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# Pharmacokinetics of repeated i.v. bolus administration of high doses of r-met-Hu interleukin-2 in advanced cancer patients

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**Summary.** We studied the pharmacokinetics of recombinant methionyl human interleukin-2 alanine (r-met-Hu IL-2 [ala 125]) given at high doses by i.v. bolus according to Rosenberg's initial schedule in seven patients with advanced cancer. Serum concentrations of IL-2 were measured by radio-immunoassay. The drug followed second-order kinetics. During the administration of repeated high doses of IL-2, we noted a progressive increase in the volume of distribution (from 5,984 ± 1,850 to 9,084 ± 4,345 ml) and a progressive decrease in the AUC (from 32,643 ± 3,817 to 22,397 ± 511 IU min ml$^{-1}$) and β-half-life (from 61 ± 14 to 48 ± 6 min); the peak serum IL-2 concentrations also decreased significantly. We attribute these findings to an expansion of the extracellular fluid space and an increase in the number of IL-2 target cells during the treatment.

## Introduction

Interleukin-2 (IL-2), a lymphokine produced by T-cells, has been shown to induce objective regression of metastatic tumors, mainly in patients with hypernephroma and melanoma [8, 9]. It works principally by inducing the formation of LAK (lymphokine-activated killer) cells, defined as cells capable of lysing natural killer (NK)-resistant, fresh human tumor cells [4]. The LAK cells induced in vivo can be expanded ex vivo in the presence of IL-2 and then reinfused to the host.

Induction of cancer regression by such a manipulation of host defenses has demonstrated that immunotherapy can be an effective approach in the management of neoplastic diseases. However, adoptive immunotherapy with IL-2 and LAK cells requires the administration of high doses of IL-2 that are associated with considerable toxicity [8, 9]. Side effects are mainly the consequences of a capillary leak syndrome that leads to hypotension, water retention, low diuresis, renal failure, pulmonary oedema and acute respiratory disease (ARDS). IL-2 therapy will have a future only if toxicity can be overcome.

From this point of view, we studied the pharmacokinetics of repeated high doses of recombinant methionyl human IL-2 alanine 125 (r-met-Hu IL-2 [ala 125]) given by i.v. bolus according to the initial Rosenberg schedule. Our purpose was to determine whether repeated IL-2 administration induces a cumulative effect on serum IL-2 levels as determined by radio-immunoassay (RIA).

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## Patients and methods

The investigation was conducted according to a protocol that had been accepted by the human research committee of the hospital.

*Patients.* To be eligible for participation in the study, patients had to have a histologically proven, advanced solid malignant tumor for which standard therapy was not available. In addition, they had to be <65 years old and have a measurable lesion, an ECOG performance status of <2 and no significant persisting toxicity from prior antineoplastic therapy. Other inclusion criteria included normal haematological (WBC, >3,500/mm$^3$; platelets, >100,000/mm$^3$), renal [serum creatinine and blood urea nitrogen (BUN), <1.25 times the upper normal range value] and hepatic (serum bilirubin, <1.25 times the upper normal range value; SGOT/SGPT, <1.5 times the upper normal range value) functions. Exclusion criteria included other major abnormalities of clinical laboratory tests or clinically significant cardiac, pulmonary, neurological, hepatic, gastrointestinal, psychiatric, endocrine, haematological or infectious disease. The absence of primary or secondary CNS tumors had to be confirmed by computerised tomographic (CT) scan. The administration of corticosteroids or cytoreductive therapy was not allowed during the study, and an interval of at least 1 month was required between prior anticancer therapy and the prestudy examination. Patients had to give informed consent. Characteristics of the seven patients analysed in the present report are shown in Table 1.

*Treatment.* R-met-Hu IL-2 [ala 125] (provided by Ortho Pharmaceutical Corp., Raritan, NJ, USA) was given i.v. at a dose of 30,000 IU/kg every 8 h (15-min infusion) from day 1 to day 5, together with the oral administration of ranitidine (cimetidine for patient 2), indomethacine (25 or 50 mg t.i.d.) and, for patients 2–4, paracetamol (500 mg every

**Table 1.** Patients' characteristics

| Patient number | Age | Sex | Malignant disease | Metastatic sites |
|---|---|---|---|---|
| 1 | 32 | F | Melanoma | Skin, liver |
| 2 | 57 | M | Renal-cell carcinoma | Lung, bone |
| 3 | 37 | F | Melanoma | Liver |
| 4 | 65 | M | Melanoma | Node |
| 5 | 21 | M | Renal-cell carcinoma | Bone-adrenal |
| 6 | 59 | M | Renal-cell carcinoma | Lung |
| 7 | 50 | F | Colon cancer | Lung |

4 h). Leukapheresis was carried out daily on days 8–12, and mononuclear cells were incubated in LAK cell activation medium containing IL-2 for 3–4 days. LAK cells harvested on days 8 and 9, on day 10 and on days 11 and 12 were reinfused on days 12, 13 and 15, respectively. IL-2 was again given by the i.v. route on days 12–20 at a dose of 30,000 IU/kg every 8 h. This last dose had to be reduced to 15,000 IU/kg in patient 5 because of renal toxicity that was probably related to prior nephrectomy. IL-2 administration had to be discontinued early in four cases due to toxicity (patients 2, 4 and 6) and treatment refusal (patient 1). Characteristics of IL-2 treatment, toxic effects, water retention and renal function are shown in Table 2.

*Blood sampling and IL-2 measurement.* Venous blood samples were collected on days 1, 3, 5, 12, 15, 17 and 19 for infusions 1, 7, 13, 16, 23, 29 and 35 at the following times: 0, 7.5, 15, 20, 30, 45, 60 and 90 min and 2, 3, 4, 5, 6, 7, 8 h. Time zero was the start of the infusion. Serum IL-2 levels were determined by a specific RIA (kindly provided by IRE-Medgenix, Fleurus, Belgium). In brief, 100 µl serum sample was incubated at room temperature with 100 µl IL-2 antiserum for 18–24 h, followed by the addition of 100 µl IL-2 tagged with iodine 125 [$^{125}$I]-IL-2). After a second 4-h incubation, 1 ml anti-rabbit-gammaglobulin antiserum mixed with PEG was added to all tubes. After a further brief incubation (20 min), the tubes were centrifuged to precipitate the [$^{125}$I]-IL-2 antibody complex, which was then counted in a gamma counter. IL-2 concentrations of the samples were determined by dose interpolation from the standard curve. The assay detection limit was 1 IU/ml. Samples from each IL-2 infusion were measured in the same assay, and the interassay coefficient of variation was 3%–7%.

*Pharmacokinetic and statistical analysis.* Pharmacokinetic analysis was performed using a computer program [12]. The significance of differences between means of paired variables was analysed by the two-tailed Student's *t*-test.

## Results

Of the seven patients included in the present study, only four were treated until day 19 (Table 2). Among the latter, patient 5 developed renal insufficiency and received 50% of the total IL-2 dose from day 14 to day 19. Thus, only three patients were fully evaluable for the last part of the treatment.

**Table 2.** IL-2 treatment characteristics and toxicity

| Patient number | Doses omitted (*n*) | Last day of treatment | Reason for early discontinuation | Maximal body weight increase (%) | Maximal serum creatinine peak (mg/100 ml) |
|---|---|---|---|---|---|
| 1 | 3 | day 19 | Refusal | 32 | 1.5 |
| 2 | 14 | day 15 | Renal toxicity | 14 | 6 |
| 3 | 0 | day 19 | – | 27 | 1.7 |
| 4 | 6 | day 18 | Cardiac toxicity | 19 | 2.4 |
| 5 | 4 | day 19 | – | 17 | 2.6 |
| 6 | 21 | day 13 | Lung toxicity (ARDS) | 6 | 2.2 |
| 7 | 3 | day 19 | – | 10 | 2.3 |

ARDS, adult respiratory distress syndrome

**Table 3.** Mean serum IL-2 levels for infusions 1, 13, 16 and 29

| Time after start of IL-2 infusion (min) | Infusion 1 (day 1) | Infusion 13 (day 5) | Infusion 16 (day 12) | Infusion 29 (day 17) |
|---|---|---|---|---|
| 0 | <1 | <1 | <1 | <1 |
| 7.5 | 302 ±212 | 293 ±128 | 408 ±418 | 46 ±1.4 |
| 15 | 921 ±361 | 561 ±143 | 842 ±295 | 564 ±100 |
| 20 | 819 ±186 | 477 ±176 | 980 ±691 | 510 ±9.5 |
| 30 | 443 ±146 | 320 ±91 | 395 ±71 | 360 ±29 |
| 45 | 304 ±36 | 211 ±66 | 256 ±91 | 200 ±47 |
| 60 | 186 ±38 | 133 ±53 | 201 ±85 | 145 ±14 |
| 90 | 112 ±23 | 76 ±36 | 102 ±36 | 60 ±9.5 |
| 120 | 111 ±61 | 46 ±23 | 60 ±19 | 44 ±22 |
| 180 | 30 ±13 | 15 ±7.6 | 23 ±10 | 7.3±3.8 |
| 240 | 18 ±7.5 | 8.3±6.4 | 9.1±4.3 | 3.3±3.2 |
| 300 | 10.4±3.8 | 4.5±4.5 | 6.2±2 | 2.2±2.7 |
| 360 | 6.8±2.8 | 2.4±3.1 | 3.3±1 | <1 |
| 420 | 3.8±2.3 | 1.5±1.3 | 1.9±0.3 | <1 |
| 480 | 3.7±0.2 | <1 | 1.5±0.6 | <1 |
| Number of patients | 7 | 7 | 5 | 3 |

[a] Data represent the mean ±SD, expressed in IU/ml

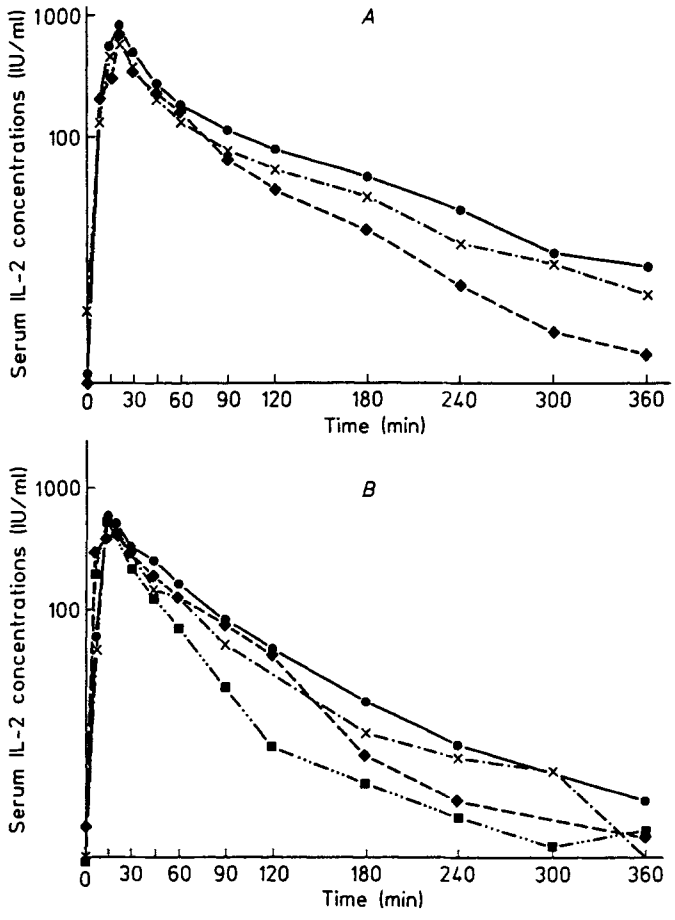


Fig. 1 A, B. Evolution of serum IL-2 concentration (semi-logarithmic scale) in patient 7. A Infusion 1 (day 1), ●——●; infusion 7 (day 3), x - - - - x; infusion 13 (day 5), ◆——◆. B Infusion 16 (day 12), ●——●; infusion 23 (day 15), x - - - - x; infusion 29 (day 17), ◆——◆; infusion 35 (day 19), ■ - - - ■

As shown in Table 3, there was a decrease in the peak serum IL-2 concentration and a more rapid return to baseline concentration in relation with the number of IL-2 doses given. On day 1, peak and trough levels were 921 ± 361 and 3.7 ± 0.2 IU/ml, respectively; these values had decreased to 561 ± 143 ($P$ <0.05) and <1.0 IU/ml by day 5. Similarly, on day 12 (just after the first infusion of LAK cells, followed by IL-2 infusion 16), peak and trough levels were 980 ± 691 and 1.5 ± 0.6 IU/ml, respectively, diminishing to 564 ± 100 and <1.0 IU/ml by day 17. Figure 1 shows an example (patient 7) of the evolution of serum IL-2 concentration curves determined for infusions 1, 7, 13, 16, 23, 29 and 35. A progressive decrease in the serum levels reached with repetition of IL-2 administration can clearly be seen.

Pharmacokinetic parameters were determined, including two half-lifes: a distribution α-half-life and an elimination β-half-life. We could not find a significant variation of the former parameter during treatment; its mean value was 16 min (range, 11–23 min).

The second phase was analysed as shown in Tables 4 and 5. Four parameters were measured. There was an increase in the distribution volume and a decrease in the half-life, the concentration at time zero and the area under the curve (AUC). Apart from the decrease in AUC between days 1 and 17, these changes did not reach statistical significance.

**Table 4.** Comparison of pharmacokinetic parameters between infusions 1 (day 1) and 13 (day 5) in 7 patients

| Pharmacokinetic parameter | Day 1 | Day 5 |
|---|---|---|
| $Vd_\beta$ (ml) | 6,000 ± 1,859 | 7,620 ± 3,550 |
| AUC (IU min ml$^{-1}$) | 37,587 ± 8,058 | 25,852 ± 8,701 |
| $t_{1/2\beta}$ (min) | 61 ± 10 | 51 ± 10 |
| $C_0$ (IU/ml) | 354 ± 114 | 300 ± 142 |

$vd_\beta$ β-distribution volume; AUC, area under the curve; $t_{1/2\beta}$, β-half-life; $C_0$, drug concentration extrapolated at time zero

**Table 5.** Comparison of pharmacokinetic parameters between infusions 1 (day 1) and 29 (day 17) in 3 patients

| Pharmacokinetic Parameter | Day 1 | Day 17 |
|---|---|---|
| $Vd_\beta$ (ml) | 5,943 ± 1,850 | 9,084 ± 4,345 |
| AUC (IU min ml$^{-1}$) | 32,643 ± 3,817 | 22,397 ± 511* |
| $t_{1/2\beta}$ (min) | 61 ± 14 | 48 ± 6 |
| $C_0$ (IU/ml) | 305 ± 90 | 220 ± 105 |

$Vd_\beta$, β-distribution volume; AUC, area under the curve; $t_{1/2\beta}$, β-half-life; $C_0$, drug concentration extrapolated at time zero
* $P = 0.05$ (Student's $t$-test)

## Discussion

We investigated the pharmacokinetics of the repeated administration of r-met-Hu IL-2 [ala 125] in seven patients. Our results show that the serum concentration, elimination half-life and AUC of IL-2 decreased with the rising number of courses given. Serum concentrations of IL-2 became undetectable 360 min after administration. The fact that the statistical tests were significant for peak serum concentration only between days 1 and 5 and for AUC only between days 1 and 17 may be explained by the small number of patients included in the study. This low accrual can be attributed to the high number of determinations of IL-2 concentrations required per patient (optimally 112 for a whole treatment and for a single patient) and to the relatively high frequency of treatment discontinuation because of life-threatening complications.

Few data on the pharmacokinetics of IL-2 are available in the literature; usually, blood levels with corresponding half-lives and concentration curves have been reported [2, 3, 6, 7, 10, 13]. Comparison between studies is made difficult by the different types of IL-2 used, by the different methods (bio-assay, RIA) of determination of serum IL-2 concentrations and by the imprecision of the reported data. To our knowledge, no pharmacokinetic analysis has yet been reported on continuous infusion of IL-2. We found an α-half-life of 16 min and a β-half-life of 61–48 min, depending on the timing of the infusion. These data are in agreement with the results reported by Lotze et al. [5], who

reported an α-distribution $T_{1/2}$ of approx. 6–7 min and a β-clearance $t_{1/2}$ of approx. 60 min, and with the study by Mitchell et al. [7], who noted a β-half-life of 60 min. The other authors report a half-life of about 30 min. We believe that our evaluation is more precise due to our use of RIA to determine the concentrations and because of the multiple doses given and the computerised analysis of serum curves.

We calculated the pharmacokinetic parameters of the drug. Our data show that high doses of r-met-Hu IL-2 given by brief i. v. infusion followed second-order kinetics, which confirms the two-compartment model described by Lotze et al. [5]. The distribution volume of IL-2 was about 6 l at the start of therapy and increased to 9 l by the end of treatment, whereas the AUC, $t_{1/2\beta}$ and decreased concentration extrapolated at time zero from about $32.10^3$ IU min $ml^{-1}$, 61 min and 303 IU/ml, respectively to corresponding values of $22.10^3$ IU min $ml^{-1}$, 48 min and 220 IU/ml. Various explanations may be given for this phenomenon.

First, the development of anti-IL-2 antibodies could have decreased the free IL-2 serum concentrations. We did not determine these antibody levels; however, if the free drug serum concentrations had indeed been decreased, the IL-2 serum concentrations on day 12 would not have increased to the levels initially measured on day 1. It has been shown that the appearance of anti-IL-2 antibodies, when it occurs, does not affect the biological activity of the drug [1]. Second, an expansion of the extracellular space takes place due to the capillary leak syndrome. This phenomenon may in part explain the increase in distribution volume and the decrease in AUC; however, the β-half-life would be expected to increase. Third, during IL-2 treatment a stimulation and proliferation of the lymphocytes occurs [11]. Such an increase in the number of target cells would indeed cause a more rapid clearance of IL-2. Finally, an increase in free, circulating IL-2 receptors shed by activated lymphocytes also could have contributed to the elimination of free IL-2. These soluble receptors were not quantified in the serum of patients tested in the present study. Our results can probably be explained by the latter hypotheses.

In conclusion, our study showed that during the repeated administration of high-dose IL-2 by brief i. v. infusion, a progressive increase in the distribution volume and a decrease in the AUC and elimination half-life of the drug occur. If these data are confirmed, the pharmacokinetic characteristics of IL-2 should be taken into account in the treatment schedule.

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