Schedule Dependency of the Antitumor Activity and Toxicity of Polyethylene Glycol-modified Interleukin 2 in Murine Tumor Models

Robert J. Zimmerman,1 Sharon Lea Aukerman, Nandini V. Katre, Jeffrey L. Winkelhake, and John D. Young

Department of Pharmacology [R. J. Z., S. L. A., J. L. W., J. D. Y.] and Department of Process and Product Development [N. V. K.], Cetus Corporation, Emeryville, California 94608

Abstract

Modification of recombinant human interleukin 2 (rhIL-2) with monomethoxy polyethylene glycol has been shown to alter its pharmacokinetic properties. Therefore, we investigated the pharmacological parameters of schedule and dose in order to assess the impact of the in vivo antitumor activity of this modification. The antitumor efficacy, as well as the toxicity, of polyethylene glycol-interleukin 2 (PEG-IL-2) was compared to that of rhIL-2 in three transplantable syngeneic murine tumor models, Meth A fibrosarcoma, B16 melanoma, and Pan-02 pancreatic carcinoma. At equitoxic dose levels, the antitumor activity of PEG-IL-2 was far superior to that of rhIL-2 in all three tumor models. This efficacy of PEG-IL-2 was dose dependent and was greatest on a Q7D x 2 schedule in Meth A and B16. When the same total doses were further divided and delivered on any of several alternative schedules, either the efficacy was reduced or the toxicity of the treatments was increased. In Pan-02, a rhIL-2-resistant tumor, PEG-IL-2 treatment on either the Q7D x 2, Q4D x 3, or Q3D x 4 schedule resulted in approximately a 200% increase in lifespan; however, the toxicity of the treatment increased as the interval between doses was shortened. Simulations of the pharmacokinetic profiles of these various regimens suggested that the toxicity of PEG-IL-2 and rhIL-2 was related to the minimum plasma concentration that was obtained and the time interval between peak levels. The efficacy of the treatment was associated with the interleukin 2 plasma peak height, since a dose response was observed; however, peak plasma concentration did not appear to be the only parameter which determined efficacy. We hypothesize that this observed schedule dependence is also affected by the kinetics of the host's biological response to rhIL-2.

Introduction

The therapeutic activity of rhIL-21 has been investigated in preclinical murine tumor models by a number of investigators (Refs. 3–12 and references therein). The clinical activity of this lymphokine has been demonstrated both as a single agent and in combination with lymphokine-activated killer cells (Refs. 13–19 and references therein). Modification of rhIL-2 with monomethoxy PEG produces a biologically active rhIL-2 with enhanced solubility and an altered pharmacokinetic profile (1). The in vivo activity of a M, 95,000 PEG-IL-2 species administered daily i.p. was investigated in the Meth A model and was shown to be enhanced in terms of both efficacy and toxicity (2). In order to more thoroughly study the influence of PEG modification on the bioactivity of rhIL-2, a series of PEG-IL-2 species have been produced and the in vivo biological activity of some of these compounds has been characterized. The degree of modification affects the plasma half-life of PEG-IL-2 (1); therefore, we hypothesized that these changes in clearance would also have an impact on the choice of the in vivo treatment schedule which would result in the greatest efficacy and least toxicity. The present series of studies was conducted using rhIL-2 modified to an average of 2 to 3 molecules of M, 7,000 PEG/rhIL-2 molecule, which produced a PEG-IL-2 species with an apparent average molecular weight of about 160,000 by size exclusion chromatography. The pharmacokinetic data suggested that the optimal treatment schedule for PEG-IL-2 would be one with less frequent administration than the schedule for rhIL-2, for example once a week compared to daily dosing. The tumor model data showed that the efficacy obtainable with PEG-IL-2 was superior to that of rhIL-2, and this improvement in efficacy was not associated with an increase in toxicity as judged by lethality.

Materials and Methods

Animals. Specific pathogen-free, 18–20 g, female BALB/c, C57BL/6, and BDF1, mice were obtained from Charles River Laboratories (Wilmington, MA). Mice were weight matched, ear notched, and randomized at the beginning of each experiment. The details of our animal husbandry conditions have been described (7).

Tumor Cell Lines. B16W10, a subline of B16 melanoma, was maintained and implanted s.c. as described (7). Briefly, cultured cells were harvested by routine trypsinization techniques and washed by centrifugation, and 5 × 106 viable cells were inoculated in the suprascapular area of BDF1 mice. The Meth A fibrosarcoma was the generous gift of Dr. Lloyd Old and was carried as an ascitic tumor stock in BALB/c mice. One million viable cells were inoculated s.c. in the suprascapular area for efficacy experiments. Pan-02 was the generous gift of Dr. W. R. Leopold and was carried s.c. in C57BL/6 mice for tumor stocks. For experiments, a brei was prepared and 100 μl were injected s.c. in the suprascapular area. All cell lines and hosts were negative for Mycoplasma and murine viral contamination (MAP test; Charles River Professional Services).

Therapeutics. Highly purified rhIL-2 from Escherichia coli was stored as a lyophilized powder, reconstituted in sterile water for injection (LyphoMed), and further diluted as necessary in 5% dextrose in water (20). The endotoxin contamination was less than 0.1 EU/mg rhIL-2. PEG-IL-2 was produced and purified as previously described (1, 2), with a degree of modification equivalent to 2 to 3 PEG M, 7,000 molecules/rhIL-2 molecule. The apparent average molecular weight of this species was M, 160,000 by size exclusion chromatography, which is a measure of the hydrodynamic radius of the molecules. The endotoxin content was 0.4 EU/mg rhIL-2. The specific activity of the PEG-IL-2 and rhIL-2 was 4 × 106 and 18.0 × 105 IU/mg respectively.

Antitumor Efficacy. Meth A and Pan-02 tumor cells were inoculated s.c. 7 days prior to the initiation of therapy. Therapy was initiated the day after s.c. injection of B16 tumor cells. Therapeutics were administered i.v. in 0.1-ml volume. In order to make meaningful comparisons of the activities of rhIL-2 and PEG-IL-2, dose levels were based on the IL-2 protein concentration of the dosing solution. Tumor volumes were calculated as the product of caliper measurements taken in three orthogonal directions. Tumor-bearing hosts with apparently complete regressions at the s.c. site of inoculation in Meth A were followed for 35 days, or in B16 for at least 60 days, to assess the durability of this response. Since the Pan-02 tumor spontaneously metastasizes from the s.c. site of injection to regional lymph nodes and lung (21), the survival of the hosts, as well as the size of the s.c. tumor mass, was used as an

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1 To whom requests for reprints should be addressed, at Department of Pharmacology, Cetus Corporation, 1400 Fifty-third Street, Emeryville, CA 94608.

2 The abbreviations used are: rhIL-2, recombinant human interleukin 2; PEG-IL-2, monomethoxy polyethylene glycol-modified recombinant human interleukin 2; QD, every day; Q7D x 2, every 7 days, 2 times; Q4D x 3, every 4 days, 3 times; Q3D x 4, every 3 days, 4 times; Q2D x 5, every 2 days, 5 times; PEG, polyethylene glycol; BDF1, C57BL/6 × DBA/2J F1; IL-2, interleukin 2.

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indication of the therapeutic benefit of the treatments.

Antitumor Efficacy Data Analysis. Data are reported as the means of replicate experiments using 5 or 6 mice/dose group/experiment. In some combinations which produced high levels of lethality, particular doses or schedules were not repeated (see tables for details). For Meth A, the T/C% was determined as the mean tumor volume at day 21 of the treated group/mean tumor volume at day 21 of the control group. For B16, the tumor growth delay parameter T—C was defined as the number of days for the mean tumor size of the treated group to reach 500 mm³ subtracted from the number of days for the mean tumor size of the controls to reach 500 mm³. Survival data for Pan-02 are expressed as the percentage increase in lifespan, calculated as the median number of survival days of the treated group/median number of survival days of the control group × 100. Statistical comparisons to controls or among various experimental groups were performed using the Quade rank analysis of covariance; P was set at 0.05.

Pharmacokinetics. Male CD mice weighing 18 to 22 g were obtained from Charles River Laboratories (Wilmington, MA). Five mice were injected/sample time with 0.25 mg PEG-IL-2/kg (total of 60 mice), and three mice were injected/sample time with 7.5 mg rhIL-2/kg (total of 36 mice). The dose was injected into a lateral tail vein, and one sample of blood was obtained from each mouse by retroorbital sinus puncture. Standards were prepared in mouse plasma and assayed with the samples. Plasma was assayed for IL-2 bioactivity by measuring the incorporation of [3H]thymidine into DNA following incubation of plasma in the presence of HT2 cells, mouse fibroblasts which are dependent on IL-2 for growth (22, 23). Mean values of rhIL-2 bioactivity and PEG-IL-2 bioactivity adjusted for dose (× 30) were analyzed to obtain the macroconstants (coefficients and rate constants) which described the plasma curves, using the microcomputer program RSTRIP obtained from MicroMath Scientific Software (Salt Lake City, UT). Coefficients A, B, and C were adjusted for dose to simulate various concentration of IL-2 attained between doses. The antitumor efficacy obtained on the Q4D x 3 and Q3D x 4 protocols, as shown by the individual hosts' tumor volume regressions were produced and no treatment-related deaths were observed within each schedule (Figs. 1-3). As shown in Table 1B, the protocol with the best therapeutic index was the one total doses were divided and delivered by the various schedules, the toxicity of the treatments was schedule dependent. At 50 mg/kg total PEG-IL-2, toxicity was observed only on the Q7D x 2 and Q2D x 5 schedules. The other combinations of dose and schedule were not lethal, with the exception of the 25 mg/kg dose on the Q2D x 5 schedule (Table 1B). As discussed below, this dependence on the frequency of administration to produce the lethality may be related to the minimum plasma concentration of IL-2 attained between doses.

The antitumor efficacy of the PEG-IL-2 treatment was also schedule dependent; in addition, dose-response relationships were observed within each schedule (Figs. 1–3). As shown in Table 1B, the protocol with the best therapeutic index was found to be 25 mg/kg given Q7D × 2, in which 8 of 10 complete regressions were produced and no treatment-related deaths were observed. A dose dependence was noted on this schedule (Fig. 1); however, at the highest dose (50 mg/kg) there were 4 of 10 deaths associated with the 5 of 6 complete regressions in the surviving animals, while at 12.5 mg/kg fewer complete regressions were obtained (3 of 10) without lethality (0 of 10). The T/C% calculated as the mean tumor volume of treated animals on day 21/mean tumor volume of control animals on day 21 × 100.

Table 1 ML-2 (A) and PEG-IL-2 (B) antitumor efficacy in Meth A

<table>
<thead>
<tr>
<th>A. Total rhIL-2 dose (mg/kg)</th>
<th>T/C%</th>
<th>Number deaths/total treated</th>
<th>Complete regressions</th>
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<tr>
<td>112.5</td>
<td>6.9'</td>
<td>1/10</td>
<td>4/9'</td>
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<tr>
<td>90</td>
<td>5.3'</td>
<td>0/10</td>
<td>3/10'</td>
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<tr>
<td>45</td>
<td>22.0'</td>
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<td>9</td>
<td>66.2'</td>
<td>0/10</td>
<td>1/10</td>
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<table>
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<tr>
<th>B. Total PEG-IL-2 dose (mg/kg)</th>
<th>Schedule</th>
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<th>Number deaths/total treated</th>
<th>Complete regressions</th>
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<td>Q7D x 2</td>
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<tr>
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<td>8/10'</td>
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<tr>
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<td>Q3D x 4</td>
<td>17.9'</td>
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<td>10/10</td>
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<tr>
<td>50</td>
<td>Q2D x 5</td>
<td>7.6'</td>
<td>0/5</td>
<td>0/5</td>
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<tr>
<td>25</td>
<td>Q2D x 5</td>
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<td>0/5</td>
<td>2/5'</td>
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<tr>
<td>12.5</td>
<td>Q2D x 5</td>
<td>14.1'</td>
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<td>2/5'</td>
</tr>
</tbody>
</table>

6522
Fig. 1. PEG-IL-2 antitumor efficacy against Meth A on the Q7D x 2 schedule, individual animal data, through day 21 post-tumor implant. A, Total dose of PEG-IL-2 of 50 mg/kg; B, 25 mg/kg; C, 12.5 mg/kg; D, phosphate-buffered saline controls. The dotted line at 20 mm³ is the limit of quantitative tumor volume determinations.

Fig. 2. As in Fig. 1, on the Q4D x 3 schedule.
the kinetics of the IL-2-induced rise in the immune effector cell populations. The influence of these biological events, and their superimposition on the pharmacokinetic parameters of scheduling, is an area under current investigation. The high toxicity associated with the Q2D x 5 schedule precluded the gathering of meaningful efficacy data on this schedule.

Schedule Dependence in B16. The B16 model was not very sensitive to rhIL-2 treatment, even at lethal doses, as shown in Table 2A. As in the Meth A studies, the maximum i.v. dose achievable was 112.5 mg/kg total rhIL-2 on the QD x 9 schedule; however, in this case, 6 of 16 animals died at this dose level. The BDF1 mice used for B16 were more sensitive to rhIL-2 than the BALB/c Meth A hosts at all but the lowest dose; 1 of 16, 5 of 16, and 6 of 16 died at 45, 90, and 112.5 mg/kg, respectively. Even at these toxic levels, there were no complete regressions obtained in the B16 tumors, and only a small mean increase of 3.5 days in the tumor growth delay parameter \( T - C \) was obtained at the highest dose (Table 2).

The antitumor efficacy of PEG-IL-2 was studied at three dose levels, 18, 12, or 6 mg/kg total dose. These levels were lower than those studied in Meth A because of the increased sensitivity of the BDF1 hosts to its lethal effects, as noted above. As shown in Table 2B, the toxicity of the treatments was again schedule dependent, in a manner similar to that observed in Meth A. The Q7D x 2 schedule did not produce lethality at these doses; however, there was a clear dose-dependent lethality associated with either the Q4D x 3 or the Q3D x 4 schedule. A single experiment at 30 mg/kg also produced no lethality on the Q7D x 2 schedule, but no greater efficacy than with 18 mg/kg was obtained. Further studies are ongoing in this model to more fully establish the doses and length of treatment which

<table>
<thead>
<tr>
<th>Total rhIL-2 dose (mg/kg)</th>
<th>( T - C ) (days)</th>
<th>Number deaths/total treated</th>
</tr>
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<tr>
<td>112.5</td>
<td>3.5</td>
<td>6/16*</td>
</tr>
<tr>
<td>90</td>
<td>2.9</td>
<td>5/16*</td>
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<td>1/16</td>
</tr>
<tr>
<td>9</td>
<td>1.5</td>
<td>0/16</td>
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</table>

<table>
<thead>
<tr>
<th>Total PEG-IL-2 dose (mg/kg)</th>
<th>Schedule</th>
<th>( T - C ) (days)</th>
<th>Number deaths/total treated</th>
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<td>Q7D x 2</td>
<td>13.6*</td>
<td>0/16</td>
</tr>
<tr>
<td>12</td>
<td>Q4D x 3</td>
<td>ND*</td>
<td>9/10*</td>
</tr>
<tr>
<td>6</td>
<td>Q3D x 4</td>
<td>10.1*</td>
<td>1/10</td>
</tr>
<tr>
<td>12</td>
<td>Q3D x 4</td>
<td>7.2*</td>
<td>10/10*</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>7.2*</td>
<td>6/10*</td>
</tr>
</tbody>
</table>

* rhIL-2 was administered QD x 9, i.v., beginning 1 day after s.c. tumor implant. Five animals/group in two experiments, six animals/group in the third experiment.

\( T - C \) is the tumor growth delay, determined as the mean number of days for the treated tumor volume to reach 500 mm³ minus the mean number of days for the control tumor volumes to reach 500 mm³. Mean value given for three experiments.

* Treatment-caused deaths.

Not significantly different than controls, as in Table 1.

PEG-IL-2 was given i.v. starting 1 day after s.c. tumor implant.

Three of six mice in the third experiment had a complete block of tumor growth through the last day of observation, day 64. These mice were excluded from the calculation of \( T - C \).

Not determined due to excessive toxicity of treatment.
produce the maximum therapeutic benefit.

The efficacy of the PEG-IL-2 on the Q7D × 2 schedule at nontoxic doses was superior to that obtained with unmodified rhIL-2 even when rhIL-2 was administered at toxic levels, as shown in Table 2B by the tumor growth delay parameter T—C. For example, at 18 mg/kg, a tumor growth delay of 13.6 days was obtained (Table 2B), which corresponded to a mean tumor growth inhibition of ≥95% compared to controls at day 28 post-implant. In addition, the third experiment in this series resulted in a complete block of tumor growth in 3 of 6 mice treated at 18 mg/kg that was durable through day 64, the final day of observation.

The increase in antitumor activity of PEG-IL-2 is even more striking if comparisons are made between the efficacy results in Table 2, A and B, at equitoxic dose levels. For example, at either 9 or 45 mg/kg rhIL-2, a T—C of 1.5 or 2.6 days was obtained, respectively. PEG-IL-2 treatment at all three doses of the Q7D × 2 schedule resulted in T—C values ranging from 8.1 to 13.6 days, a 3- to 9-fold increase in activity, depending on the comparison made (P < 0.05). Further, the total doses of PEG-IL-2 required to obtain these levels of activity were reduced in comparison to those of rhIL-2.

Although the antitumor activity of PEG-IL-2 was also increased on the Q4D × 3 schedule compared to the rhIL-2 at equitoxic dose levels, the lethality observed was greater than that observed with Q7D × 2 studies (Table 2B). The excessive toxicity of the Q3D × 4 schedule resulted in efficacy measurements of limited meaning.

Schedule Dependence in Pan-02. Pan-02 spontaneously metastasizes from the s.c. site of inoculation and has shown resistance in vivo to some 50 chemotherapeutic agents (21). In our hands, it has also proven refractory to several treatment regimens, including combination therapy studies with chemotherapeutics and various lymphokines or cytokines. Since the tumor metastasizes, the increase in lifespan (%ILS) of the C57BL/6 hosts can be used as an indication of therapeutic benefit, as well as the size of the s.c. tumor implant. As a single agent, rhIL-2 produced limited antitumor efficacy against Pan-02 even at toxic levels when benefit was measured by either of these two parameters (Table 3A). The C57BL/6 hosts were also more sensitive than BALB/c mice to the lethal effects of rhIL-2, as was found for BDF, mice.

The three doses of PEG-IL-2 used in the Pan-02 model were 12, 6, or 3 mg/kg, as shown in Table 3B. Although the toxicity of PEG-IL-2 was reduced at these dose levels compared to those used in the B16 model, there was a schedule-dependent increase in toxicity as the interval between doses was decreased. No deaths were recorded on the Q7D × 2 schedule, 1 of 30 mice died on Q4D × 3, 5 of 30 died on Q3D × 4, and 12 of 15 died on the three doses of the Q2D × 5 schedule (Table 3B). An additional single study has shown that PEG-IL-2 at 24 mg/kg on the Q7D × 2 schedule also was not lethal.

The antitumor efficacy obtained with PEG-IL-2 was not markedly dependent on the schedule of administration, in contrast to the results obtained with the other two models. The activity observed at nonlethal doses with PEG-IL-2, however, was again superior to that obtained with rhIL-2 at lethal doses. At the s.c. site of tumor implant, the Q7D × 2 schedule resulted in about a 5-day mean decrease in the rate of growth to 500 mm³ (range, 3.2-5.7 days) when compared to controls (T—C) (P < 0.05). The mean T—C on the other schedules ranged from about 1.5 to 4 days at nonlethal doses (Table 3B). The Q2D × 5 schedule was once again quite toxic and of limited therapeutic value.

<table>
<thead>
<tr>
<th>Total rhIL-2 dose (mg/kg)</th>
<th>Schedule</th>
<th>T—C (days)</th>
<th>%ILS</th>
<th>Number deaths/total treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>112.5</td>
<td>0.4</td>
<td>&lt;100</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>0.5</td>
<td>143</td>
<td>0/10</td>
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</tr>
<tr>
<td>18</td>
<td>0.8</td>
<td>127</td>
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</table>

* rhIL-2 treatment started 7 days following s.c. tumor implant, i.e., QD × 9. Five mice/group/experiment.
* T—C calculated as in Table 2.
* %ILS is the percentage increase in lifespan, calculated as the median day of death of the treated group/median day of death of the controls, × 100.
* Treatment-caused deaths.
* Significantly different than controls, as in Table 1.
* PEG-IL-2 treatment started 7 days post-implant, i.e., on the schedules indicated.
* Tumor-bearing hosts which did not survive treatment were excluded from calculations of survival. Days post-tumor inoculation.

When the treated animals' survival was followed, there were many instances of an increased lifespan observed, some as much as 200%. However, there was neither a strong dose nor schedule dependence to this effect, which may reflect the limited number of animals/group. Nevertheless, the results obtained were encouraging. Additional experiments are ongoing with more prolonged treatment regimens at higher doses, in order to reach the maximally tolerated dose in this very resistant tumor.

Pharmacokinetics of PEG-IL-2 and rhIL-2 in Mice. Plasma curves were constructed from the mean concentrations of IL-2 bioactivity at each of the time points shown in Fig. 4 for rhIL-2 and PEG-IL-2-treated mice. The prolonged lifetime PEG-IL-2 compared to rhIL-2 suggested that the optimum dosage schedules would produce different plasma concentration pro-
files for the two proteins. Therefore, the curves shown in Fig. 4 were used to predict the plasma curves for some of the effective dosage schedules used in the efficacy studies.

Both curves were fit to a three-exponential function, with parameter values shown in Table 4 along with the ratio of PEG-IL-2 to rhIL-2 values. Although the plasma curves both extrapolated to the same starting concentration (equal C0 values), the major difference in the two curves was found in the contribution of the terminal phase of clearance to the overall clearance of the proteins. The value of C and the area under the curve contributed by the C phase (C/r) were increased by 8.23- and 30.14-fold, respectively, for PEG-IL-2. The half lives for all three phases were longer for PEG-IL-2, and the total area under the plasma curve (AUC) for PEG-IL-2 increased 11.76-fold over that of rhIL-2. The parameters from Table 4 were used to simulate plasma curves for the four PEG-IL-2 schedules which were studied in the Meth A efficacy model at 25 mg/kg (Fig. 5).

The simulations shown in Fig. 5 demonstrate that peak concentrations among the schedules for PEG-IL-2 ranged from 0.6 to 1.6 x 10^6 IU/ml, less than 3-fold. However, the minimum concentration achieved between doses or the duration below 10 IU/ml between doses varied greatly among the schedules and appeared to correlate with toxicity. For example, when the 25 mg/kg total dose was given Q7D x 2 the time below 10 IU/ml was almost 4 days, whereas the Q4D x 3 schedule reduced this time to 1 day and the Q3D x 4 schedule further reduced this time to only a few hours. The Q2D x 5 schedule produced a minimum concentration between doses of 94 IU/ml and was 100% lethal in the Meth A model (Table 1B). For rhIL-2 given at 12.5 mg/kg/day (approximately the LD50), the daily schedule allowed about 6 h/day below 10 IU/ml.

**DISCUSSION**

In the Meth A, B16, and Pan-02 tumor models, the antitumor efficacy of PEG-IL-2 was found to be superior to that of rhIL-2 at equitoxic doses. Further, in these three murine tumor models the toxicity and the antitumor efficacy of PEG-IL-2 was found to be dependent on the schedule of administration (Tables 1B, 2B, and 3B). The Q7D x 2 schedule was the least toxic at the doses studied and resulted in the best antitumor activity. More closely spaced dosing regimens were increasingly toxic as the interval between doses was decreased. Further, no enhancement of the efficacy was obtained by decreasing the interval between doses and in many instances the antitumor activity was reduced in comparison to the Q7D x 2 schedule.

Meth A was somewhat sensitive to rhIL-2, as 3 of 10 and 4 of 10 complete regressions were obtained at the two highest dose levels. The PEG-IL-2 was more active, however, as 8 of 10 complete regressions were observed at equitoxic, or even less than equitoxic, doses (Table 1). In B16, which was marginally sensitive to rhIL-2 even at lethal dose levels, PEG-IL-2 treatment at nonlethal doses resulted in a 2-fold increase in tumor growth delay (T=T) compared to rhIL-2 at lethal doses (Table 2). If the highest nonlethal doses were compared, an increase of over 9-fold in T=T was obtained with PEG-IL-2 treatment: T=T of 13.6 days for PEG-IL-2 Q7D x 2 at 18 mg/kg, compared to 1.5 days for rhIL-2 at 9 mg/kg, QD x 9. A complete block of tumor growth in 3 of 16 treated animals was also observed with PEG-IL-2 at this dose. In Pan-02, a tumor resistant to rhIL-2 treatment, PEG-IL-2 increased the T=T to as much as 5.7 days (6 mg/kg, Q7D x 2). In addition, an increase in the lifespan of the tumor hosts of from 150% to nearly 200% was obtained with PEG-IL-2 treatment at nonlethal doses (Table 3).

The response of other methylcholanganthrene-induced fibrosarcomas (5) and of murine mammary adenocarcinomas (8, 12) to rhIL-2 or rhIL-2 in combination with lymphokine-activated killer cells (3, 4) has been found to correlate with the degree of immunogenicity of the tumor. Meth A has also been shown to be immunogenic (25), which probably contributed to its sensitivity to rhIL-2 and PEG-IL-2. The B16 and Pan-02 tumors apparently are nonimmunogenic, however, as defined by reimplantation studies and as confirmed by their resistance to rhIL-2. The PEG-IL-2 treatments did result in a significant amount of antitumor activity, however, which suggested that perhaps even resistant nonimmunogenic tumors could respond to IL-2 if a more optimum manner of therapy was used. It should also be clear that for these more resistant tumors, two weekly treatments may not be the most therapeutic schedule possible but it was chosen to match those used in Meth A in order to develop the comparisons made in this series of experiments. Additional weekly treatments might increase the degree of antitumor activity obtained in these more resistant models.

In earlier studies with a PEG-IL-2 species modified to a lesser extent that the material used in the present studies, five daily i.p. treatments of 80 μg/kg (0.4 mg/kg total dose) resulted in a marked decrease in the growth of Meth A at day 10 (2). Additional investigations revealed that this schedule and route was a comparatively toxic method of PEG-IL-2 administration. Therefore, we began to investigate the protocols described in the present study to establish less toxic and more efficacious regimes. In addition, pharmacokinetic studies have demonstrated that modification of rhIL-2 with PEG prolonged the half-life in proportion to the degree of modification of the rhIL-2 and that the clearance can be predicted from the hydrodynamic radius of the modified species (1). We hypothesized from this work that perhaps different degrees of modification would sufficiently alter the pharmacokinetics such that determination of the protocol with the best therapeutic index would depend on these clearance characteristics. Preliminary in vivo tumor studies comparing a PEG-IL-2 species with apparent molecular weight of 93,000-95,000 with the results presented here have confirmed these expectations.

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**Table 4 Pharmacokinetic parameters in mice given 7.5 mg PEG-IL-2 or IL-2/kg**

Male CD mice were dosed once i.v. with 7.5 mg/kg rhIL-2 or with 0.25 mg/kg PEG-IL-2. Five mice/time point, times of collection are shown in Fig. 4. One plasma sample was collected from each mouse by retroorbital sinus puncture. IL-2 bioactivity, measured by the methods of Gillis et al. (22) and Watson (23), was adjusted for dose.

<table>
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<tr>
<th>Parameter</th>
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<th>PEG-IL-2/rhIL-2</th>
</tr>
</thead>
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<td>A (KU/ml)</td>
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<td>769</td>
<td>1.17</td>
</tr>
<tr>
<td>B (KU/ml)</td>
<td>219</td>
<td>149</td>
<td>0.693</td>
</tr>
<tr>
<td>C (KU/ml)</td>
<td>62.0</td>
<td>75.3</td>
<td>1.21</td>
</tr>
<tr>
<td>C0 (KU/ml)</td>
<td>937</td>
<td>925</td>
<td>1.01</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>10.0</td>
<td>1.6</td>
<td>6.25</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>82.8</td>
<td>12.9</td>
<td>6.42</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>327.9</td>
<td>89.4</td>
<td>3.67</td>
</tr>
<tr>
<td>A1/2 (KU/ml/h)</td>
<td>158</td>
<td>29.7</td>
<td>5.32</td>
</tr>
<tr>
<td>B/β (KU/ml/h)</td>
<td>436</td>
<td>46.1</td>
<td>9.45</td>
</tr>
<tr>
<td>C/r (KU/ml/h)</td>
<td>489</td>
<td>16.2</td>
<td>30.14</td>
</tr>
<tr>
<td>AUC (KU/ml/h)</td>
<td>1083</td>
<td>92.0</td>
<td>11.76</td>
</tr>
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</table>

* Parameters were defined as follows: A, B, and C are the zero-time intercepts of each clearance phase obtained from curve-stripping; C0 is the zero-time intercept of the plasma curve; the T1/2 values (half lives) were calculated from ln(0.5)/rate constant; A1/2, B/β, and C/r are the areas under each phase of the plasma curve; and AUC is the area under the plasma curve from time zero to infinity.
Fig. 5. Pharmacokinetic simulations of PEG-IL-2 in mice dosed i.v. with 25 mg/kg total dose on the four schedules studied in the Meth A model; IL-2 bioactivity is expressed in IU/ml plasma. A, Q7D x 2 schedule (12.5 mg/kg/dose); B, Q4D x 3 schedule (8.33 mg/kg/dose); C, Q3D x 4 schedule (6.25 mg/kg/dose); D, Q2D x 5 schedule (5 mg/kg/dose).

The toxicity of PEG-IL-2 is hypothesized to be related to the minimum plasma concentration attained between doses, as illustrated by the striking schedule dependency of PEG-IL-2 toxicity in these studies (Tables 1B, 2B, and 3B). In addition, the duration of low plasma concentrations also appeared to be important. For example, at 25 mg/kg on the Q7D x 2 schedule (a nontoxic regimen in Meth A), the calculated minimum plasma levels reached approximately $6 \times 10^{-5}$ IU/ml, a theoretical number of questionable biological significance. In addition, however, the interval between which measurable levels (1–10 IU/ml) could be reliably detected was about 4 days, the longest of the schedules tested (Fig. 5A).

On the Q3D x 4 schedule at 18 mg/kg or 12 mg/kg, toxic deaths were recorded in the B16 (10 of 10) or Pan-02 (5 of 10) models, respectively. The minimum plasma levels in this case were approximately 6 IU/ml, which meant there was no time interval during which the PEG-IL-2 levels were below measurable concentrations (Fig. 5C). The Q2D x 5 schedule proved to be essentially 100% lethal; in this case, the lowest levels achieved between doses were about 94 IU/ml (Fig. 5D).

Further, high single doses of PEG-IL-2 were toxic, which also suggested that the time IL-2 stays above a particular plasma concentration can contribute to toxicity. Data obtained using an unmodified rhIL-2 with improved solubility have indicated that 75 mg/kg as a single i.v. bolus is not lethal in Meth A tumor-bearing BALB/c mice. The clearance of this material is very similar to the rhIL-2 shown here. Therefore, these data also support the hypothesis that the peak plasma concentration alone does not determine the toxicity of IL-2 but that the clearance characteristics are also major contributory factors to IL-2 toxicity.

The efficacy obtained is hypothesized to be related to the peak plasma IL-2 concentration, as well as the manner in which the levels fall off, since in all three tumor models there was a dose dependence to the efficacy obtained. The Q7D x 2 schedule resulted in the best therapeutic index, since there was little or no lethality associated with the treatments and substantial antitumor activity was observed (Tables 1B, 2B, and 3B). The degree of antitumor activity obtained was also schedule dependent, however, which suggested that while higher peak IL-2 concentrations improved the antitumor response the doses had to be delivered at appropriate intervals in order to reduce the toxicity of the treatment. One pharmacokinetic factor that did not appear to be significant was the area under the curve. Since the same total dose was delivered on each of the different schedules, the area under the curve would be equivalent at each dose level.

While the treatment schedule and pharmacokinetic parameters appeared to be important factors in the therapeutic responses obtained, one must also consider the hosts’ biological responses to IL-2 and the kinetics of these responses. We are investigating the hypothesis that the once-a-week treatment schedule was superior to the other schedules in part due to a coordination of these biological responses with the PEG-IL-2 administration. For example, it has been shown in clinical trials that the peripheral WBC population increases dramatically (up
to 16-fold) on days 6–8 following 5 days of rhIL-2 therapy (Refs. 13–19 and references therein). It may be that the Q7D × 2 schedule takes advantage of such cycles, which are poorly defined at this time, either to enhance the efficacy or reduce the toxicity of PEG-IL-2. In addition, there may be subtle differences in the pharmacokinetics or biological consequences of PEG-IL-2 in tumor-bearing animals, compared to non-tumor-bearing, or in different strains of mice. Studies to address the relationships between these biological responses and the treatment schedules are ongoing in order to more fully understand their impact on the toxicity and antitumor efficacy of PEG-IL-2.

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Schedule Dependency of the Antitumor Activity and Toxicity of Polyethylene Glycol-modified Interleukin 2 in Murine Tumor Models

Robert J. Zimmerman, Sharon Lea Aukerman, Nandini V. Katre, et al.


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