ELSEVIER

International Journal of Pharmaceutics 113 (1995) 73–81

international journal of pharmaceutics

Sustained-release interleukin-2 following intramuscular injection in rats

Pao-Li Wang, Thomas P. Johnston *

Department of Pharmaceutics and Pharmacodynamics (M / C 865), University of Illinois at Chicago, College of Pharmacy, 833 South Wood Street, Chicago, IL 60612–7231, USA

Received 14 October 1993; modified version received 3 June 1994; accepted 7 June 1994

Abstract

A potential sustained-release recombinant interleukin-2 (rIL-2) formulation was evaluated following intramuscular (i.m.) injection in rats. Poloxamer 407 (Pluronic® F-127) is a block copolymer comprised of polyoxyethylene and polyoxypropylene segments which exhibits the property of reverse thermal gelation. Thus, an rIL-2/poloxamer 407 preparation was injected i.m. in rats as a viscous mobile solution with subsequent gelation in vivo. Resultant plasma rIL-2 concentration-time data indicated absorption rate-limited disposition of rIL-2 following i.m. injection. The mean values of the absorption rate constant (k_a) were 0.64 ± 0.073 and 0.21 ± 0.019 h^{-1} following i.m. injection of an rIL-2 aqueous solution and the rIL-2 gel formulation, respectively. The mean values of the elimination rate constant (k_{elim}) were 1.76 ± 0.22 and 1.21 ± 0.079 h^{-1} following administration of rIL-2 as an aqueous solution or gel formulation, respectively. The blood sampling time point at which the greatest plasma rIL-2 concentration (C_{max}) was observed was 2 h for rats injected i.m. with rIL-2 formulated in poloxamer 407 compared to 1 h for rats injected i.m. with an rIL-2 aqueous solution. The mean value of the C_{max} was significantly ($p < 0.05$) less in rats injected i.m. with the rIL-2 gel formulation ($C_{max} = 12500 \pm 1450$ pg/ml) compared to rats injected i.m. with an aqueous solution of rIL-2 ($C_{max} = 19600 \pm 2650$ pg/ml). The bioavailability of rIL-2 when injected i.m. as an rIL-2/poloxamer gel formulation relative to an i.m. injection of an rIL-2 aqueous solution was approx. 1.0. Cumulative amounts of rIL-2 recovered in the urine 48 h after an i.m. injection of either an rIL-2 aqueous solution or rIL-2 gel formulation were less than 1 percent of the administered dose. Since the rIL-2/poloxamer formulation evaluated in this study resulted in a decrease in the maximum blood concentration of rIL-2 achieved and an increase in the time required to reach a maximum blood concentration, without a reduction in the bioavailability of the protein, the rIL-2 gel formulation may represent an alternative, sustained-release mode of rIL-2 administration.

Keywords: Biological response modifier; Cancer immunotherapy; Pluronic F-127; Poloxamer 407; Recombinant interleukin-2

1. Introduction

Exogenous administration of interleukin-2 (IL-2) has been shown to activate natural killer (NK) cells (Hefeneider et al., 1983) or lymphocyte-

* Corresponding author. Tel. (312) 996-2365; Fax (312) 996-0098.

SSDI 0378-5173(94)00179-9

activated killer (LAK) cells (Rosenberg et al., 1985), enhance alloantigen responsiveness (Hefeneider et al., 1983), restore immune function in acquired immunodeficient states (Merluzzi et al., 1981) or nude mice (Stotter et al., 1980), and enhance antitumor effects, either alone (Rosenberg et al., 1985) or with adoptively transferred effector cells such as long-term cultured T cells (Cheever et al., 1982) or LAK cells (Mazumder and Rosenberg, 1984). Antitumor treatment using IL-2 and killer cells has met with encouraging results for several cancers in both experimental animals models and humans (Grimm et al., 1982). The primary limitation with the use of high-dose IL-2 therapy is the associated toxicity (Richards, 1989). In addition, the use of IL-2 is hampered due to the protein's rapid clearance from the systemic circulation following administration, with detectable serum levels lasting for only several hours following parenteral administration to mice (Donohue and Rosenberg, 1983; Cheever et al., 1985). Since it has been reported that the greatest therapeutic or antitumor effects of exogenously administered IL-2 are more positively correlated with the length of time IL-2 is detectable in the serum rather than the peak serum level achieved, a sustained-release preparation of IL-2 would be anticipated to enhance therapeutic efficacy. Hence, to possibly minimize the problem of systemic toxicity and increase the therapeutic efficacy of IL-2 by prolonging the serum levels, we have evaluated a sustained-release, injectable formulation of IL-2 designed for extravascular administration.

It has recently been reported that poloxamer 407 (Pluronic® F-127) may be a suitable vehicle for incorporation into sustained-release, parenteral formulations designed to deliver proteins following extravascular administration (Fults and Johnston, 1990; Johnston et al., 1992; Pec et al., 1992). Poloxamer 407 belongs to a chemically similar family of ABA block copolymers which are composed of poly(oxyethylene) [POE] and poly(oxypropylene) [POP] groups. The physicochemical properties of these nonionic surface active agents may be modified by varying the proportions of the POE and POP groups in the final molecule. Many of the poloxamers have been used in the food, cosmetic and pharmaceutical industries because they are nonionic surface-active agents which possess excellent wetting, solubilizing, and antifoaming properties. Certain members of the poloxamer family exhibit the phenomenon of reverse-thermal gelation, i.e., the polymer exists a mobile viscous liquid at reduced temperatures, but forms a rigid semisolid gel network with an increase in temperature (Wang and Johnston, 1991). It was previously reported that the rate of gelation and the temperature of the sol-to-gel transition for poloxamer 407 are concentration dependent (Wang and Johnston, 1991). As measured using pulse shearometry, poloxamer 407 at a 30% w/w concentration begins the gelation process at 7.8 ± 0.7°C and reaches the completely gelled state at 15.8 ± 0.6°C when a chilled solution is allowed to passively warm at 22.0°C (Wang and Johnston, 1991). The property of reverse thermal gelation exhibited by poloxamer 407 has been exploited to sustain the delivery of a macromolecular (nonprotein) compound as well as biologically active macromolecules (proteins) to rats and mice following intramuscular and intraperitoneal injection, respectively (Johnston and Miller, 1989; Johnston et al., 1992; Pec et al., 1992). Poloxamer 407 has also been reported to be inert with regard to myotoxicity when administered as single or multiple intramuscular injections to rabbits (Johnston and Miller, 1985). The lack of myotoxicity and the rapid sol-to-gel transition (Wang and Johnston, 1991) make poloxamer 407 an attractive vehicle with which to sustain the delivery of interleukin-2 or other biological response modifiers following extravascular administration.

Previously, we reported the sustained release of human recombinant rIL-2 from a poloxamer 407 gel matrix in vitro (Johnston et al., 1992) and demonstrated a zero-order (constant) release rate of rIL-2 from both 30 and 35% w/w poloxamer 407 matrices (Fig. 1). It was reported that the release of rIL-2 following intraperitoneal injection of the gel formulation to mice was potentially sustained in vivo due to enhanced cytotoxicity of harvested NK cells towards murine YAC-1 cells in vitro (Johnston et al., 1992). However, we now provide a quantitative determination of

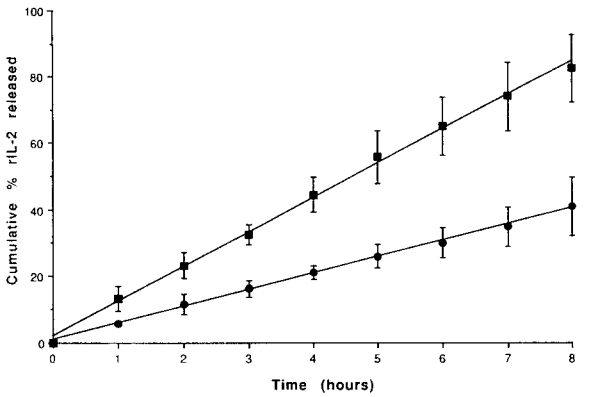

Fig. 1. Cumulative percentage of rIL-2 released from rIL-2/poloxamer 407 matrices in vitro at 22°C as determined by ELISA. (■) rIL-2/poloxamer 407 matrices containing 30% (w/w) poloxamer 407; (●) rIL-2/poloxamer 407 matrices containing 35% (w/w) poloxamer 407. From Johnston et al. (1992).

plasma rIL-2 concentrations in rats following intramuscular (i.m.) administration of an rIL-2/poloxamer 407 formulation.

2. Materials and methods

Poloxamer 407, NF (Pluronic® F-127, NF) was generously provided by BASF (Parsippany, NJ) and used as received. Sterile normal saline and heparin sodium (5000 U/ml) were purchased from Elkins Sinn (Cherry Hill, NJ). Tuberculin syringes were 1 ml and fitted with 1 inch, 23 gauge needles purchased from Becton and Dickinson (Rutherford, NJ). All recombinant interleukin-2 (rIL-2) was generously provided by Hoffmann La-Roche, Inc. (Nutley, NJ). Each 1 ml vial of rIL-2 contained 10^6 units of rIL-2 (spec. act. = 1.6×10^7 U/mg), 5 mg mannitol, and 25 mg of human serum albumin. Rats (male, Sprague Dawley, 275–300 g) used to assess the pharmacokinetics of rIL-2 after intramuscular injection were purchased from Harlan Laboratories (Indianapolis, IN). Urine was collected using metabolism cages provided by the Biological Resources Laboratory at the University of Illinois (Chicago, IL) and centrifuged in 1.5 ml plastic polypropylene Eppendorf centrifuge tubes obtained from Fisher Scientific (Chicago, IL). Assay kits (Intertest-2™) for the quantitation of rIL-2 in plasma and urine were enzyme-linked immunosorbent assays (ELISA) and were obtained from the Genzyme Corp. (Boston, MA).

2.1. Instruments

Plasma obtained from blood samples collected into 1.5 ml heparinized polypropylene eppendorf centrifuge tubes was centrifuged using a model 235C micro-centrifuge purchased from Fisher Scientific (Chicago, IL). All rIL-2 quantitation was determined using a model 450 E, 96-well microplate reader purchased from Bio-Rad (Richmond, CA).

2.2. Preparation of sustained-release injectable IL-2 formulations

Recombinant interleukin-2 was homogeneously dispersed in poloxamer 407 as previously described (Johnston et al., 1992). In brief, a vial of lyophilized recombinant interleukin-2 (1×10^6 U/vial; ≈ 62.5 μg/vial) was reconstituted with pH 7.2 sterile phosphate-buffered saline (PBS) under aseptic conditions. The sterile PBS was added to the rIL-2 at 18:00 h and poloxamer 407 was added to achieve a 30% w/w concentration. The entire mixture was then placed on ice overnight to facilitate dissolution of the poloxamer 407 by the 'cold-method' of incorporation (BASF, 1975).

2.3. Interleukin-2 administration

Since the pharmacokinetics of rIL-2 administered intravenously as an aqueous solution to rodents have been reported in the literature (Donohue and Rosenberg, 1983; Cheever et al., 1985; Matory et al., 1985), we made no attempt to duplicate those results in the present investigation. Our primary objectives were to assess whether the time required to reach a maximum concentration of rIL-2 in the blood (t_{max}) was increased, whether the maximum blood rIL-2 concentration achieved (C_{max}) was decreased, and whether the bioavailability of rIL-2 when administered i.m. as a gel formulation was decreased

relative to an i.m. injection of an aqueous solution of rIL-2. Therefore, we used only two groups of rats in our studies. The first group of six rats was administered rIL-2 i.m. as an aqueous solution at a dose of 8.3×10^5 U/kg (≈ 52.1 μg/kg). Injections were made into the easily palpable M. gluteus superficialis muscle of one hind leg. The second group of six rats was each administered a dose of rIL-2 formulated in poloxamer 407 equal to 8.2×10^5 U/kg (≈ 51.3 μg/kg). A blood sample (0.5 ml) was obtained from each rat in both groups by partial tail amputation (Johnston and Miller, 1989) prior to all rIL-2 dosing and then at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h following i.m. injections. All blood samples were immediately placed on ice until they could be centrifuged. Following centrifugation of blood samples, the plasma was frozen immediately at −70°C until the time of rIL-2 assay.

The amount of rIL-2 excreted into the urine following i.m. administration of the rIL-2 to rats was also evaluated in the present investigation. This experiment was conducted with two groups of rats similar to the groups described above which examined the time course of rIL-2 in the plasma of rats. However, the dose of rIL-2 administered to rats injected with an rIL-2 solution and an rIL-2/poloxamer 407 formulation was 1.16×10^6 U/kg (≈ 72.9 μg/kg) and 1.13×10^6 U/kg (≈ 70.7 μg/kg), respectively. The reason for the slightly different doses of rIL-2 administered as either an aqueous solution or as a gel formulation in both the rIL-2 plasma concentration-time and urinary excretion studies resulted from syringes being weighed prior to and following i.m. injection of the rIL-2/poloxamer formulation. The selection of a larger dose for the urinary excretion experiments compared to the studies which evaluated the time course of rIL-2 in the plasma (≈ 72 μg/kg vs ≈ 51.5 μg/kg) was based on expected urine volumes from rats using the 'free-catch' method of urine collection. A group of four rats was administered an aqueous solution of rIL-2 i.m. as described above and then placed in individual glass metabolism cages. The second group of four rats was injected i.m. with the rIL-2/poloxamer formulation and then placed in similar individual metabolism cages. All animals were allowed food and water ad libitum and urine was collected from each animal in each group over the following urine collection intervals: 0–3, 3–6, 6–12, 12–24, and 24–48 h. The volume of urine from each rat for each collection interval was accurately measured using a graduated cylinder. In addition, two rats were allowed to remain in a metabolism cage for 48 h and urine collected over the same time periods as rats injected with rIL-2 to assess any effects that blank urine might have on the quantitation of rIL-2 using the ELISA assay.

2.4. Quantitation of rIL-2 in plasma and urine

The amount of rIL-2 was determined in each plasma sample at each sampling time point using a commercially available ELISA kit. In brief, the rIL-2 assay was a solid-phase enzyme immunoassay which employed a multiple antibody sandwich principle. A 96-well microtiter plate, pre-coated with mouse monoclonal antibody specific for human rIL-2, was used to capture rIL-2 present in standard and unknown samples. An anti-rIL-2 polyclonal antibody which binds to multiple epitopes on the captured rIL-2 was added. Next, a peroxidase-conjugated goat anti-rabbit polyclonal antibody was added to each well. Addition of a peroxide/tetramethylbenzidine substrate solution initiated a peroxidase catalyzed color change which was stopped within 5 min by acidification with a 1 M sulfuric acid solution. The absorbances of the standards and the unknowns were then measured at 450 nm. The absorbance was proportional to the concentration of rIL-2 present in the sample. The concentration range over which the absorbance was directly proportional to the rIL-2 concentration was 150–4050 pg/ml. The limit of detection of rIL-2 in both plasma and urine using the ELISA was 150 pg/ml.

The manufacturer's instructions included with the assay kit suggest a 1:2 dilution of the biological matrix (plasma or urine) with the buffer contained in the kit. However, the plasma samples in the present study required a 1:10 dilution with the buffer (20 μl plasma; 180 μl buffer) to allow quantitation of rIL-2 from the calibration curve

constructed with rIL-2 standards contained in the assay kit. The urine samples were diluted 1:2 with the buffer (100 μl urine; 100 μl buffer) contained in the assay kit subsequent to filtration with 0.22 μm diameter syringe filters. To ensure that rIL-2 contained in urine specimens from rats injected i.m. with rIL-2 was not bound to the filter cartridges, several known concentrations of freshly reconstituted rIL-2 were prepared and filtered prior to their assay using the ELISA kit. Blank (control) urine was used to determine the recovery of rIL-2 in urine when assayed using ELISA by adding varying known amounts of rIL-2 to the urine. The typical recovery of rIL-2 in urine when assayed using the ELISA procedure was 98% and the assay was linear through the concentration range of 150–4050 pg/ml.

2.5. Data treatment following i.m. injection of rIL-2

The plasma concentrations of rIL-2 determined for each rat in each group were plotted at each sampling time point on semi-logarithmic paper. Thus, there were a total of 12 plasma rIL-2 concentration-time profiles analyzed. Using the method of residuals (Gibaldi and Perrier, 1982), the absorption and elimination rate constants were determined from the individual plasma rIL-2 concentration-time profiles for both treatment groups. The time point at which the maximum plasma rIL-2 concentration was observed experimentally was designated the apparent t_{max}. The corresponding plasma rIL-2 concentration at the apparent t_{max} was designated the apparent C_{max}. Since plasma rIL-2 concentrations were approximately baseline for both treatment groups 12 h after the i.m. injection, the values for the area under the plasma concentration-time curves were truncated at 12 h ($AUC|_{0\rightarrow 12}$). The values for the $AUC|_{0\rightarrow 12}$ for each rat in both treatment groups were calculated using the trapezoidal rule. The bioavailability ($F_{rel.}$) of rIL-2 when administered i.m. as a gel formulation relative to an i.m. injection of an rIL-2 aqueous solution was defined as $AUC|_{0\rightarrow 12\ gel}/AUC|_{0\rightarrow 12\ soln.}$. All statistical comparisons between mean values of pharmacokinetic parameters listed in Table 1 were performed using the Student's t-test and deemed significantly different if $p < 0.05$ (Snedecor and Cochran, 1980).

Table 1
Pharmacokinetic parameters following intramuscular injection of rIL-2 in rats

Parameter	rIL-$2_{soln.}$	rIL-2_{gel}
k_a [a] (h^{-1})	0.64 ± 0.073 ($n = 6$)	0.21 ± 0.019 ($n = 6$)
$k_{elim.}$ [a] (h^{-1})	1.76 ± 0.22 ($n = 6$)	1.20 ± 0.079 ($n = 6$)
$AUC|_{0\rightarrow 12}$ (pg h ml^{-1})	60300 ± 5820 ($n = 6$)	62900 ± 6170 ($n = 6$)
App. t_{max} [a] (h)	1.0 ($n = 6$)	2.0 ($n = 6$)
App. C_{max} [a] (pg/ml)	19600 ± 2650 ($n = 6$)	12500 ± 1450 ($n = 6$)

All values represent the mean value ± standard error of the mean (SE).
[a] Significant ($p < 0.05$) difference in the mean value compared to the rIL-$2_{soln.}$ group using the Students' t-test.

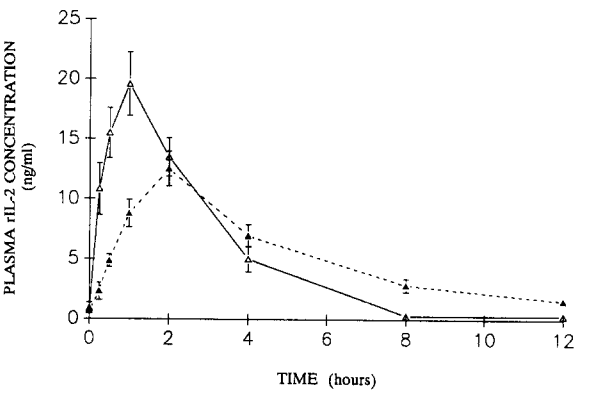

Fig. 2. Plasma rIL-2 concentrations following intramuscular injection of rIL-2 in rats. (△,▲) Measured plasma rIL-2 concentration-time profiles following intramuscular injection of an rIL-2 aqueous solution and rIL-2/poloxamer 407 sustained-release formulation, respectively. Plasma rIL-2 concentrations are the mean value ± standard error of the mean (SE) ($n = 6$).

3. Results

3.1. Disposition of rIL-2 following i.m. injection

The mean (± SE) plasma rIL-2 concentration-time profiles for each treatment group are illus-

trated in Fig. 2. As listed in Table 1, the mean values of the absorption rate constant (k_a) were 0.64 ± 0.073 and 0.21 ± 0.019 h^{-1} following i.m. injection of an rIL-2 aqueous solution and the rIL-2 gel formulation, respectively. In addition, the mean values of the elimination rate constant (k_{elim}) were 1.76 ± 0.22 and 1.21 ± 0.079 h^{-1} following i.m. administration of rIL-2 as an aqueous solution or gel formulation, respectively (Table 1). There was a significant ($p < 0.05$) decrease in the mean value of the k_a and k_{elim} when rIL-2 was administered i.m. as a gel formulation compared to the corresponding mean values of k_a and k_{elim} when rIL-2 was administered i.m. as an aqueous solution.

The blood sampling time point at which the maximum plasma rIL-2 concentration (C_{max}) was observed was 2 h for rats injected i.m. with rIL-2 formulated in poloxamer 407 compared to 1 hour for rats injected i.m. with an rIL-2 aqueous solution. The mean value of the C_{max} was significantly ($p < 0.05$) less in rats injected i.m. with the rIL-2 gel formulation ($C_{max} = 12\,500 \pm 1450$ pg/ml) compared to rats injected i.m. with a solution of rIL-2 ($C_{max} = 19\,600 \pm 2650$ pg/ml). The bioavailability of rIL-2 when injected i.m. as an rIL-2/poloxamer gel formulation relative to an i.m. injection of an rIL-2 aqueous solution was approx. 1.0.

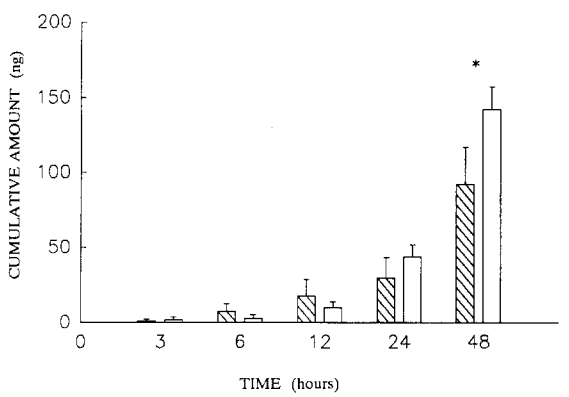

Fig. 3. Cumulative amount of rIL-2 excreted in the urine for rats injected intramuscularly with either a rIL-2 aqueous solution (empty bar) or an rIL-2/poloxamer 407 sustained-release formulation (hatched bar). Cumulative amounts of rIL-2 in the urine are the mean value ± SE ($n = 4$). * Significant ($p < 0.05$) difference in the mean values.

3.2. Urinary excretion of rIL-2 following i.m. injection

Fig. 3 illustrates the cumulative amount of rIL-2 detected in the urine of rats injected i.m. with either an rIL-2 solution or an rIL-2/poloxamer gel formulation. The mean value of the cumulative amount of rIL-2 excreted in the urine after 48 h was significantly ($p < 0.05$) less for rats injected i.m. with the rIL-2 gel formulation compared to rats injected i.m. with an aqueous solution of rIL-2. However, cumulative amounts of rIL-2 recovered in the urine after 48 h for both treatments were less than 1% of the administered dose.

4. Discussion

Biologic response modifiers such as interleukin-2 are demonstrating therapeutic potential for use in human malignancies especially when combined with various cellular therapies. However, rIL-2 and other recombinant-derived proteins continue to be administered primarily by the intravenous route of administration. Since it is well documented that the length of time that therapeutic levels of rIL-2 are maintained in the blood is more positively correlated with increased antitumor efficacy than transient high blood levels of rIL-2 (Cheever et al., 1985), a sustained-release formulation of rIL-2 for parenteral administration would be beneficial. In addition, further prolongation of therapeutic blood levels of rIL-2 is possible by extravascular dosing. We have determined quantitatively the time course of rIL-2 in the plasma and urine of rats injected i.m. with an rIL-2/poloxamer 407 formulation compared to the same parameters following i.m. injection of an rIL-2 solution.

Several attempts have been made to sustain the blood levels of rIL-2 to increase therapeutic efficacy. As an example, Donohue and Rosenberg (1983) used a natural polymer (gelatin) to attempt to prolong the serum levels of rIL-2 following subcutaneous (s.c.) and i.p. injection of an rIL-2/gelatin formulation to mice. More recently, Dunn et al. (1988) reported the slow re-

lease of rIL-2 from an ethylene-vinyl-acetate (EVA) copolymer. Similarly, slow release of rIL-2 from an ALZET miniosmotic pump was more effective in augmenting the therapeutic efficacy of immune spleen cells in adoptive chemoimmunotherapy (ACIT) than a single injection of the same total dose of rIL-2 (Nishimura et al., 1986). A more practical approach for the sustained administration of rIL-2 involved implantation locally into the tumor site of a biodegradable rIL-2 minipellet fabricated with atelocollagen derived from natural bovine skin (Fujiwara et al., 1990). Others have employed Pluronic® F-127 as a slow release system for rIL-2 because of the ease of administration of an rIL-2/poloxamer formulation into any site of the body by use of a syringe (Morikawa et al., 1987) and the fact that the polymer depot need not be removed from the body at the completion of therapy. However, no measurements of plasma/blood rIL-2 concentrations were performed and hence, no pharmacokinetic parameters were reported (Morikawa et al., 1987).

Matory et al. (1985) have shown that an i.v. bolus dose of an rIL-2 aqueous solution to rats has an elimination half-life of 3.0 min. In contrast, Colburn et al. (1987) have demonstrated using human recombinant interleukin-2 identical to rIL-2 used in our studies that the apparent elimination half-life in rats following an intravenous bolus dose was approx. 27 min. In the present study, the apparent elimination half-life of rIL-2 was approx. 24 min. In addition, the mean value of k_a was less than that of k_{elim} for both the rIL-2/poloxamer 407 formulation and the aqueous rIL-2 solution when injected i.m. in rats. This suggests that the slope of the terminal phase of the plasma rIL-2 concentration-time curve was affected by the relatively slow absorption of rIL-2 when administered i.m. either as a solution or as a gel formulation. Thus, it appears from our study that rIL-2 absorption was rate-limiting with regard to overall disposition following i.m. injection in rats. The high molecular mass of rIL-2 (15.5 kDa) compared to conventional drug substances is a likely contributor to this absorption rate-limited process.

Since an equivalent dose of rIL-2 was administered i.m. to rats as either an rIL-2 aqueous solution or rIL-2/poloxamer formulation, an estimate of $F_{rel.}$ of rIL-2 for the gel formulation can be made. Our study has demonstrated that rIL-2 was completely available from the proposed sustained-release formulation when compared to the extent of absorption observed for rIL-2 injected i.m. as an aqueous solution. This is of paramount concern, especially with rIL-2, since prolonged exposure of replicating lymphocytes to rIL-2 at a predetermined therapeutic concentration is desired. To date, we are not familiar with any reports in the literature concerning an optimal blood rIL-2 concentration with which to eradicate a human tumor implanted in rats. This results primarily from the extensive use of athymic nude mice in cancer immunotherapy. Since we did not evaluate the pharmacokinetics of rIL-2 in rats following intravenous administration, the absolute bioavailability was not determined. However, Colburn et al. (1987) using rIL-2 identical to ours demonstrated a bioavailability of 0.16 in rats following i.m. injection when compared to plasma concentration-time profiles observed following intravenous infusion of rIL-2.

Although the mean cumulative amount of rIL-2 excreted in the urine after 48 h of rats injected i.m. with an rIL-2/poloxamer formulation was significantly ($p < 0.05$) less than the value for rats administered an rIL-2 aqueous solution, we do not feel that the results are reliable, since less than 1% of the injected dose was recovered. To date, the authors are not familiar with any reports in the literature that quantitatively describe either the rate or extent to which human rIL-2 is excreted in the urine of rats following parenteral administration. However, 8–10% of the dose of iodinated recombinant human interleukin-6 (^{125}I-rhIL-6) was shown to accumulate in the kidneys of rats following an intravenous injection (Castell et al., 1990). These authors were able to detect only [^{125}I]iodide in the urine, not intact ^{125}I-rhIL-6 (Castell et al., 1990). This may, in part, explain why we observed the low recovery of rIL-2 in the urine of rats injected i.m. with the rIL-2 preparations. Presumably, either intrarenal storage of rIL-2 and/or intrarenal metabolism (catabolism) of the protein may have contributed

to the low urinary recovery. Based on values obtained for the plasma clearance and volume of distribution in rats following systemic administration of rIL-2 identical in origin to the rIL-2 we used, it was suggested that human rIL-2 must be degraded in the vascular space or adsorbed to intravascular components of this species (Colburn et al., 1987). Previously it was reported that the principle route of serum IL-2 clearance in mice was the kidney (Donohue and Rosenberg, 1983). These authors demonstrated an almost 30-fold increase in the serum half-life of elimination when the kidneys were removed from the circulation by ligation of the renal vascular pedicle (Donohue and Rosenberg, 1983). Donohue and Rosenberg (1983) also reported that no IL-2 could be detected in dialyzed urine from animals with measurable serum IL-2 levels and suggested that this finding was consistent with the minimal excretion of similar sized proteins known to be degraded in the kidney (Strober and Waldmann, 1974). Further evidence that IL-2 is degraded in the kidney rather than excreted in the urine was obtained by demonstrating that bilateral ureteral ligation had little effect on the short half-life of IL-2 in mice with normal renal perfusion (Donohue and Rosenberg, 1983). Donohue and Rosenberg (1983) suggested that aggregation of IL-2 or conjugation of IL-2 to another molecule may be possible means of reducing renal clearance of IL-2 from the serum. Conjugation of IL-2 to another molecule in the renal tissue would suggest intrarenal storage of this protein.

The therapeutic implications of the sustained-release formulation we have evaluated are numerous. Specifically, other biological response modifiers could be formulated with the polymer and injected extravascularly. The sustained-release poloxamer depot is easily administered without the need for surgical implantation and contains no toxic additives. In the case of rIL-2, this is significant because it results in a diminution of peak plasma rIL-2 concentrations and allows for extended exposure of replicating lymphocytes to rIL-2. This, in turn, should maximize lymphocyte proliferation and result in enhanced tumor lysis. Since we have previously demonstrated that poloxamer 407 does not cause irreversible inactivation (denaturation) of rIL-2 (Johnston et al., 1992) as well as alter the protein's secondary structure (Wang and Johnston, 1993), the sustained-release rIL-2/poloxamer system should potentially increase the therapeutic efficacy of rIL-2 immunotherapy.

Acknowledgements

This research was supported by research grants from the Illinois Cancer Council and the American Association of Colleges of Pharmacy awarded to T.P.J. The authors are extremely grateful to Dr Maurice K. Gately, Hoffmann-La Roche, Inc., for generously providing all recombinant interleukin-2 used in this research.

References

BASF Wyandotte Corp., Technical data on Pluronic polyols, Publication No. 0S-796, Wyandotte, MI (1975).

Castell, J., Klapproth, J., Gross, V., Walter, E., Andus, T., Snyers, L., Content, J. and Heinrich, P.C., Fate of interleukin-6 in the rat. *Eur. J. Biochem.*, 189 (1990) 113–118.

Cheever, M.A., Greenberg, P.D., Fefer, A. and Gillis, S., Augmentation of the anti-tumor therapeutic efficacy of long-term cultured T lymphocytes by in vivo administration of purified interleukin 2. *J. Exp. Med.*, 155 (1982) 968–980.

Cheever, M.A., Thompson, J.A., Kern, D.E. and Greenberg, P.D., Interleukin 2 (IL 2) administration in vivo: Influence of IL 2 route and timing on T cell growth. *J. Immunol.*, 134 (1985) 3895–3900.

Colburn, W.A., Hakimi, J. and Bekersky, I., Plasma concentration profiles of human recombinant interleukin-2 (HrIL-2) in the rat following administration by various systemic routes. *Drug Metab. Dispos.*, 15 (1987) 429–431.

Donohue, J.H. and Rosenberg, S.A., The fate of interleukin-2 after in vivo administration. *J. Immunol.*, 130 (1983) 2203–2208.

Dunn, C.J., Staite, N.D. and Hardee, M.M., Slow release of interleukin-2 from polymer implants. Letters and Corrections in *Ann. Intern. Med.*, Nov. 1 (1988) 761–762.

Fujiwara, T., Sakagami, K., Matsuoka, J., Shiozaki, S., Uchida, S., Fujioka, K., Takada, Y., Onoda, T. and Orita, K., Application of an interleukin 2 slow delivery system to the immunotherapy of established murine colon 26 adenocarcinoma liver metastases. *Cancer Res.*, 50 (1990) 7003–7007.

Fults, K.A. and Johnston, T.P., Sustained release of urease from a poloxamer gel matrix. *J. Parenter. Sci. Technol.*, 44 (1990) 58–65.

Gibaldi, M. and Perrier, D., First order absorption. In Gibaldi, M. and Perrier, D. (Eds), *Pharmacokinetics*, 2nd Edn, Dekker, New York, 1982, pp. 33–43.

Grimm, E.A., Mazumder, A., Zhang, H.Z. and Rosenberg, S.A., Lymphokine-activated killer phenomenon. Lysis of natural killer resistant fresh solid tumor cells by interleukin-2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med.*, 155 (1982) 1823–1841.

Hefeneider, S.H., Conlon, P.J., Henney, C.S. and Gillis, S., In vivo interleukin 2 administration augments the generation of alloreactive cytolytic T lymphocytes and resident natural killer cells. *J. Immunol.*, 130 (1983) 222–227.

Johnston, T.P. and Miller, S.C., Inulin disposition following intramuscular administration of an inulin/poloxamer gel matrix. *J. Parenter. Sci. Technol.*, 43 (1989) 279–286.

Johnston, T.P. and Miller, S.C., Toxicological evaluation of poloxamers for intramuscular use. *J. Parenter. Sci. Technol.*, 39 (1985) 83–88.

Johnston, T.P., Punjabi, M.A. and Froelich, C.F., Sustained delivery of interleukin-2 from a poloxamer 407 gel matrix following intraperitoneal injection in mice. *Pharm. Res.*, 9 (1992) 425–434.

Matory, Y.L., Chang, A.E., Lipford, E.H., III, Braziel, R., Hyatt, C.L., McDonald, H.D. and Rosenberg, S.A., Toxicity of recombinant human interleukin-2 in rats following intravenous infusion. *J. Biol. Response Mod.*, 4 (1985) 377–390.

Mazumder, A. and Rosenberg, S.A., Successful immunotherapy of natural killer resistant established pulmonary melanoma metastasis by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin 2. *J. Exp. Med.*, 159 (1984) 495–507.

Merluzzi, U.J., Walker, M.M. and Faanes, R.B., Inhibition of cytotoxic T-cell clonal expansion by cyclophosphamide and the recovery of cytotoxic T-lymphocyte precursors by supernatants from mixed-lymphocyte cultures. *Cancer Res.*, 41 (1981) 3663–3665.

Morikawa, K., Okada, F., Hosokawa, M. and Kobayashi, H., Enhancement of therapeutic effects of recombinant interleukin-2 on a transplantable rat fibrosarcoma by the use of a sustained release vehicle, Pluronic® gel. *Cancer Res.*, 47 (1987) 37–41.

Nishimura, T., Togashi, Y., Goto, M., Yagi, H., Uchiyama, Y. and Hashimoto, Y., Augmentation of the therapeutic efficacy of adoptive tumor immunotherapy by in vivo administration of slowly released recombinant interleukin-2. *Cancer Immunol. Immunother.*, 21 (1986) 12–18.

Pec, E.A., Wout, Z.G. and Johnston, T.P., Biological activity of urease formulated in poloxamer 407 following intraperitoneal injection in the rat. *J. Pharm. Sci.*, 81 (1992) 626–630.

Richards, J.M., Therapeutic uses of interleukin-2 and lymphokine-activated killer (LAK) cells. *Blood Rev.*, 3 (1989) 110–119.

Rosenberg, S.A., Mule, J.J., Spiess, P.J., Reichert, C.M. and Schwarz, S.L., Regression of established pulmonary metastasis and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. *J. Exp. Med.*, 161 (1985) 1169–1188.

Snedecor, G.W. and Cochran, W.G., *Statistical Methods*, 7th Edn, Iowa State University Press, Ames, IA, 1980, pp. 64–79.

Stotter, H., Ruede, E. and Wagner, H., T-cell factor (interleukin 2) allows in vivo induction of T helper cells against heterologous erythrocytes in athymic (nu/nu) mice. *Eur. J. Immunol.*, 10 (1980) 719–722.

Strober, W. and Waldmann, T.A., The role of the kidney in the metabolism of plasma proteins. *Nephron*, 13 (1974) 35–39.

Wang, P. and Johnston, T.P., Enhanced stability of two model proteins in an agitated solution environment using poloxamer 407, *J. Parenter. Sci. Technol.*, 47 (1993) 183–189.

Wang, P. and Johnston, T.P., The kinetics of the sol-to-gel transition for poloxamer polyols. *J. Appl. Polym. Sci.*, 43 (1991) 283–292.