous addition of IFN-α2a (100 IU/ml) for the whole experiment (Figure 3A) or IFN-α2a+cisplatin (0.2 μg/ml for 1 h, Figure 3B) followed by IFN-α2a until the end of experiment. Figure 3A shows that, in the absence of ATRA, 100 IU/ml IFN-α nearly halved survival of the colonies (45%). In the absence of IFN-α2a, 0.2 μg/ml cisplatin halved survival of the colonies (Figure 3B). As shown in Figure 3(A and B), 8 h pre-exposure was sufficient to potentiate IFN-α2a cytotoxicity by ATRA or that of cisplatin by IFN-α2a. While this potentiation increased for ATRA in a time-dependent manner until 48 h, for

Table 1. Cytotoxicity of cisplatin, IFN-α2a and ATRA on five human ovarian carcinoma cell lines

	Cisplatin (μg/ml)			ATRA (10^{-7} M)			IFN-α2a (UI/ml)		
	IC_{20}	IC_{50}	IC_{80}	IC_{20}	IC_{50}	IC_{80}	IC_{20}	IC_{50}	IC_{80}
$OVCCR_1$	0.4±0.05	1±0.2	2.25±0.3	2.5±0.2	5±0.5	20±0.3	6±2	30±2	150±7
$NIHOVCAR_3$	0.04±0.01	0.20±0.03	0.4±0.03	0.2±0.3	0.83±0.10	2.5±0.3	25±2	100±2	1000±15
2008	0.20±0.04	0.70±0.08	2.2±0.04	NE	NE	NE	200±18	3000±120	NE
2008/C13*	1±0.3	5±0.3	9.5±0.5	5±0.4	50±0.5	NE	150±15	750±54	3000±135
$IGROV_1$	0.06±0.01	0.4±0.04	1±0.1	NE	NE	NE	NE	NE	NE

Cytotoxicity was evaluated by clonogenic assay on plastic, after a continuous incubation in the presence of various ATRA and IFN-α2a concentrations. For cisplatin cytotoxicity only a 1 h incubation was performed. Values represent the average of at least four independent determinations carried out in triplicate. Means ± SD are given. IC_{80}=80% inhibitory concentration, IC_{50}=50% inhibitory concentration, IC_{20}=20% inhibitory concentration. NE, not evaluable.

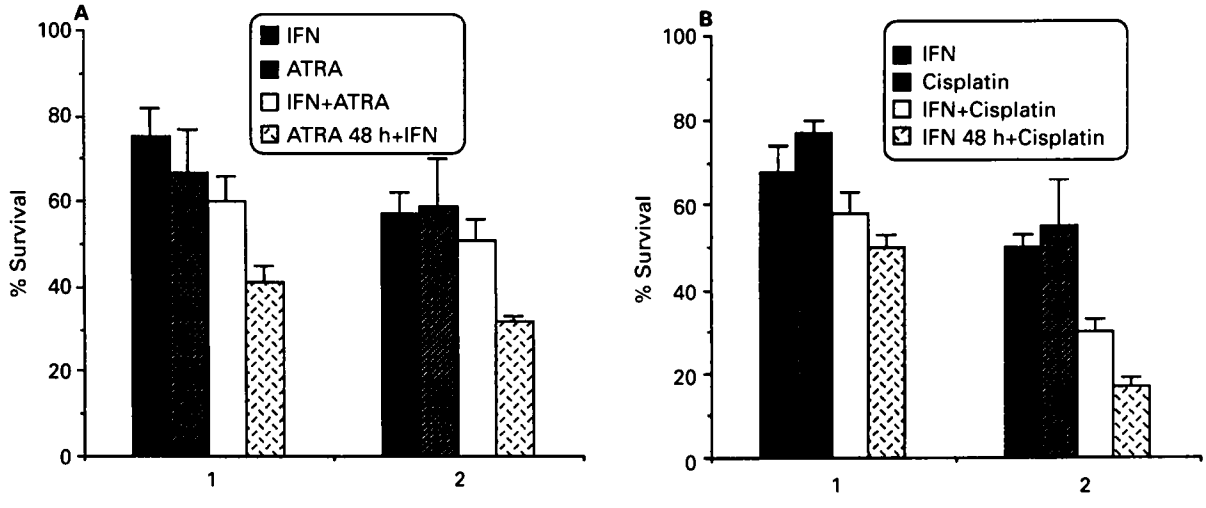


Figure 2. Effect of various administration sequences of either ATRA–IFN-α2a (A) or IFN-α2a–cisplatin (B) on the respective IFN-α2a and cisplatin cytotoxic sensitization in $NIHOVCAR_3$ cells. Cytotoxicity was evaluated by clonogenic assay on plastic. ATRA was delivered either before or after IFN-α2a and for 48 h (A), and the cells were then exposed to both drugs until the end of the experiment. For IFN-α2a and cisplatin (B) the administration schedule was similar, except that cells were exposed to cisplatin for 1 h. Experiments were conducted with low (IC_{20},'1') or high (IC_{50},'2') concentrations of the drugs. The results are mean ± SD of four experiments and are expressed as the percentage of the control survival number.

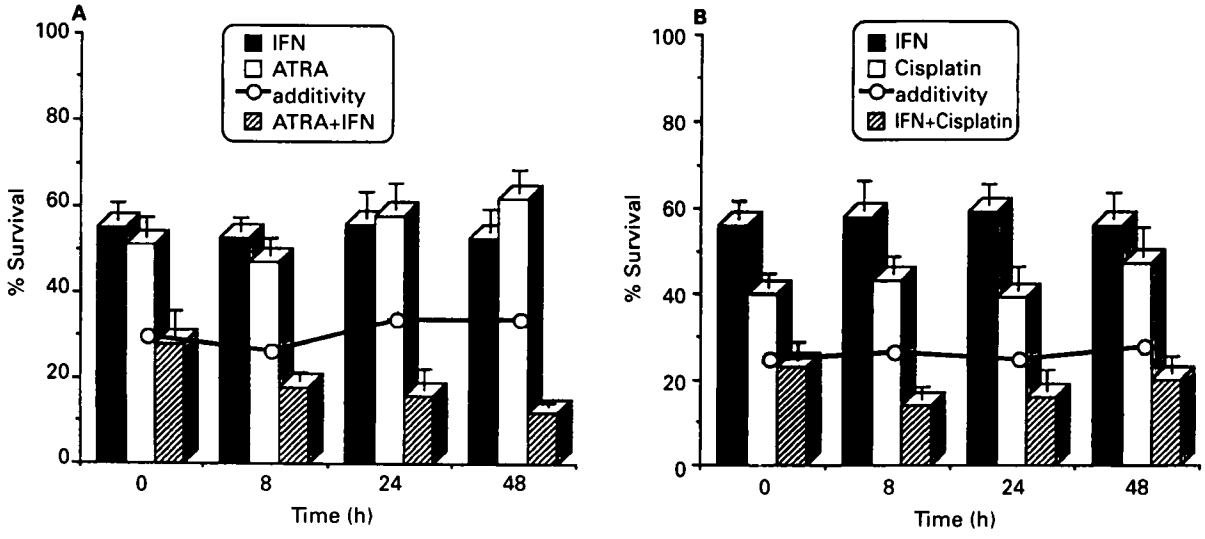


Figure 3. Time course of IFN-α2a (A) or cisplatin (B) sensitization by, respectively, ATRA or IFN-α2a, in NIHOVCAR$_3$ cells. Two days after plating, the cells were pre-treated for different times (0, 8, 24 or 48 h) with either 10^{-7} M ATRA (A) or 100 IU/ml IFN-α (B) followed by a continuous administration of either 100 IU/ml IFN-α (A) or 1 h exposure to 0.2 μg/ml cisplatin (B) as described in Materials and methods. Each histogram represents the percentage of control survival. The lines drawn on each histogram show the expected additive result of the ATRA–IFN-α or IFN-α–cisplatin combination, calculated from the corresponding ATRA and IFN-α or the IFN-α and cisplatin survivals at the corresponding times. The results are the mean ± SD of four separate experiments.

IFN-α2a, the potentiation of cisplatin cytotoxicity reached a peak at around 8 h. A line drawn on the two experimental histograms indicates the additive inhibition expected for the ATRA-IFN-α2a and IFN-α2a-cisplatin combinations at the corresponding times. This inhibition was calculated from the percentage survival obtained from each drug at 0, 8, 24 and 48 h.

Nature of interactions either IFN-α2a and ATRA or IFN-α2a and cisplatin

In NIHOVCAR$_3$ cells, ATRA enhanced IFN-α2a cytotoxicity if delivered first. Likewise IFN-α2a enhanced cisplatin cytotoxicity when given first. To throw more light on the interactions between these two agents, we investigated the influence of ATRA-IFN-α2a and IFN-α2a-cisplatin on five human ovarian carcinoma cell lines. To take into consideration the different doubling times of each cell line, we decided to analyze the nature of interactions between these agents after a pre-incubation of either ATRA or IFN-α for their respective doubling time followed by a simultaneous exposure to the two drugs until the end of the experiment (*cf.* Figures 4 and 5). It can be seen that there was a synergism for all the fractions (CbI < 1) with a combination index 50 (CbI_{50}) of 0.51 ± 012. This indicates that in these cells, the combined amount of the two drugs required to kill 50% cells is only 0.51 as much as would be required if they demonstrated purely additive behavior. Figures 4 and 5 show the average plot of CbI as a function of fraction affected from four experiments conducted in the five cell lines. Concerning the ATRA-IFN-α combination (Figure 4A and B) in OVCCR$_1$ and 2008/C13* cells, a strong synergism was observed at a lower level of cell kill, and a marked antagonism at a higher level. For the 2008 cell line, an antagonism was observed at both low and high fractions affected, whereas a synergism was noted for the intermediate fractions. For IGROV$_1$ cells, an antagonism was observed for almost all the fractions. The interaction between IFN-α2a-cisplatin (Figure 5A and B) produced similar results: in NIHOVCAR$_3$ cells, a synergism was observed with a CbI_{50} of 0.80 ± 0.12. For the other cell lines, we observed curves with two parts: one synergistic and one antagonistic, except for IGROV$_1$ cells where an antagonism was observed for all the fractions.

Discussion

Cytotoxic treatment in ovarian cancer rapidly loses efficacy due to acquired resistance.[2] A large number of clinical trials have been conducted using

combinations of drugs designed to augment the activity of the individual drugs and attenuate resistance.

To date, among several classes of molecules, the BRM (IFN-α2a) and the differentiating agents (ATRA) have been shown to have an antiproliferative action on tumor cells. Unfortunately, differentiation inducers or BRM have limited activity on their own in advanced cancer.[20,21] Furthermore, differentiation induction of tumors is not always an irreversible process. These findings have prompted a search for other therapeutic strategies against solid tumors such as combinations of these agents with conventional chemotherapeutic drugs. In ovarian cancer, there is experimental evidence that such combinations enhance cellular sensitivity to

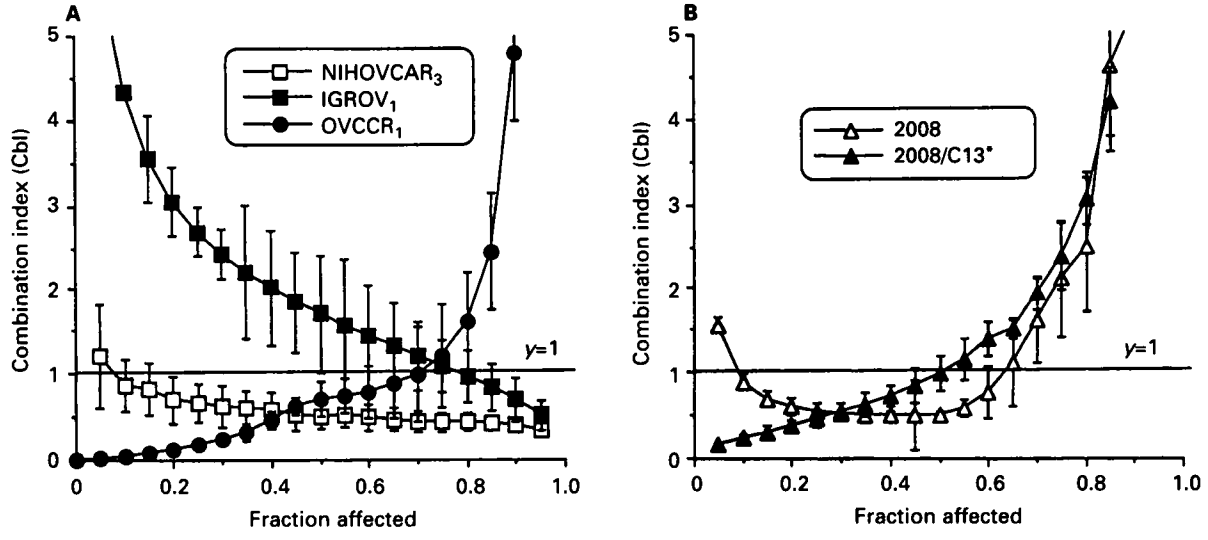


Figure 4. Nature of interaction between ATRA and IFN-α in five human ovarian carcinoma cell lines. The combination index plots (Cbl) were calculated according to Chou and Talalay from the clonogenic assay as described in Materials and methods. Cbl > 1 indicates an antagonism, Cbl < 1 indicates a synergy. Each curve represents the average of four separate experiments using triplicate cultures from each data point. The representation was split into two parts, A and B, for the sake of clarity.

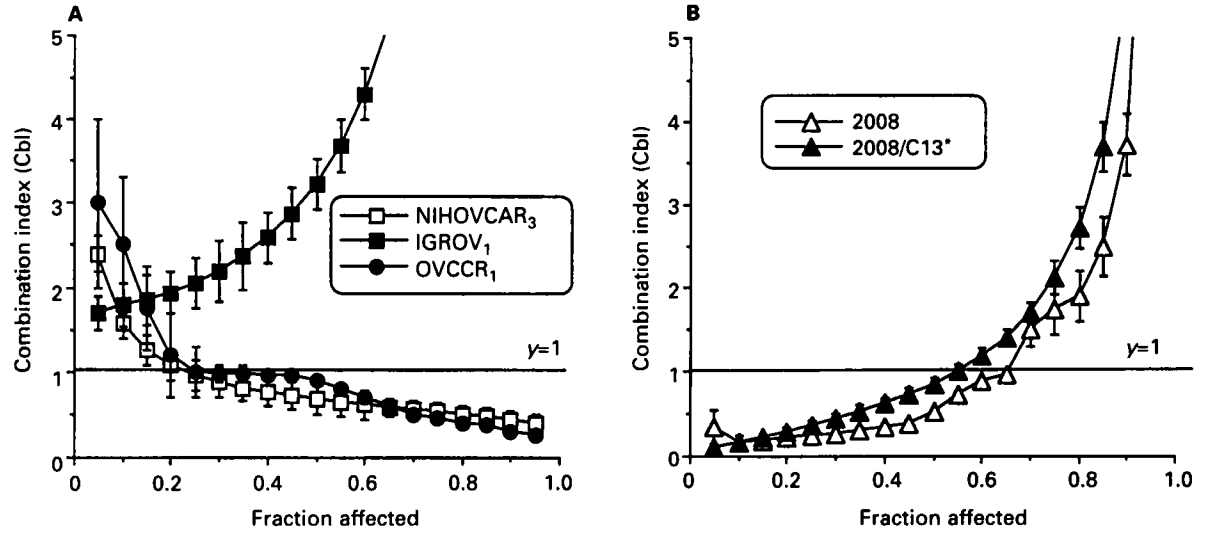


Figure 5. Nature of interaction between IFN-α and cisplatin in five human ovarian carcinoma cell lines. The combination index plots were also calculated according to Chou and Talalay from the clonogenic assay as described in Materials and methods. Each curve represents the average of four separate experiments using triplicate cultures from each data point. The figures were split into two parts, A and B, for the sake of clarity.

conventional chemotherapeutic agent,[8,19] although the results of clinical trials have been somewhat disappointing.[15,16]

In the present *in vitro* study, we observed a cytotoxic synergism between IFN-α2a and ATRA or between IFN-α2a and cisplatin in three out of five human ovarian carcinoma cell lines. Median effect analysis showed that efficacy was a function of the schedule of delivery. Indeed only the addition of ATRA before IFN-α, or of IFN-α before cisplatin, gave rise to a potentiation in these cell lines. This aspect has received scant attention and clinical trials have often been conducted with simultaneous administration of the two drugs: 13-*cis*-retinoic acid and IFN-α2a in renal cell carcinoma,[2] and IFN-α2b and cisplatin in non-small cell lung cancer (NSCLC).[23] However, in one clinical trial[24] a schedule of IFN-α2a three times a week before the 5 day cisplatin infusion was found to have antitumor activity, which is in line with preliminary results against NSCLC.[14] In a discussion of the results obtained in this pathology with different regimens of administration, these authors[14] noted that sequential administration of IFN-α2b and cisplatin[25] was superior to simultaneous delivery of the two agents.[23].

We also showed that synergism was only observed with the ovarian carcinoma cell lines that were responsive to the antiproliferative effect of either ATRA or IFN-α. This is in line with other work showing that synergism is only observed in tumors that are sensitive to both agents.[11] However, it was of note that the magnitude of the synergistic effect was similar in $OVCCR_1$ combination, although these two cell lines had quite different sensitivities to ATRA and IFN-α. 2008/C13* cells were, respectively, 10- and 25-fold more resistant than $OVCCR_1$ cells to ATRA and IFN-α. This latter result is not readily accounted for, but may explain the conflicting results observed in clinical trials of combinations of these drugs against tumors of the same histological type.

Due to the similar sensitivity of these cell lines to IFN-α or ATRA, we failed to note any further influence of IFN-α2a on the action of ATRA–IFN-α. The synergistic action of cisplatin in the IFN-α–cisplatin combination was found to be influenced by the cells' sensitivity to cisplatin. Indeed, the combination index for $OVCCR_1$ and $NIHOVCAR_3$ was comparable to that for 2008/C13* and 2008, although $OVCCR_1$ and 2008/C13* were more sensitive than $NIHOVCAR_3$ and 2008 to IFN-α. Comparing their IC_{50}s, $OVCCR_1$ and 2008/C13* cells were more resistant than $NIHOVCAR_3$ (5-fold) and 2008 (8-fold) cells to cisplatin. These results are in line with those observed in the tamoxifen (TAM)–cisplatin combination[26] for which the synergistic effect of TAM was shown to be critically dependent on the sensitivity of the cells to it, whereas cisplatin resistance had less influence on the degree of synergism.

Finally, we showed that the time to maximum potentiation depended on the drug: for $NIHOVCAR_3$ cells, this effect was observed at 48 h with ATRA, whereas it was observed within 8 h for IFN-α2a. These differences in time course point to the existence of different mechanisms of potentiation. ATRA may trigger a differentiation pathway involving a cascade of events taking at least 48 h to reach a peak effect, whereas IFN-α2a may modulate cisplatin cytotoxicity (accumulation, cellular detoxification or DNA repair) in a more direct way. Preliminary results obtained in our laboratory are not in favor of an effect on the intracellular accumulation of this cytotoxic agent by IFN-α (data not shown) and we are currently investigating other mechanisms.

The differences in shape of the median effect curves highlight the difficulties involved in designing schedules of administration of such drugs. For $NIHOVCAR_3$, the most sensitive cell line to ATRA, IFN-α and cisplatin, a synergism was observed for nearly all the fractions affected, with a greater effect in the highly affected fractions. The same type of synergism has also been reported for the combinations of IFN-γ and cisplatin,[27] IL-1α–cisplatin[28] or ATRA–cisplatin in the same cell line. These results suggest that interactions between cisplatin and BRM or a differentiating agent and BRM are cell line dependent. For $IGROV_1$ cells, which are insensitive to ATRA and IFN-α2a, an antagonism was observed for all the affected fractions, irrespective of the combination, which is in agreement with other reports[8,27] on the ATRA–cisplatin and IFN-γ–cisplatin combinations in this cell line. For all the other cell lines examined here, we found that synergism depended on the concentration of the two drugs used: sometimes it was obtained for the weakly affected fractions, and sometimes for the highly affected fractions. This could explain some of the discrepancies in the results of clinical trials using the same drug combinations against tumors of the same histological type.

Conclusions

These results suggest that a judicious combination of drugs is rather difficult to devise, indeed two cell lines with different sensitivities to two drugs individually may show a similar response to a combination of the two. Moreover, the present findings demonstrate for the first time the impor-

tance of the schedule of administration of the two drugs. Our results also confirmed the importance of the intrinsic sensitivity of the cell lines to the first administered drug and the concentration of the drug. As such information is difficult to obtain *in vivo*, a better understanding of the mechanisms involved in potentiation should help in the design of efficient schedules of administration. However, it should be borne in mind that the results are complicated by the multifactorial nature of the interactions between biological response modifiers, differentiating agents and standard chemotherapeutic drugs.[8]

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