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 2.13×10^8 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 $1 \times 10^6 \text{ ml}^{-1}$. Aliquots (0.2 ml) were then incubated in microculture wells at 37°C, in 5% CO_2 , 85% humidity, for 3 days. Cells were cultured alone or with IFN- α at concentrations of 10, 10^2 , 10^3 and 10^4 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. =

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

% reduction in [^3H]dT incorporation =

$$\frac{[\text{H}]d\text{T count from cells in IFN containing medium}}{[\text{H}]d\text{T count from cells in control medium}} \times 100$$

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 I). Inhibition was always dose dependent, 50% inhibition being observed at IFN concentrations of 10^3 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

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.

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

^aFirst 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 $< 10^3$ units ml⁻¹ were

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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