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Original Paper

Clinical and Immunomodulatory Effects of Repetitive 2-day Cycles of High-dose Continuous Infusion IL-2

M. Engelhardt,[1] K. Wirth,[1] R. Mertelsmann,[1] A. Lindemann[1] and U. Brennscheidt[2]

[1]Department of Internal Medicine I, University of Freiburg, Hugstetter Strasse 55, 79106 Freiburg; and
[2]Goedecke/Parke Davis, Mooswaldallee 1, 79090 Freiburg, Germany

High-dose interleukin-2 (IL-2) treatment has demonstrated promising antitumour activity in renal cell carcinoma (RCC) and malignant melanoma (MM) and has been shown to induce broad immunological effects. The optimal IL-2 dose and schedule, however, still remain to be defined. We studied a treatment protocol consisting of five repetitive cycles of high-dose recombinant (rh) IL-2 (24×10^6 U/m^2/day) administered weekly on two consecutive days by continuous intravenous infusion. 17/19 were RCC patients, 2 of whom responded with a complete remission (CR) and 3 with a partial response (PR) (CR + PR: 29%; median response duration of 11.5+ months (range: 3–14 months)). IL-2 induced a pronounced increase of lymphocytes and pro-inflammatory cytokines IL-8, IL-5, γ-IFN, TNF-α and TNF-β ($p < 0.05$) that peaked in cycle 3. With subsequent therapy, serum levels of these cytokines, NK, T cells and eosinophils decreased, whereas serum IL-10 levels progressively increased with maximum levels achieved after the fifth week of treatment, suggesting that it may be involved in dampening the inflammatory response induced by IL-2. Absolute numbers of activated T cells and NK cells remained elevated as compared to baseline for at least 4 weeks after treatment cessation. Based on these observations, future scheduling of IL-2 will be done at 3 weekly 2-day cycles separated by a week 4 treatment-free interval in order to increase further the 29% objective response rate achieved in this study. © **1997 Elsevier Science Ltd.**

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INTRODUCTION

IN CONJUNCTION with lymphokine-activated killer (LAK) cells or alone, interleukin-2 (IL-2) has been broadly studied as an immunostimulatory agent in tumour therapy [1–3]. In selected chemotherapy resistant tumours, particularly in malignant melanoma (MM) and renal cell carcinoma (RCC), it has shown encouraging results with response rates of 15–30% [1–3]. IL-2 is known to activate natural killer (NK) and T cells and has been reported even in low or ultra-low doses to induce profound immunological effects [4, 5]. However, with lower doses, clinical efficacy decreases considerably [4, 5]. High-dose (HD) IL-2 regimens have been developed based on the model that tumour response correlates with dose intensity [1, 2, 6]. Numerous clinical trials have since been completed using HD IL-2 regimens in conjunction with LAK cells [1, 2], tumour infiltrating lymphocytes (TIL) [6] or interferons [7] without improving response rates as compared to single-agent IL-2. To date, the optimal schedule, balancing efficacy and toxicity still remains to be defined. Here we used a HD IL-2 schedule in RCC and MM patients that has been evaluated previously with LAK cells or interferon-γ [8, 9], to treat patients in standard care units. This treatment schedule (24×10^6 U/m^2/day) was chosen to reduce treatment toxicity since the most severe side-effects have usually been observed with prolonged treatment [10].

Correspondence to U. Brennscheidt.
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PATIENTS AND METHODS

Patients

19 patients (aged 28–64 years) were treated with HD IL-2 from July 1993 to December 1995. All patients eligible to receive the therapy fulfilled the following criteria: histologically confirmed RCC or MM, age <70 years, measurable sites of disease, Karnofsky performance status >70%, white blood cell count (WBC) > 3000/μl, platelet count >100 000/μl, normal renal (creatinine <1.5 mg/dl) and hepatic function

(liver enzymes <3 times normal value, negative HBsAg). Patients with central nervous system metastases, severe cardiac dysfunctions (<45%), respiratory disorders, active systemic infections and autoimmune diseases were excluded. No additional treatment such as chemotherapy, radiotherapy or immunotherapy were administered for at least 4 weeks before study entry and during HD IL-2 treatment. The study was approved by the institutional review board and all patients gave informed consent before treatment started.

Study design

Recombinant IL-2 was administered by continuous intravenous infusion for two consecutive days with a daily dose of 24×10^6 U/m^2. This was repeated for five weeks. For the first two treatment weeks, IL-2 was administered by means of a central access line catheter that was replaced by a peripheral access if therapy was tolerated without major side-effects. Concomitant medication was paracetamol (1 g every 6 h), continuous dopamine administration (200 mg/24 h), and ranitidine (150 mg). Tumour response was evaluated 4 and 8 weeks after IL-2 therapy. Another IL-2 cycle was recommended for those patients that exhibited a complete or partial response or had stable disease after 8 weeks rest.

Laboratory assessment

Serum chemistry, complete blood cell count, thyroid function test, antinuclear antibody screening, surface marker studies and serum collections to measure cytokine and soluble receptors were done before, during and after HD IL-2. Enzyme-linked immunoabsorbed assays were used to measure soluble CD8 (sCD8, Biermann, Bad Nauheim, Germany), soluble tumour necrosis factor (TNF)-receptor I, tumour necrosis factor (TNF)-β, IL-5, IL-4 (all Amersham Life Science, Illinois, U.S.A.), TNF-α, interleukin (IL)-10, IL-8 and interferon-γ (all Medgenix Diagnostics, Ratingen, Germany). The sensitivity of these assays is 25 pg/ml for sTNF-RI, 16 pg/ml for TNF-β, 2 pg/ml for IL-4, 3 pg/ml for TNF-α, 1 pg/ml for IL-10, 18.1 pg/ml for IL-8, and 0.03 IU/ml for γ-IFN. Blood samples were obtained before, during (first, third and fifth IL-2 cycle, day 2 and 4) and after IL-2 therapy.

In order to characterise lymphocyte subpopulations, surface marker studies were performed by three-colour immunofluorescence analysis using flow cytometry and fluoresceinated (FITC), phycoerythrin (PE) or peridinchlorophyll-alpha protein (PerCP)-conjugated monoclonal antibodies (MAb)) to the following clusters: p75-IL-2R (Endogen INC., Boston, Massachusetts, U.S.A.), CD3, CD4, CD16 (Leu 11 a) (Becton Dickinson BD, Heidelberg, Germany), CD56 (NKH-1), CD25 (IL-2RI) (Coulter Electronics, Krefeld, Germany), HLADR, CD44, CD45RO, and CD8 (Becton Dickinson BD, Heidelberg, Germany). Cells for surface marker studies were obtained before, during (first and fifth IL-2 treatment cycle, day 1 and 4) and after IL-2 therapy.

Response criteria

The response was evaluated using WHO criteria: complete response (CR) was defined as the disappearance of all measurable tumour lesions for at least 4 weeks, partial (PR) as a reduction of at least 50% in the sum of the products of the perpendicular diameter of all lesions and minor response (MR) if 25–50% objective tumour regression occurred. Unchanged tumour size for at least 4 weeks was termed stable disease (SD), and progressive disease (PD) was defined by an increase in tumour size of 25% or more or detection of occurrences of new tumour sites.

Statistical analysis

The data were analysed by the Wilcoxon matched-pairs test and the Student's *t*-test (Statworks, Cricket Software, Philadelphia, Pennsylvania, U.S.A.). A p value of less than 0.05 was considered significant.

RESULTS

Patient characteristics and antitumour activity

17 out of 19 patients suffered from metastatic RCC and 2 from stage IV malignant melanoma. 8 of 17 RCC patients responded to HD IL-2, with 2 CR, 3 PR, 1 MR (13 months) and 2 SD (3+, 14 months), with a median response duration of 11.5+ months (range 3–114 months). One patient with lung metastases achieved CR (13+ months) of pulmonary and lymph node metastases after the first IL-2 cycle. Another, showing histologically verified lymph node metastases 13 years after the diagnosis of RCC, achieved CR (11+ months) after two IL-2 cycles. A partial response was observed in 3 patients with lung, bone and adrenal tumour manifestations lasting 12, 11 and 6 months, respectively. 4 other patients with PR after the first 5 weeks of HD IL-2 were retreated with the regimen, resulting in CR in one and improving the PR in the others. The remaining 9 RCC patients and both MM patients, with extensive disease showing lung and lymph node metastases in one and lung, liver and subcutaneous metastases in the other, had disease progression.

"Toxicity"

Despite high doses of IL-2, treatment was generally well tolerated (Table 1). Common but mild side-effects included fever, weight gain and cutaneous erythema in all patients (Table 1). Grade 3 and 4 toxicity consisted mainly of fever (26%), weight gain (21%), hyperbilirubinaemia (16%) and hypercreatininaemia (16%), tachycardia (16%) and arrhythmia (11%) (Table 1). One patient developed a transient arrhythmia that required digitalis medication. Another had an i.v. catheter-related sepsis that responded promptly to appropriate i.v. antibiotic treatment. Moderate pulmonary oedema and a transient renal failure due to intravascular fluid depletion developed in one patient each. All patients responded well to specific treatment. No dose reduction was needed except in the one patient who developed pulmonary oedema after the first week of IL-2 and was subsequently treated with 75% of the initial IL-2 dose. Thyroid function tests did not reveal any impairment of endocrine function. The time from the end of each 2-day IL-2 cycle to hospital discharge was 1 day (median).

Changes in peripheral blood cell count

The blood cell count was measured before, during (day 1 to day 4 of each treatment cycle) and after IL-2 therapy. The leucocyte count increased progressively during IL-2 therapy and remained elevated despite intermittent discontinuation of therapy. Marked eosinophilia was found during IL-2 therapy that increased progressively despite a treatment-free interval of five days and maximum levels were

Table 1. Summary of clinical toxicities in 19 patients receiving high-dose IL-2 treatment (classified according to WHO criteria (I– IV) where applicable)

Toxic effect	WHO grade I	II	III	IV
Fever	13*	1	5	0
Weight gain	13	2	4	0
Skin rash/erythema	18	0	1	0
Nausea	6	0	0	0
Diarrhoea	6	0	0	0
Hypotension	10	0	0	0
Neurotoxicity (confusion/ depression)	5	0	0	0
Dyspnoea	2	0	1†	0
Bilirubin	3	0	3	0
Creatinine	2	0	3	0
Arrhythmia Tachycardia	2	0	5	0
Infection‡	0	0	1	0
Severe leucopenia§	0			
Anaemia§	0			
Thrombocytopenia§	0			
Admission to ICU¶	0			

*Number of patients experiencing at least one episode of toxic effect.
†Treatment schedule change from 100% HD IL-2 to 75% due to moderate pulmonary oedema.
‡Gram-positive central line-related sepsis.
§Leucopenia defined as <1000 leucocytes/mm^3, anaemia requiring erythrocyte transfusions, thrombocytopenia <30000/μl platelets.
¶ICU denotes intensive care unit.

reached after the third week of treatment with median eosinophil numbers of 4200/μl (range, 900–15750), corresponding to an increase of >1500% relative to baseline levels ($p < 0.0001$).

Cytokines

Serum samples were taken before treatment and thereafter and on day 2 and day 4 of the first, third and fifth IL-2 therapy cycle. Significantly increased levels of IL-8, TNF-β, γ-IFN, IL-5, TNF-α, sTNF-R and sCD8 were detected after the third week (cycle 3) of IL-2 ($p < 0.05$) (Figure 1(a)). No progressive increase was seen with subsequent therapy. Contrasting these kinetics, IL-10 increased further in response to IL-2 with maximum levels after the fifth week of IL-2 (mean increase in week 3 and 5 of 36-fold and 77-fold respectively) ($p < 0.01$) (Figure 1(a)).

Surface marker studies

The relative changes of T and natural killer (NK) cell subsets during IL-2 treatment are shown in Figure 1(b). CD56 + NK cells increased during therapy as detected by subsets (CD56 + /p75 IL-2R + , CD56 + /CD3–, CD56 + / CD16 + , CD56 + /HLADR+). The relative increase of CD56 + /CD3– cells was similar to that of CD56 + /CD16+ cells, while activated NK cells (CD56 + /HLADR+) expanded to a higher degree ($p < 0.001$). The rise in NK cells during IL-2 therapy was significant, with a 6–13-fold increase, and maximum levels of NK and T cell populations were detected after the third week of IL-2 ($p < 0.01$). After termination of IL-2 therapy, NK cells (CD56 + /p75IL-2R + , CD56 + , CD3– /CD56 + /CD16 + , CD56/HLADR) decreased, but remained elevated compared to baseline levels 4 weeks after therapy. The same phenomenon was observed with T cell subpopulations CD4 + /CD25 + , CD8 + /25 + , CD4 + /45RO + , CD8 + /45RO+ that showed a marked increase during IL-2 treatment, decreased after therapy, but also remained elevated after discontinuation of IL-2 ($p < 0.01$). Activated T cells (CD3 + / CD25 + , CD4 + /CD25 + , CD8 + /CD25+) also exhibited a more pronounced expansion than the bulk of T cells ($p < 0.001$).

DISCUSSION

Patients with advanced RCC and MM have a particularly dismal prognosis with a median survival duration of 2–12 months and a 5 year survival of less than 5% using conventional therapy [11, 12]. Studies exploring biological therapies, such as IL-2 treatment, have described objective responses in 15–30% [1–3]. In an attempt to study tumour regression and immunological changes of HD IL-2, we used a protocol in which five repetitive cycles of IL-2 were administered on two consecutive days by continuous intravenous infusion. This differed from related treatment schedules previously described using IL-2 in combination with LAK cells or IFN-γ [8, 9]. Of the RCC patients, 12% (2/17) achieved a complete response, 18% (3/17) partial responses, with an objective response rate (CR + PR) of 29% and a median response duration of 11.5+ months. The side-effects of our treatment schedule were moderate compared to that reported elsewhere [8, 9, 11, 12], and therapy was given in standard care units. The mean stay for each 2-day cycle was 3 ± 1 days.

In line with previous reports [11, 13], we found considerable increases in circulating eosinophils, NK cells and T cells. Maximum counts were observed after the third week of HD IL-2 followed by a subsequent plateau or decrease. A similar course was observed for a variety of cytokines including IFN-γ, TNF-α, TNF-β, IL-8 and IL-5, as well as sTNF-RI, and sCD8. Contrasting these kinetics of pro-inflammatory Th1 and Th2 cytokine levels, eosinophil and lymphocyte counts, IL-10 levels progressively increased with subsequent HD IL-2 treatment showing maximum levels in

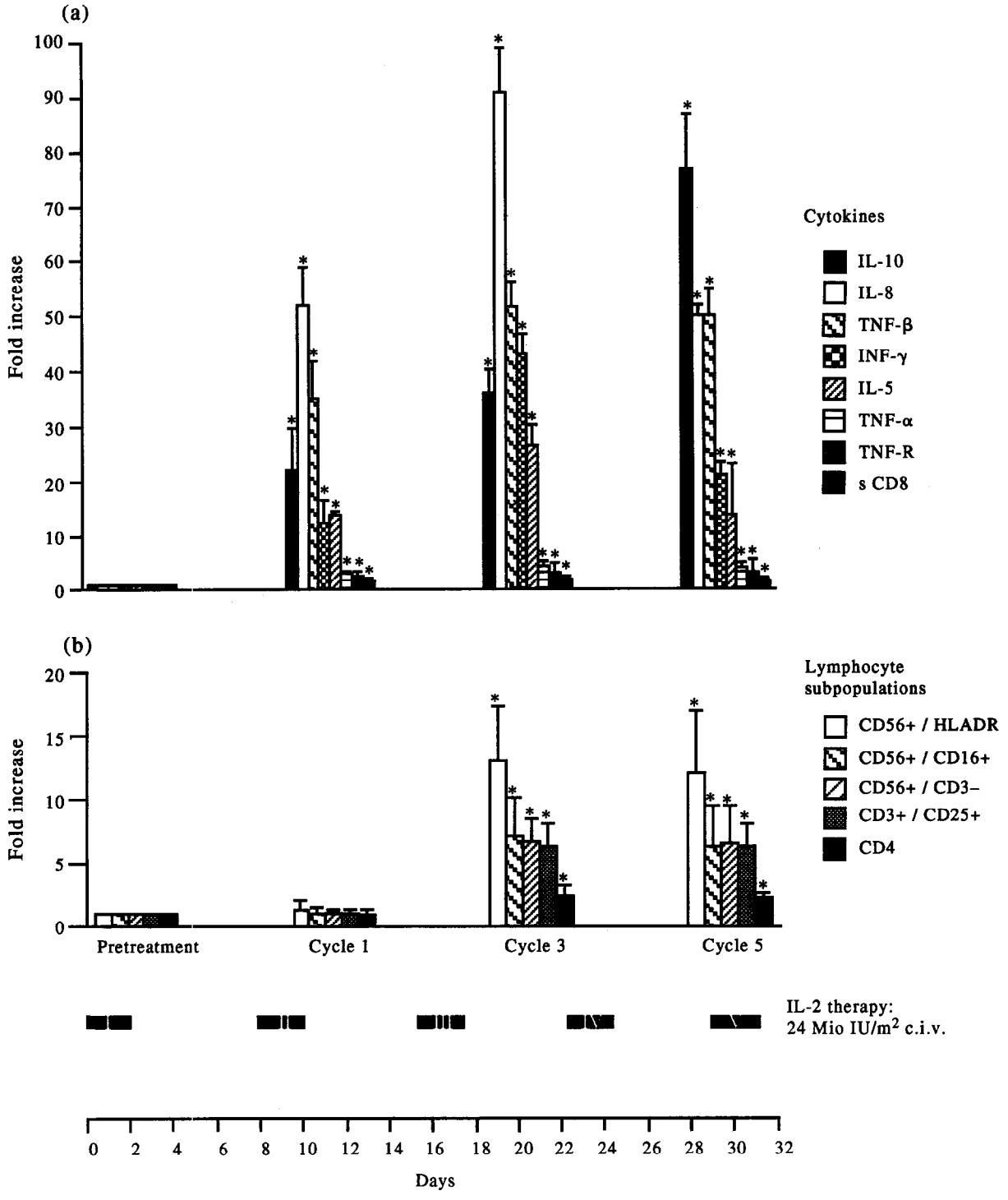

Figure 1. Changes in serum cytokine levels (a) and in lymphocyte subpopulations (b) in response to IL-2 treatment. (a) The fold increase relative to baseline (=1) of IL-10, IL-8, TNF-β, IFN-γ, IL-5 (fold increase × 100), TNF-α, TNF-R and sCD8 at day 2 of each cycle is depicted (values represent means of 5 patients ± SEM). Absolute numbers (mean ± SEM) in response to cycle 3 were 25 ± 3 pg/ml IL-10, 24 ± 5.3 pg/ml IL-8, 51.5 ± 4.6 pg/ml TNF-β, 6.2 ± 0.5 U/ml IFN-γ, 80 ± 10 pg/ml IL-5, 35 ± 7 TNF-α, 10 ± 5 ng/ml sTNF-R and 383 ± 53 U/ml sCD8. (b) The fold increase relative to baseline (=1) of NK (CD3-/CD56+, CD56+/CD16+, CD56+/HLADR+) and T cell subpopulations (CD3+/CD25+, CD4+) at day 1 of each cycle is shown (values represent means of 10 patients ± SEM). Absolute numbers (mean ± SEM) in response to cycle 3 were 2011 ± 768/ml CD3-/CD56+, 1850 ± 796/ml CD56+/CD16+, 300 ± 100/ml CD56+/HLADR+, 500 ± 140/ml CD3+/CD25+ and 2250 ± 832/ml CD4+. A statistical significant increase as compared to baseline ($p < 0.05$) is designated with an asterisk (*).

cycle 5. IL-10 has formerly been described as an anti-inflammatory cytokine downregulating the production of others such as TNF, IFN-γ, IL-1 or IL-6 and decreasing T-cell activation [14]. Elevated levels have also been found in sepsis [15]. Therefore, the consistent rise in IL-10 during subsequent treatment may resemble an attempt of the immune system to protect and control inflammatory responses induced by HD IL-2. While serum cytokine levels decreased to baseline within a few days after discontinuation of treatment, numbers of activated NK and T cells remained elevated for at least 4 weeks.

In summary, we have shown that side-effects with this IL-2 protocol compared favourably to that published by others [11, 12]. Two-day cycles of HD IL-2 were well tolerated while being as effective as the more standard 5-day regimen. This is supported by the profound immunological effects. Interestingly, the latter are dampened with repetitive treatment, paralleled by increasing concentrations of IL-10 showing an excessive increase with the fifth cycle. Therefore, further protocols will include three weekly 2-day cycles followed by a 4-week treatment-free interval before therapy continues. This may be reasonable given the fact that activated NK and T cells remain elevated for approximately 4 weeks.

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