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Interferon Alpha and 5′-Deoxy-5-fluorouridine in Colon Cancer: Effects as Single Agents and in Combination on Growth of Xenograft Tumours

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Interferon-α (IFN-α) enhances the activity of the 5-fluorouracil (5-FU) prodrug 5′-deoxy-5-fluorouridine (5′-dFUrd) in colorectal cancer cells *in vitro* by upregulating the enzyme pyrimidine nucleoside phosphorylase (PNPase), which is responsible for converting 5′-dFUrd to 5-FU. We examined whether such enhancement also occurs *in vivo* using human colorectal xenografts in nude mice. The Co-115 line has high basal levels of PNPase and the enzyme level was increased in tumours from mice treated for 3 weeks with 50 000 IU/day (5 days/week) of IFN-αA/D. The prodrug 5′-dFUrd (200 mg/day, 5 days/week) had a much greater antitumour activity than 5-FU had when it was used at an approximately equitoxic dose (20 mg/day, 5 days/week). However, because of the high activity of 5′/dFUrd as a single agent, no enhancement by IFN-αA/D was observed. Studies on xenografts of WiDr cells indicated that this line is much less sensitive to 5′-dFUrd. However, treatment of animals with IFN-αA/D at doses of 75 000 IU/day or 150 000 IU/day resulted in significant inhibition of WiDr tumour growth. Combination treatment with 75 mg/kg/day or 150 mg/kg/day of 5′-dFUrd resulted in enhanced antitumour activity, particularly at the higher dose of IFN-αA/D. Synergy of this drug combination was confirmed by isobologram analysis. Analysis of PNPase levels in WiDr tumours, excised from mice treated with IFN-αA/D, demonstrated that the enzyme activity was increased by IFN-α in a dose-dependent manner. Slight increases were also seen in normal liver and intestine from the same animals. Our results indicate that modulation of converting enzymes for anticancer prodrugs by cytokines could be a novel therapeutic strategy for combination therapy of colorectal cancer.

Key words: colon cancer, chemotherapy, interferon, nude mice, xenografts, enzymes, pyrimidine nucleoside phosphorylase, prodrug

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INTRODUCTION

CHEMOTHERAPY OF advanced colorectal carcinomas remains limited due to the remarkable resistance of this tumour to cytotoxic agents. The most effective chemotherapeutic agent is 5-fluorouracil (5-FU), but only about 5–15% of patients with disseminated colorectal cancer respond objectively. Several new treatment strategies have been developed that focus on enhancing the activity of 5-FU by combining it with modulators of its metabolism. The effect of 5-FU depends upon the ability of cells to form the active nucleotides, 5-fluorouridine-5′-triphosphate, 5-fluoro-2′-deoxyuridine-5′-triphosphate and 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP). The first two compounds compete with their normal counterparts for incorporation into RNA and DNA, respectively; while the third, FdUMP, prevents DNA synthesis by inhibiting thymidylate synthetase.

The rationale for combining 5-FU with other agents is to facilitate the pathway to one or other of these nucleotides. As an example, combination therapy with leucovorin has shown promising results due to the stabilisation of the FdUMP-TS complex. Interferon-α (IFN-α) also modulates the therapeutic effects of 5-FU in patients [1–4] as well as in some [5], but not all [6], colorectal cell lines.

However, the systemic toxicity of 5-FU limits its therapeutic application. Another approach has been to synthesise new derivatives exhibiting lower toxicity. One interesting compound is 5′-deoxy-5-fluorouridine (5′-dFUrd), which is clinically used in Japan and in Korea for treating colorectal, gastric, breast, bladder and cervical cancers. Results of recent clinical trials comparing the activity of 5′-dFUrd to that of 5-FU have been reported [7]. In animal models, 5′-dFUrd has a higher therapeutic index [8] than most other fluorinated pyrimidines. It is also less immunosuppressive [9, 10] and induces lower grade leucopenia than 5-FU [11]. The reason it has a higher therapeutic index is that it is a prodrug that must be enzy-

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matically cleaved into 5-FU by pyrimidine nucleoside phosphorylases (PNPase), uridine phosphorylase (EC 2.4.2.3) and thymidine phosphorylase (EC 2.4.2.4) before it becomes active. Thymidine phosphorylase (TdR Pase) is the predominant enzyme present in human tumour cells [12].

In a previous report [6], we showed that IFN-α potentiates the effects of 5'-dFUrd, and not those of 5-FU in five human colorectal cancer cell lines *in vitro*. We correlated this observation with a significant dose-dependent increase in PNPase activity in cells previously treated with IFN-α. Potentiation of the 5'-dFUrd effect by IFN-α is thus explained by an enhancement of its conversion to 5-FU through stimulation of PNPase. Similar results have been obtained with interferon-γ, tumour necrosis factor α and interleukin-1α, demonstrating upregulation of TdR Pase and subsequent increased efficacy of 5'-dFUrd [12].

We report here the results of *in vivo* assays using xenografts of two colon cell lines in nude mice. We analysed the effect of IFN-α alone or combined with 5'-dFUrd, and measured PNPase activity in nude mice previously treated with IFN-α.

MATERIALS AND METHODS

Cells and cell culture

The human colon cell line WiDr [13] was obtained from the ATCC, Rockville, Maryland, U.S.A. (CCL 218) and the Co-115 line was kindly provided by Dr B. Sordat (Epalinges, Switzerland) [14]. The cells were cultured in EF medium, a 1 : 1 mixture of enriched Dulbecco's modified Eagle's medium (EMED) and modified Ham's F-12 nutrient mixture (FMED) [15–17], containing 7.5×10^{-5} M α-thioglycerol and 10 mM HEPES. This was further supplemented with 5% fetal calf serum (FCS, Seromed, Fakola, Basel, Switzerland), 1 mg/ml of delipidated and deionised [18] bovine serum albumin (BSA, Cohn fraction V, Fluka, Buchs, Switzerland), 8 μg/ml of transferrin, 3 μg/ml of insulin, 1.2 μg/ml of ethanolamine, 2.8 μg/ml of linoleic acid, 2.6 μg/ml of cholesterol, plus nucleosides (uridine, adenosine, cytidine, guanosine, thymidine, 2'-deoxyadenosine, 2'-deoxycytidine HCl, and 2'-deoxyguanosine; 10 μg/ml each). The medium was supplemented with L-glutamine (204 mg/ml) immediately before use. The cells were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 in air as subconfluent monolayers in 25 cm^2 flasks (Nunclon, Nunc, Roskilde, Denmark). The cells were subcultured once a week by treatment with 0.05% trypsin in 0.02% EDTA (Seromed, Berlin, Germany). The cell lines were tested periodically for the presence of mycoplasma using the Myco Test assay system (Gibco/BRL, Paisley, U.K.) and were consistently found to be free from contamination throughout the studies presented.

Factors and chemicals

The prodrug 5'-deoxy-5-fluorouridine (MW 214.1) was supplied by Hoffmann-La Roche (Basle, Switzerland) and was dissolved in sterile water, aliquoted, and stored at −20°C. The recombinant human hybrid interferon rHuIFN-αA/D, which was constructed by joining the N-terminal segment of rIFN-αA and the C-terminal segment of rIFN-αD at the common BgL 1 site amino acid position 63 [19], was kindly provided by Dr M.J. Brunda (Hoffmann-La Roche, Nutley, New Jersey, U.S.A.). This type of IFN acts on both human cells or murine cells [20]. The specific activity was between 6.4 and 7.9×10^7 IU/mg protein as assayed by inhibition of the cytopathic effect of vesicular stomatitis virus on murine L cells [21]. It was diluted in 0.9% NaCl solution just prior to injection.

Pyrimidine nucleoside phosphorylase assays

The assays to measure enzyme activity with Co-115 cultured cells and xenograft tumours were performed as described [6, 22]. Briefly, tissue culture cells were scraped from the flasks in Ca^{2+}, Mg^{2+}-free phosphate buffered saline, centrifuged and resuspended in ice-cold homogenisation buffer (0.05 M potassium phosphate buffer supplemented with 0.01 M dithiothreitol and 0.01 M EDTA, pH 7.4). Tumours were dissected free of necrotic tissue, washed three times with ice-cold homogenisation buffer, cut into small pieces with a sterile scalpel before being homogenised in a Dounce homogeniser with a Teflon pestle for 2 min at 4°C. Aliquots of 800 μl containing cell extracts were incubated with 4 μmol of 5'-dFUrd for 12 h at 37°C. After incubation, the reaction was stopped by adding 800 μl of ice-cold buffer and 370 μl of 2.2 M perchloric acid. The samples were centrifuged at 15 000 rpm for 10 min and the absorbance of the supernatants was measured at 305 nm. The results were normalised on the basis of protein content as measured using a BioRad kit (Richmond, California, U.S.A.).

A different assay technique was used to measure normal and tumour tissues from nude mice bearing WiDr tumours because it appeared to be more sensitive for measuring low enzyme levels. The tissues were dissected, washed with buffer, and snap frozen before being sent to the Nippon Roche Research Centre in Kamakura, Japan. The PNPase activity was measured as described [12]. Samples were sonicated in 10 mM TRIS-HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM $MgCl_2$ and 50 mM potassium phosphate. Each sample was centrifuged at 105 000*g* for 90 min and dialysed overnight against 20 mM potassium phosphate buffer (pH 7.4) containing 1 mM of 2-mercaptoethanol. The substrate 5'-dFUrd was added to give a concentration of 10 mM and the samples were incubated at 37°C for 60 min. The reaction was terminated by adding methanol. An aliquot of the reaction mixture was separated by high performance liquid chromatography and the elutant was monitored by UV absorbance at 280 nm.

Mice

Male athymic mice (Swiss *nu+/nu+*), 10 weeks of age, were bred at the Swiss Institute for Experimental Cancer Research (Lausanne, Switzerland) under specific pathogen-free conditions. They were fed on a standard rodent diet (U.A.R., Villemoisson-sur-Orge, France) and water *ad libitum*. Mice were age-matched in all experiments.

Human tumour xenograft experiments

Subcutaneous tumours were initiated from cultured cells by injecting them in the left dorsal flank region of nude mice. A total of 1.5×10^6 Co-115 cells (passage 207) or 1×10^6 WiDr cells (passage 183 or 196) were given per mouse in 0.1 ml of EF medium. Palpable tumours (about 10 mm^3) developed within 7 days in 80% of the animals, and tumour-bearing animals were randomly assigned to treatment or vehicle control groups. Treatment was initiated on days 7 or 11 and drugs were given by intraperitoneal injection. The tumour size was measured in three dimensions (L, W and H) and volume was calculated by the formula: $V = \pi/6\,(L \times W \times H)$.

Calculation of drug interaction index

In order to estimate the degree of interaction between 5'-dFUrd and IFN-αA/D with WiDr xenografts, the interaction index [23] was calculated by the formula:

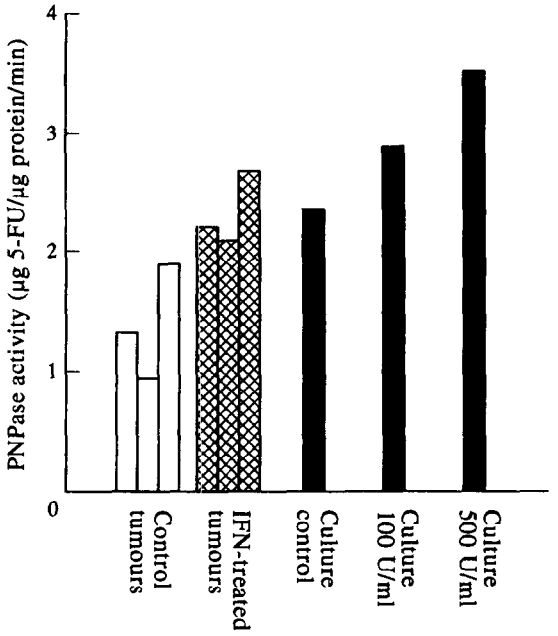

Figure 1. Pyrimidine nucleoside phosphorylase activity of Co-115 xenograft tumours from animals treated with vehicle (open bars) or IFNα (hatched bars) compared with cells treated with IFNα *in vitro* (solid bars). Cultured cells were treated with various concentrations of IFN-αA/D for 5 days. For the *in vivo* tumours, each bar represents an individual tumour.

$$I = \frac{d_{5'\text{-dFUrd}}}{D_{5'\text{-dFUrd}}} + \frac{d_{\text{IFN}}}{D_{\text{IFN}}},$$

where $d_{5'\text{-dFUrd}}$ and d_{IFN} are the doses of these drugs in the combination and $D_{5'\text{-dFUrd}}$ and D_{IFN} are the doses of each that would result in the same effect if they were used alone. The data in Figure 4 for 5'-dFUrd and IFN-αA/D alone at day 30 was used to calculate $D_{5'\text{-dFUrd}}$ and D_{IFN} by least squares regression analysis of the data plotted as log growth inhibition against drug dose. Growth inhibition was calculated by the formula: 100% (treated tumour volume/control tumour volume) × 100. Values of the interaction index (I) below 1 indicate synergy.

Statistics

Results are expressed as mean ± 1 S.E.M. Comparison of different treatment groups was determined by one-way Anova. All statistical calculations were performed using SigmaStat™ software (Jandel, San Rafael, California, U.S.A.).

RESULTS

The first human colorectal line selected for study in the xenograft system was Co-115. This line has the highest basal level of PNPase of the lines we had previously measured and is relatively sensitive to the effect of IFN-α [6]. As can be seen in Figure 1 (solid bars), a dose of 100 IU/ml of IFN-α increased the enzyme activity by 23%, which compares with the level of upregulation seen in our previous experiment. At 500 IU/ml, it was increased by about 50%. In order to determine whether IFN-α also increased PNPase levels *in vivo*, Co-115 tumours growing in nude mice were treated with 50 000 IU of IFN-αA/D per day, 5 days a week for 3 weeks starting on day 7. The tumours were excised on day 27 and PNPase levels were determined. The enzyme levels in tumours from IFN-αA/D animals were elevated compared to tumours from vehicle treated control animals (Figure 1).

As seen in Figure 2, IFN-αA/D (50 000 IU/mouse) had only a small, non-significant effect on Co-115 tumour growth in nude mice. The doses of the fluoropyrimidines in this experiment were selected to be approximately equitoxic at levels near their maximum tolerated doses. This means that the dose of 5'-dFUrd (200 mg/kg) used was 6-fold higher on a molar basis than that of 5-FU due to its lower toxicity. The dose of 5-FU had no effect on growth of tumours and combination with IFN-αA/D did not influence this. In contrast, 5'-dFUrd was highly effective in this model, leading to a marked reduction in tumour volume. In the combination with 5'-dFUrd, IFN-αA/D did not appear to enhance the activity of 5'-dFUrd, but this could not be accurately determined because of the high efficacy of 5'-dFUrd by itself.

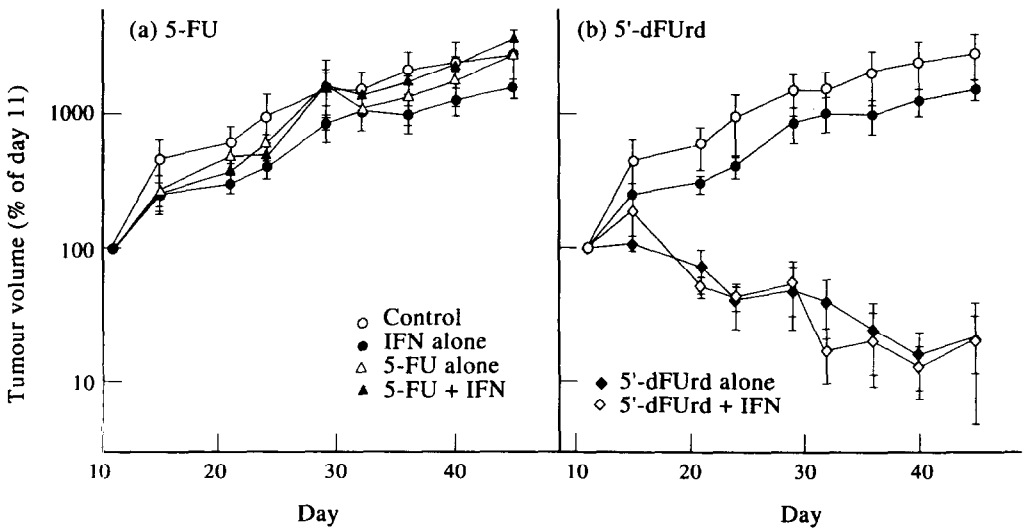

Figure 2. Effect of interferon-α on growth inhibition by (a) 5-FU or (b) 5'-FUrd of Co-115 xenografts in nude mice. Tumour cells were inoculated on day 0 and treatment was started on day 7. Interferon-αA/D was given three times/week at a dose of 50 000 IU/mouse. The other drugs, 5-FU (20 mg/kg) and 5'-dFUrd (200 mg/kg), were given five times/week. The results were normalised to the mean tumour volume of each group (four mice) as measured on day 11. The growth curves for the controls and interferon alone groups are reproduced in both panels for comparison. Points represent mean values and horizontal bars represent ±1 S.E.M.

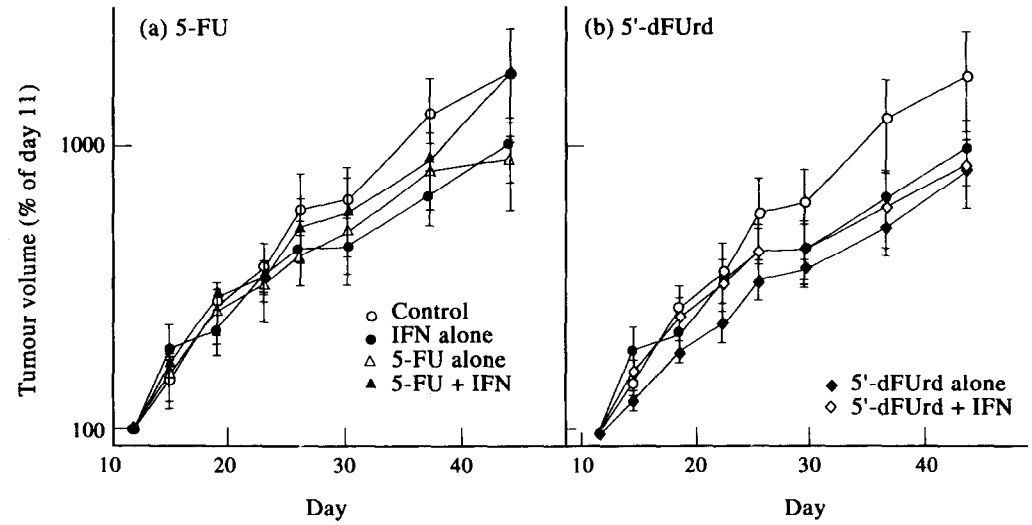

Figure 3. Effect of interferon-α on growth inhibition by (a) 5-FU or (b) 5'-dFUrd of WiDr xenografts in nude mice. Tumour cells were inoculated on day 0 and treatment was started on day 7. Interferon-αA/D was given three times/week at a dose of 50 000 IU/mouse. The other drugs, 5-FU (20 mg/kg) and 5'-dFUrd (50 mg/kg), were given five times/week. The results were normalised to the mean tumour volume of each group (four mice) as measured on day 11. The growth curves for the controls and interferon alone groups are reproduced in both panels for comparison. Points represent mean values and horizontal bars represent ±1 S.E.M.

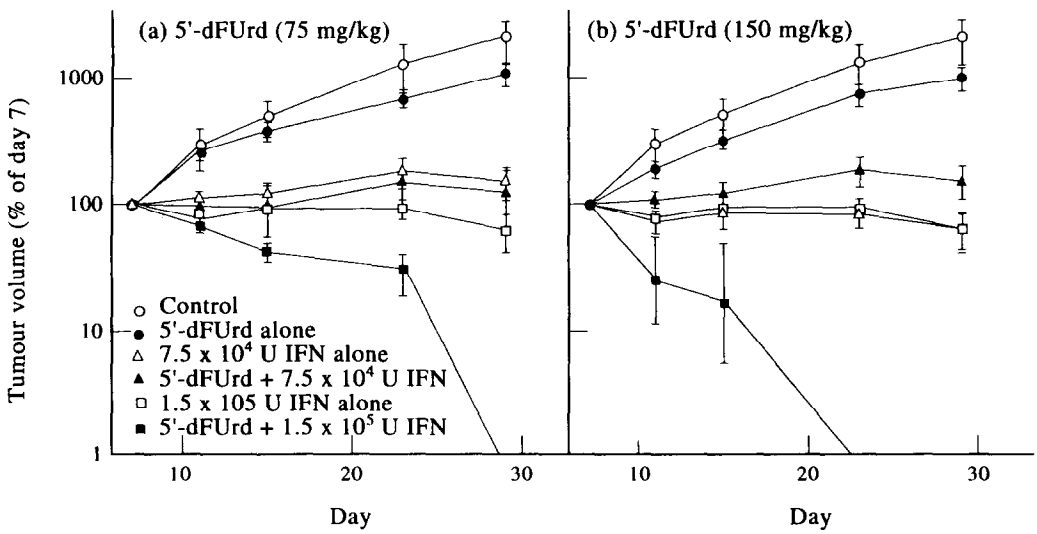

Figure 4. Effect of interferon-α on growth inhibition by 5'-dFUrd of WiDr xenografts in nude mice. Tumour cells were inoculated on day 0 and treatment was started on day 7. Interferon-αA/D was given intraperitoneally (i.p.) daily at two different doses, 75 000 IU/mouse and 150 000 IU/mouse. The 5'-dFUrd was also given i.p. daily at two different doses (a) 75 mg/kg and (b) 150 mg/kg. The results are normalised to the mean tumour volume of each group (five mice) as measured on day 7. The growth curves for the controls and interferon alone groups are reproduced in both panels for comparison. Points represent mean values and horizontal bars represent ±1 S.E.M.

Similar experiments were conducted with the WiDr cell line. In previous studies, this line revealed a relatively low basal level of PNPase activity, but this was increased by 25% with treatment with 100 IU/ml of IFN-α, and by 90% with 1000 IU/ml [6]. A comparison of the effects of 5-FU and 5'-dFUrd showed that, on a molar basis, the dose of 5'-dFUrd (0.23 mmole/kg) was approximately 50% higher than that of 5-FU (0.15 mmole/kg) (Figure 3). Neither drug had a significant effect on tumour growth, and IFN-αA/D did not significantly influence this. Growth inhibition in the 5'-dFUrd combination treatment group tended to be slightly greater than in the groups treated with 5'-dFUrd and IFN-αA/D as single agents.

In order to determine whether there was an interaction between 5'-dFUrd and IFN-αA/D in this model, two doses of each drug were tested as single agents and in combination. This allowed an evaluation of the interaction index [23]. The doses selected were higher than those used in the experiment shown in Figure 3 so that any effects would be clearly evident. The results are shown in Figure 4. There was very little effect at either dose of 5'-dFUrd, whereas IFN-αA/D was quite effective, exhibiting a statistically significant tumoristatic effect at 75 000 IU/mouse and resulting in some tumour reduction at 150 000 IU/mouse. Combination treatment was clearly more effective than single agent treatment, particularly those combinations with the higher dose of 5'-dFUrd. Isobologram analysis (Figure 5) showed clear synergy between the two drugs. The interaction indices calculated for day 30 tumour volumes were 0.47 and 0.85 for the low and high IFN-αA/D dose combinations indicating

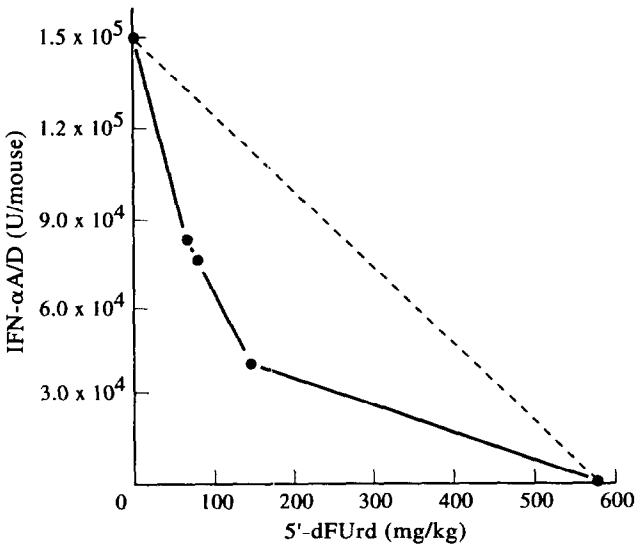

Figure 5. Isobologram of combination treatment with 5'-dFUrd and interferon-αA/D for 90% reduction in growth rate of WiDr tumours. The values are calculated from the data obtained at day 30 as presented in Figure 4.

synergistic interactions. The values for the low and high 5'-dFUrd groups were 1.1 and 0.34, suggesting that the low dose combinations were at best additive, but the high dose combinations were strongly synergistic. Even in the group of mice given the high doses of both drugs, no symptoms of diarrhoea or significant weight loss were observed.

The PNPase activity of the WiDr tumours in this experiment was examined. As shown in Table 1, treatment with IFN-αA/D significantly increased the enzyme activity in a dose-dependent manner, confirming our previous *in vitro* results. The effect of IFN-αA/D on enzyme levels in normal intestine and liver of these nude mice is also shown in Table 1. In mice, a combination of uridine phosphorylase and thymidine phosphorylase is responsible for conversion of 5'-dFUrd [24]. The small intestine contains very high levels of pyrimidine nucleoside phosphorylase activity, but this is not significantly augmented by treatment with IFN-αA/D. In the liver, the levels of enzyme activity were comparable with those in WiDr tumours, and increased slightly but not sigificantly after IFN-αA/D treatment.

DISCUSSION

Human colorectal carcinoma is a highly resistant tumour type. Over the last 30 years, no agent has been found to surpass the modest, mainly palliative effect of 5-FU. In recent clinical trials, the response rate for 5-FU as a single agent is 5–15% at best. The major problem is that colon carcinoma is intrinsically refractory to the lethal effects of all known cytotoxic drugs. By combining 5-FU with biochemical or biological modulators, the response rate can be raised to 20–40%. Several studies have shown IFN-α to be one such agent capable of improving the response rate to 5-FU in patients [1–4].

The mechanism by which IFN-α enhances the activity of 5-FU *in vivo* is not clear. As a biochemical response modifier, IFN-α can act directly on the tumour cells or it can act indirectly by activating cells of the immune system. Studies with human colorectal cell lines *in vitro* have yielded clear evidence of synergy between 5-FU and IFN-α in only a limited number of cell lines [5, 6]. Our previous results have indicated direct enhancement in only one of eight cell lines tested. These findings suggest that immune effects may be a major contributor to the clinical efficacy of this combination. We used the hybrid IFN-αA/D because it has high activity on both murine and human cells, so that if an immune effect were mediated by macrophages or natural killer cells, it would be detected in the nude mouse xenograft model [19, 25]. However, these mice have a deficiency in terms of the T-lymphocyte compartment [26] and, thus, a lack of interaction between IFN-α and 5-FU in this model might be an indication of T-cell involvement. Interestingly, the WiDr tumour appeared to be relatively sensitive to the antitumour activity of IFN-α/AD used as a single agent.

With the aim of improving the anti-tumour efficacy of 5-FU, several prodrugs have been synthesised. As such, 5'-deoxy-5-fluorouridine has a better therapeutic activity than 5-FU on murine tumours [11, 27], xenografts of head and neck squamous cell carcinomas [28], as well as on ovarian carcinomas [29]. Our results with the Co-115 xenografts confirm the high efficacy of this drug compared with 5-FU when the compounds were used at doses that were approximately equitoxic. This tumour is very sensitive to 5'-dFUrd due to the high basal levels of PNPase it expresses, thereby resulting in generation of high concentrations of 5-FU within the tumour cells. WiDr cells, which have much lower PNPase activity, were much less sensitive to 5'-dFUrd.

Our previous work with human colorectal cancer cell lines *in vitro* demonstrated that the effect of 5'-dFUrd was synergistically enhanced by IFN-α in five of eight lines tested, because of increased PNPase activities [6]. The results measuring the enzyme activities in xenografted tumours from animals treated with IFN-αA/D show that our *in vitro* findings are applicable *in vivo*. It has been possible to demonstrate a therapeutic effect in

Table 1. Effects of IFN-αA/D in vivo treatment on PNPase activity in grafted WiDr tumour and murine tissues

	Enzyme activity (% increase)		
IFN-α dose (IU)	Small intestine	Liver	Tumour
0	205 ± 33	21 ± 3	15 ± 1
75 000	221 ± 29 (8%)	27 ± 4 (28%)	19 ± 3* (20%)
150 000	246 ± 59 (20%)	28 ± 1 (33%)	24 ± 1* (60%)

* $P < 0.05$ compared with controls without IFNαA/D.

WiDr cells (passage 13) were injected on day 0 and were treated by i.p. injections 3 times a week for 3 weeks. Results are mean ± standard deviation based on 3 independent determinations (μg 5-FU/mg protein/h).

this model, but this depended upon finding the optimal doses of each drug. With the Co-115 model, the high efficacy of 5'-dFUrd prevented observation of any contribution by IFN-α. Use of lower doses of 5'-dFUrd in this tumour model might provide evidence that combination treatment with IFN-α can be synergistic, but this would not necessarily result in maximal therapeutic benefit. However, with the WiDr tumour it was possible to demonstrate true synergy *in vivo*, especially with higher doses of 5'-dFUrd. This line possesses a lower PNPase basal activity and a higher percentage increase when treated with IFN-α *in vitro* [6, 30].

Of course the important question is whether or not this combination would show improved clinical therapeutic efficacy. Of particular concern is the effect of IFN-α on PNPase levels of normal tissues, particularly normal intestine, which has the highest endogenous levels of activity ([31] and Table 1). This explains the high level of gastrointestinal side effects seen in patients treated with 5'-dFUrd [7, 32]. In our experiment, there appeared to be a slight increase in intestinal enzyme activity in tissue from animals treated with IFN-α, but this was not statistically significant and was proportionally smaller than the increase in tumours. However, one approach to overcome this potential disadvantage of 5'-dFUrd would be to use a prodrug of 5'-dFUrd itself, such as galactocytabine (trimethoxybenzoyl-5'-deoxy-5-fluorocytidine) [33]. Galactocytabine must first be converted by an acylamidase to 5'-deoxycytidine, which is further converted by cytidine deaminase to 5'-dFUrd. Compounds of this type have extremely high therapeutic indices with respect to haematopoietic, lymphopoietic [33] and intestinal toxicities [34].

In conclusion, we have shown that a cytokine, IFN-α, can modulate the activation of a prodrug *in vivo*, resulting in increased antitumour efficacy. Several other cytokines have been shown to upregulate PNPase *in vitro* to an even higher extent [12] and could potentially be considered for combination therapy as well, particularly with a prodrug of 5'-dFUrd having less intestinal toxicity.

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Soluble Intercellular Adhesion Molecule-1 (ICAM-1) is Released into the Serum and Ascites of Human Ovarian Carcinoma Patients and in Nude Mice Bearing Tumour Xenografts

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We have demonstrated that patients with ovarian carcinoma have higher levels of soluble intercellular adhesion molecule-1 (ICAM-1) in their serum and ascitic fluids than serum from normal individuals and non-neoplastic gynaecological disease or ascites from patients with cirrhosis. In order to investigate the source of the ICAM-1, and to study the mechanisms which regulate ICAM-1 relase in ovarian carcinoma, we have employed the nude mouse model system. Three different human ovarian carcinoma (HOC) cell lines were grown as ascitic tumours in the peritoneal cavity of nude mice. HOC xenografts harvested from nude mice expressed comparable levels of ICAM-1 on their cell surface. Human ICAM-1 was detected, with a species-specific ELISA, in serum and ascitic fluid of tumour-bearing mice, confirming that the tumours were the source of the ICAM-1. The three HOC xenografts showed different levels of ICAM-1 release, but within each xenograft model the level of ICAM-1 in serum and ascitic fluid correlated with the tumour burden. The level of ICAM-1 released by the HOC xenografts could be increased by *in vivo* treatment with interferon gamma (IFNγ). Interleukin 1 (IL-1), tumour necrosis factor (TNF) and IFNγ increased the cell surface expression of ICAM-1 and caused the release of soluble ICAM-1 from HOC cells established *in vitro*. The nude mouse provides a useful system in which to study the effects of modulating ICAM-1 release on the progression of ovarian carcinoma and suggests that measuring ICAM-1 levels in the blood or ascites of patients may provide an indication of tumour burden.

Key words: ovarian carcinoma, intercellular adhesion molecule-1, interferon, nude mouse
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INTRODUCTION

CELLULAR ADHESION molecules have been implicated in homotypic and heterotypic cell interactions. Intercellular adhesion molecule-1 (ICAM-1) (CD-54), an adhesion ligand for β2 integrins LFA-1(CD11a/CD18) and MAC-1(CD11b/CD18), has been shown on various cell types, including vascular endothelial cells and leucocytes, and its expression can be modulated by cytokines [1]. ICAM-1 plays a role in inflammatory and immune responses [2, 3], and it has been implicated in the migration of tumour and normal cells. While ICAM-1 has been extensively studied on melanomas, where its expression has been associated with tumour progression and metastasis [4, 5], restricted examples of other tumour types expressing ICAM-1 have been reported [6].

Recently soluble forms of circulating sICAM-1 have been described in serum of normal donors, with elevated levels in serum of patients with various diseases [7–10]. Circulating sICAM-1 has been shown in blood of cancer patients, with higher levels being associated with malignant disease [11–13]. We have shown that human melanoma cells release sICAM-1, and high levels of sICAM-1 were found in the serum of nude mice bearing human melanoma [14].

In advanced human ovarian carcinoma, tumour cells spread throughout the peritoneal cavity [15]. We have previously