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ORIGINAL ARTICLE

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Characterizations of irofulven cytotoxicity in combination with cisplatin and oxaliplatin in human colon, breast, and ovarian cancer cells

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Abstract *Purpose*: This study assessed the cytotoxic effects of irofulven in combination with oxaliplatin and cisplatin in a panel of human cancer cell lines. *Methods*: Growth inhibition studies were performed using the human HT29 colon cancer cell line, irofulven-resistant derivative HT29/IF2, breast cancer cell line MCF7, and ovarian cancer line CAOV3. Irofulven–oxaliplatin combinations were compared with irofulven–cisplatin combinations in the same cell lines using similar experimental settings. Cells were exposed for 1 h to irofulven and then for 24 h to oxaliplatin or cisplatin and vice versa. *Results*: Single agent irofulven displayed cytotoxic effects against human colon HT29 cells, human breast cancer cell lines including MCF7, SKBR3, and ZR-75-1, and human ovarian cancer cell lines CAOV3, OVCAR3, and IGROV1, with OVCAR3 being the most sensitive cancer cell line (IC_{50}: 2.4 μM). In all tested cell lines the oxaliplatin–irofulven combination led to clear evidence of synergistic activity. In HT29 and HT29/IF2, the sequence oxaliplatin followed by irofulven appears to be the most effective whereas in MCF7 cells, irofulven given prior to or simultaneously with oxaliplatin is more effective than the other schedule. The combination displays additive activity toward CAOV3 ovarian cells when irofulven was administered prior to or simultaneously with oxaliplatin and partially synergistic when oxaliplatin was followed by irofulven. In most of the cell lines, the sequence oxaliplatin followed by irofulven appears to be the most effective as compared to other schedules. A combination of irofulven with cisplatin has the same efficacy as with oxaliplatin for the same cell lines. Cell cycle studies show that irofulven increases the proportion of cells in the S phase. Cisplatin–irofulven and oxaliplatin–irofulven combinations block cells in G1/S and potently induce apoptosis. *Conclusion*: Irofulven displays synergistic antiproliferative and pro-apoptotic effects when combined with oxaliplatin over a broad range of concentrations in human colon and breast cancer cells. Acquired resistance to irofulven has limited impact on the effects of cisplatin–irofulven and oxaliplatin–irofulven combinations. Based on these data, irofulven–oxaliplatin and cisplatin–irofulven combinations will be further explored in clinical trials, favoring the use schedules of oxaliplatin given prior to irofulven in patients with cancer.

Keywords MGI 114 · Illudin S · Combination index · Synergy · Preclinical pharmacology

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Introduction

Irofulven (6-hydroxymethylacylfulvene, MGI 114) (Fig. 1) is a novel DNA-interacting agent derived from the mushroom product illudin S. Recent studies have suggested that irofulven inhibits DNA synthesis, arrests

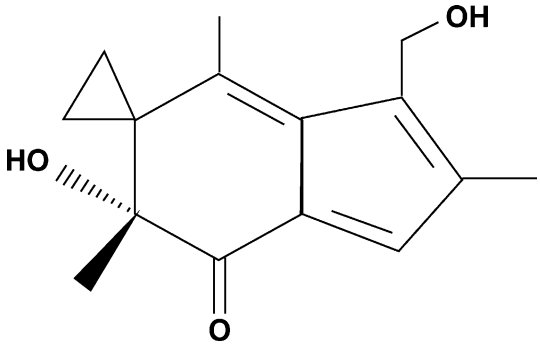

Fig. 1 Chemical structure of irofulven

cell cycle in S phase, and induces apoptosis via multiple mechanisms. Irofulven also induces DNA strand breaks without inducing DNA intrastrand cross-links or DNA-protein cross-links [1, 2]. Unlike many other alkylating agents, the activity of irofulven is not affected by loss of p53 or mismatch repair (MMR) function. In addition, the drug is not a substrate for multidrug transporters such as the P-glycoprotein and MRP1 that could limit the activity of other anticancer agents [3]. The nucleotide excision repair pathway was shown to play a key role in the cellular sensitivity to irofulven [4]. Expression of XPG endonuclease is correlated with cytotoxicity for the drug [4, 5]. Irofulven activates the ATM-CHK2 DNA damage-signaling pathway, and CHK2 activation contributes to S phase cell cycle arrest induced by irofulven [6]. Apoptosis induction by irofulven is independent of Bcl-2 and caspase-3 whereas the MAPK signaling pathway and caspases-8 and caspases-9 are activated by irofulven treatment [7–9]. Irofulven displays activity against a variety of human tumors in preclinical and clinical trials, both as a single agent and in combination with several other anticancer drugs. Preclinical single agent antitumor activity of irofulven has been demonstrated in breast, colon, gastric, lung, ovarian, prostate, and renal cell lines. Additionally, combinations of irofulven with radiation or chemotherapeutic agents such as paclitaxel, irinotecan, 5-fluorouracil (5-FU), mitomycin C, thiotepa, topotecan, and cisplatin have produced additive and/or synergistic inhibition of cellular proliferation in a variety of tumor types [10–13].

Oxaliplatin is a diaminocyclohexane (DACH)-platinum compound that displayed a unique spectrum of activity and is widely used in the treatment of colorectal carcinoma in combination with 5-FU/folinic acid in both adjuvant and metastatic settings [14–16]. Like cisplatin, oxaliplatin acts as an alkylating agent on DNA, forming platinated intrastrand cross-links. Recent studies have suggested that alterations in MMR activity can lead to intrinsic resistance to cisplatin and carboplatin. Preclinical data suggest that resistance mechanisms that discriminate between cisplatin and oxaliplatin are defects in MMR complex and enhanced replication bypass mechanisms. For example, oxaliplatin retains its cytotoxic activity in MMR-deficient cells, while this characteristic leads to cisplatin resistance [17]. Nucleotide excision repair also plays a role in oxaliplatin sensitivity, through XPA and ERCC1 [15]. Thus, cytotoxic effects of oxaliplatin have been demonstrated in vitro and in vivo against a broad range of tumor tissues including cisplatin-resistant cell lines or tumors [18]. In preclinical models oxaliplatin is active against a broad range of human cancer cell lines including colon, ovarian, as well as breast carcinoma.

Irofulven has been used either alone or in combination with other anticancer drugs in several phases I and II clinical trials with promising results [19–23]. To further optimize the clinical use of this compound, we decided to explore the antiproliferative activity of irofulven in combination with oxaliplatin and cisplatin using several drug concentrations and sequences in a panel of human colon (HT29), breast (MCF7), and ovarian (CAOV3) cancer cell lines characterized for p53, MDR, and MMR expressions. In addition, we investigated the effects of those combinations in HT29/IF2, an irofulven-resistant cancer cell line selected for acquired resistance to continuous irofulven exposure. Our preclinical experiments were designed to provide insight on how this novel agent in combination with cisplatin and oxaliplatin might be useful in heavily pretreated patients who may have acquired multidrug resistance due to exposure to previous treatment with chemotherapy.

Materials and methods

Cell lines and reagents

HT29 colon cell lines, SKBR3, BT-474, MDA-MB-361, ZR-75-1, MCF7 breast cancer cell lines, and CAOV3, OVCAR3, IGROV1 ovarian cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained according to its recommendations. The HT29/IF2 irofulven-resistant cell line was obtained from Dr. Larsen's laboratory (IGR, Paris). All cell lines were regularly tested for mycoplasma contamination by PCR using a Stratagene kit (La Jolla, CA, USA).

Purified irofulven was supplied by MGI Pharma Inc. (Bloomington, MN, USA). A 5 mg/ml stock solution was prepared in ethanol; oxaliplatin (purchased from Sanofi Synthelabo, France) 10 mg/ml was prepared in water; cisplatin (Sigma, Saint-Quentin Fallavier, France) 10 mg/ml was prepared in PBS.

In vitro growth inhibition assay (MTT assay)

The MTT assay was carried out as described previously (Hansen et al. 1989). In brief, cells were seeded in 96-well tissue culture plates at a density of 2×10^3 cells/well. Cell viability was determined after 120 h incubation by the colorimetric conversion of yellow, water-soluble tetrazolium MTT (3−[4, 5-dimethylthiazol-2-yl]−2, 5-diphenyltetrazolium bromide; Sigma) into purple, water-

insoluble formazan. This reaction is catalyzed by mitochondrial dehydrogenases and is used to estimate the relative number of viable cells (Mosmann 1983). Cells were incubated with 0.4 mg/ml MTT for 4 h at 37°C. After incubation, the supernatant was discarded; the cell pellet was re-suspended in 0.1 ml of DMSO; and the absorbance was measured at 560 nm by the use of a microplate reader (Dynatech, Michigan). Wells with untreated cells or with drug-containing medium without cells were used as positive and negative controls, respectively. Growth inhibition curves were plotted as a percentage of untreated control cells.

Simultaneous and sequential exposure of irofulven with oxaliplatin or cisplatin

For simultaneous drug exposure (Fig. 2c), cells were seeded at 2×10^3 cells/well in 96-well plates and treated 24 h later with increasing concentrations of irofulven alone or with concentrations of oxaliplatin or cisplatin corresponding to the IC_{20}, IC_{40}, or IC_{60} values. The drugs were added simultaneously for 24 h. For sequential exposure (Fig. 2a, b), the cells were incubated with different concentrations of irofulven for 1 h prior to or after oxaliplatin or cisplatin treatment. The cells were then washed and post-incubated in drug-free medium for 72 h Growth inhibition was then determined by the MTT assay.

Statistical analysis and determination of synergistic activity

Effects of drug combinations were evaluated using the Chou and Talalay method, which is based on the median-effect principle (Chou and Talalay 1984). This involves plotting dose-effect curves for each drug and for multiple diluted, fixed-ratio combinations, using the equation: $fa/fu = (C/Cm)m$, where C is the drug concentration, IC_{50} the concentration required for a half-maximal effect (i.e., 50% inhibition of cell growth), fa the cell fraction affected by the drug concentration C (e.g., 0.9 if cell growth is inhibited by 90%), fu the unaffected fraction, and m the sigmoidicity coefficient of the concentration–effect curve. On the basis of the slope of the curve for each drug in a combination, it can be determined whether the drugs have mutually nonexclusive effects (e.g., independent or interactive modes of action). The combination index (CI) is then determined by the equation:

$$CI = [(C)1/(Cx)1] + [(C)2/(Cx)2] + [\alpha(C)1(C)2/(Cx)1(Cx)2],$$

where $(Cx)1$ is the concentration of drug 1 required to produce an x percent effect of that drug alone and $(C)1$ 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 nonexclusive then α is 0 or 1, respectively. A CI values were calculated by solving the equation for different values of fa (i.e., for different degrees of cell growth inhibition). A CI values of < 0.8 indicate synergy, the values between 0.8 and 1.2 indicate additive effects, and values > 1.2 indicate antagonism. Data were analyzed on an IBM-PC computer using concentration–effect analysis for microcomputer software (Biosoft, Cambridge, UK). For statistical analysis and graphs we used Instat and Prism software (GraphPad, San Diego, USA). Results were expressed as the Mean + SD of at least three experiments performed in duplicate. Means and SD were compared using Student's t-test (two-sided p value).

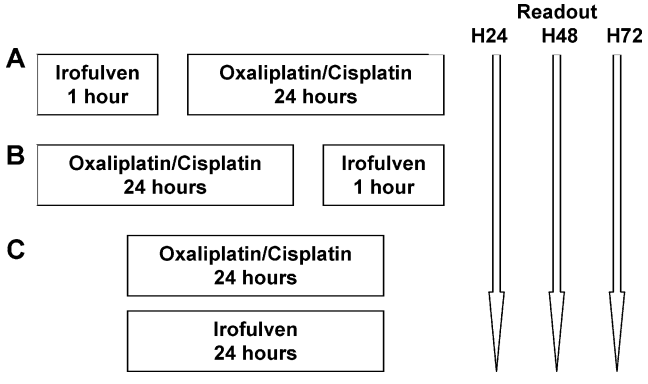

Fig. 2 Sequences evaluating the effects of irofulven-based combination. A and B schedules: sequential exposure to irofulven given either prior to (**a**) or after (**b**) oxaliplatin or cisplatin; C: simultaneous exposure to irofulven and cisplatin or oxaliplatin

Cell cycle analysis and apoptosis

Cell cycle analysis and the measurement of the percentage of apoptotic cells were assessed by flow cytometry. In brief, cells were seeded onto 25 cm^3 flasks and treated with various concentrations of irofulven, oxaliplatin, and cisplatin. At various time-points adherent and nonadherent cells were recovered, washed with PBS, fixed in 70% ethanol, and stored at +4°C until use. Cells were dehydrated in PBS, incubated for 20 min at room temperature with 250 µg/ml RNAse A with Triton X-100 and 20 min at +4°C with 50 µg/ml propidium iodide in the dark. The cell cycle distribution and percentage of apoptotic cells were determined with FACScan flow cytometer.

Results

Single agent studies

Activity of irofulven in the panel of cancer cell lines

In HT29 cells, irofulven shows time-dependent cytotoxic effects with IC_{50}s of 284 ± 32, 6 ± 1, and

2.9 ± 0.2 μM for exposure of 15 min, 1, and 24 h, respectively (Fig. 3). In irofulven-resistant HT29/IF2 cells, IC_{50}s of irofulven were 1000 ± 130, 406 ± 50, and 32 ± 4 μM for exposure of 15 min, 1, and 24 h, respectively. Based on our results, showing a rapid degradation of irofulven in culture medium at 37°C and previous pharmacokinetic data [24], 1 h exposure was selected for further evaluations of irofulven in our panel of cancer cell lines. Table 1 shows the activity of irofulven as a single agent against a panel of tumor cell lines. Irofulven displayed cytotoxic effects against human colon HT29 cells and several human breast and ovarian cancer cells, OVCAR3 being the most sensitive cancer cell line. Human breast cancer cells BT-474 and MDA-MB-361 were more resistant to irofulven compared to other breast cancer cells. As expected, HT29/IF2 was significantly more resistant to irofulven than other cancer cell lines used in this panel.

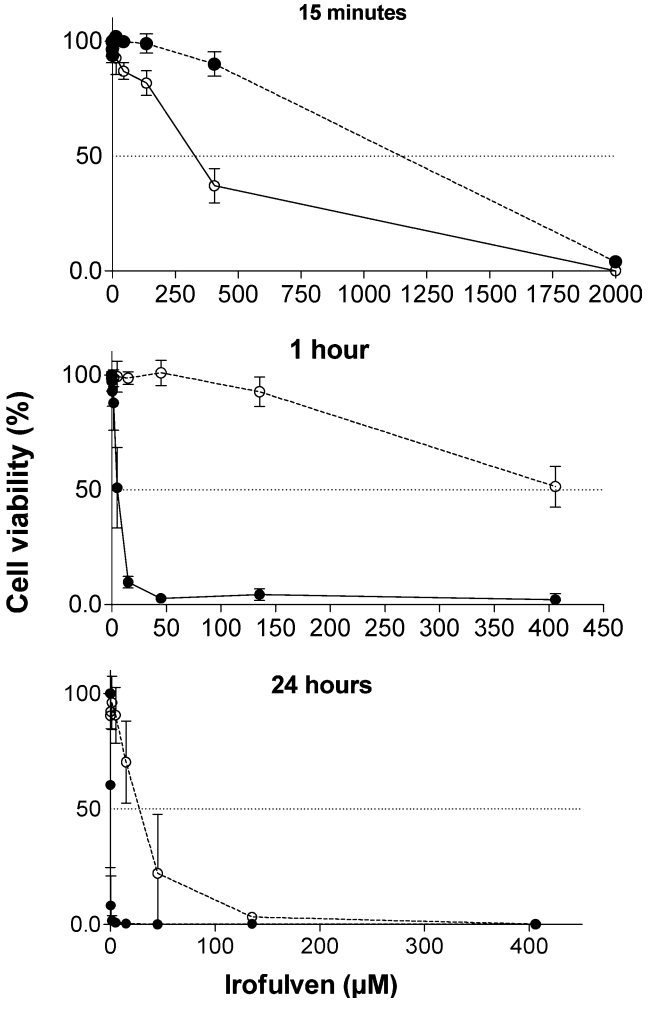

Fig. 3 Time course experiments of irofulven in human colon cancer cells. Cytotoxicity of irofulven exposure for 15 min, 1, and 24 h in HT29 human colon cancer cells (●- and its derived counterpart selected for resistance to irofulven HT29/IF2 (⊖)

Table 1 Activity of irofulven, oxaliplatin, and cisplatin given as single agents in a panel of selected human cancer cell lines

Cell line	Genetic characteristics			IC_{50} (μM)		
	P53	MDR	MMR	Irofulven	Oxaliplatin	Cisplatin
HT29	mut	Low	High	5.9 ± 0.9	5.0 ± 0.8	20 ± 5
HT29/IF2	mut	Low	High	406 ± 50	5.0 ± 0.7	18 ± 4
MCF7	wt	Low	Low	8.1 ± 0.8	8.1 ± 1.3	30 ± 6
SKBR3	mut	Low	Low	30 ± 4	50 ± 9.5	43 ± 7
MDA-MB-361	mut	Low	Low	109 ± 21	30 ± 8.0	60 ± 7
BT-474	mut	Low	High	85 ± 11	150 ± 20	75 ± 12
ZR-75-1	mut	Low	High	16 ± 2	65 ± 12	63 ± 11
CAOV3	mut	Low	High	3.2 ± 0.8	4 ± 0.8	2.5 ± 0.5
OVCAR3	mut	Low	High	2.4 ± 0.6	21 ± 3	15 ± 3
IGROV1	wt	Low	Low	6.1 ± 0.7	7 ± 1.5	21 ± 4

Values are given as means of at least three individual experiments (each done in duplicate) ± SD

Activity of oxaliplatin and cisplatin in the panel of cancer cell lines

Cytotoxic effects of oxaliplatin and cisplatin toward colon, breast, and ovarian cancer cell lines are shown in Table 1. Results are comparable to those previously published [25]. The ovarian CAOV3 cell line and HT29 and HT29/IF2 colon cancer cell lines were the most sensitive, while BT-474, ZR-75-1, and SKBR3 displayed more marked resistance to oxaliplatin. Interestingly, no cross-resistance was observed for the platinating agents in the HT29/IF2 cell line with experimentally acquired resistance to irofulven.

Combination agent studies

The effect of sequential (schedules A and B) and simultaneous (schedule C) exposure to irofulven with oxaliplatin or cisplatin was determined using combination indexes that represent an affected fraction for the concentration of drugs corresponding to IC_{50}.

In HT29, the combination of oxaliplatin and irofulven leads to various degrees of synergistic activity (Fig. 4, Table 2). As shown in Fig. 4, oxaliplatin given either prior to or after irofulven led to CI values < 1 demonstrating synergistic effects between those two compounds. Similarly, simultaneous exposure to irofulven and oxaliplatin was associated with synergism in this cell line. From these experiments, we concluded that the combination of irofulven with oxaliplatin yields synergistic effects over a broad range of concentrations with no clear evidence of schedule dependency.

Similar experiments were done in HT29/IF2 cells. As shown in Fig. 4, acquired resistance to irofulven slightly decreased the sensitivity of this cell line to the combination irofulven–oxaliplatin. At lower concentrations, only additive effects were observed between irofulven and oxaliplatin in this cell line. Synergism was achieved only at concentrations leading to a fraction affected > 0.75. From those experiments, the sequence oxalipla-

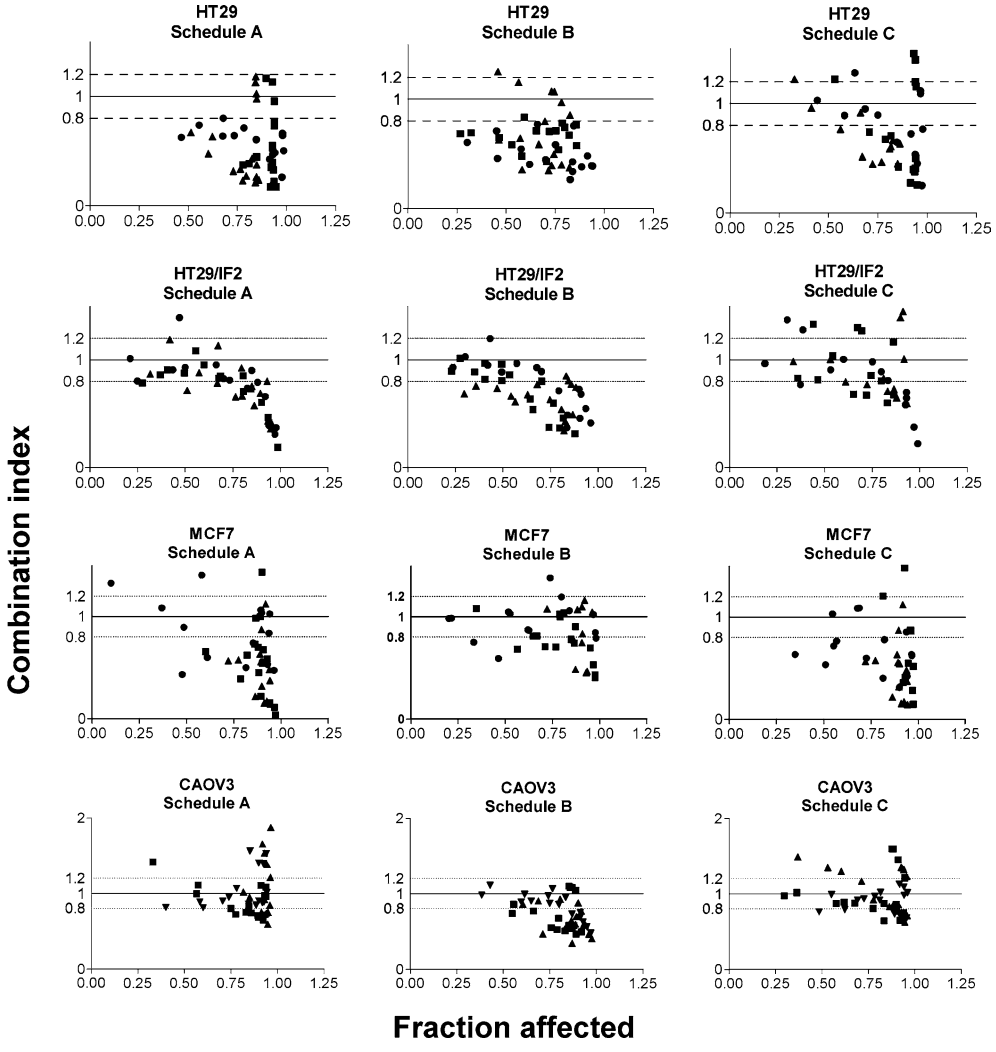

Fig. 4 Isobolograms showing the interaction of irofulven and oxaliplatin in the HT29, HT29/IF2, MCF7, and CAOV3 human cancer cell lines. Schedule A irofulven followed by oxaliplatin, Schedule B oxaliplatin followed by irofulven, Schedule C simultaneous administration of irofulven and oxaliplatin. *Each spot* represents one experiment performed in triplicate (symbols distinguish three separate sets of experiments). Calculation of a combination index (*CI*) below 0.8 indicates synergy, above 1.2 shows antagonisms, while a combination index between 0.8 and 1.2 corresponds to an additive effect

Table 2 Summary of the effects of irofulven–oxaliplatin combinations in human colon, breast, and ovarian cancer cell lines

Cell lines	Irofulven/Oxaliplatin combinations		
	Schedule AI + O	Schedule B O + I	Schedule C concurrent
HT29	Synergy	Synergy	Additive/Synergy
HT29/IF2	Additive/Synergy	Additive/Synergy	Additive
MCF7	Synergy	Additive/Synergy	Synergy
CAOV3	Additive	Additive/Synergy	Additive

tin followed by irofulven (schedule B) appears to be the most active compared to other schedules.

We explored the effects of irofulven–oxaliplatin combinations in MCF7 breast cancer cells and CAOV3 ovarian cancer cells. As shown in Fig. 4, additive and synergistic effects were observed in all groups. In MCF7 breast cancer cells, irofulven given prior to or simultaneously with oxaliplatin (schedules A and C) was more effective than schedule B.

In CAOV3 ovarian cells, the combination displays additive activity when irofulven was administered prior to or simultaneously with oxaliplatin (schedules A and C) whereas synergism was observed when oxaliplatin was followed by irofulven (schedule B).

Overall, the effects of irofulven/cisplatin combinations are comparable to that of irofulven/oxaliplatin combination (Fig. 5, Table 3); however, combinations with oxaliplatin were slightly more synergistic in HT29 colon cancer cell lines.

Induction of cell cycle arrest and apoptosis

We examined irofulven/oxaliplatin and irofulven/cisplatin effects on cell cycle progression by flow cytometry. The HT29 cells were incubated with 10 μM irofulven for 1 h, then 24 h with 7 μM oxaliplatin or 30 μM cisplatin. Irofulven treatment caused the accumulation of cells in

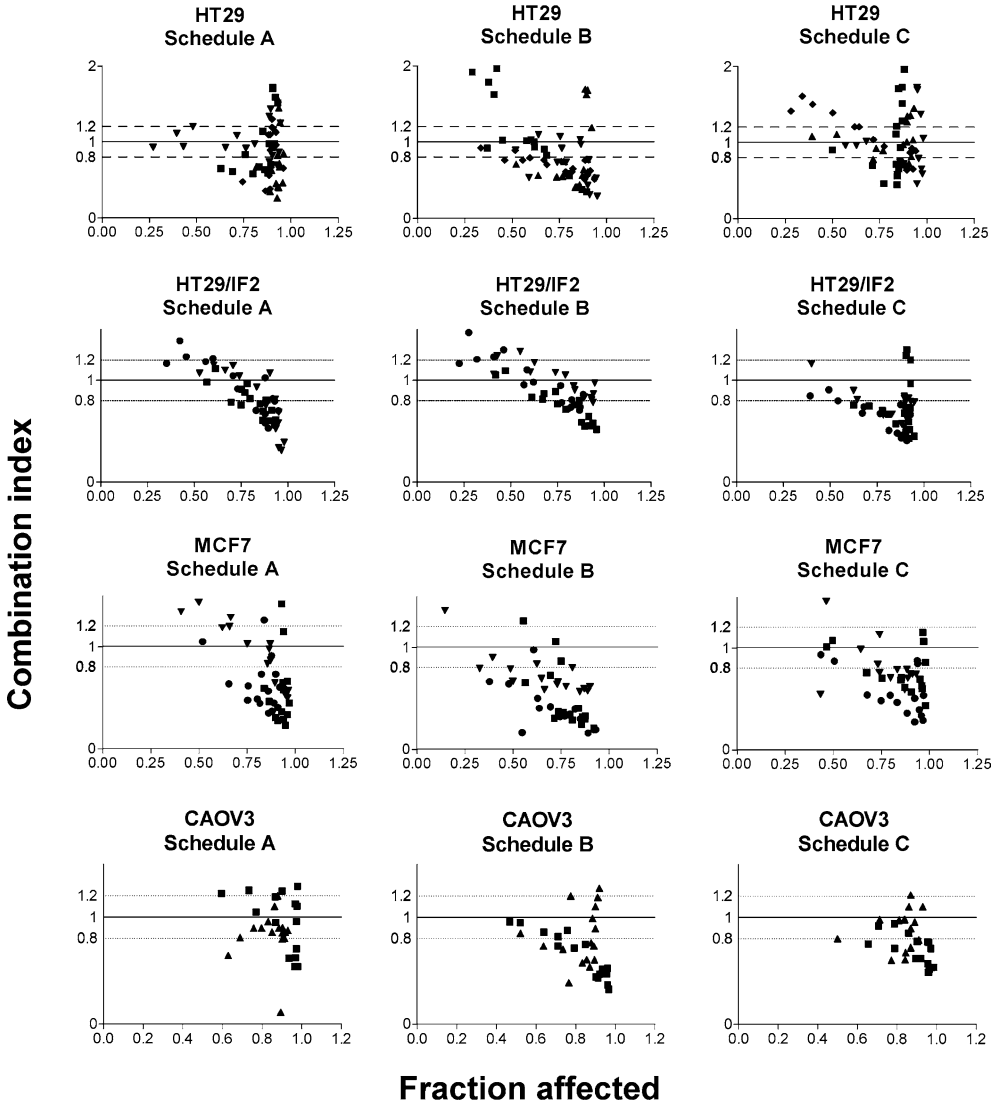

Fig. 5 Isobolograms showing the interaction of irofulven and cisplatin in the HT29, HT29/IF2, MCF7, and CAOV3 human cancer cell lines. Schedule A irofulven followed by cisplatin, Schedule B cisplatin followed by irofulven, Schedule C simultaneous administration of irofulven and cisplatin. *Each spot* represents one experiment performed in triplicate (symbols distinguish three separate sets of experiments). Calculation of a CI below 0.8 indicates synergy, above 1.2 shows antagonisms, while a CI between 0.8 and 1.2 corresponds to an additive effect

Table 3 Summary of the effects of irofulven–cisplatin combinations in human colon, breast, and ovarian cancer cell lines

Cell lines	Irofulven/Cisplatin combinations		
	Schedule A I+O	Schedule B O+I	Schedule C concurrent
HT29	Additive/Synergy	Additive/Synergy	Additive
HT29/IF2	Additive/Synergy	Additive/Synergy	Synergy
MCF7	Synergy	Synergy	Synergy
CAOV3	Additive/Synergy	Synergy	Additive/Synergy

the S phase; in combination with oxaliplatin or cisplatin, all cells were blocked in the G1/S (Fig. 6).

Irofulven also induced the progressive generation of particles corresponding to hypoploid DNA content (sub-G1 fraction) that is characteristic of apoptosis. Quantification of apoptotic fraction is plotted as a percentage of total cell number (Fig. 7). Our results clearly show that combinations of irofulven/oxaliplatin and irofulven/cisplatin increase percentage of cells undergoing apoptosis as compared to the extent produced by each individual agent.

Discussion

Although useful cancer treatment regimens were generated with first generations of platinum-compounds including cisplatin and carboplatin, many cancers re-

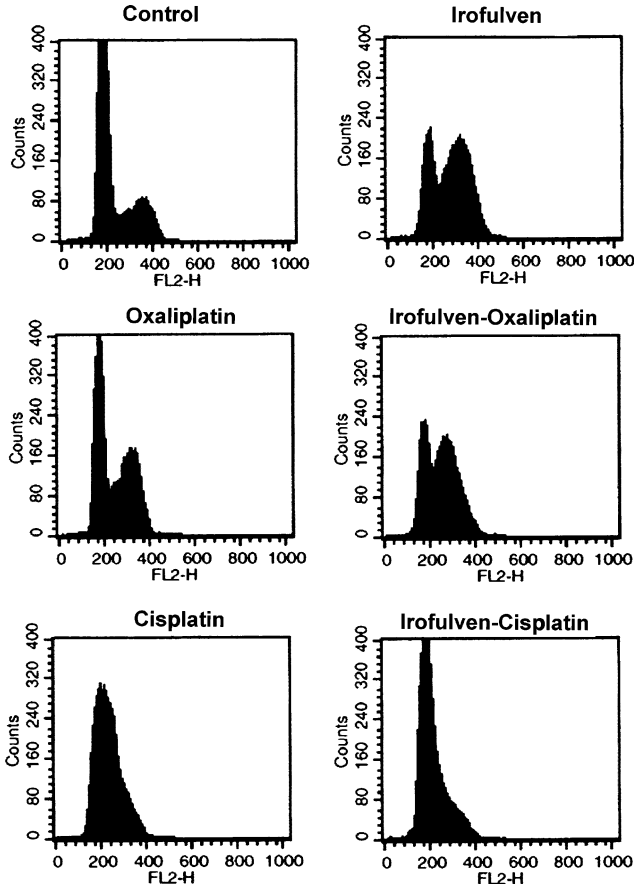

Fig. 6 Effects of irofulven combinations on cell cycle in HT29 cells detected by flow cytometry. Irofulven exposure resulted in the accumulation of cells in S-phase of the cell cycle and irofulven–platinum combinations led to G1/S cell cycle blockage

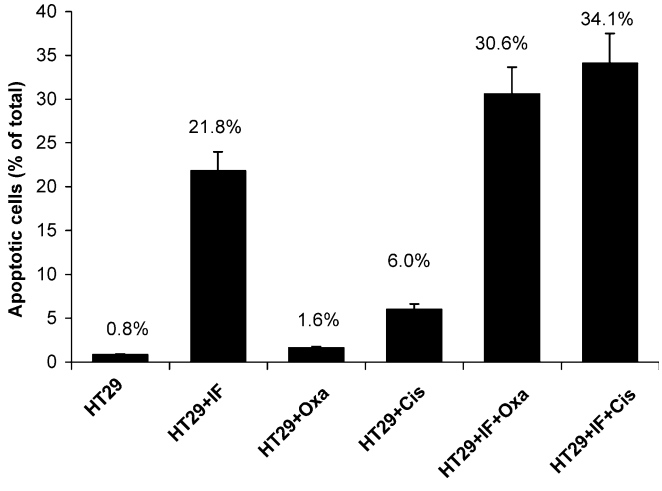

Fig. 7 Flow cytometry histograms for HT29 cancer cells exposed to irofulven, cisplatin, and oxaliplatin, and irofulven–platinum-based combinations. Flow cytometry shows the quantity of sub-G1 material indicating apoptotic cells calculated as a percentage of total cell number (*I* irofulven, *O* oxaliplatin, *C* cisplatin)

main resistant to cisplatin and carboplatin either given as single agents or in combination with other cytotoxics. The DACH-platinum drug oxaliplatin was shown to be only partially cross-resistant with cisplatin and carboplatin, and oxaliplatin-based combinations have offered new possibilities to overcome cisplatin resistance in cancer patients [26]. It has proven a mainstay in colorectal cancer treatment when combined with either intensive or acquired 5-FU ± folinic acid, and has shown impressive activity in combination with other agents such as irinotecan, taxanes, and gemcitabine [27–29].

Irofulven, a novel DNA-interacting agent, may represent an interesting alternative to currently available alkylating agents. Previous studies have shown that unlike many alkylating agents, irofulven cytotoxicity is not dependent on p53, MMR, and MDR expressions. Irofulven, oxaliplatin, and cisplatin were first evaluated as single agents in a panel of cancer cell lines. Exposure to 5–10 μM irofulven leads to strong antiproliferative effects in colon (HT29) and ovarian (CAOV3, OVCAR3, and IGROV1) cancer cell lines. Irofulven spectrum of activity in those cell lines was comparable to that of oxaliplatin and cisplatin. Breast cancer cell lines SKBR3, BT-474, MDA-MB-361, and ZR-75-1 were shown to be more resistant than other cell lines tested for irofulven, cisplatin, and oxaliplatin in our panel. The MCF7 appears to be the only breast cancer cell line that displayed sensitivity to irofulven, cisplatin, and oxaliplatin. As suggested previously [3], there was no clear correlation between sensitivity to irofulven and p53 status in our panel of cancer cells. As previously described the HT29/IF2 irofulven-resistant cell line remained sensitive to cisplatin. Furthermore, HT29/IF2 was also not cross-resistant with oxaliplatin. In all cell lines but CAOV3, the activity of single agent cisplatin varied less than irofulven or oxaliplatin. Interestingly, irofulven, and oxaliplatin cytotoxicity was not affected by inherent cisplatin resistance in HT29, MCF7, and IGROV1. This suggested that mechanisms involved in cisplatin resistance are different from those involved in irofulven-resistance in human cancer.

In our study, we have also determined the activity of irofulven in combination with oxaliplatin toward colon (HT29 and HT29/IF2), breast (MCF7) and ovarian (CAOV3) cancer cell lines using several drug concentrations and schedules. Combinations of irofulven with oxaliplatin induce synergistic activity over a broad range of concentrations in cancer cells. Additive and synergistic effects were observed when irofulven was combined with oxaliplatin in HT29/IF2 suggesting that acquired resistance to irofulven has little impact on the effects of the combination. Schedules using oxaliplatin prior to irofulven appear slightly more synergistic than other sequences in both sensitive and HT29/IF2-resistant cell lines. Although no direct comparison is possible, combinations of irofulven with oxaliplatin appeared at least as effective as the combination of irofulven with cisplatin in our selected cancer cell lines. Our data showing synergism between irofulven and cisplatin are

consistent with those previously published by ourselves and other authors [11, 30]. Overall, these data clearly demonstrated the potential of combining irofulven with first and second generations of platinum drugs.

Compared with other alkylating agents, irofulven is a strong inhibitor of S-phase progression [1, 2]. Our results showed that under irofulven exposure the proportion of cells in S-phase increases dramatically. Combination with oxaliplatin and cisplatin was shown to strongly block cancer cells at G1/S-phase of cell cycle. One possible explanation of this effect may be related to the mechanism by which these agents induce apoptosis. Irofulven apoptosis was shown to be mediated by caspase-8 and caspase-9 activation and was independent of caspase-3. Conversely, platinum compounds induce caspase-3 activation and this process is the major contributor to subsequent induction of apoptosis. Consequently, irofulven–oxaliplatin and irofulven–cisplatin combinations could be expected to induce apoptosis by activation of multiple caspase pathways, resulting in a synergistic effect.

In summary, our data strongly suggest that combinations of irofulven with oxaliplatin and cisplatin should be further explored in clinical trials using a schedule that preferably gives oxaliplatin or cisplatin immediately prior to irofulven. Platinum-based irofulven combinations were clearly synergistic in colon and ovarian cancer cell lines. Interestingly, acquired resistance to irofulven did not affect the synergy between cisplatin/oxaliplatin and irofulven. Similarly, the synergy between platinum drugs and irofulven was retained in cancer cells that were only marginally sensitive to cisplatin. This information will be incorporated in the design of current phase I/II clinical trials using irofulven in combination with oxaliplatin and cisplatin.

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