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Antitumour Effect of Intratumoral Injection of Human Recombinant Interleukin-2 in Patients with Hepatocellular Carcinoma: a Preliminary Report

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Five hepatoma patients with small resectable tumour received an intratumoral injection of recombinant interleukin-2 (rIL-2) once weekly over 2–4 weeks (1.05×10^6–3.6×10^6 U in total per patient). Tumour regressions of 32% and 57% were observed in two patients at day 42 after the first rIL-2 injection. No response was observed in two patients and disease progressed in one. Lymphokine-activated killer (LAK) activity was enhanced and Leu-11$^+$ cells increased in the peripheral blood in the patients with 32% and 57% tumour regression after rIL-2 therapy. However, LAK activity, Leu-7$^+$ cells were reduced in the patient who progressed. No consistent changes in Leu-2a$^+$ cells and Leu-3a$^+$ cells were demonstrated. In the three patients showing no response or 32% tumour regression, hepatic segments containing tumour were resected; histologically the tumour showed severe necrosis and lymphocytic infiltration in the patient with 32% tumour regression but mild or moderate changes in the other two. IL-2 mediated tumour killing can be induced in tumours by intratumoral injection of rIL-2, leading to tumour regression.
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INTRODUCTION

INTERLEUKIN-2 (IL-2) can activate and expand T lymphocytes that are specially cytotoxic for autologous tumour cells *in vitro* [1]. Natural killer (NK) activity is mediated almost exclusively by Leu-11-bearing cells [2] and incubation of Leu-11$^+$ cells with IL-2 for over 24 h induces lymphokine-activated killer (LAK) activity [3]. Animal experiments indicate a potential role for IL-2 in cancer therapy; local injections have sometimes resulted in cures [4–6]. Some trials of IL-2, with or without LAK cells, have reported promising results in patients with, for example, renal or colorectal cancer or malignant melanoma [7–11] and Pizza *et al.* [12] demonstrated complete tumour regression in 3 of 6 bladder cancer patients with intralesional injection of IL-2.

We describe preliminary results of the intratumoral injection of recombinant IL-2 (rIL-2) in patients with early resectable hepatocellular carcinoma (HCC).

PATIENTS AND METHODS

Five patients with histologically proven and resectable HCC (trabecular pattern, well differentiated) were studied (Table 1). All had one hepatic tumour less than 3.5 cm in diameter and underlying liver cirrhosis (functionally well compensated). No patient had positive serum markers for hepatitis B infection. Three patients (2, 3 and 4) were alcoholics and the other two had clinical signs of non-A, non-B hepatitis. No patient had received previous therapy. On entry to the trial, Karnofsky scores ranged from 80 to 100. No extra-hepatic carcinoma invasion was found. No patients received anti-cancer therapy or drugs known to cause immunological changes during the study. Informed consent was obtained from each patient.

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rIL-2 was supplied by Takeda Chemical Industries, Osaka [10, 11]. The patients received an intratumoral injection of 4.5×10^5–9.0×10^5 U by percutaneous transhepatic cholangiography (PTC) over a few minutes once weekly for 2–4 weeks under ultrasonographic control (1.05×10^6–3.6×10^6 U in total per patient). The effect of rIL-2 on immune function (LAK activity and surface phenotype of peripheral blood mononuclear cells [PBMC] was examined 1–3 days before the first injection, during treatment, and 3 days after the final injection.

Full blood counts were obtained and serum biochemistry was monitored at least twice weekly during treatment and thereafter once weekly for 3 months. Alpha-fetoprotein (AFP) measurement and computed tomography (CT) scan were done 1–2 weeks before the first injection and 2–3 weeks after the final injection. Tumour volume was estimated in three dimensions by reconstruction of the tumour portion of CT films (IBAS, Zeiss). Tumour regression was defined as 0–24% = no response, 25–50% = minor response and 51–99% = partial response.

Tumour segments were resected in three patients (1, 4 and 5), and liver biopsy was done in patient 2 at 2–3 weeks after the final rIL-2 injection. Two patients refused consent for operation.

PBMC were prepared by centrifugation on Ficoll–Hypaque gradients. After two washes in RPMI 1640, the PBMC were resuspended at 5×10^6/ml in complete medium consisting of RPMI 1640 supplemented with 5% human AB serum, antibiotics and glutamine. rIL-2 (1.5×10^4 U/ml) was added to cell suspensions, which were then incubated for 72 h at 37°C in 5% CO_2. After the incubation, the lymphocytes were washed three times and were resuspended at 5×10^6/ml in complete medium.

LAK cytotoxic activity of the cells was evaluated against Daudi cells by the 4 h ^{51}Cr release assay [12]. The effector:target ratio was 50:1. The plates were incubated for 4 h at 37°C in 5% CO_2 and culture supernatants were harvested (Skatron-Titertek) and counted in a gamma counter. Spontaneous and maximum ^{51}Cr releases were measured by incubating targets in medium and in 1 mol/l HCl, respectively. Each assay was in triplicate,

Table 1. Antitumour effect of rIL-2 and histopathology

Case (age/sex)	Dose of rIL-2 (U)	Tumour regression (%)	AFP: before/after (ng/ml)	Tumour necrosis*	Lymphocyte infiltration*
1 (74/M)	4.5×10^5 and 6×10^5	11	1345/1220	±	+
2 (59/M)	6×10^5 3 times	−47	10/9	−	±
3 (42/M)	4.5×10^5 4 times	57	60/30	NE	NE
4 (61/M)	4.5×10^5 2 times and 6×10^5 2 times	3	19/24	+	++
5 (70/F)	6×10^5 4 times	32	4780/4200	++	+++

*− = none, ± = very mild, + = mild, ++ = moderate and +++ = severe. NE = not examined.

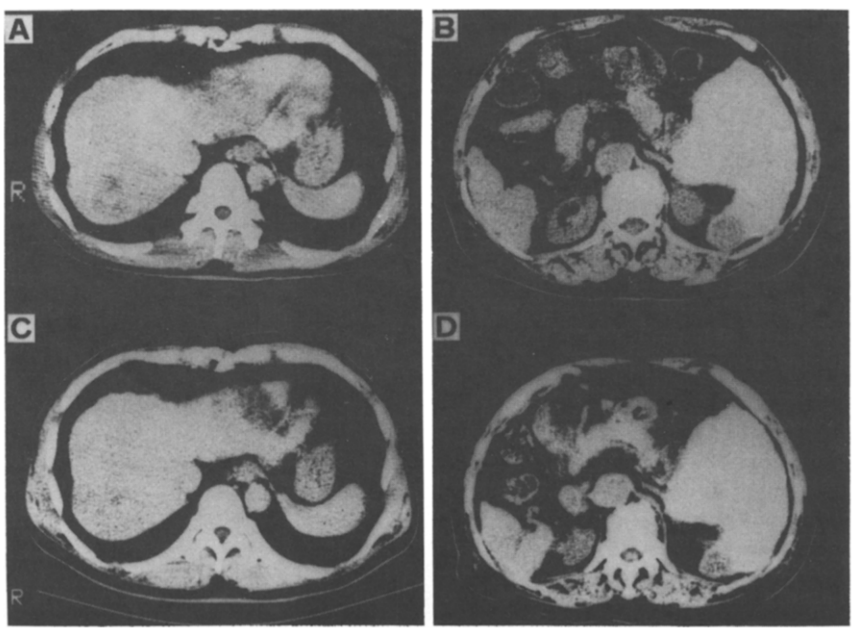

Fig. 1. CT before (A,B) and after (C,D) treatment with rIL-2 in cases 3 and 5, respectively. Scan shows significant regression of low density area (cancer) 2 weeks after treatment.

and cytotoxicity values (% specific lysis) were calculated by: (experimental release − spontaneous release) × 100 ÷ (maximum release − spontaneous release).

PBMC were stained with fluorescein isothiocyanate conjugated monoclonal antibodies to CD3 (Leu-1, pan T), CD4 (Leu-3a, helper/inducer T cell), CD8 (Leu-2a, cytotoxic/suppressor T cell), CD16 (Leu-11 NK cells and neutrophils, Fc receptor), HNK1 (Leu-7, reactive on most large granular lymphocytes, NK cell) and CD25 (p55 chain of IL-2 receptor [IL-2R]) (Becton Dickinson). The cells were analysed in a FACS IV [3].

RESULTS

Tumour volume showed no response in cases 1 and 4, minor response in case 5, partial response in case 3 and rapid disease progression in case 2 at days 35, 42, 42, 42 and 35, respectively, after the first rIL-2 injection (Table 1). Serum AFP at day 42 was decreased in case 3 but not at all or only slightly decreased in the other patients. CT images in case 3 before and after treatment with rIL-2 are shown in Fig. 1. The tumorous shadow had regressed on CT 2 weeks after the treatment.

Histological examination disclosed that the hepatoma nodes, injected with rIL-2 showed little to massive necrosis (approximately 15, 40 or 70% of the area of maximum tumour cut surface in cases 1, 4 and 5, respectively), with a parallel increase in lymphocytic infiltration (Table 1, Fig. 2). There was no tumor necrosis and very mild lymphocytic infiltration in case 2.

The effect of rIL-2 injection on LAK generation in PBMC *in vivo* and *in vitro* by culture with rIL-2 was examined (Fig. 3). The LAK cytotoxicity of fresh PBMC before *in vitro* incubation with rIL-2 was low in all patients before rIL-2 therapy (less than 8%) and enhanced in cases 3, 4 and 5 after therapy (higher than normal range). The LAK activity generated *in vitro* by rIL-2 was small in cases 4 and 5 before therapy. After the final injection of rIL-2, enhancement of LAK activity in response to rIL-2 *in vitro* was more striking in cases 3 and 5 (within normal range), with a parallel increase in tumour regression rates. However, the enhancement of activity was small in case 1, and decrease of activity was observed in case 2 after treatment.

Cell surface antigens of PBMC were assessed by flow cytometry (Fig. 4). Leu-2a$^+$ cells were decreased in all patients, compared with the normal range, and showed no apparent change after rIL-2 therapy. For Leu-3a$^+$ cells, three patients (1, 2 and 5) showed decreased levels and two patients had high levels before therapy. In case 3, Leu-3a$^+$ cells and Leu-3a$^+$/Leu-2a$^+$ cell ratio were increased after the therapy (greater than 10% and

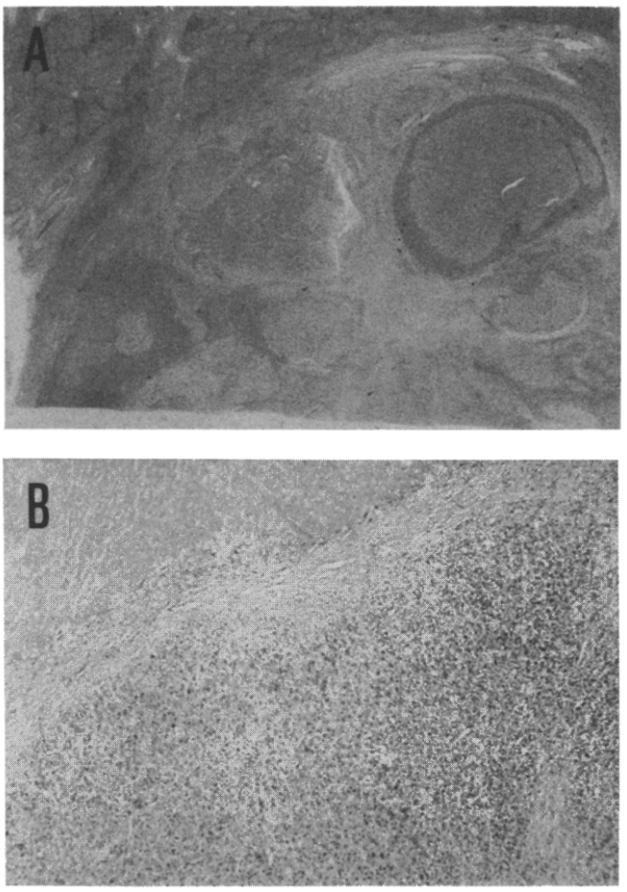

Fig. 2. Histology of resected cancer injected with rIL-2, in case 5. Severe necrosis of cancerous tissue with lymphocytic infiltration was observed. Haemotoxylin eosin; A = × 2, B = × 100.

1.5 change, respectively). The percentage of Leu-7$^+$ cells and Leu-11$^+$ cells increased by more than 10% in response to rIL-2 in case 5. Case 3 showed increased Leu-11$^+$ cells after rIL-2 injection (increase of over 5%). In case 2, Leu-7$^+$ cells and Leu-11$^+$ cells were decreased after therapy (by more than 5%). The IL-2R$^+$ cell fraction was increased in cases 3, 4 and 5 after treatment (more than 10% increase). The increase in IL-2R$^+$ cell fraction in response to rIL-2 was almost consistent with

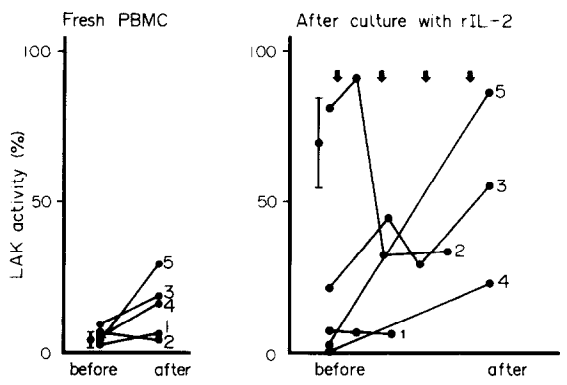

Fig. 3. LAK activity in fresh PBMC and in PBMC incubated with rIL-2 *in vitro* for 72 h before, during and after rIL-2 therapy. Normal range (▮) is mean (S.D.) of 30 healthy volunteers. Case numbers shown and arrow = rIL-2 injection.

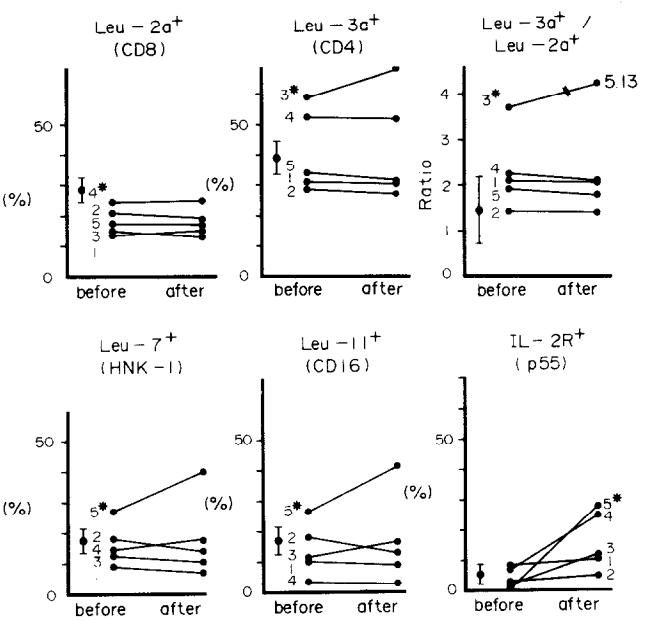

Fig. 4. Flow cytometry of PBMC before and after rIL-2 injection. Normal range (▮) is mean (S.D.) of 80 healthy volunteers.

induction of LAK activity *in vivo* and *in vitro* after therapy, especially in cases 3 and 5.

In none of the patients treated were any adverse effects observed, except for increased temperature (often more than 38°C) and mild anorexia several hours after the injection. No increase of serum aminotransferases was seen (Table 2). There was slight leukocytosis and lymphocytosis in cases 3, 4 and 5.

DISCUSSION

Injection of IL-2 alone into a tumour had no significant effect on the tumour growth [14], although several investigators have reported that local injection of IL-2 inhibited the growth of tumour [4, 5, 8, 15, 16]. HCC is a solid tumour, easy to approach under ultrasonographic control. We previously reported the clinical and immunological efficacy and antiviral activity of rIL-2 in patients with chronic hepatitis B [17]. In the present study, we investigated the effects of rIL-2, injected into the hepatic tumour, on IL-2 activated immunity *in vivo*. Therapeutic effects were obtained safely with repeated intralesional injections of rIL-2. A possible explanation for the tumour regression seen in our patients could be the activation of LAK and their infiltration into the tumour.

Tumour cells unsuppress lymphocyte reactivity [18]. Diminished NK (Leu-11$^+$ cell or Leu-7$^+$ cell) cytotoxicity in patients with HCC has been reported [19, 20]. In our study, tumour regression was associated with enhanced LAK activity and increased numbers of Leu-11$^+$ cells. Deficient LAK activity was seen even in patients with small tumours before treatment. Depletion in LAK precursors (Leu-11$^+$ or Leu-7$^+$) was thought to be one of causes for deficient activity in cases 1 and 4. It is possible that simultaneous IL-2 stimulation of different classes of cells enhances cellular incorporation in the tumour. However, cytotoxic T cells were diminished in all the patients, and were not increased after therapy. Helper T cells were induced in case 3 but not in case 5. The enhancement in the level of IL-2 receptor expression following IL-2 therapy is thought to be partly due to the increase of Leu-7$^+$ cells in case 4, Leu-3a$^+$ and Leu-11$^+$ cells in case 3, and of Leu-7$^+$ and Leu-11$^+$ cell in case

Table 2. Laboratory data before and after rIL-2 therapy

Case	White cells (/μl) (5300–7900)*	Lymphocytes (/μl) (2000–4900)*	AST (U/l) (1–40)*	ALT (U/l) (1–35)*	Prothrombin time (%) (80–125)*
1	4900/4700†	1560/580	31/47	27/31	96/77
2	2700/2700	900/1040	58/71	35/35	65/60
3	4200/5000	1230/2000	216/59	217/52	32/43
4	4300/5100	1650/2460	179/53	175/71	133/127
5	2400/2700	1220/1300	113/83	76/41	79/75

*Normal values in parentheses. †Before/after. AST = aspartate aminotransferase and ALT = alanine aminotransferase.

5, although the increase of IL-2 receptor-positive cells was linked to enhancement of LAK activity in cases 3, 4 and 5.

The major side-effect of rIL-2 was increased body temperature, often to more than 38°C. We saw no adverse changes in liver function or other blood variables.

In our study, intra-tumoral injection of rIL-2 was effective in one of five patients with significant augmentation of LAK activity. Moreover, severe or moderate tumour necrosis was observed in two other cases, histologically. Higher doses of rIL-2 given over longer periods in combination with other therapies, such as adoptive immunotherapy [21, 22], transhepatic arterial embolisation or intratumoral injection of ethanol, may make widespread use of this therapeutic approach possible.

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