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RECOMBINANT LEUKOCYTE A INTERFERON AS SINGLE AGENT THERAPY OR IN COMBINATION WITH CIMETIDINE IN PATIENTS WITH ADVANCED COLO-RECTAL CARCINOMA

A phase II investigation

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Abstract

Recombinant leukocyte A interferon used as single agent therapy (7.5×10^6 units/m^2 three times weekly intramuscularly) showed very limited clinical efficacy in patients with advanced colo-rectal carcinoma. No objective tumour regressions were documented in 15 evaluable patients, although three patients demonstrated a stable disease status after 12 weeks of treatment. Neither were any objective tumour regressions registered in 13 patients during subsequent combined therapy with Recombinant Leukocyte A Interferon and cimetidine (1 000 mg/day orally). Two of the 3 patients maintained their stable disease status. No change in natural killer (NK) cell activity or in antibody dependent cellular cytotoxicity (ADCC) of peripheral blood mononuclear cells was seen during the two treatment periods.

In Sweden, like in most Western industrialized countries, the incidence of colo-rectal carcinoma is high and ranks second only to carcinoma of the prostate (males) and breast (females) in terms of annual new cases (6). Since more than half of the patients have advanced disease at the time of diagnosis (42), colo-rectal carcinoma is also one of the most common causes of death in malignant disease. The primary treatment is surgery. There is no established case for adjuvant treatment of colo-rectal carcinoma, although chemotherapy and/or radiation therapy are being evaluated in clinical trials (22, 26, 29, 34). Systemic treatment of advanced and metastatic disease includes chemotherapy with 5-FU as a single agent or as part of multiple agent regimens, but reported objective response rates are usually less than 20 per cent (25, 41). Hence there is no effective standard therapy of metastatic or irresectable local disease and thus it is justified to evaluate novel treatment modalities. Interferon was originally described as an anti-viral agent (23), but further investigations showed that it consisted of a group of structurally related proteins, possessing anti-proliferative and immunomodulating properties (1, 19, 43), which pointed to a possible usefulness in cancer therapy. Early studies, most often using partially purified human leukocyte interferon (7), indicated therapeutic efficacy in some malignant diseases such as multiple myeloma, breast carcinoma and lymphomas (3, 20, 28, 43) but not in others, i.e malignant melanoma (24). However, progress in the understanding of anti-tumour effector mechanisms and in defining the role of interferons in cancer treatment has so far been restricted by the relative impurity, low specific activity, the high cost and the scarcity of available preparations.

Recent development in recombinant DNA technology (16, 31) has now made it possible to produce

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virtually pure leukocyte interferon in such large quantities that some of these restrictions have been overcome. Phase I investigations have been carried out to determine maximal tolerable dose, safety and biologic properties of recombinant interferon in patients with various malignancies (21,39). Although these studies were not designed to evaluate treatment efficacy, some tumour regressions were noticed. On the basis of these results a number of larger clinical trials were initiated evaluating the anti-neoplastic activity of recombinant interferons in different malignant diseases.

The first aim of this investigation was to study the possible therapeutic effects of a highly purified recombinant leukocyte interferon in locally advanced or metastatic colo-rectal carcinoma. A previous study in patients with metastatic malignant melanoma had indicated that combined therapy with partially purified leukocyte interferon and cimetidine, a histamine-2 receptor antagonist and thus a suppressor T cell inhibitor, could lead to complete tumour regressions (15). Therefore, a second aim of the present study was to investigate whether patients with colo-rectal carcinoma, non-responsive to treatment with IFL-rA, would benefit from combined therapy with IFL-rA and cimetidine. A third aim was to evaluate sequentially possible changes in natural killer cell cytotoxicity (NK cell activity) and antibody dependent cellular cytotoxicity (K cell activity) during interferon and combined interferon-cimetidine therapy.

Material and Methods

Between June 1982 and September 1983, 16 patients entered the study. Patient selection criteria included histologically confirmed locally non-resectable or metastatic colo-rectal carcinoma, measurable marker lesion(s) histologically or cytologically confirmed as colo-rectal carcinoma, no concurrent anti-neoplastic therapy, Karnofsky index >70, expected survival more than 3 months without treatment and informed consent. Patients with prior chemotherapy had to show progression of the disease before entry. Baseline evaluation consisted of a complete history and physical examination, ECG, complete blood count, blood chemistries, and serum CEA. Metastatic survey included chest radiography, abdominal CT scan, ultrasound of the liver and liver/spleen and skeletal isotope investigations. Patients with symptomatic heart disease, impaired renal function (serum creatinine >200 μmol/l), impaired hematologic function (WBC $<3\times10^9$/l, granulocytes $<1\times10^9$/l, platelets $<100\times10^9$/l) and impaired hepatic function (total serum bilirubin >30 μmol/l) were excluded from the study, as were patients with equivocal marker lesion(s).

Patient characteristics, including site(s) and size(s) of marker lesion(s), are given in Table 1. One patient (No. 5) entered the study at the time when his non-resectable rectal carcinoma was first diagnosed, while the other 15 were included after a diagnosis of recurrent disease. As expected, liver and lungs were organs most frequently involved in the metastatic process. Five patients with primary rectal carcinoma also had local pelvic recurrent disease at the start of the treatment.

Treatment. Recombinant leukocyte A interferon (IFL-rA), an alpha-2 interferon with a specific activity of $2\text{-}4\times10^8$ units/mg protein, was supplied by Hoffmann-La Roche, Nutley, N. J., USA. It was administered intramuscularly 3 times weekly at a dose of 7.5×10^6 units/m^2 per injection. In the initial protocol the scheduled dose of IFL-rA was set at 20×10^6 units/m^2 to be given 3 times weekly. Due to the registered fatigue and marked loss of appetite in the first patient of the series, the rest of the patients received the lesser dose. During the first week of treatment the patients were hospitalized, but from then on the intramuscular injections were given on an out-patient basis at the Department of Oncology.

Response to treatment was evaluated by appropriate tumour measurements after a period of 6 weeks, or earlier when clinically indicated. Patients with stable disease or tumour regression received continued IFL-rA therapy for a second period of 6 weeks. Patients with tumour progression, or patients not attaining at least a partial remission, after 12 weeks of interferon therapy and who were fit for further treatment, were continued on the same IFL-rA dose schedule supplemented with 1 000 mg cimetidine (Tagamet; Smith, Kline & French, Philadelphia, Pa., USA) per day orally in 4 divided doses.

For safety reasons, reiterated physical and neurologic examinations, ECG, routine blood counts and hepatic and renal function tests were performed throughout the treatment period.

Evaluation of response. The following response categories were used: complete remission was defined as disappearance of all evidence of tumour; partial remission was defined as >50 per cent reduc-

Table 1

Patient characteristics, sequence and result of therapy in 16 patients with advanced colo-rectal carcinoma

Case No.	Age/ sex	Site of primary tumour	Prior relapse therapy	Site and size of tumour extension on entry*	Sequence of treatment			
					IFL-rA		IFL-rA+cimetidine	
					Duration (weeks)	Response**	Duration (weeks)	Response**
1	67/F	Colon	Surgery	Local recurrence (5×6)	12	SD	6	SD
2	73/F	Colon	Surgery	Lung (2×2), liver (3×3, m), abd. wall (2×2)	12	PD	6	PD
3	62/M	Rectum	Surgery	Lungs (5×5, m), liver (2×2, m)	8	PD	4	PD
4	66/F	Rectum	Surgery, radiation therapy	Lungs (1×2, m)	12	PD	12	PD
5	50/M	Rectum	–	Rectum, non-resect. (14×15)	12	SD	10	SD
6	56/M	Rectum	–	Lungs (5×4, m), liver (1×1, m), local recurrence (3×3)	6	PD	12	PD
7	45/M	Colon	Surgery	Lungs (1×1, m)	17	SD	8	PD
8	53/F	Colon	Surgery	Liver (7×8)	6	PD	2	PD
9	49/F	Colon	–	Liver (5×5, m)	6	PD	3	PD
10	59/M	Colon	–	Abd. wall (4×3)	2	PD	–	–
11	64/M	Rectum	–	Liver (5×5, m)	6	PD	7	PD
12	62/M	Rectum	Oncovin CCNU 5-FU	Small pelvis (7×6), liver (2×3), supraclav. (2×3)	1	PD	–	–
13	66/F	Colon	5-FU	Lungs (2×2, m)	6	PD	6	PD
14	57/F	Colon	5-FU	Liver (2×2, m)	6	PD	6	PD
15	55/M	Rectum	–	Local recurrence (4×6), perineum (1×1, m), abd. wall (4×6)	3	SD vertigo	–	–
16	71/F	Colon	Surgery	Liver (10×10)	6	PD	6	PD

* Size of lesion, or size of largest lesion when multiple (m), in cm.
** SD = stable disease. PD = progressive disease.

tion in the product of longest perpendicular diameters of indicator lesions or a >30 per cent reduction in liver measurements with no progression in any lesion or appearance of any new lesions; stable disease was defined as failure to qualify for complete remission, partial remission or progressive disease. Progression was defined as the appearence of new lesions or >25 per cent increase in the product of longest perpendicular diameters of indicator lesions or in liver measurements.

Assays of natural killer cell cytotoxicity and antibody dependent cellular cytotoxicity. Tests for NK cell and K cell activity were performed before start of therapy (baseline) and were repeated at intervals of 4 to 6 weeks during interferon and combined interferon-cimetidine therapy. During treatment, blood samples for the tests were drawn 48 to 72 hours after the preceding IFL-rA injection. Two sets of effector cells were used in the NK and ADCC tests. One of them (designated L 0) consisted of peripheral blood mononuclear cells, isolated by density gradient centrifugation (5) and containing less than 2 per cent granulocytes. The second set of effector cells (L 1) was prepared from the L 0 fraction by passage through a gelatin bead column (40). This procedure effectively removes adherent cells leaving less than 0.5 per cent monocytes in the eluate. The in vitro influence of cimetidine, IFL-rA and IFL-rA in combination with cimetidine was studied on NK and K cell activity. Before being

Table 2

Example of a test performed to evaluate natural killer cell activity and antibody dependent cellular cytotoxicity in a patient before start of therapy. Influence of preincubation of effector cells with cimetidine, IFL-rA and IFL-rA + cimetidine

Effector cell fraction*	Preincubation**	Natural killer cell activity				Antibody dependent cellular cytotoxicity			
		Per cent specific release***			'Weighed' specific release 16:1	Per cent specific release***			'Weighed' specific release 4:1
		32:1	16:1	8:1		8:1	4:1	2:1	
L 0	Medium	44.1±2.3	27.1±2.1	15.8±1.9	29.0	49.2±2.2	46.5±3.1	44.7±3.2	46.8
	Cimetidine	35.9±2.7	22.9±2.3	14.8±1.3	24.5	59.1±2.3	44.8±3.0	41.9±2.2	48.6
	IFL-rA	63.2±3.3	43.9±3.1	28.4±2.7	45.2	57.9±2.9	51.7±3.0	41.8±2.1	50.5
	IFL-rA + cimetidine	54.9±3.0	40.7±2.7	27.6±2.3	41.1	57.7±2.4	52.3±2.5	45.1±2.2	51.7
L 1	Medium	13.4±1.9	10.4±1.4	6.3±1.1	10.0	67.7±4.3	55.3±2.9	40.9±2.7	54.6
	Cimetidine	20.5±1.9	13.2±1.3	4.1±1.0	12.6	84.4±3.3	66.7±2.3	45.7±2.7	65.6
	IFL-rA	46.5±2.1	38.7±1.9	18.5±1.5	34.6	72.3±3.1	64.5±2.1	41.3±2.2	59.4
	IFL-rA + cimetidine	50.1±2.3	37.4±2.0	21.5±1.4	36.4	85.0±3.2	59.7±2.0	35.9±2.1	60.2

* L 0 = peripheral blood mononuclear cells. L 1 = monocyte-depleted peripheral blood mononuclear cells.
** Cimetidine was used at 10^{-5} mol/l concentration, IFL-rA at 10^3 units/ml.
*** Per cent specific ^{51}Cr release ± SEM at indicated effector to target cell ratios.

used in the cytolytic assays effector cells were incubated for 2 hours in either medium (control), cimetidine (10^{-5} mol/l), IFL-rA (10^3 units/ml) or a combination of the same concentrations of IFL-rA and cimetidine. During the NK and ADCC assays IFL-rA was omitted but the cimetidine concentration was maintained.

The NK tests were performed as ^{51}Cr release assays using K 562 cells as labelled target cells. The details of the test procedure have been reported previously (27). In short, 5×10^3 labelled cells were pipetted in 0.1 ml aliquots into V-shaped 96-well, microtiter plates (Flow Laboratories, Solna, Sweden) to which varying numbers of effector cells (L 0 or L 1) had been added giving final effector to target cell (E/T) ratios of 32:1, 16:1 and 8:1. Each E/T ratio was run in triplicates. After incubation for 4 hours the plates were centrifuged (150×g/5 min) and the supernatants were harvested with a Titertek Supernatant Harvester (Flow Laboratories, Solna Sweden). Radioactivity of the samples was determined in a Beckman 310 Gamma Counter (LKB Beckman, Bromma, Sweden). Spontaneous release was estimated by addition of medium to labelled target cells and maximal release was obtained by addition of a solution of distilled water and zapoglobin (Coulter Electronics Ltd, Harpenden England). Percentage specific release was calculated as follows: (cpm release with lymphocytes—cpm spontaneous release)/cpm maximum release—spontaneous release) ×100.

The assay for ADCC has been described previously (27). Thymus cells from 4-week-old Wistar-Furth rats, labelled with ^{51}Cr and exposed to rabbit antiserum to rat brain, were used as target cells. The release assays were performed in U-shaped 96-well, microtiter plates to which L 0 or L 1 effector cells and target cells (1×10^4) were added in 0.1 ml aliquots per cavity giving final E/T ratios of 8:1, 4:1 and 2:1. Each ratio was run in triplicates. The plates were centrifuged (40×g/5 min) after 0.5 hour and again after a total incubation time of 4 hours (150×g/5 min). Supernatants were then harvested as in the NK tests and the percentage specific release was calculated according to the same formula. Table 2 gives an example of a test performed to evaluate the levels of NK cell and K cell activities in a patient before start of therapy. The influence on cytotoxicity by preincubation of effector cells with cimetidine, IFL-rA and IFL-rA plus cimetidine is also presented in the table.

The ^{51}Cr release increases linearly with the E/T ratio in the range tested but the slope of the increase varies from patient to patient and is dependent on

Table 3

Efficacy of therapy with IFL-rA as a single agent or in combination with cimetidine

Therapy	No. of evaluable patients	No. of patients with progressive disease relative to duration of therapy		No. of patients with stable disease after 12 weeks
		0–6 weeks	7–12 weeks	
IFL-rA	15	9	3	3
IFL-rA + cimetidine	13	7	4	2

prior effector cell separation and exposure to interferon and cimetidine. The results are therefore not presented as lytic units but rather as a 'weighed' per cent release value of the middle ratio (16 : 1 for NK and 4 : 1 for ADCC assays) being equal to the mean release of ratios 32 : 1, 16 : 1 and 8 : 1 for NK and of ratios 8 : 1, 4 : 1 and 2 : 1 for ADCC.

Statistical analysis. Statistical significance of data obtained in the cytotoxicity tests was determined by using the Mann-Whitney U and the Wilcoxon matched-pairs signed-rank test.

Results

Clinical efficacy of IFL-rA therapy. Sixteen patients were included in the IFL-rA study (Table 1). In patient No. 15 treatment was discontinued in stable disease three weeks after entry due to occurrence of severe vertigo, possibly related to the interferon medication. Therefore, 15 patients are evaluable for response. Due to obvious disease progression, therapy was discontinued in two patients (Nos 12 and 10) after one and 2 weeks, respectively. Thirteen patients received IFL-rA injections for a minimum of 6 weeks, at which time progressive disease was demonstrated in 7. Thus, interferon therapy failed in 9 patients within 6 weeks (Table 3). The disease was classified as stable in the remaining 6 patients. This group of patients continued IFL-rA therapy for another period of 6 weeks. After a total treatment duration of 12 weeks disease progression was demonstrated in 3 of these 6 patients, while the disease was stable in the remaining 3.

Clinical efficacy of combined IFL-rA-cimetidine treatment. All of the 13 patients who received at least six weeks of interferon therapy were given a trial period of combined IFL-rA-cimetidine treatment ranging from 2 to 12 weeks (Table 3). Eleven of the patients demonstrated tumour progression during the combined therapy, while the treatment was stopped in stable disease due to extreme fatigue in one case (No. 1) and due to another treatment option in one further case (No. 5). Both of these 2 patients, who thus maintained a stable disease status during IFL-rA therapy as a single agent and in combination with cimetidine, had pelvic tumours only but no distant metastases. Off all treatment, patient No. 1 developed hepatic and pulmonary secondaries after a period of 3 months, while external radiation therapy induced a partial tumour regression in patient No. 5.

Side effects. All patients experienced episodes of fever and chills with a duration of 4 to 8 hours in connection with the initial 2 or 3 interferon injections. After 6 weeks of treatment most of the patients reported increasing fatigue necessitating a bed rest in the afternoons. Half of the patients also reported loss of appetite. Although these side effects continued throughout the remaining treatment period, and during the following period of combined interferon-cimetidine therapy, the adverse reactions were of minor importance and did not require any change in planned therapy.

Interferon medication was discontinued in patient No. 15 due to the development of severe vertigo, the cause of which could not be established despite extensive investigations and which gradually completely reversed on cessation of therapy. During prolonged interferon and combined interferon-cimetidine treatment a fall in hemoglobin levels (maximally 15%) was registered in most patients. A slight decrease was also observed in WBC and platelet counts. There were no laboratory signs of hepatic or renal toxicity on either treatment schedule, nor were any signs of cardiac toxicity encountered.

Influence of treatment on NK cell and K cell activity. Table 4 gives the results of the sequential tests performed to evaluate the effect of therapy on

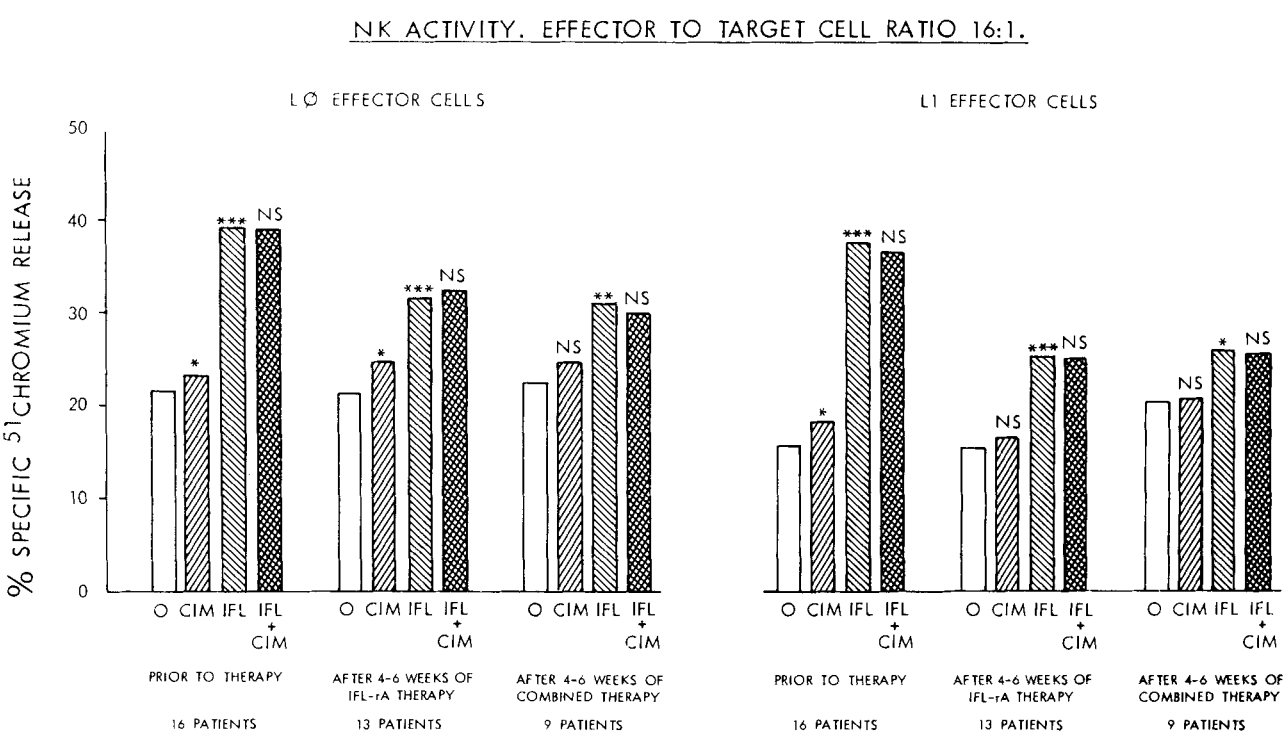

Fig. 1. Natural killer (NK) cell activity of peripheral blood mononuclear (L 0) and monocyte-depleted blood mononuclear (L 1) effector cells before start of therapy and during treatment with IFL-rA as a single agent or in combination with cimetidine. Before being used in the NK assays, effector cells were exposed to control medium □, cimetidine (10^{-5} mol/l) ▨, Recombinant Leukocyte A Interferon (10^3 units/ml) ▩ or Recombinant Leukocyte A Interferon and cimetidine ▩ for two hours. The influence of preincubation of effector cells with cimetidine and IFL-rA is compared with NK cell activity registered after preincubation of effector cells with medium, while the effect of combined preincubation with IFL-rA and cimetidine is compared with results obtained by exposure to IFL-rA alone. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, NS = not significant (Wilcoxon matched-pairs signed-rank test, two-tailed).

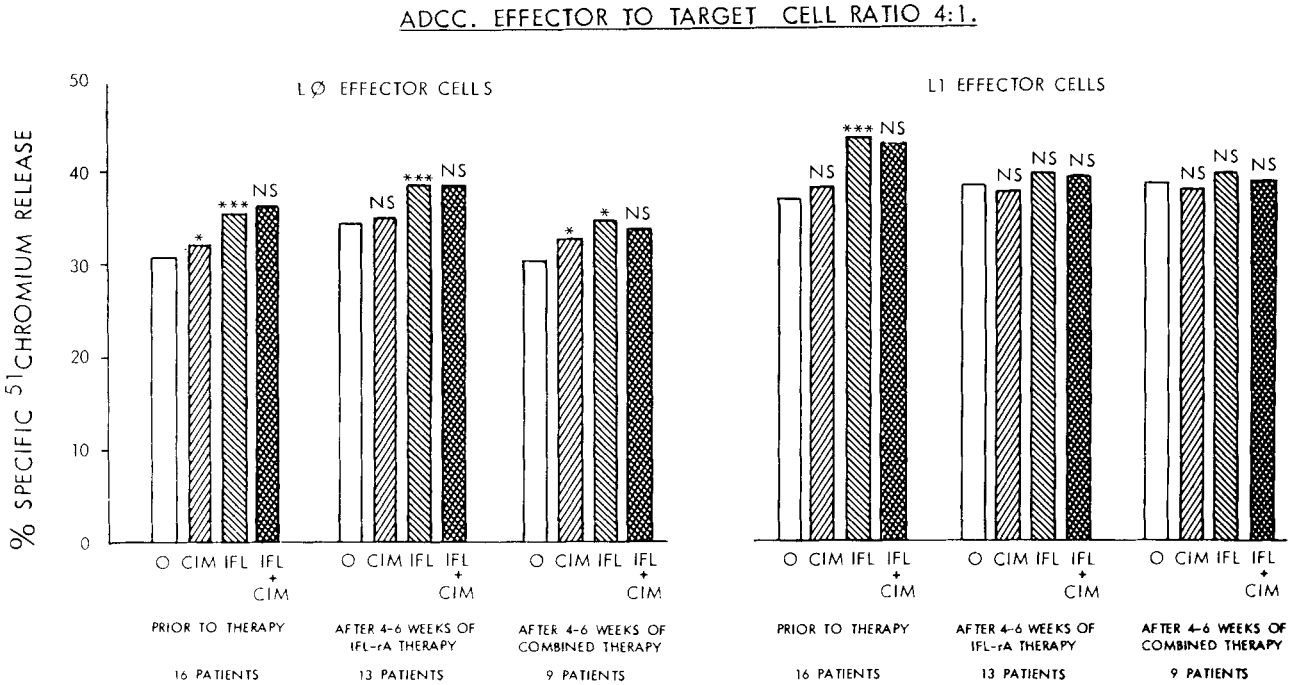

Fig. 2. Sequential tests performed to evaluate antibody dependent cellular cytotoxicity (ADCC). The symbols and statistical methods used are the same as in Fig. 1.

Table 4

Analysis of natural killer cell activity and antibody dependent cellular cytotoxicity. Effect of therapy with IFL-rA and IFL-rA + cimetidine (cim)

Effector cell fraction	No. of patients tested**	Before start of therapy	Natural killer cell activity***		Antibody dependent cellular cytotoxicity****		
			Treatment duration (weeks)		Before start of therapy	Treatment duration (weeks)	
			IFL-rA	IFL-rA+cim		IFL-rA	IFL-rA+cim
			4–6	4–6		4–6	4–6
L0	13	20.3±2.2	21.7±3.4 NS	–	32.5±3.2	34.5±3.2 NS	–
	9	22.6±2.3	22.5±3.8 NS	22.8±2.8 NS	34.5±3.4	38.4±3.2 NS	30.3±3.7 NS
L1	13	14.4±2.0	15.7±1.7 NS	–	38.8±5.2	38.7±4.4 NS	–
	9	15.9±2.7	16.2±1.8 NS	20.9±4.6 NS	45.1±4.3	44.0±4.1 NS	39.0±6.5 NS

* L0 = peripheral blood mononuclear cells. L1 = monocyte-depleted peripheral blood mononuclear cells.
** Number of identical patients tested at indicated time intervals.
*** Per cent 'weighed' specific ^{51}Cr release at an effector to target cell ratio of 16 : 1 ± SEM.
**** Per cent 'weighed' specific ^{51}Cr release at an effector to target cell ratio of 4 : 1 ± SEM.

NS = differences not significant compared with level before start of therapy ($p>0.05$, Mann-Whitney U, two tailed).

NK and K cell activities of peripheral blood mononuclear cells without or with monocyte depletion. Levels of cytotoxic activities evaluated before start of therapy and during treatment with IFL-rA alone or in combination with cimetidine did not differ significantly using any type of effector cells. The NK and K cell activities of the 6 patients with slower tumour progression or with stable disease did not differ significantly from the patients showing a more rapid progresssion when compared before or after 4 to 6 weeks of therapy (data not shown). Neither did the in vitro response of their mononuclear blood cells to interferon and/or cimetidine differ from the other group of patients.

Before start of therapy, preincubation of effector cells with cimetidine as well as interferon resulted in a significant increase in specific ^{51}Cr-release in both NK and ADCC tests compared with preincubation with medium, except in ADCC tests using the cimetidine-exposed L1 fraction as effector cells (Figs 1, 2). Preincubation with interferon and cimetidine in combination did not further potentiate the in vitro interferon-induced enhancement of NK and K cell activities before start of therapy or later during the 2 treatment periods. After 4 to 6 weeks of interferon therapy significant enhancement of cytolytic activities by preincubation with cimetidine was seen only in NK tests using the L0 fraction as effector cells. Augmentation of ADCC by interferon was lost in the L1 fraction. During combined therapy with interferon and cimetidine precincubation of L0 effector cells with cimetidine caused a slight but significant increase in NK and K cell activities, but not in L1 effector cells. Again the L1 fraction was unresponsive to interferon in ADCC tests. Significantly reduced levels of group cytolytic activity by any type of preincubation were never demonstrated.

Discussion

The present investigation was carried out to explore the therapeutic efficacy of Recombinant Leukocyte A Interferon in advanced colo-rectal carcinoma. Besides mediating a possible direct inhibitory effect on the proliferation of some neoplastic cells (2, 10, 18, 30, 38, 43), interferons may cause tumour regression indirectly through potentiation of the activity of cytolytic cells belonging to the immune system. While high interferon concentrations are usually needed to achieve a direct cell growth inhibitory effect, much lesser concentrations are required to influence cytolytic cells of the immune system. There, interferons seem to inhibit sensitising and proliferative pathways while enhancing the activity of any effector cells produced (1). Administered in large doses interferons will give side effects that are comparable to the ones encountered in cancer treatment with regular cytostatic agents (fatigue, loss of hair and appetite, bone marrow depression). To

avoid such adverse reactions, an interferon dose schedule substantially below the maximal tolerable dose was chosen for this investigation. This treatment schedule would also have the potential benefit of being less suppressive on the immune system. The reason for the addition of cimetidine (histamine-2 receptor antagonist) to continued interferon therapy in non-responsive patients was to investigate whether the efficacy of the interferon treatment could be enhanced by concomitant inactivation of suppressor T lymphocytes. These cells carry histamine-2 receptors on the plasma membrane and have been shown to produce a histamine-induced suppressor factor, which can abrogate various activities of both T and B lymphocytes (36). Previous studies have indicated therapeutic benefit of such a combined treatment in patients with cutaneous/subcutaneous metastatic malignant melanoma, but not in patients with visceral melanoma metastases (4, 15).

Our results show very limited efficacy in advanced colo-rectal carcinoma using Recombinant Leukocyte A Interferon at a dose of 7.5×10^6 units/m^2 intramuscularly 3 times per week. After 12 weeks of interferon therapy tumour progression was found in 12 of 15 patients, while the 3 remaining patients were classified as having a stable disease status. Thus no objective tumour regressions were observed on interferon treatment.

These findings are in agreement with other reported investigations using various types of alpha interferons with varying dose schedules. In a report by FIGLIN et coll. (14) all 18 evaluable patients treated with human leukocyte interferon (HuIFN-α(Le)) at a dose of 3×10^6 units/day intramuscularly 5 days/week demonstrated progression of disease during therapy. In another study, 18 of 19 patients showed progressive disease within a 12-week treatment period using lymphoblastoid interferon (HuIFN-α(Ly)) at a dose of 3×10^6 units/m^2 intramuscularly 3 times/week. In the remaining patient a stable disease status was scored at 12 weeks (8). Using IFL-rA administered at a dose of 50×10^6 units/m^2 intramuscularly 3 times per week, NEEFE et coll. (32) observed progression of the disease in 17 of 18 evaluable patients. In one patient a transient complete disappearance of lung metastases was observed. Dose reduction was required for 13 of the patients in the series.

Furthermore, in our study no objective tumour regressions were demonstrated on combined therapy with IFL-rA and cimetidine. On the contrary, 11 of 13 patients had disease progression during periods of treatment ranging from 2 to 12 weeks. In the 2 remaining patients the disease was stable when combined treatment was discontinued at 6 and 10 weeks, respectively, as it had also been during the 12 weeks of therapy with IFL-rA as a single agent. This finding indicates lack of additive anti-tumour activity by cimetidine. The results of combined treatment in this group of patients with, in most cases, heavy and widespread tumour burdens are in accordance with our experience with combined leukocyte interferon-cimetidine therapy in metastatic malignant melanoma, which indicated lack of effectiveness in patients with visceral metastases as opposed to patients with cutaneous/subcutaneous metastases.

There are conflicting reports on the influence on NK activity and ADCC of peripheral blood mononuclear cells by in vivo treatment with interferons. Some authors (11, 12, 13) have demonstrated augmented NK activity, maintained throughout prolonged treatment periods, using varying types of interferons and dose regimens, while others (3, 17, 35) have not found that to be the case. This discrepancy can probably be accounted for by differences in the preparation of effector cells, type of target cells in the assays and by interferon-related factors such as dose, mode of administration and species used in the therapy. In the present investigation we did record intraindividual changes in NK activity during the 2 treatment phases, but group analyses did not show any significant differences, compared with baseline levels, for the 2 types of effector cells used. As a group the 6 patients with a slower tumour progression or with stable disease did not differ significantly from the patients with a more rapid progression. In vitro exposure of both L0 and L1 effector cell populations to IFL-rA persistently induced increased NK cell activity. This means that prolonged, low dose IFL-rA therapy did not result in abrogation of NK cell responsiveness to leukocyte interferon although the response tends to be weaker. Despite the fact that cimetidine preincubation in vitro of both types of effector cells caused a slight, but significant, increase in spontaneous cytotoxicity before start of therapy, and in L0 cells throughout the treatment period, cimetidine exposure did not further augment the interferon-induced increase in NK cell reactivity. The significance of this finding is unclear at present, and is in part at

variance with our observations in other groups tested (FLODGREN et coll., unpublished).

In the present investigation we found no significant changes in ADCC during treatment with IFL-rA alone or in combination with cimetidine. This finding is in agreement with EINHORN et coll. (11) who were unable to detect any augmentation of K cell activity during prolonged therapy with partially purified leukocyte interferon. In fact, over time a decrease in ADCC was observed. In the present study the enhancement of the ADCC activity recorded after in vitro interferon exposure of L 1 effector cells obtained before therapy could no longer be demonstrated when retested in the course of treatment. Some authors have previously reported that ADCC can be augmented in vitro by various interferon preparations (9, 33) while others have found that not to be the case (37). The significance of these discrepancies in results is also unclear at present. However, the same limitations as discussed in comparing results in NK tests performed in different laboratories are also applicable in ADCC testing.

Conclusion

This phase II investigation of Recombinant Leukocyte A Interferon, without and with concomitant cimetidine medication, in patients with advanced colo-rectal carcinoma indicates very limited therapeutic efficacy at the dose levels used. However, the fact that stabilisation of the disease was recorded in 3 patients during prolonged treatment with IFL-rA and in 2 of these patients during further IFL-rA therapy combined with cimetidine should be underlined. Since these patients had progressive disease on entry to the study lack of further tumour growth might point at a possible antitumour effect of this type of interferon on colo-rectal carcinoma.

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