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Original Paper

Interferon Gamma Induces Cell Cycle Arrest and Apoptosis in a Model of Ovarian Cancer: Enhancement of Effect by Batimastat

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Locoregional human IFN-γ may have activity against refractory ovarian cancer. We investigated this further in an ovarian cancer xenograft model. Administered at clinically relevant doses, intraperitoneal IFN-γ prolonged the survival of mice bearing multiple established peritoneal tumours, with optimal treatment giving a 3–6-fold increase in median survival time. Daily dosing, which was superior to intermittent treatment, decreased DNA synthesis and induced apoptosis in tumour cells with maximal effects after 7–21 days treatment. This was preceded by an increase in p53 protein at 48 h. The effect of IFN-γ was not enhanced by sequential treatment with carboplatin. However, the matrix metalloprotease inhibitor, batimastat, further incrased mouse survival when given after IFN-γ. Thus IFN-γ is cytotoxic to ovarian epithelial cells *in vivo* and intensive locoregional dosing over short periods is effective. Sequential administration of novel agents that perturb the host/tumour relationship may be of benefit. © **1997 Elsevier Science Ltd.**

Key words: IFN-γ, ovarian cancer, animal model, batimastat, apoptosis

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INTRODUCTION

INTERFERON GAMMA (IFN-γ) has antiproliferative and antiviral activities, as well as a role in immune and inflammatory responses [1]. This cytokine inhibits the growth of a range of tumour cell lines including those derived from ovarian cancer [2–5]. Clinical trials of IFN-γ in advanced cancer have generally been disappointing [6–8], although it is possible that optimal routes and schedules of administration have not yet been determined. There is some evidence for activity of intraperitoneal (i.p.) IFN-γ in human epithelial ovarian cancer [9]. Of 98 patients with residual disease at second look laparotomy, 23% achieved a complete response and 9% a partial response, with patients under 60 years of age, with tumours less than 2 cm, most likely to respond. However, the i.p. route of administration was associated with a certain number of adverse effects, including peritonitis, which were often related to the length of treatment (E. Pujade-Lauraine, Haoitai Hotel-Dieu, France).

We previously reported that human IFN-γ also had antitumour activity in ascitic and solid intraperitoneal human ovarian cancer xenografts, significantly increasing survival of nude mice [10]. The ovarian cancer xenograft models were considered a suitable model for further investigation of the action of IFN-γ since the cytokine pharmacokinetics were comparable to those achieved in clinical trials in ovarian cancer [9], and the mice bore established multiple intraperitoneal tumours with histological resemblance to the human disease [10]. Moreover, the use of a species-specific IFN in a xenograft model allowed us to study direct effects of this cytokine on the tumour cells and to dissociate these effects from any action on host cells.

The aims of the present study were to determine optimal treatment schedules and to determine whether higher doses and/or shorter treatment schedules were as effective as those used previously. Mechanisms of direct action of IFN-γ were further investigated and the ability of conventional and novel therapies to increase this action was assessed. The efficacy of some tumour therapies may be associated with

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the intrinsic ability of an individual tumour cell to respond by apoptosis [11, 12]. In cells damaged by radiation of other trauma, such as survival factor depletion, apoptosis may be preceded by molecular events such as the induction of p53 and the cdk inhibitor $p21^{cip1waf1}$, and hypophosphorylation of pRb. We therefore studied tumour cell cycle, apoptosis, and some of the molecular events preceding this, during IFN-γ therapy of the experimental ovarian tumours.

Finally, we investigated the ability of other therapies, both conventional and novel, to enhance the activity of IFN-γ. If IFN-γ was capable of directly damaging the tumour cells, they may subsequently be more responsive to other therapies. We therefore treated mice with carboplatin or batimastat after IFN-γ therapy. We chose carboplatin because the efficacy of IFN-γ in combination with either this agent or cisplatin had been investigated previously in both animal models and cell lines with beneficial results [13–15]. Batimastat, a matrix metalloprotease inhibitor (MMPI), was chosen because it too had previously been shown to have antitumour activity against the ascitic form of the ovarian cancer xenograft, but alone had no activity on solid tumours ([16], and unpublished data). Batimastat has also been used successfully in other animal models [17, 18].

Our results suggest that clinically achievable doses of IFN-γ, given over a short period of time, reduce tumour cell proliferation, induce apoptosis and upregulate p53 protein expression. The action of IFN-γ is not improved by sequential chemotherapy, but can be enhanced by sequential therapy with the MMPI, batimastat.

MATERIALS AND METHODS

Mice and xenografts

Six to 12-week old specific pathogen free female nu/nu (nude) mice of mixed genetic background were maintained as described previously [19]. The ovarian cancer xenograft HU was established from a 23-year-old woman with a moderately differentiated serous cystadenocarcinoma [20]. At the start of each experiment, mice were injected i.p. with tumour cells, and after 7 days mice were treated i.p. with 2 μg recombinant human tumour necrosis factor (rhTNF-α). This induced intraperitoneal solid tumour formation and eradicated most of the ascites [21]. At the end of this time the mice bore multiple solid tumours with well-developed stroma and good histological resemblance to the human disease [21]. After a day's rest, IFN-γ treatment was commenced (day 16). Eight mice per group were used for survival experiments and three mice per group for other experiments. Two mice were subjected to post mortem investigation prior to IFN-γ treatment to confirm that i.p. tumours were established. The tumour weight was approximately 5 g when experimental therapy started. All drugs in this study were injected i.p.

Immunoblotting

Sample preparation of total protein lysates for polyacrylamide gel electrophoresis. Tumour material was lysed by homgenisation in 2 × Laemmli buffer with apoprotein (1 μg/ml) and phenylmethylsufonyl fluoride (PMSF) (100 μg/ml) as described previously [22]. Protein estimations were carried out on each sample and 100 μg of total protein loaded on to a PAGE gel. Samples were boiled for 2 min and stored on ice prior to loading. Electrophoresis was performed using standard conditions. A 12.5% denaturing gel was made up for detection of $p21^{cip1}$, and a 10% gel for p53 detection. The protein lysates were run at 120 V for 1.5 h on a AE-6400 Dual Mini slab kit (Atta, Genetic Research, Essex, U.K.). Rainbow markers (Amersham, U.K.) were used to estimated product size. Protein was transferred to nitrocellulose according to the method of Towbin and associates [23] using a Trans-Blot electrophoretic transfer cell (Biorad) for 2–3 h at 300 mA. Filters were stained with 0.1% w/v Ponceau S Solution (Sigma, U.K.) prior to blocking to confirm equal transfer. Membranes were incubated in blocking reagent (10% non-fat milk powder) overnight. Proteins were detected using the Amersham ECL™ protocol.

Antibodies used for protein detection. All antibodies were used at a concentration of 1 μg/ml. Antibody to p21 was obtained from Oncogene Science (Cambridge, Massachusetts, U.S.A.); p53 (which recognises wild type and mutant) was from Transduction laboratories (Lexington, Kentucky, U.S.A.).

Measurement of BrdU in situ. To assess cell proliferation *in vivo*, mice were injected with the thymidine analogue bromodeoxyuridine (BrdU). Mice bearing established tumours were treated on a daily basis with either IFN-γ or with control diluent. At appropriate timepoints (24 h, 4 d, 7 d and 14 d), BrdU, diluted in saline immediately before use, was injected into the mice for 45–60 min at a concentration of 50 mg/kg in 0.1 ml. The tumours were excised, placed in formal saline, embedded in paraffin wax and immunohistochemistry was subsequently performed on tissue sections using standard procedures.

Specific labelling of DNA fragmentation. In situ DNA fragmentation was measured by end labelling as described previously [24], to detect evidence of apoptosis. Mice bearing solid tumours were treated on a daily basis with either IFN-γ or with control diluent. At appropriate timepoints (24 h, 4 d, 7 d and 14 d), tumours were excised, placed in formal saline and embedded in paraffin wax. Briefly, the sections were protease treated to permeate the tissue and immersed in an appropriate buffer (39 mM trizma base, pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride, all provided in kit form by Boehringer Mannheim, Germany). Terminal deoxytransferase and biotinylated deoxyuridine were added to the sections and incubated for 60 min. The reaction was terminated by immersing slides in TB buffer (300 mM sodium chloride, 300 mM sodium citrate) for 15 min. After rinsing in distilled water, 2% BSA sections were incubated in bovine serum albumin for 10 min at room temperature, followed by conventional immunohistochemical procedures.

Electron microscopy. Excised tumour samples were immediately fixed in 2.5% glutaraldehyde in Sorensen's phosphate buffer and processed for electron microscopy using standard procedures. One micrometre toluidine blue sections were used to select representative areas for ultrastructural investigation.

Cytokines and other treatments

Tumour necrosis factor. rhTNF (recombinant human tumour necrosis factor) was provided by BASF/Knoll (Maidenhead, Berkshire, U.K.) and was more than 99% pure. Endotoxin levels were less than 0.036 ng/mg and the specific activity was 6.6×10^6 units/mg. rhTNF was diluted in calcium and magnesium free phosphate buffered saline

plus 3 mg/ml BSA and stored in single dose aliquots at −70°C until required.

Interferon. Two equally active but different preparations of human IFN-γ were used in this study. Recombinant human interferon gamma, IFN-γ (RU 42369), was provided by Roussel UCLAF (Romainville, France) as a lyophilised preparation (20×10^6 U/vial) more than 95% pure. The endotoxin levels were less than 0.24 U/vial and the specific activity was 2×10^7 units/mg protein. IFN was diluted in sterile distilled water and stored in aliquots at −70°C. Alternatively, recombinant human IFN-γ was provided by Boehringer Ingelheim, Research and Development, Vienna, Austria. It was more than 99% pure, had a specific activity of 3×10^7 U/mg and was diluted in 3 mg/ml BSA/PBSA prior to use. The IFN-γ was diluted to give a concentration of 1.5×10^6 U/ml. Mice were injected with 1.5×10^5 as 0.1 ml of the 1.5×10^6 U/ml solution. The lower dose of IFN-γ given daily to the mice (1.5×10^5 U) achieved similar i.p. levels to those found in ovarian cancer patients receiving i.p. IFN-γ [10]. This dose of IFN-γ was comparable to the i.p. dose of 20×10^6 U that was given to patients when adjusted for differences in surface area : volume ratios between mouse and man [25]. Earlier studies have shown that to achieve the same i.p. exposure in mice as that obtained in clinical trials with twice weekly dosing in patients, daily exposure is required in mice. Peak i.p. levels of IFN-γ, as detected by radioimmunoassay, were similar to those achieved in patients, but the clearance was much slower in humans [10].

Batimastat was provided by British Biotech Ltd (Oxford, U.K.). It was used at a dose of 40 mg/kg/day. It was supplied in a 25 ml volume (500 mg) in 2.5% ethanol, 2.5% PEG400 and 1% methyl cellulose. It was diluted 1 in 8 with dextrose for injection. Placebo injections contained the batimastat diluent. Carboplatin was purchased from Bristol-Myers Pharmaceuticals (Middlesex, U.K.), diluted in saline and stored at −20°C prior to use.

Statistical analysis. The log rank test was used to assess survival and Student's *t*-test was used for analysis of the BrdU and end-labelling experiments.

RESULTS

Schedule of IFN-γ therapy in the HU xenograft growing as a solid tumour

In previous experiments, we treated established i.p. xenografts daily for 56 days. In this study, we investigated the effects of shorter treatment periods and higher doses of IFN-γ on mouse survival (Figure 1), in particular whether higher doses given over fewer days with rest periods would be effective.

Two protocols were used: a 5 day treatment followed by a 7 day rest cycle (Figure 1a) and a 3 day treatment followed by a 7 day rest cycle (Figure 1b). Both these protocols were used at a low (1.5×10^5 U) and high (7.5×10^5 U) dose. The above regimes were compared to the previously used schedule of 1.5×10^5 U daily for 56 days. Survival times in all treatment groups were significantly better than controls ($P = 0.0009$–0.009). However, daily therapy with the lowest dose was equivalent to, or better than, other treatment schedules. For instance, treatment with 7.5×10^5 U IFN-γ for 5 days followed by a rest period of 7 days (for four cycles) was non-significantly different from daily therapy of 1.5×10^5 U for 56 days ($P = 0.66$), although the mice in the latter group received less IFN-γ (8.4×10^6 U compared to 15×10^6 U) (Figure 1a). Survival was significantly better ($P = 0.007$) in the daily dose group compared with groups receiving 3 or 7.5×10^5 U/day for 3 days of each treatment cycle (Figure 1b). Daily therapy with 1.5×10^5 U was also better than a dose of 3×10^5 U for 5 days (7 rest days/four cycles) ($P < 0.01$) or 5 daily doses of 7.5×10^5 ($P < 0.003$) (data not shown).

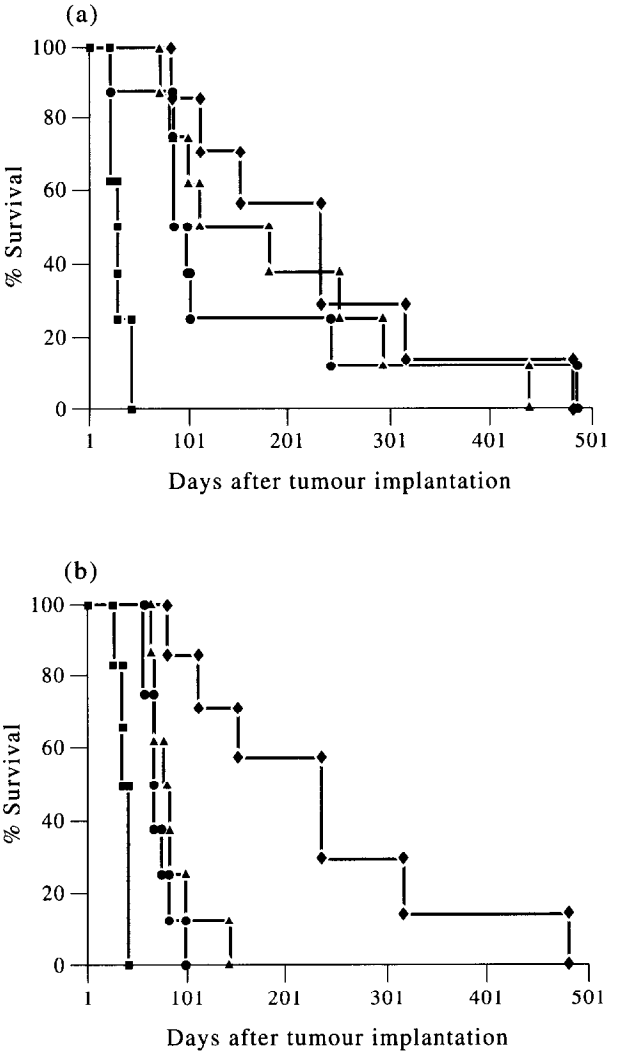

Figure 1. The antitumour effect of IFN-γ against human ovarian cancer xenografts. (a) five day cycles versus daily therapy: ■, control diluent 5 days, rest 7 days, four cycles (MST = 29 days); ●, HU IFN-γ 1.5×10^5 U, 5 days, rest 7 days, four cycles (MST = 85 days); ▲, HU IFN-γ 7.5×10^5 U, 5 days, rest 7 days, four cycles (MST = 112 days); ◆, HU IFN-γ 1.5×10^5 U, daily, 56 days (MST = 234 days). (b) Three day cycles versus daily therapy: ■, control diluent, 3 days, rest 7 days, four cycles (MST = 40 days); ●, HU IFN-γ 1.5×10^5 U, 3 days, rest 7 days, four cycles (MST = 70 days); ▲, HU IFN-γ 7.5×10^5 U, 3 days, rest 7 days, four cycles (MST = 78 days); ◆, HU IFN-γ 1.5×10^5 U, daily, 56 days (MST = 234 days). Therapy started 16 days after tumour implantation.

Proliferation status of the cells following IFN-γ therapy

To assess the rate of proliferation of the tumour cell during IFN-γ therapy, the mice were injected with BrdU and immunohistochemistry carried out on the excised tumours. Figure 2a shows the percentage of epithelial cells

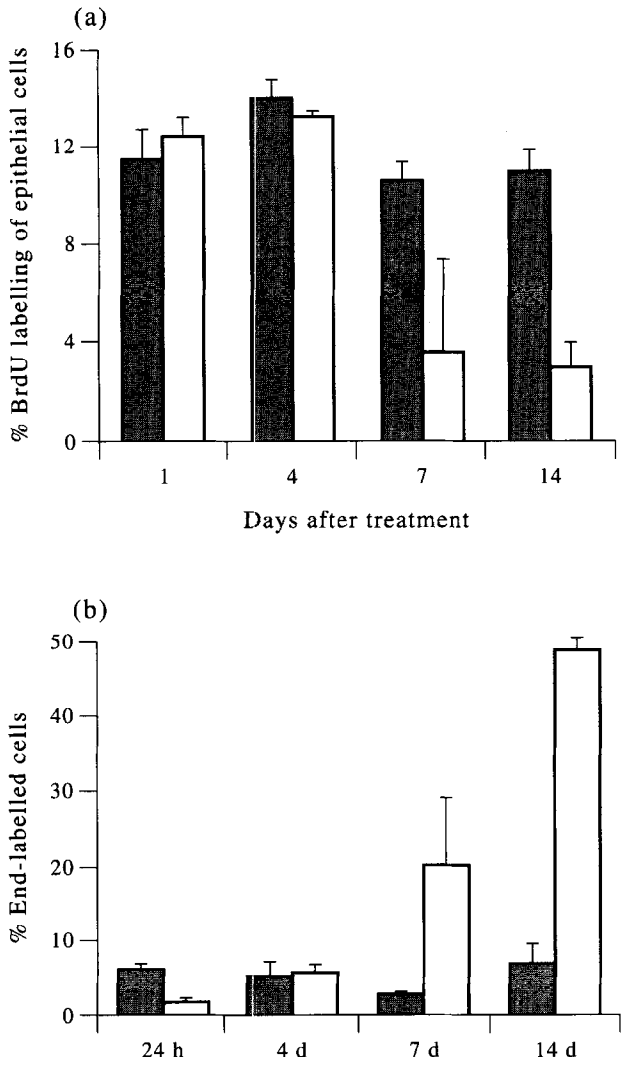

Figure 2. Measurement of cell cycle and cell death in treated tumours. (a) BrdU analysis of tumours: □, control diluent, HU IFN-γ 1.5×10^5 U i.p. daily. (b) End-labelling studies of examining apoptosis during IFN-γ therapy: ■, control diluent, HU IFN-γ 1.5×10^5 U i.p. daily. Therapy started 16 days after tumour implantation.

labelled as daily treatment progressed. At the start of treatment, 10–13% of cells in control or treated tumours were labelled. This percentage did not change noticeably in controls, but a marked decline in labelling was noted in IFN-γ treated tumours by 7 days (control versus IFN-γ, $P = 0.02$) which decreased further by 14 days (control versus IFN-γ, $P = 0.001$).

In situ *DNA fragmentation assay*

An *in vivo* end-labelling technique was used to detect cells undergoing apoptosis. Figure 2b shows the percentage of epithelial cells with positive staining. As treatment progressed, there was an increase in the number of cells with fragmented DNA, which is indicative of apoptosis. This reached a maximum after 14 days therapy where nearly 50% of the epithelial cells were positive in the IFN-γ treated group compared with a mean of 7% in the control group ($P = 0.0001$). Also, at the later timepoints (21, 28 and 35 days), the number of cells present in IFN-γ treated tumours was greatly reduced in some areas (data not shown).

Electron microscopy of treated tumours

Electron microscopic analysis confirmed light microscope evidence of acellularity in the treated tumours. Throughout the treatment period, the control xenografts consisted of dense clumps of tumour cells showing evidence of organisation with junctions and microvilli (Figure 3a). These cells were surrounded by a dense capsule of host collagen containing fat, blood vessels and a variety of infiltrating cells. The treated xenografts presented a very different picture. After 4 days of IFN-γ treatment, the clumps of tumour cells had become smaller, and in some areas of the tumour there were fewer cells. This change was associated with a number of apoptotic cells characterised by densely marginated chromatin and apoptotic bodies (Figure 3b). With increasing IFN-γ treatment areas of tumour cells were reduced and the number of apoptotic cells increased. Isolated tumour cells were surrounded by apoptotic cells underoing secondary necrosis (Figure 3c). Eventually, in many places, tumour cells were replaced by sparsely scattered host lymphocytes and granulocytes presumably migrated from the capsule, which showed signs of collagen degradation (Figure 3d).

Transplantability of treated tumours

The transplantability of the control and treated tumours was also investigated. Solid tumours from control (22 day established tumours) or IFN-γ treated (35 day established tumours) were transplanted into mice without tumours. Fewer mice developed tumours when transplanted from IFN-γ treated material compared to control (3/8 mice versus 7/8 mice developed tumours). Moreover, the survival of mice was significantly longer if their xenografts had been isolated from mice who had previously received IFN-γ (median survival time, MST, of mice given IFN-γ—pretreated tumours = 347 days, MST of mice given control tumours = 32 days).

7–21 *days versus* 56 *day treatment protocol*

Both the proliferation index and the end-labelling assay suggested that the effects of IFN-γ on cell survival were maximal at 14 days. Therefore, shorter time schedules (7, 14 and 21 days) were compared with 56 days daily therapy. Figure 4 shows that survival of mice given a shorter treatment period of the same dose for 21 days (1.5×10^5 U) was not significantly different from survival of mice given 56 daily doses ($P = 0.2$). Both significantly increased mouse survival ($P = 0.02$) compared with controls.

Post mortem analyses of mice

The majority of mice (>96%) died of multiple solid tumours within the peritoneal cavity. Often a watery peritoneal fluid was also present. The other 4% of mice died from subcutaneous needle track tumours of disorders not related to their malignancies. The post mortem appearance of the peritoneum was similar in IFN-γ related and control mice. Thus, IFN-γ significantly enhanced the survival of mice bearing solid tumour xenografts, but did not usually cure them of their disease.

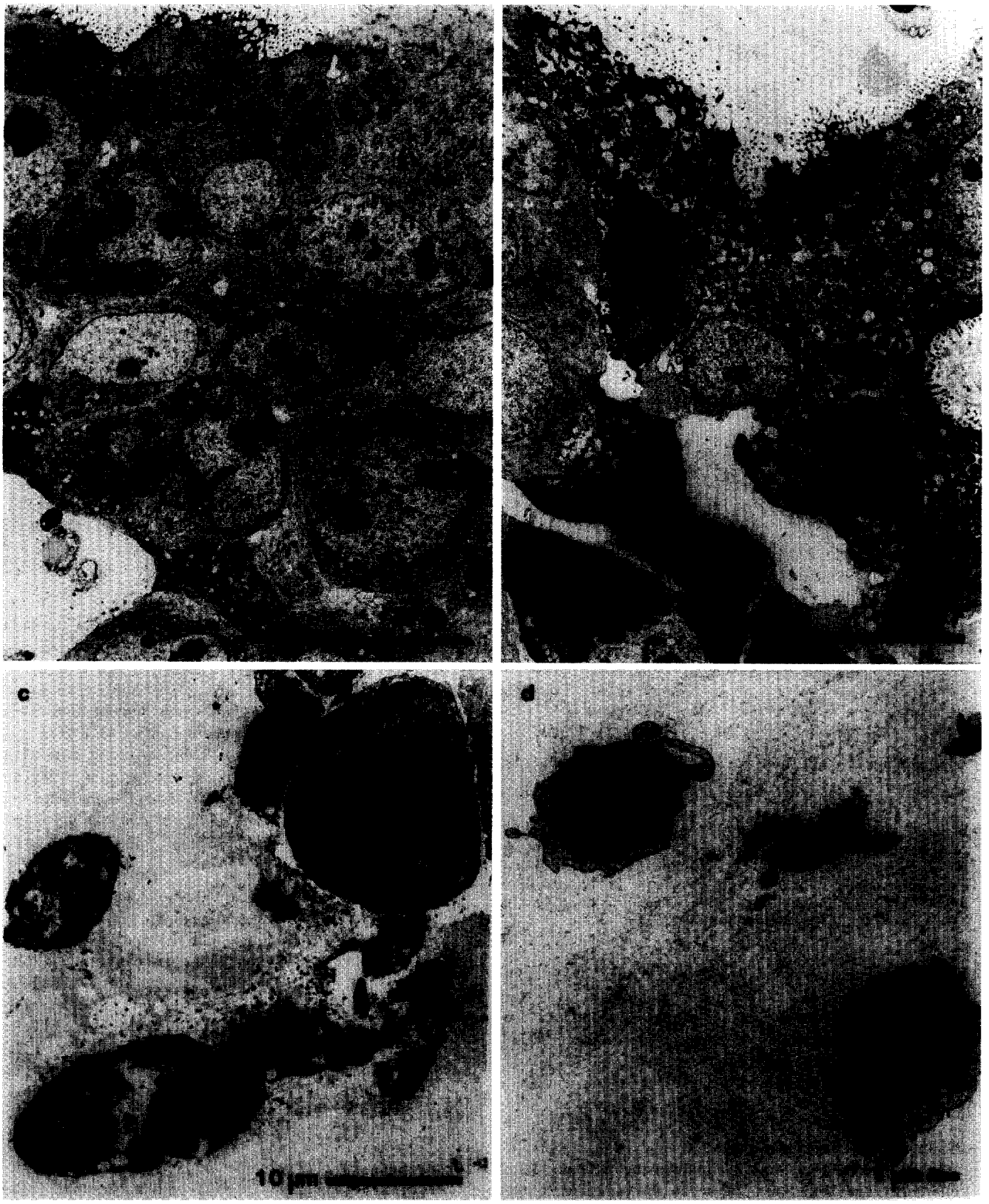

Figure 3. Electron microscopy of treated tumours. (a) Thirty day established tumour treated with control diluent for 14 days. (b) Twenty day established tumour treated with HU IFN-γ for 4 days showing tumour mass and apoptotic cells. (c) Twenty day established tumour treated with HU IFN-γ for 4 days showing isolated tumour cells and apoptotic cells undergoing secondary necrosis. (d) Twenty-three day established tumour treated with HU IFN-γ for 7 days showing apoptotic cells with a host lymphocyte.

Sequential treatment with other therapies

Following 14 days therapy with IFN-γ, after solid tumour implantation, carboplatin was administered once a week for four weeks (i.e. at days 35, 42, 49 and 56 after initial tumour injection). This regime increased survival when given to mice with ascitic tumours (data not shown).

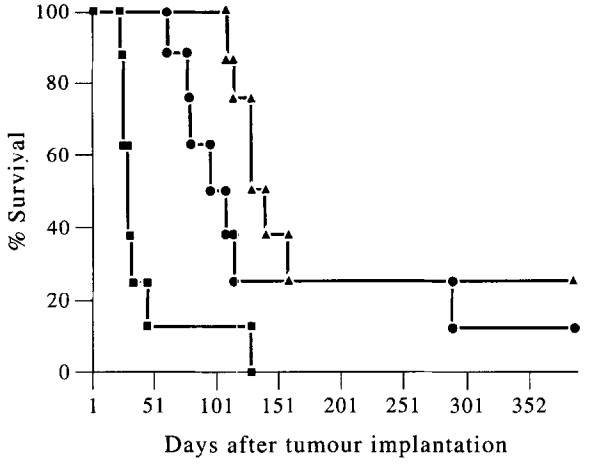

Figure 4. Comparison of effect of 21 and 56 days treatment with IFN-γ on survival. ■, control diluent, 56 days (MST, 30 days); ●, HU IFN-γ 1.5×10^5 U daily for 21 days (MST, 109 days); ▲, HU IFN-γ 1.5×10^5 U daily for 56 days (MST, 140 days). Therapy started 16 days after tumour implantation.

However, this treatment did not enhance the survival of mice when compared with 14 day IFN-γ therapy alone (Figure 5a). Batimastat given i.p. daily for 35–60 days after IFN-γ treatment, significantly improved survival compared with 14 days IFN-γ therapy alone (Figure 5b); ($P = 0.03$, results of three experiments combined).

There was a discrepancy between median survival times in the controls and daily treated groups in the above experiments, with the mice described in Figure 1a,b surviving much longer than those described in Figures 4 and 5. This was probably due to a variation in the number of tumour cells originally injected in the different experiments. It was not possible to count the tumour cells prior to injection into the nude mice because the tumour is comprised of large cell clumps.

Molecular changes associated with cell cycle arrest and death in treated tumours

As shown in the Western blot in Figure 6, the HU xenograft expressed low levels of p53. During IFN-γ therapy, p53 was upregulated. This was first noted 48 h after the start of therapy and remained at this level throughout the 7 day period studied. p21 was also present in the HU xenograft but we could detect no consistent change in levels during therapy. In some experiments, there was evidence of p21 mRNA induction, but this was not consistently related to a change in p21 protein levels, which were detectable in all samples at all time points (data not shown).

DISCUSSION

Human IFN-γ, given at doses that are achievable in patients, directly inhibited the growth of xenografted solid tumours of human ovarian cancer. This growth inhibition developed gradually over a period of 14 days and led to apoptosis and secondary necrosis of the tumour cells. Daily exposure was necessary for optimal antitumour activity.

p53 was detected in control and treated tumours, but was upregulated 48 h after the start of IFN-γ therapy and remained at these levels throughout the 7 day treatment period. There have been reports that the growth suppression caused by the inhibitory cytokine, transforming growth factor-β (TGF-β) involves the cdk inhibitor p21 [26, 27]. In this study, p21 protein was present in the tumour cells irrespective of levels of DNA synthesis and IFN-γ treatment, and no consistent relationship with growth inhibition could be determined.

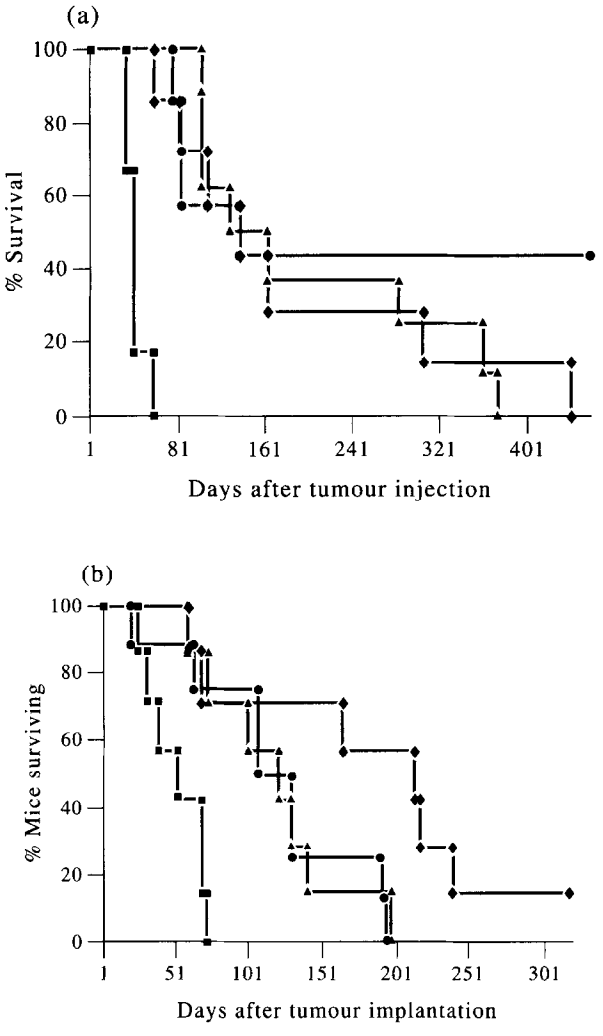

Figure 5. Treatments sequential to IFN-γ. (a) IFN-γ in combination with carboplatin. ■, control diluent, 56 days (MST, 38 days); ●, HU IFN-γ 1.5×10^5 U daily for 14 days (MST, 136 days); ▲, HU IFN-γ 1.5×10^5 U daily for 14 days, carboplatin (10 mg/kg), weekly for 4 weeks (MST, 136 days); ◆, HU IFN-γ 1.5×10^5 U daily for 14 days, carboplatin (20 mg/kg), weekly for 4 weeks (MST, 145 days). (b) IFN-γ in combination with Batimastat. ■, control diluent, 56 days (MST, 52 days); ●, HU IFN-γ 1.5×10^5 U daily for 14 days (MST, 118 days); ▲, HU IFN-γ 1.5×10^5 daily for 14 days, placebo from 30–95 days (MST, 121 days); ◆, HU IFN-γ 1.5×10^5 U daily for 14 days, batimastat for 30–95 days (MST, 212 days). Therapy started 16 days after tumour implantation.

A critical time period between 7 and 21 days, which was sufficient for IFN-γ to achieve maximal effect was identified. Treatment of the xenografts for a shorter period was comparable to the 56 days schedule used in previous experiments [10]. Consistent with these findings are observations from a clinical trial of ovarian cancer patients with IFN-γ, where a number of patients had their treatment terminated early due to adverse effects [9]. In this trial, there was no correlation between length of treatment and response to

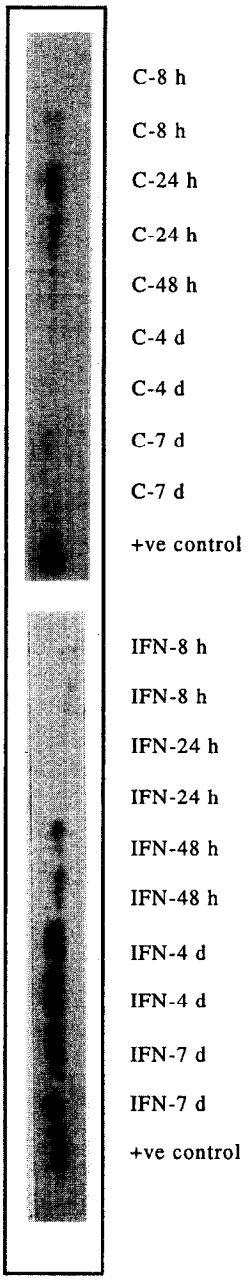

Figure 6. p53 changes in IFN-γ treated tumours. Samples were run in duplicate following 8 h, 24 h, 48 h, 4 days, 7 days of IFN or control treatment. OVCAR-433 cell line, positive control.

therapy (Pujade-Lauraine, Haoitai Hotel-Dieu, France). As adverse effects of i.p. IFN-γ were often related to problems with i.p. administration, a shorter period would be a distinct advantage.

It is not certain whether the effects described here are direct or act via other IFN-γ induced intermediates. We have recently defined, by RT-PCR, a partial cytokine network in ovarian cancer, many components of which are still present in the HU xenograft [28]. Within this network, there is potential for several autocrine loops that may regulate the growth and survival of ovarian epithelial cells. IFN-γ could modulate this network by affecting the production of, or response to, growth and survival cytokines, as has been suggested for IFN-α action in hairy cell leukaemia [29]. Removal of survival cytokines can precipitate cell death. Thus, modulation of growth and/or survival cytokine loops may be a first step in the growth inhibitory/cytotoxic action of IFN-γ. The HU xenograft cells are passaged from mouse to mouse and do not survive well *ex vivo*. However, we have recently found that 3/4 ovarian cancer cell lines grown in our laboratory are sensitive to the cytostatic/cytotoxic action of IFN-γ. These lines are now being used to identify the early events that culminate in the death of ovarian cancer cell after IFN-γ treatment.

Another explanation for the cell cycle arrest and extensive apoptosis seen in IFN-γ treated tumours is that the therapy was destructive to tumour vasculature. While the human IFN-γ should not directly affect the murine vasculature of these tumours, it may inhibit the production of angiogenic factors in the tumours or stimulate the production of angiostatic/toxic factors by the tumour cells.

The effects of IFN-γ used sequentially with two other therapeutic agents were also examined. If IFN-γ is able to inhibit growth and/or induce apoptosis, then treating tumours with an 'apoptotic' dose, followed by other agents, may be of benefit. It is perhaps not surprising that carboplatin failed to enhance the effect of IFN-γ when given after the 14 day treatment. If apoptosis and necrosis were already well advanced in the tumour, it is unlikely that another DNA damaging agent would give additional benefit. Batimastat, a MMPI, that has already been used clinically in ovarian cancer [30] and has activity in the ascitic form of the xenograft model [16], as well as other animal models [17, 18], significantly extended mouse survival when given after IFN-γ. However, it has been previously shown to have no effect on established solid tumours [16]. The mechanisms of antitumour action of this agent are not yet known. Inhibition of MMPs, allowing development of a host capsule around the tumour, is one possibility [16]. In this respect, it is of interest that the collagen capsule surrounding the IFN-γ treated tumours showed signs of degradation. Earlier studies in other models have also shown that IFN-γ inhibits collagen synthesis [31, 32]. This may have been suppressed by MMPI therapy, essentially encapsulating the dead and dying tumour cells. Others have implicated effects of MMPIs on tumour neovasculature [33]. Inhibition of the release of autocrine or paracrine survival/growth factors such as TNF-α [34] may also play a role. Preliminary histological examination of tumours treated with sequential IFN-γ and batimastat have shown an increase in the areas of necrosis compared with the other groups (data not shown). Whatever the mechanism, the important point is that this treatment is targeted to stromal–tumour interactions rather than the tumour cells themselves. Our result suggest that MMPIs such as batimastat (or indeed anti-angiogenic factors) may be active against solid tumours if the tumours are first compromised by cytotoxic therapies, whether cytokine or chemotherapy based. It is also possible that therapies which disrupt host tumour interactions may act when given with cytotoxic therapies, although disruption of the tumour blood supply early in the treatment may prevent optimal access of the cytotoxic agent.

The xenograft model system described here allows the direct effects of IFN-γ on the ovarian cancer cells to be determined, and as we have already shown that this human cytokine has no measurable activity on the nude mouse host [35] (although it is possible that IFN-γ treatment induces

production of other members of the cytokine network that can act across species barriers). In an autologous system, this direct cell damage may be accompanied by, or followed with, non-specific host mediated events such as macrophage and natural killer cell activation and effects on tumour vasculature, ultimately resulting in a specific host immune response to the tumour.

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