Short Communication

Growth inhibitory effects of interferon on blast cells from patients with acute myelogenous leukaemia

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The growth inhibitory effect of Interferon was shown to be dose dependent by Gresser, studying mouse Interferon and L1210 leukaemia (Gresser et al., 1970). Similar findings have subsequently been reported for the antiproliferative activity of human lymphoblastoid, leukocyte and fibroblast Interferons on human leukaemic myeloblasts in short-term liquid culture (Balkwill & Oliver, 1977; Lundgren et al., 1980).

On the basis of these findings, clinical evaluation of human lymphoblastoid Interferon (IFN-α) was undertaken at St. Bartholomew's Hospital in patients with haematological malignancies. A Phase I study was performed to determine whether doses high enough to achieve serum levels known to be growth inhibitory to myeloblasts in vitro (Balkwill & Oliver, 1977) could be administered in vivo (Rohatiner et al., 1982). This having been demonstrated, a Phase II study of high dose IFN-α, administered by continuous intravenous infusion was commenced in patients with acute myelogenous leukaemia (AML) (Rohatiner et al., 1983). Myeloblasts from patients with AML who received IFN in these studies were cultured with different concentrations of IFN-α to determine whether any correlation existed between in vitro growth inhibition and clinical response. The in vitro results and clinical findings form the basis of this report.

Myeloblasts were obtained from the bone marrow (BM) of 23 patients with AML. Nineteen patients were in relapse and 4 had failed to enter remission with conventional combination chemotherapy. The degree of BM infiltration was >50% in all patients studied.

IFN-α (Wellcome Research Laboratories) derived from the Namalwa lymphoblastoid cell line had a specific activity of 2.13 x 10⁸ units mg⁻¹ protein.

Samples of bone marrow were collected into RPMI 1640 and Heparin (preservative-free, 500 units ml⁻¹). After washing in culture medium (RPMI 1640 supplemented by 10% foetal calf serum) the Buffy coat was removed and the cells resuspended at 1 x 10⁶ ml⁻¹. Aliquots (0.2 ml) were then incubated in microculture wells at 37°C, in 5% CO₂, 85% humidity, for 3 days. Cells were cultured alone or with IFN-α at concentrations of 10, 10², 10³ and 10⁴ units ml⁻¹.

Cell growth was assessed by two methods: viable cells were enumerated using phase contrast microscopy, 200 cells from each microculture well being counted after 72 h; 0.1 μCi of tritiated thymidine [³H]dT specific activity 5 Ci mmol⁻¹ (The Radiochemical Centre, Amersham) was added to each well after 72 h. Labelled cells were harvested and counted after 18 h.

Growth inhibition was expressed as follows:

% reduction in cell no. =

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\frac{\text{No. cells in control medium} \times 100}{\text{No. cells in IFN containing medium}}
\]

% reduction in [³H]dT incorporation =

\[
\frac{\text{[³H]dT count from cells in control medium} \times 100}{\text{[³H]dT count from cells in IFN containing medium}}
\]

All assays were performed in triplicate and the mean of three samples taken for each parameter.

IFN-α decreased the number of viable cells and inhibited the uptake of tritiated thymidine in all samples (Table 1). Inhibition was always dose dependent, 50% inhibition being observed at IFN concentrations of 10³ units ml⁻¹ in most samples, although there was considerable between-patient variability in the degree of growth inhibition observed at different IFN concentrations.

The mean peak levels of IFN-α achieved in the peripheral blood of individual patients in the clinical studies and the dose received are shown in

Received 7 February 1984; accepted 5 March 1984.
Table I. The effect of IFN-α on cell growth and thymidine incorporation in cultures of bone marrow myeloblasts. Pharmacokinetic and clinical data are included.

| Patient | % Reduction in cell number | % Reduction in [3H]dT incorporation | Daily IFN dose (× 10^-6 units m^-2) | Mean peak serum IFN level (units ml^-1) | Clinical response |
|---------|-----------------------------|-------------------------------------|-------------------------------------|----------------------------------------|------------------|
| (1)     | 2 18 42 69                 | 9 15 58 63                          | 5a                                  | 10^3                                   | NE               |
| (2)     | 0 10 35 53                 | 0 12 42 50                          | 5a                                  | 8.5 × 10^2                             | NE               |
| (3)     | 3 32 58 84                 | 0 29 54 85                          | 5                                   | 2.0 × 10^2                             | NR               |
| (4)     | 0 21 51 65                 | 4 27 46 61                          | 5                                   | 2.3 × 10^2                             | NR               |
| (5)     | 7 27 60 85                 | 5 31 65 79                          | 5                                   | 3.3 × 10^2                             | NE               |
| (6)     | 0 13 22 45                 | 0 11 33 60                          | 7.5                                 | 1.1 × 10^3                             | NR               |
| (7)     | 12 32 54 68                | 0 27 65 80                          | 10                                  | 1.8 × 10^3                             | NR               |
| (8)     | 3 24 48 59                 | 0 19 49 62                          | 10                                  | 2.7 × 10^3                             | NR               |
| (9)     | 10 28 59 74                | 5 26 65 80                          | 15                                  | 1.1 × 10^3                             | NR               |
| (10)    | 0 11 40 62                 | 3 18 54 69                          | 20                                  | 4.3 × 10^3                             | decrease in circulating blasts |
| (11)    | 2 11 26 39                 | 7 19 36 60                          | 20                                  | 5.5 × 10^2                             | NR               |
| (12)    | 3 19 51 62                 | 11 17 56 70                         | 25                                  | 1.3 × 10^3                             | decrease in circulating blasts |
| (13)    | 12 29 69 85                | 11 34 89 30                         | 30                                  | 3.9 × 10^3                             | NR               |
| (14)    | 4 30 51 66                 | 3 21 56 69                          | 50                                  | 3.6 × 10^3                             | BM 99% to <5% blasts |
| (15)    | 0 18 38 49                 | 2 21 48 62                          | 50                                  | ND                                     | NR               |
| (16)    | 2 10 30 60                 | 0 19 54 78                          | 100                                 | 1.3 × 10^3                             | NE               |
| (17)    | 0 17 47 61                 | 8 17 47 81                          | 100                                 | 1.4 × 10^3                             | decrease in circulating blasts |
| (18)    | 0 28 70 84                 | 6 10 49 59                          | 100                                 | 2.7 × 10^3                             | NE               |
| (19)    | 3 19 52 78                 | 0 13 32 56                          | 200                                 | 6.4 × 10^3                             | NR               |
| (20)    | 4 29 34 41                 | 14 33 48 66                         | 100                                 | ND                                     | NR               |
| (21)    | 0 1 54 62                  | 13 34 61 78                         | 100                                 | 8.5 × 10^3                             | NR               |
| (22)    | 3 35 42 58                 | 12 22 48 55                         | 100                                 | 7.6 × 10^3                             | NR               |
| (23)    | 0 2 28 58                  | 7 28 54 69                          | 100                                 | 1.07 × 10^4                            | NR               |

*First 2 patients in the Phase I study received 5 × 10^6 m^-2 by i.v. push on 1 and 2 days respectively.
NR = No response.
NE = Not evaluable for response due to early death.
ND = Not done.

Table I. These studies have been reported in full elsewhere (Rohatiner et al., 1982; Rohatiner et al., 1983). It can be seen that there was no correlation between the serum levels achieved and response or between the degree of growth inhibition in vitro and response. However, only patients in whom peak serum levels > 10^3 units ml^-1 were achieved showed any indication of response, which suggests that minimum serum levels of 10^3 units ml^-1 are required for any antiproliferative activity in vivo.

These results confirm that IFN has a dose dependent growth inhibitory effect on human leukaemic myeloblasts in short term liquid culture, 50% inhibition occurring at an IFN concentration of 10^3 units ml^-1. This appears to be reversible, inactivation of the IFN by incubation with trypsin using the same liquid culture system having been shown to virtually abrogate the growth inhibitory effect (Balkwill & Oliver, 1977).

In contrast to the findings with myeloblasts reported above, when normal bone marrow was cultured with IFN-α (Balkwill & Oliver, 1977), thymidine incorporation was suppressed but the effect on cell survival was less marked.

There was no significant response to therapy in patients in whom serum levels < 10^2 units ml^-1 were
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achieved during IFN therapy and even above this level the results were very disappointing.

It is clearly possible that higher concentrations of IFN might have greater antiproliferative activity both in vitro and in vivo. However, the achievement of higher serum levels for prolonged periods is associated with intolerable toxicity. It must therefore be concluded from both the in vitro and in vivo data that high dose infusion IFN therapy will not play a major part in the primary therapy of acute myelogenous leukaemia. Whether an alternative role will be found remains to be seen.

I am indebted to Dr F. Balkwill for her advice and experience regarding in vitro culture of myeloblasts, and to Dr T.A. Lister for helpful advice and criticism. I am grateful to Mr R. Sewell for technical assistance and to Mrs J. Ashby for preparing the manuscript.

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