Pharmacokinetics of Recombinant Interleukin 2 in Humans

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ABSTRACT

This report summarizes the pharmacokinetics in humans of recombinant interleukin 2 (IL-2) given as an i.v. bolus, i.v. or i.p. infusion, and i.m. or s.c. injection. Immediately after an i.v. bolus the serum IL-2 level equals the dose divided by the plasma volume, in a typical human 650 units/ml for a dose of 10^6 units/m^2. The level initially decreases with a half-life of 12.9 min, followed by a slower phase with a half-life of 85 min out to 4 h after the bolus. The median steady state level during an i.v. infusion of 10^6 units/ml over 6 h is 41 units/ml. A clearance rate of approximately 120 ml/min is obtained from either the i.v. bolus or infusion data and is consistent with the renal filtration being the major route of clearance. Serum levels remain fairly constant for about 8 h after s.c. or i.m. injection but are approximately 2% of the level seen immediately after an i.v. bolus. The area under the time-concentration curve suggests that about 30% of the IL-2 activity is transported from the site of an i.m. injection to the blood. After i.p. infusion IL-2 is only slowly transported to the blood. The median serum IL-2 levels are 430-fold lower than levels in the i.p. fluid and decrease with a median half-life of 6.3 h.

INTRODUCTION

Early clinical studies using native IL-21 provided limited information on the pharmacokinetics of this protein. The small amounts of material available, compared to the sensitivity of the assay used to follow serum levels, allowed only the early part of the serum clearance curve to be measured. The rapid drop in serum level seen immediately after an i.v. bolus, which is due to transport of IL-2 from the blood into tissue, could be followed, but the second, steady state phase of elimination was beyond the sensitivity of the assay (1, 2). This early, rapid drop was sometimes interpreted as being due to receptor mediated endocytosis and degradation by T-cells, although Muhlradt and Opitz (2) concluded that this was unlikely to be the case since IL-2 pharmacokinetics was unchanged in athymic mice. The second, steady state phase can also be missed if serum levels are not followed for a sufficient length of time (3, 4). During the last 4 years more than 1000 patients have been given recombinant IL-2 to determine toxicity and efficacy against cancer and acquired immunodeficiency virus. Single doses of between 0.001 and 100 million units/m^2 body surface (MU/m^2) have been administered by four routes utilizing a number of schedules. Some pharmacokinetic data have been presented in descriptions of individual clinical trials (5, 6), and an abstract of a part of these results has appeared (7). The present report will attempt to provide a more detailed and quantitative summary of these data, compare the results obtained by different investigators using various routes, and relate the behavior of IL-2 to proteins of similar molecular size.

MATERIALS AND METHODS

Assay of IL-2 Activity. Serum or plasma was collected, frozen, and shipped on dry ice by overnight air express to Cetus Corporation, Emeryville, CA. Samples were kept at −20°C until time of assay. No difference was noted in the stability or recovery of IL-2 from plasma or serum. The bioassay (8) consists of measuring [3H]thymidine incorporation by the murine IL-2 dependent HT2 cell line. Cells are incubated in microtiter wells (10^4 cells/well) in 3-fold dilutions of the serum for approximately 18 h. One μCi of [3H]thymidine is added to each well for 3 h, the cells are harvested, and the incorporated radioactivity is measured by a liquid scintillation counter. Details of IL-2 quantitation and typical performance of the assay are discussed in the result section which follows. All activities are reported in Cetus units. One Cetus unit is equal to 3–6 biological response modifier program units or International Units.

Recombinant IL-2. The Proleukin recombinant human IL-2 used in these clinical trials was produced by Cetus. The nonglycosylated protein is 132 amino acids long, is missing the alanine present at the NH2 terminus of the native molecule, and has a serine instead of cysteine at position 125 (9). The average specific activity is 4 × 10^6 Cetus units/mg protein.

RESULTS

IL-2 Bioassay. The results of a typical assay of 2-fold serial dilutions of a standard preparation of native IL-2 isolated from the gibbon ape cell line MLA-144, are presented in Fig. 1. The log of the IL-2 concentration has been plotted on the horizontal axis. The percentage of cpm is defined as the percentage of observed [3H]thymidine incorporation compared to the asymptotic maximum observed at high IL-2 concentrations. The probit (the inverse of the cumulative normal distribution) of percentage of incorporation is plotted on the vertical axis, while the percentage scale is indicated to the right of the axis for visual comparison. The original definition of 1 unit/ml of IL-2 was the amount of IL-2 that produced half-maximal incorporation. However, in order to eliminate the variability caused by changes in the IL-2 dependent assay cells, IL-2 titers are now defined in terms of standard IL-2 preparations. The background incorporation observed in the absence of exogenous IL-2 was 0.5%; the lower line at 4 times this background is the lower useful limit for this assay. As incorporation approaches 100%, it becomes increasingly difficult to distinguish differences in IL-2 levels, and we consider 80% percent as the maximum useful upper limit. Thus, in the assay described in Fig. 1, the lower useful limit (in the actual wells containing the cells) was about 0.08, while the upper useful limit was about 4.8 units/ml, a 60-fold range. As observed by Gillis et al. (8) the log-probit plot of the incorporation results in a remarkably linear line. Cantrell and Smith (10, 11) have pointed out that this is consistent with the observed log-normal distribution of IL-2 receptors among IL-2 requiring cells. The use of the probit...
function to linearize the standard curve in this way is a convenient method to utilize the entire range of the assay. Hooton et al. (12) obtained satisfactory results using the logit function, which is very similar in shape to the probit, and is often used in the construction of standard curves for enzyme linked immunosorbent assay data. Since 3-fold serial dilutions of sera were assayed and since the range of the assay is approximately 60-fold, several serum dilutions often produced useful values for the IL-2 titer. After multiplication by the serum dilution factor and a weighting factor, these values were averaged to produce the final serum titer. The weighting factor used was zero at the two extremes of the useful range and increased linearly (in probit space) to 1.0 at the midpoint. The factor which usually limited the sensitivity of the assay for the purposes of this study was toxicity of the human serum to the assay cells. There have been several reports in the literature of "IL-2 inhibitors" in serum; however, the pattern of toxicity observed by us was always consistent with a direct effect on the cells, not on the IL-2. Serum at modest dilutions, e.g., 3- or 9-fold, often reduced [³H]thymidine incorporation to a level much lower than the background seen without any added IL-2, and even massive amounts of IL-2, e.g., 10,000 units/ml, had but modest effect in reversing this toxicity. The toxicity could usually be reduced 10-fold by heat inactivating the serum to 50°C for 30 min. Our observations are thus consistent with those of Pruett and Lackey (13), who concluded that this toxicity was mediated by antibody and complement action on the cells. The temperature and time of heating were critical, inasmuch as a lower temperature or time was ineffectual in eliminating the toxicity, while a higher temperature or time could inactivate the IL-2 by a factor of >2. In each assay, a known amount of IL-2 was added to preinjection serum, and series in which more than a 2-fold inactivation due to the heat treatment was seen were not used in these analyses. In addition, the incorporation among a dilution series was scanned and wells in the series were ignored if the incorporation was decreased (due to toxicity) by an increase in serum concentration.

In Vitro Stability of IL-2 in Whole Blood. Blood was drawn from a normal volunteer into a heparinized tube and used for this experiment within 2 h. IL-2 was added to achieve a concentration of about 3 units/ml and the blood was divided into aliquots in siliconized polyethylene tubes (1.5 ml; Eppendorf) and placed at 4°C or 37°C. The tubes held at 37°C were in a roller bottle incubator, rotating slowly to keep cells in suspension. At the times indicated in Fig. 2, tubes were removed, plasma was obtained by centrifugation, and the IL-2 activity was determined in quadruplicate. The means ± SD of the activities are plotted in Fig. 2.

The activity decreased by a little less than a factor of 2 in 20 h at 37°C. At 4°C the activity has decreased even less, and at higher concentrations the percentage of decrease in activity is even lower at both temperatures (data not shown). The decrease appears to be progressive, or at least there is not a disproportionally rapid drop in activity in the first 5 h. We thus have some confidence that modest variations in the preparation and storage of clinical samples will have only small effects on the measured levels of IL-2 activity and that the clearance of IL-2 activity from blood of the patient is the result of interaction of IL-2 with tissues other than the blood itself. In an earlier study, Paetkau et al. (14) showed that the biological activity of native, murine IL-2 had a half-life of at least 2 h at 37°C in murine blood.

Serum Levels after an i.v. Bolus. The clearance of IL-2 from the serum of a patient receiving 1.0 MU/m² by an i.v. bolus is presented as a semilog plot in Fig. 3. A curve representing the sum of two decreasing exponentials,

\[ Y = A \exp(-t/t_1) + B \exp(-t/t_2) \]

where \( t \) is time (min) after bolus, \( A \) is the magnitude of the fast component, \( t_1 \) is the characteristic time of fast component (half-life, \( T_T = \log2/t_1 = 0.69/t_1 \)), and \( B, t_2 \) are the corresponding parameters for the slow component, has been fitted to the data so that the square of the deviation of the curve and the data in this plot, i.e., \( \log(\text{curve}) - \log(\text{observed}) \)², is a minimum (the assay utilizes serial dilutions; thus errors are proportional to a percentage of the observed value). The curve is seen to be a reasonable representation of the observed IL-2 titers, at least over the time period studied.

The curve fitting process was repeated for 51 patients receiving doses between 0.25 and 14 MU/m², with the serum levels...
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Fig. 3. IL-2 serum clearance after an i.v. bolus. A dose of 1.0 MU/m² was given as a 5-min i.v. bolus. Serum samples were taken and the IL-2 bioactivity was determined. A biexponential curve has been fitted to the assay values, minimizing the sum of the squares of the percentage of deviation of the curve from the data.

Table 1 Summary of i.v. bolus pharmacokinetic parameters

This summary was prepared from data obtained from 52 patients given a 5-min i.v. bolus injection of from 0.25 to 14 MU/m². Thirteen serum samples were taken at times up to 4 h after the end of the bolus. The serum levels were normalized to dose, and a biexponential curve was fitted. A and B are the sizes of the fast and slow exponentially decaying terms, with half-lives of $T_a$ and $T_b$, respectively. $V_c$ is the apparent volume of the central compartment (the volume in which the IL-2 is diluted immediately after injection), while $V_m$ is the apparent volume of distribution at steady state. AUC is the area under the normalized serum curve, and $Cl$ is the clearance. The three rate constants $K_{12}$, $K_{31}$, and $K_{10}$ are those of the pharmacokinetic model in Fig. 10. Means ± SD are given only for the parameters determined directly from the data.

<table>
<thead>
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<th>Parameter</th>
<th>Units</th>
<th>Median</th>
<th>Mean ± SD</th>
</tr>
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<tr>
<td>Dose</td>
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</tr>
<tr>
<td>$A + B$/dose</td>
<td>(units/ml)/dose</td>
<td>400</td>
<td>540 ± 490</td>
</tr>
<tr>
<td>$B/(A + B)$</td>
<td>%</td>
<td>13.4</td>
<td>18.1 ± 14.2</td>
</tr>
<tr>
<td>$V_c$</td>
<td>ml</td>
<td>4,300</td>
<td></td>
</tr>
<tr>
<td>$V_m$</td>
<td>ml</td>
<td>7,900</td>
<td></td>
</tr>
<tr>
<td>$T_a$</td>
<td>min</td>
<td>12.9</td>
<td>13.8 ± 7.7</td>
</tr>
<tr>
<td>$T_b$</td>
<td>min</td>
<td>85</td>
<td>86 ± 34</td>
</tr>
<tr>
<td>AUC</td>
<td>(units/ml)min/dose</td>
<td>14,500</td>
<td>18,200 ± 15,900</td>
</tr>
<tr>
<td>$Cl$</td>
<td>ml/min</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>$K_{12}$</td>
<td>L/min</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>$K_{31}$</td>
<td>L/min</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>$K_{10}$</td>
<td>L/min</td>
<td>0.031</td>
<td></td>
</tr>
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</table>

normalized to a dose of 1.0 MU/m². A summary of values for the four parameters describing the 52 clearance curves is presented in Table 1. The three rate constants for a standard two compartment pharmacokinetic model were calculated from the median values of these parameters and are also presented in Table 1 and Fig. 10.

There was no significant correlation between the dose level and the two half-lives or between the dose level and the ratio $A/B$ (data not shown). However, there is a slight correlation (coefficient, 0.5) between dose level and dose level normalized $A + B$ (the normalized serum level extrapolated back to $t = 0$). This relationship is seen in Fig. 4. The level to be expected if there were no drop rapid enough to be hidden by the time necessary to sample the blood would be 650 units/ml, since a typical human with a body surface of 1.7 m² has a plasma volume of about 2600 ml. The intercepts at doses above 6 MU/m² are close to this value, while the intercepts seen below this dose level are typically 2–3-fold lower. The integral of the serum IL-2 level over time (AUC), normalized to the dose, is an important pharmacokinetic parameter in a system where elimination is only from the blood compartment, since its reciprocal is proportional to the rate of clearance from the blood. Most of the variation in the dose normalized integral is due to variation in the intercept at $t = 0$. This can be seen from the scatter diagram (Fig. 5), where the integral and the intercept are found to be approximately proportional to each other, with a correlation coefficient of 0.9.

Only a very limited amount of data are available at times later than 4 h after the bolus. However, the few series going past 4 h all indicate that extrapolation of the curves fitted to data to 4 h out to later times produces an underestimate of the actual IL-2 level. An example of this is seen in Fig. 6, where a measurement at 8 h and the curve fitted to all the points (heavy line) is compared to the curve fitted to data from the first 4 h (thin line).

i.v. Infusion. The serum IL-2 levels seen in patients receiving i.v. infusions at a constant rate for 6 h are listed in Table 2, and data from a typical patient are presented in Fig. 7. The steady state level appears to be reached by 2 h and, as would be expected from the i.v. bolus results, falls off rapidly after the infusion has been halted. The dose range covers only a factor of 2 in this group, but the serum levels seen after i.v. bolus injections suggest that the steady state level should be linearly proportional to dose. The median steady state level was found to be 41 units/ml for an infusion rate of 1 MU/m² over 6 h, (4700 units/min). At a typical plasma volume of 2600 ml, this infusion rate corresponds to 1.8 units/min/ml of plasma. Thus the steady state elimination rate from the central plasma com-

Fig. 4. Normalized initial IL-2 serum level, $(A + B)/$dose level, after i.v. bolus administration, versus dose level. The dose level normalized intercept of the fitted curve at $t = 0$ has been plotted versus dose level for each of 52 patients. The normalized IL-2 level expected if the total dose is initially distributed in the blood plasma is 650 units/ml (---).
Fig. 5. Normalized initial IL-2 serum level versus normalized area under clearance curve. The normalized AUC is seen to be highly correlated with the dose level at $t = 0$. Thus a major part of the variation in the normalized AUC values for these 52 patients is due to variation in initial levels.

Fig. 6. Biexponential curves fitted to data extending to 4 or 8 h after the i.v. bolus. Serum levels at 8 h were available for a few of the 52 patients summarized in Table 1. As shown in this representative patient, the biexponential curve fitted to data for the first 4 h (---), underestimates the level found at 8 h and line fit to all data (•••).

Table 2 Steady state serum levels during i.v. infusions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum Level (units/ml)</th>
<th>Mean units/ml</th>
<th>Mean/dose level</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Dose level (MU/m²/6 h)</td>
<td>2 h</td>
<td>4 h</td>
</tr>
<tr>
<td>14</td>
<td>1.0</td>
<td>44</td>
<td>66</td>
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<td>1</td>
<td>1.0</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>16</td>
<td>1.0</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>17</td>
<td>2.0</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>19</td>
<td>2.0</td>
<td>ND</td>
<td>114</td>
</tr>
<tr>
<td>23</td>
<td>1.0</td>
<td>48</td>
<td>54</td>
</tr>
<tr>
<td>25</td>
<td>1.0</td>
<td>20</td>
<td>24</td>
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<tr>
<td></td>
<td>1.0</td>
<td>48</td>
<td>44</td>
</tr>
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</table>

Median 40.5  
Minimum 20.0  
Maximum 65.3  
Mean ± SD 39.2 ± 13.8

Fig. 7. Serum levels during and after a 6-h i.v. infusion. This patient received 1 MU/m² over 6 h, and the steady state level of about 28 units/ml is close to the dose normalized median level seen in all patients (Table 2). Since the first blood sample was taken 60 min after the start of the infusion, the rising phase of the curve was not accurately determined, and the curve seen here is somewhat symbolic of the actual time course expected.

The IL-2 levels seen 2, 4, and 6 h after the start of infusion show no consistent pattern of increasing or decreasing, and thus all are used to define the mean steady state level. ND, not determined.
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Table 3 Peak serum levels after i.m. injections and comparisons of areas under i.m. and i.v. clearance curves

Serum levels were determined in all patients at 30, 60, 90, 120, 180, and 240 min and at higher doses also at 360, 480, 720, and 1440 min after the injection. As can be seen from the entries in the Peak time column, the peak levels were often not reached until several h after the injection and had not decreased much, if any, from the peak level by 240 min. Thus, estimates for the final half-life and the AUC were not possible except for those patients receiving the larger doses.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose level (MU/m²)</th>
<th>Last time point (min)</th>
<th>Peak time (min)</th>
<th>Peak level (U/ml)</th>
<th>Peak/dose</th>
<th>AUC i.m./dose</th>
<th>AUC i.v./dose</th>
<th>AUC i.m./AUC i.v.</th>
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<td>16</td>
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<td>160.0</td>
<td>40.0</td>
<td>10.4</td>
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</tr>
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</table>

Mean ± SD 0.37 ± 0.20

Fig. 8. Serum levels after an i.v. bolus and an i.m. injection of 1 MU/m².

Serum levels, occurring 90 to 240 min after IL-2 administration, ranged from 1.3 to 160 units/ml. Serum levels in patients receiving less than 0.25 MU/m² by the i.m. route were mostly below the sensitivity of our assay, 0.3 unit/ml.

As would be expected, peak levels are approximately proportional to dose, and the peak level divided by dose level is presented in Table 3, Column 5. However, as was the case for the i.v. bolus data, even these dose normalized peak levels have a tendency to be higher with higher doses (correlation coefficient, 0.5).

Data out to 600 min or longer was available from 8 patients receiving IL-2 i.m. It was possible in these cases to calculate an estimate for the areas under the activity-time curves. This was done using the trapezoidal rule, estimating the area after the last time point by a linear (on a semilog plot) extrapolation of the last two time points (this extrapolated area constituted less than 15% of the total). These estimates are presented in Table 3, Column 7. The median value of 7900 units/min/ml for the dose normalized area is about one-half of the median value of 14,500 for this parameter seen in Table 1 for the i.v. bolus injections.

In addition, pharmacokinetic data after i.v. bolus administration are available in 4 of the 8 patients receiving i.m. injections. The dose normalized areas for these clearance curves are presented in Table 3, Column 8. The ratios of i.m. to i.v. areas (Table 3, Column 9) are less than 1.0 in all 4 cases, with a median of 0.34. This ratio would suggest that only 34% of the IL-2 injected i.m. is transported to the serum. However, to the extent that the AUC after an i.v. bolus is not completely proportional to dose level, as seen in Fig. 5, the clearance rate may be dependent on serum IL-2 concentration. Such a concentration dependence would make uncertain the interpretation of the ratio of AUC for two routes of administration as defining the ratio of bioavailability by the two routes.

s.c. Injection. The data on serum levels seen after s.c. administration of IL-2 (as a single agent) are somewhat limited; however, serum levels for 6 patients are presented in Table 4. The times to peak level of 120 to 360 min are in the range seen after i.m. administration, and the median peak level of 10.7 units/ml (normalized to a dose level of 1 MU/m²) is essentially the same as the median value of 14 units/ml reported for the i.m. route.

i.p. Infusion. IL-2 has been administered to patients with ovarian cancer as a 30-min i.p. infusion of 500 ml. The initial dose was 1 MU/m², which if well tolerated, was increased to 10 MU/m² in the same volume. Data on activity levels in the i.p. fluid and serum were obtained during 8 infusions of 6 patients, although the data were only partially complete in the case of three infusions. A summary of the data from all infusions is presented in Table 5, while the time courses of IL-2 levels for 24 h after the infusion in one patient are seen in Fig. 9.

The most striking characteristic of these kinetics is the long half-life of IL-2 in both peritoneal fluid and serum, medians of 22 and 6.3 h, respectively, and the high ratio of activity in the
peritoneal fluid to serum, the median being 430. In many cases the values reported here for the half-lives must be taken as first approximations or lower limits, since the IL-2 activity decreases by only a small factor over the period of observation [the decrease in activity seen in Fig. 9 is actually the most rapid seen among this group of patients (6)]. In addition, the total volume of fluid in the peritoneal cavity may well change during 24 h, so that changes in peritoneal IL-2 concentration may not give an accurate picture of the amount of IL-2 leaving the cavity. However, even with these limitations on the precise accuracy of the parameters listed in Table 5, the general pattern is clear. IL-2 is being transported slowly (relative to the rate of elimination from the blood) from the peritoneal cavity into the blood, so that the rate at which the serum level decreases mainly reflects depletion of IL-2 from the peritoneal cavity. Under these conditions, when the serum IL-2 level is almost at a steady state, the kinetics is analogous to an almost constant i.v. elimination from the blood) from the peritoneal cavity into the blood.

where \( C_4 \), \( V_4 \), and \( K_{s} \) are IL-2 concentration, fluid volume, and rate of IL-2 transport from the peritoneal cavity, and \( C_1 \), \( V_1 \), and \( K_{o} \) are the corresponding parameters for the plasma (using the notation of Fig. 10). Using 500 and 2600 ml for \( V_4 \) and \( V_1 \), 430 for \( C_4/C_1 \), and 0.045 min\(^{-1} \) for \( K_{o} \) (from i.v. infusion data) we obtain \( K_{s} = 5.4 \times 10^{-4} \text{ min}^{-1} \).

In most patients serum IL-2 levels did not decrease sufficiently during the time they were followed to allow a half-life, and therefore the area under the clearance curve, to be estimated. However, for the data presented in Fig. 9, the area under the serum clearance curve can be calculated and was found to be 28,900 units min/ml. Since the dose was 10 MU/m\(^2\), the dose normalized AUC would be 2,800 which is actually twice the median AUC observed after i.v. bolus administration (Table 1).

Summary of Values of Pharmacokinetic Parameters. In Fig. 10 we have summarized the routes of IL-2 administration described in this report, and the values of first order rate constants which are consistent with observed serum levels and a two compartment model. Defining these rate constants assumes the transport process to be first order in concentration, which certainly may be an approximation, particularly for transport from the site of an i.m. injection or from the peritoneal fluid. The description of interstitial fluid as one compartment is of course a great simplification, but the inaccuracy of the IL-2 bioassay and the somewhat limited time scale over which levels were measured obscure the presence of multiple exponential curves that would most likely be needed to fit accurate data.

### DISCUSSION

In this report we have presented and summarized data from several clinical trials and institutions on the biological activity of IL-2 in serum after administration of recombinant IL-2 by bolus i.v., i.m., and s.c. injections and i.v. and i.p. infusions. The fundamental pharmacokinetic parameters are best determined from i.v. bolus and infusion data. After an i.v. bolus the serum IL-2 activity curve from 0 to 240 min after injection is concave upward when the log of activity is plotted versus time and can be approximated by the sum of two exponentially decaying components. In the dose range of 0.25 to 14 MU/m\(^2\), the median half-life for the fast component, representing initially 86.6% of the total clearance curve, was 12.9 min, and the median half-life of the slow component was 85 min.

The pharmacokinetics of IL-2 is approximately linear, in the sense that serum levels are linearly proportional to dose, while the shape of the serum clearance curve is independent of dose. However, the dose normalized initial serum levels do decrease by a factor of 3 from the highest to lowest dose. The level expected immediately after i.v. injection is the total dose divided by the plasma volume, and this level is seen at the higher doses. The failure of the fitted curves to extrapolate to this value at the lower doses could be due to an unobserved, initial, rapid drop in IL-2 level, present only for these low doses. However,
PHARMACOKINETICS OF IL-2

over 30 min. Data from all patients studied are found in Table S.

concentrations were very much higher in these locations compared to the plasma

K\textsubscript{io} = 5.4 \times 10^{-3} \text{ min}^{-1} since serum IL-2

The apparent steady state volume of distribution determined from

s.c. injection, it enters the plasma compartment directly. After i.m. or

as an i.v. bolus or infusion enters the plasma compartment directly. After i.m. or

here only because the indicated transport rates were determined while the IL-2

levels after i.p. infusions are 43 times lower than IL-2 levels in the i.p. fluid and

times the plasma volume (15). this suggests that the concentration of IL-2 in the

ratios of plasma to i.p. volumes is 2600/500 = 5.2 (see text). Transport from

Fig. 9. Serum levels in serum and in peritoneal fluid after i.p. infusion. Serum

and i.p. levels of IL-2 for a 24-period in a patient receiving 10 MU/m\textsuperscript{2} in 500 ml

over 30 min. Data from all patients studied are found in Table 5.

Fig. 10. Summary of pharmacokinetic parameters values. IL-2 administered

as an i.v. bolus or infusion enters the plasma compartment directly. After i.m. or

s.c. injection, it enters the plasma from the injection site, C3, and after i.p.

infusion it enters the plasma from the peritoneal cavity, C4. We assume here that

IL-2 is eliminated only from the plasma compartment. The rate constants, K\textsubscript{ip},

indicate the fraction of IL-2 in compartment I transported to compartment J per

min. K\textsubscript{io} = 0.031 \text{ min}^{-1} from i.v. bolus data, using a two component model with

an apparent initial volume of distribution of 4300 ml; 0.045 \text{ min}^{-1} from steady

state levels during 6-h i.v. infusions, assuming an initial volume of distribution

equal to a typical plasma volume of 2.600 ml. K\textsubscript{3i} = 0.017 \text{ min}^{-1} and K\textsubscript{2i} = 0.014

\text{ min}^{-1} using i.v. bolus data. Since the volume of the interstitial fluid is 3 to 7

times the plasma volume (15), this suggests that the concentration of IL-2 in the

interstitial fluid is considerably less than is seen in the plasma at steady state.

The fact that the apparent steady state volume of distribution determined from

the bolus data is only 7900 ml, about twice instead of 4 to 8 times the plasma

volume, is an alternate way of reporting the same calculation. K\textsubscript{3i} = 6.2 \times 10^{-4}

\text{ min}^{-1} since peak levels after i.m. or s.c. injections are about 2% of the initial level

seen immediately after an i.v. bolus. K\textsubscript{2i} = 5.4 \times 10^{-4} \text{ min}^{-1} since serum IL-2

levels after i.p. infusions are 430 times lower than IL-2 levels in the i.p. fluid and

the ratio of plasma to i.p. volumes is 2600/500 = 5.2 (see text). Transport from

the site of injection and from the peritoneal cavity is indicated as unidirectional

here only because the indicated transport rates were determined while the IL-2

concentrations were very much higher in these locations compared to the plasma

and thus transport was essentially in one direction.

The biexponential curve fit to observed serum levels after an

i.v. bolus is useful for interpolation between serum sampling

times and as a device for generating summary phenomenologi-

cal parameters. However, the use of these parameters to calcu-

late rate constants of a two compartment pharmacokinetic

model, with the blood plasma being the central compartment

and the total body interstitial fluid being the second compart-

ment, can be misleading. The uncertainty in the bioassay and

the resulting scatter in activity measurements means that the

fit of the biexponential curve to the data is not a very stringent

test of the model, and more accurate measurements might well

require a larger number of terms in the fitting equation. In

particular, the modest accuracy of the data results in its being

a poor indicator of pharmacokinetic structures with time con-

stants comparable to or longer than the 240 min to which the

data extend. Thus, when using model parameters to compare

different clearance curves, it is essential that the parameters

come from data covering the same time span. The fact that the

limited data available at 480 min are all higher than the "predicted" values using curves fitted to only the earlier time points

is not surprising and reveals the dangers of extrapolation of

this type of model. A potential danger of associating a specific

compartmental model with the data could be the temptation to

view the model as a real structure which could be used for

prediction at times far outside the range used to construct the

model.

One of the assumptions implicit in the interpretation of a

multiexponential curve as a compartment model is that the

injected material is a single, homogeneous species and that the

assay used to produce the clearance curve is sensitive only to

this species. Most heterogeneities in the protein or complica-
tions due to the sensitivity of the assay to protein fragments

would generate clearance curves that are convex upward, since

the rapidly cleared or inactivated species are by definition

eliminated earlier than more stable species. Any curvature due
to heterogeneity would be added to that due to compartments.

The median area under the i.v. bolus clearance curves (AUC)

was 14,500 min units/ml, normalized to a dose of 1 MU/m\textsuperscript{2}. The clearance

(dose/AUC) for an average human with a surface area of 1.7 m\textsuperscript{2} would thus be

117 ml/min. Donohue and Rosenberg (3) have shown in mice that the kidney is the major

organ of elimination for IL-2. While the fractional filtration

rate of a protein is not completely determined by its molecular

weight, a protein the size of IL-2 (assuming that the hydrody-
namic size is approximately that predicted by its covalent

structure) would be expected to be only slightly retained by the

kidney (16). Since a typical kidney filtration rate in humans is

130 ml/min (17), the observed clearance rate is consistent with

kidney filtration being the major mechanism for removal of IL-

2.

The most relevant data in the literature to compare with

results presented here are those reported by Lotze et al. (18).

The IL-2 used by them was the same material used in clinical

trials described in the present paper, and serum levels were

followed by an essentially identical biological assay. The initial

and final half-lives were reported in the text to be 6.5 and 60

min. The shorter half-lives observed by Lotze et al. results in a

large value for the elimination rate constant, K\textsubscript{io}, of 0.10 \text{ min}^{-1},

compared to our value of 0.031 \text{ min}^{-1}. While a second half-life
of 60 min is consistent with their data out to 240 min, the last two data points in Fig. 3 of Lotze et al., at 4 and 24 h, define a half-life of about 260 min. Thus, they also observe that the “final” half-life becomes longer as the data extends to longer times. They find as we do that half-lives are independent but absolute levels are proportional to dose.

Lotze et al. (18) also determined the steady state IL-2 serum levels during i.v. infusions of 3000 units/kg/h. Assuming a body weight of 70 kg and surface area of 1.7 m², this would correspond to an infusion rate of 0.74 MU/m² over a 6-h period. Our experience with i.v. infusions, summarized in Table 2, would predict a serum level of 30 units/ml for this infusion rate, which is about 4-fold higher than the “5 to 10 units/ml” reported by Lotze et al. (18).

In a recent report Gustavson et al. (19) describe the pharmacokinetics of another recombinant human IL-2 (Teceleukin) after 2- and 24-h i.v. infusions and s.c. administration. The clearance rates for the two infusion times were similar but were 2-3 times higher for the lower doses compared to the higher doses (Ref. 19, Tables 3 and 4). This apparent increase in clearance at lower doses is consistent with our observation that the dose normalized initial level and AUC after an i.v. bolus are lower at low doses (Figs. 4 and 5). The mean of the clearance rates reported in Tables 3 and 4 of Gustavson et al. was 6.20 liters/h, or 103 ml/min, which is very close to the value of 117 ml/min reported by us in Table 1. The peak level of 40.8 units/ml seen by Gustavson et al. after s.c. administration of a total dose of 3 MU is about 4 times the level reported here in Table 4 for a dose about one-half as large, 1 MU/m².

The pharmacokinetics of native IL-2 produced by the Jurkat cell line has been described by Lotze et al. (20). After an i.v. bolus they report an initial half-life of 5-7 min and a final half-life of 30-120 min, with data presented in a figure extending to 500 min, and the initial fast component representing about 90% of the total curve. This pattern is similar to their results with recombinant IL-2 and to the results presented here. However, they report a serum level of 20 units/ml after 6 h in a patient receiving 1 MU of IL-2 over 24 h, which is about 7 times higher than one would predict from their experience, and twice the level expected from our experience with i.v. infusions of recombinant IL-2.

Siegel et al. (21) presented data on serum levels after a 2-h i.v. infusion of 0.25 MU of IL-2. Two patients received 0.25 MU of recombinant IL-2 and three patients received the same amount of lymphocyte-derived IL-2. The biphasic nature of the curve was discussed by these authors, who concluded as we do that it represents evidence for a multicompartment pharmacokinetic model. The mean levels seen at the end of the infusions were about 4 units/ml for both types of IL-2. An infusion rate of 0.25 MU over 2 h corresponds to about 0.44 MU/m² over 6 h; thus we would have predicted a steady state level of 18 units/ml from our data in Table 2. Thus our observed levels are approximately 4-fold higher than those seen by Siegel et al. (21). These authors observed a faster decay in IL-2 levels after the end of infusion with recombinant IL-2 as compared to native material, which would suggest a real difference in the two IL-2 preparations if the pattern were confirmed in a larger number of patients.

Reports on the i.v. bolus pharmacokinetics of IL-2 can be misleading by implying that there is only one half-life, emphasizing or only describing the first, short one (3, 4, 22, 23). In discussions of the implications of the pharmacokinetics of IL-2 to its therapeutic effectiveness, it should be noted that the rapid initial drop in serum concentration of a drug exhibiting biphase clearance is conventionally ascribed to transport of the drug from the serum into the interstitial space, where it is often most effective. However, even the steady state half-life of 85 min, using our data to 240 min, may seem too short for ideal clinical effect. While the availability of recombinant IL-2 in large amounts should allow high serum levels to be maintained by merely increasing the dose, it may be thought to be undesirable to have such high peak levels immediately after the i.v. bolus. However, the data on i.v. infusions presented here and by Lotze et al. (20) demonstrate that it is possible to maintain predictable and constant IL-2 serum levels. The i.m., s.c., and i.p. routes also may be promising in this regard. Finally, pharmacokinetics of recently developed polyethylene glycol derivatives of IL-2 shows greatly extended steady state half-lives (24, 25). The fact that the curve of clearance rate versus hydrodynamic size of the IL-2 derivative has an inflection at the size of an 80-kDa protein (25) is consistent with renal filtration being a major route of elimination.

Data on serum levels after i.v. bolus injections of small doses of native IL-2 have been reported by Bindon et al. (1). The amounts injected varied between 5,000 and 67,000 units total, which resulted, as would be expected, in serum levels in the range of 1 to 10 units/ml immediately after injection. Since the assay sensitivity was about 0.2 unit/ml, only a hint of a second phase could be seen. The authors had expected IL-2 levels to fall to a steady state level equal to the dose divided by plasma plus interstitial fluid volume. This pattern would not be expected for a protein the size of IL-2 that was being efficiently filtered by the kidney and is not the pattern seen by us and others using larger doses of IL-2. However, the pharmacokinetics of very low doses of IL-2 may well be different than those of larger doses, in that saturable pathways, such as binding to minor serum proteins, receptors, and internalization might become significant or even dominate. Low doses of 15S labeled IL-2 were administered to mice by Koths and Halenbeck (26, 27) who reported a biphase clearance of bioactivity and radioactivity from the serum, and saturable binding to serum proteins to produce complexes 350 kDa and larger. These authors also reported a large fraction of radioactivity in the cortical region of the kidney by 20 min after injection, consistent with renal filtration of IL-2.

Administration of IL-2 could itself produce changes which would alter the pharmacokinetics of IL-2. Proliferation of lymphocytes and increases in the density of receptors on their surface should increase the receptor mediated metabolism of IL-2 (28). Renal function appears to be depressed after several days of administration of high doses of IL-2 (29). In some cases IL-2 induces the formation of antibodies in patients (30), and the pharmacokinetics of IL-2 in immune complexes could well be different than that of IL-2 in normal serum. The presence of neutralizing antibodies could mask the biological activity of circulating IL-2 (31). High doses of IL-2 appear to increase capillary permeability (32), and this could also alter its pharmacokinetics.

Detectable (0.1-1 unit/ml) IL-2 serum levels are not normally seen in human serum in the absence of administration of exogenous IL-2. Thus, endogenous IL-2 produced during modest immunological responses must act locally, presumably providing a kind of immunological cell-cell communication. However, a mean IL-2 level of 42 units/ml was seen in a group of 25 patients with chronic, progressive multiple sclerosis, a disease thought to be autoimmune (33). A medium level of about 15 units/ml was observed in the serum of 18 patients suffering from the autoimmune disease systemic lupus erythematosus.
(34), and a mean IL-2 serum level of 79 ng/ml (about 600 units/ml) was reported in a group of 17 patients experiencing acute renal transplant rejections (35). Additional investigation will be needed to determine if these endogenous IL-2 levels are merely symptoms of an intense and pathological inflammatory response (perhaps useful in its diagnosis), or if they participate in its generation.

While any model of the “micropharmacokinetics” important to normal cellular immunological events would be different from the compartmental models considered in this report, $K_{10}$ provides an estimate of the upper limit of the amount of IL-2 that must be removed from plasma to keep the systemic concentration below 0.3 unit/ml. Using $K_{10}$ determined by the 6-h infusion data, (0.3 units/ml) (2600 ml) (0.045/min) = 35 units/min. Of course more IL-2 could be produced by the immune system as long as it was removed by receptor mediated or some other local mechanism. On the order of 3,500–70,000 units/ml of IL-2 must be produced to obtain the serum levels of 30–600 units/ml seen in the autoimmune situations discussed above.

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Pharmacokinetics of Recombinant Interleukin 2 in Humans

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