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Interferon-α and interferon-γ combined with chemotherapy: *in vitro* sensitivity studies in non-small cell lung cancer cell lines

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Non-small cell lung cancers (NSCLC) are often resistant to chemotherapy. Cisplatin has shown the most activity against all the histological subtypes and is now used in most combined treatment programmes. Interferon (IFN)-α has been shown to potentiate cisplatin and other drugs experimentally and in clinical trials involving NSCLC. We are looking at the responses of different NSCLC cell lines to cisplatin (P), etoposide (VP-16) and IFN [recombinant human IFN-α2c (IFN-α) and IFN-γ 1b (IFN-γ)], individually and in combination. We then compare the results with those from a clinical trial of etoposide and cisplatin with interferon in advanced NSCLC. We report here the results from the first of our cell lines, established from a large cell anaplastic carcinoma. We have confirmed earlier findings that NSCLC cell lines are not sensitive to either IFN-α or IFN-γ alone. However a combination of IFN-α and IFN-γ does reduce cell proliferation in our cell lines. This IFN combination potentiates the response of the cells to etoposide far more than to cisplatin. There is a trend towards greater activity when a combination of cisplatin and etoposide is used, compared with the activity of either drug alone. This effect is further increased by the interferon combination.

Key words: Chemotherapy, interferon, non-small cell lung cancer.

Introduction

The prognosis for non-small cell lung cancer (NSCLC) is poor: 90% of patients will die within one year of diagnosis. NSCLC is relatively resistant to chemotherapy in previously untreated patients and, since most patients already have metastatic disease at the time of diagnosis, the basis of any successful treatment program must be systemic.[1] Programs of treatment need to be developed in which the effectiveness of the systemic chemotherapy element is maximized.

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Platinum-based chemotherapy produces the best response rates, 30–60% when two or three drug combinations are used;[2,3] but only minor improvements in survival have been documented.

Biological response modifiers, especially interferons (IFNs), have been shown to augment responses to chemotherapeutic agents in various cancers, in clinical and experimental studies.[4] Neither NSCLC[5,6] nor human squamous cell or adenocarcinoma xenografts[7] respond to IFN in single agent therapy. However, doses of recombinant IFN-α2b, which had no effect alone, have been shown to potentiate cisplatin in these xenografts. In patients with advanced NSCLC, recombinant IFN-α2b also potentiated cisplatin.[9] In that study the response rate for squamous cell carcinoma was significantly higher (46%) than for adenocarcinoma (25%) or large cell anaplastic carcinoma (17%).

We are studying the effects of a combination of recombinant human interferon-α2c (IFN-α) and recombinant human IFN-γ1b (IFN-γ) on the responses of a large cell anaplastic carcinoma cell line and an adenocarcinoma cell line to cisplatin, etoposide and cisplatin plus etoposide. The results are then compared with the effect of similar therapy on patients with advanced NSCLC.[8] We present here the preliminary findings.

Materials and methods

Two human NSCLC lines were used in these experiments, both of which had been established in our laboratory from fresh tumor tissue samples taken at thoracotomy. Neither patient had received any treatment before surgery. The results reported here are from cell line LCA 123, which was

established from a large cell anaplastic carcinoma of the lung.

Cells (200 000) were plated into 6-well plates (10 cm^2) in 3 ml of RPMI 1640 medium, supplemented with 3% fetal calf serum, 0.03% L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 ng/ml epidermal growth factor, 0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml selenite (Sigma, St Louis, MO, USA). The cells were incubated at 37°C for 48 h to establish exponentially-growing cultures. The medium was then replaced by fresh medium supplemented with IFN and/or drugs, and incubated for a further 72 h. The cells were detatched with 0.05% trypsin–EDTA, centrifuged and stained with Trypan Blue. The numbers of viable cells were counted.

The responses of the cells to the following agents and combinations were tested: IFN-α (Berofer®, Boehringer Ingelheim), IFN-γ (Imukin®, Boehringer Ingelheim, Germany) and IFN-α plus IFN-γ; cisplatin (Platinol, Lääkefarmos, Finland), etoposide (Vepesid, Bristol Myers, UK) and cisplatin plus etoposide; the same drugs and a drug combination with 0.05 μg/ml of both IFN-α and IFN-γ. The range of concentrations tested for IFN was 0.0001–0.5 μg/ml and for the drugs was 0.01–100 μg/ml. The inter-experimental variation was less than 10%. The results are presented as the mean percentage cell survival from two or more independent experiments of duplicate cultures.

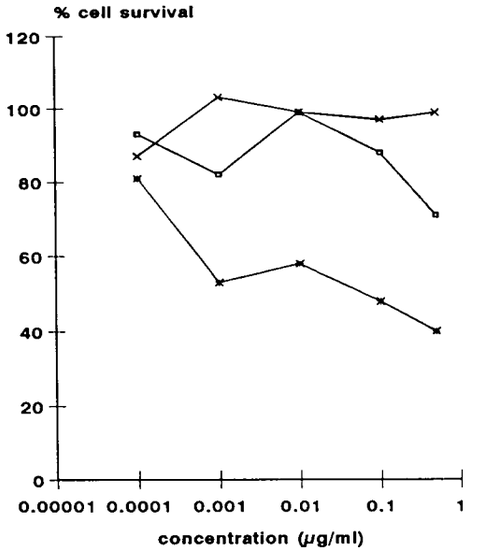

Figure 1. The effects of recombinant human IFN-α (×), IFN-γ1b (□) and IFN-α2c plus IFN-γ (*) on cell line LCA 123.

Results

The cells did not respond to either of the recombinant IFNs alone, but showed a 50% reduction in survival using IFN-α and IFN-γ together, in concentrations greater than 0.001 μg/ml (Figure 1).

Using a concentration of 0.05 μg/ml of the IFN combination we were able to show a trend towards potentiation of cisplatin (Figure 2) and etoposide (Figure 3). The effect was more pronounced in the case of etoposide. Use of the IFN combination with the cisplatin/etoposide combination produced similar results (Figure 4). The quantity of etoposide needed to produce a 50% reduction in survival [50% toxic concentration (TC_{50})] was 48 μg/ml on its own, 0.075 μg/ml in combination with 50 μg/ml of both IFN-α and IFN-γ, and 0.1 μg/ml with cisplatin and the IFN combination. The corresponding figures for cisplatin were 5, 2 and 0.1 μg/ml. Cisplatin was therefore the more effective drug on its own, but etoposide was potentiated by the IFN combination, and the most effective combination was that of etoposide and both IFNs together.

In the clinical trial, the details of which are reported elsewhere,[8] the patients who responded to chemotherapy with IFN-γ achieved longer periods of remission than patients in the other arms of the study (Table 1). However, there was no improve-

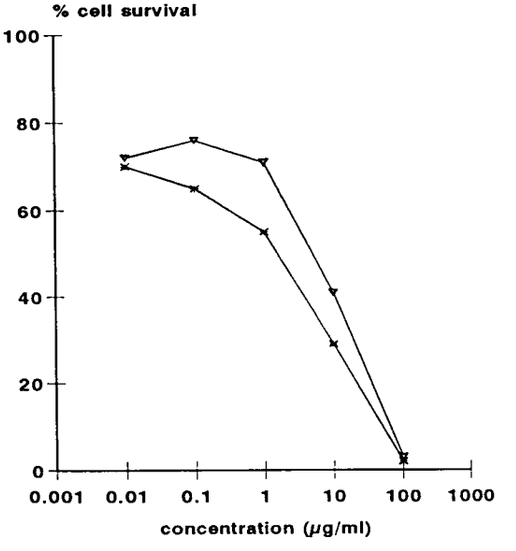

Figure 2. The effects of cisplatin alone (▽) and in combination with 0.05 μg/ml of both recombinant human IFN-α2c and IFN-γ1b (*) on cell line LCA 123.

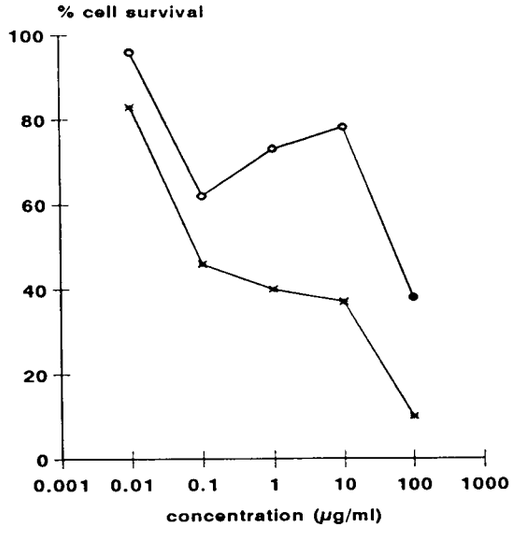

Figure 3. The effects of etoposide alone (○) and in combination with 0.05 μg/ml of both recombinant human IFN-α2c and IFN-γ1b (*) on cell line LCA 123.

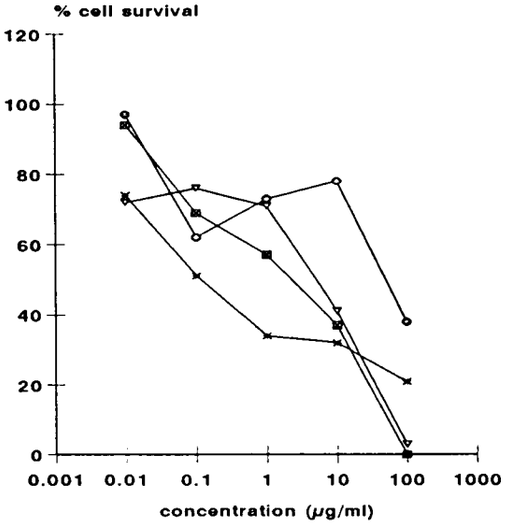

Figure 4. The effects of etoposide (○) and cisplatin (▽) as individual agents, in combination (□) and the combination with 0.05 μg/ml of both recombinant human IFN-α2c and IFN-γ1b (*) on cell line LCA 123.

ment in median survival time for the patients undergoing treatment by chemotherapy plus IFN-γ.

Discussion

Despite the low response rates achieved with the chemotherapy available for the treatment of NSCLC, it is usually administered.[10] A large number of experimental studies have shown that IFN potentiates many chemotherapeutic drugs, in a variety of tumor models.[4] However, few of these positive results have translated into successful clinical trials, probably because the interactions of IFN and drugs are complex, and very little is known as yet about the biochemistry involved. On the other hand, clinical studies using etoposide against NSCLC have revealed the importance of schedule and route in the administration of this drug,[11] and IFN-α has been shown to potentiate cisplatin-based chemotherapy in advanced NSCLC.[9] Tsai *et al.*[12] have demonstrated that drug sensitivity testing in cell lines can be relevant to the clinical situation, by predicting response rates to chemotherapy and the eventual survival of lung cancer patients.

In the Bowman *et al.* study,[9] a mean response rate of 30% was achieved in a randomized trial of cisplatin plus recombinant IFN-α2b in patients with various forms of advanced NSCLC, as compared with 12–19% for cisplatin as a single agent in historical controls. We were able to demonstrate a small increase in cisplatin activity by the simultaneous use of recombinant IFN-α2c and IFN-γ1b.

Table 1. Response rates and survival of patients with advanced NSCLC treated with etoposide and cisplatin alone (I), or combined with either recombinant human IFN-γ (II) or recombinant human IFN-γ and IFN-α (III)

	Total	I	II	III
No. patients	61	22	21	18
Partial response	17 (28%)	6 (27%)	5 (24%)	6 (33%)
Stable disease	25 (41%)	10 (46%)	6 (29%)	9 (50%)
Progressive disease	19 (31%)	6 (27%)	10 (47%)	3 (17%)
Median duration of response (weeks)	28	27	36	28
Median time to progression (weeks)	16	17	12	17
Median survival (months)	6.5	7	6	7

In our system the IFN combination potentiated etoposide to a greater extent than it did cisplatin.

We have not observed any significant improvement in the response rate or survival of patients with advanced NSCLC, treated by a similar regime.[8] However, of the patients who responded to chemotherapy, those who had received IFN-γ in addition to chemotherapy enjoyed a longer period of remission. This observation is supported by a report of high-dose recombinant IFN-γ activity in single agent induction therapy for NSCLC.[13] The activity of the IFN combination in our study does not contradict these observations. The activity of IFN-γ against NSCLC should be confirmed, both experimentally and in the clinical setting.

The preliminary results which we present here, and which appear to be confirmed in an adenocarcinoma cell line (unpublished data), support the available clinical observations. Our study also suggests a role for cell line systems in the development of chemotherapy for NSCLC.

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