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Pharmacokinetics of the recombinant fusion protein $DAB_{486}IL\text{-}2$ in animal models

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Summary. The kinetics of the in vitro cytotoxicity of $DAB_{486}IL\text{-}2$, a genetically engineered fusion protein containing a portion of diphtheria toxin and human interleukin-2, were examined in the C91/PL cell line, which constitutively expresses IL-2 receptors. Maximal inhibition of protein synthesis was observed by 4–6 h after DAB_{486} IL-2 addition at a concentration of 300 ng/ml. The tissue distribution, urinary excretion, and plasma pharmacokinetics of $DAB_{486}IL\text{-}2$ in the rat and its plasma pharmacokinetics in the monkey were also examined. In rats the primary site of distribution of [^{35}S]-$DAB_{486}IL\text{-}2$ outside the vasculature appears to be the liver, followed by the kidney, spleen, and lung. Persistence of radioactive material in the liver and urinary excretion of metabolic degradation products suggest that labeled protein is metabolized by hepatic tissue. Following i. v. bolus administration of $DAB_{486}IL\text{-}2$, the initial serum half-life for both the rat and the monkey was approximately 5 min. The overall clearance rate of drug for the two species differed, with $DAB_{486}IL\text{-}2$ being cleared from circulation 2–3 times more rapidly in the monkey. Presence of high levels of neutralizing antibodies to diphtheria toxin in the rat significantly influenced the clearance of bioactive $DAB_{486}IL\text{-}2$. However, the question as to whether the presence of in vitro biological activity for the molecule is masked by the presence of antibodies cannot be clearly answered.

Introduction

The surface of many neoplastic cells contains elements critical to their continued survival and unregulated growth. Thus, many neoplastic cells express unique, altered, or high concentrations of specific surface antigens or cellular receptors for endogenous growth factors. Targeting the surface of neoplastic cells to attain selective cytotoxicity over normal cells has been a goal of a variety of research groups [10, 15, 16]. Various targeting strategies use either monoclonal antibodies to target surface antigens or specific ligands to target cell-surface receptors.

Cell-surface receptors offer an attractive pharmacological target. A variety of peptide growth factors and steroid hormone antagonists have been used for therapy of solid tumors to interrupt hormone-driven neoplastic growth [7, 12, 14]. An alternative approach has involved the coupling of peptide hormones to specific toxins, thereby creating a hybrid protein capable of both binding to specific cell-surface receptors via the ligand-binding domain and causing cell death by virtue of its toxin component. Over the past several years, several hybrid toxins have been designed and constructed using recombinant DNA methodology [6, 13, 18]. One such hybrid toxin, $DAB_{486}IL\text{-}2$, is a genetically engineered protein that contains the enzymatic and membrane translocation portions of diphtheria toxin (DT). The receptor-binding domain of the native DT molecule has been deleted and replaced with human interleukin-2 (IL-2) sequences.

Previous studies have shown that $DAB_{486}IL\text{-}2$ is selectively cytotoxic to cells expressing the high-affinity IL-2 receptor [1, 17]. The cytotoxicity of $DAB_{486}IL\text{-}2$ is the result of an interruption of protein synthesis that follows ribosylation and inactivation of protein-chain-elongation factor-2 [1]. As part of the preclinical evaluation of this agent, we examined its tissue distribution, urinary excretion, and pharmacokinetics in the rat and its pharmacokinetics in the monkey following i. v. administration.

Materials and methods

Hybrid toxin. $DAB_{486}IL\text{-}2$ was purified from extracts of *Escherichia coli* (pSI100-31) via immunoaffinity and high-pressure liquid chromatography, essentially as previously described [18]. Bioactive [^{35}S]-DAB_{486} IL-2 was purified from *E. coli* cultures grown in a labeled mixture of [^{35}S]-methionine and [^{35}S]-cysteine (Trans[^{35}S]-Label; ICN Pharmaceuticals, Irvine, Calif.) at a concentration of 0.1 mCi/ml culture

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medium. The specific activity of [^{35}S]-DAB$_{486}$IL-2 used in these studies was approximately 0.15 μCi/μg.

Cell culture. C91/PL cells derived from human T-cell leukemia-virus type I (HTLV-I)-transfected human umbilical-cord blood lymphocytes [9] were subcultured at 2- to 4-day intervals in RPMI 1640 medium (Gibco, Grand island, N.Y). supplemented with 2 m*M* glutamine (Gibco) and 15% fetal calf serum (Hazleton Biologicals, Inc., Lenexa, Kan.) These cells constitutively express high-affinity IL-2 receptors and were obtained from Dr. Robert Schwartz (New England Medical Center, Boston, Mass.).

Animals. Female Sprague-Dawley rats (200–250 g) were obtained from Harlan Sprague-Dawley Inc. (Indianapolis, Ind.). Adult feral cynomolgus monkeys (*Macaca fascicularis*) of both sexes weighing approximately 3–4 kg were purchased from Charles River Research Primates Corp. (Port Washington, N.Y.) and were housed at EG & G Mason Research Institute (Worcester, Mass.). All animals were maintained in accordance with the guidelines of the individual Institutional Animal Care and Use Committees and with those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources of the National Research Council. Preimmunized rats used in this study were immunized with i.m. injections of 100 μg diphtherin toxoid (DT) (Massachusetts State Laboratories, Boston, Mass.) in 0.1 ml PBS at 5, 8, and 10 weeks prior to the start of the study. At study initiation, preimmunized rats had anti-DT neutralizing antibody titers in the range of 3.8–7.7 IU/ml.

Neutralizing antibody assay. Neutralizing antibody titers were measured essentially as described by Zucker and Murphy [20]. Briefly, 2-fold dilutions of serum were prepared in Dulbecco's modified Eagle's medium (MEM) supplemented with 10% fetal calf serum, 1% nonessential amino acids, and 2 m*M* glutamine. Each dilution was incubated with an equal volume of DT (List Biological Laboratories, Campbell, Calif.) at a concentration of 310 ng/ml for 1 h at 37°C. A 100 μl aliquot of the preincubated mix was added to duplicate flat-bottom microtiter wells containing 10^5 Chinese hamster ovary cells (CCL 61; American Type Culture Collection, Rockville, Md.) in 100 μl complete medium. The cells were then incubated at 37°C in an atmosphere containing 5% CO_2. After 3 days, the cells were inspected visually for color change, indicative of cell growth, and microscopically for monolayer formation. The last dilution of serum that protected the cells from death was used as the end point and converted to international units (IU); 1 IU was defined as being the amount of antibody required to neutralize the cytotoxic effects of 2.5 μg DT.

Cytotoxicity assay. C91/PL cells were seeded in 96-well V-bottomed plates at a concentration of 10^5/well in 100 μl complete medium. DAB$_{486}$IL-2 or serum samples were added to triplicate wells in 100 μl complete medium at varying concentrations. Cells cultured in medium alone were included as a control. After an 18-h incubation at 37°C in an atmosphere containing 5% CO_2, plates were centrifuged for 5 min at 825 *g* to pellet the cells. Medium was aspirated from each well and then replaced with 100 μl leucine-free MEM (Gibco) containing 2.5 μCi/ml L-[^{14}C]-leucine (300 mCi/mmol; Dupont-New England Nuclear, boston, Mass.). After 90 min, cells were collected on glass-fiber filters using a Skatron cell harvester. Filters were washed, dried, and counted using 3 ml Ecolume scintillation fluid (ICN). Radioactivity in the treated cells was compared with that in control cells incubated with media alone. The IC_{50} was defined as being the concentration of DAB$_{486}$IL-2 required to inhibit 50% of control levels of radioactive leucine incorporation into protein. Serum concentrations of bioactive DAB$_{486}$IL-2 were determined from a comparison of the serum-sample IC_{50} to that of the starting test material. Using this technique, the limit of detection in serum samples is approximately 50–100 ng/ml.

Exposure time and DAB$_{486}$IL-2 cytotoxicity. A modification of the above assay was used to determine the exposure time required for DAB$_{486}$IL-2 to induce maximal inhibition of protein synthesis. In all, 5×10^5 C91/PL cells were incubated for 1, 3, 5, 15, 30, 60, or 90 min in 0.5 ml complete medium with 300 ng/ml DAB$_{486}$IL-2. After exposure to DAB$_{486}$IL-2, the cells were centrifuged in a microcentrifuge (1,310 *g*) for 5 s and the cell pellets were washed once with 0.5 ml warm PBS. Cells were then resuspended in 1 ml fresh medium and seeded into V-bottomed microtiter plates at 10^5 cells/well. For measurement of the maximal inhibition of protein synthesis, control cells were incubated in tubes with DAB$_{486}$IL-2 for 90 min, pelleted, resuspended twice, and then transferred directly to V-bottomed microtiter plates. All cells were incubated for 18 h at 37°C in an atmosphere containing 5% CO_2 and the level of protein synthesis inhibition was assessed using the [^{14}C]-leucine incorporation assay described above.

Time course for DAB$_{486}$IL-2 cytotoxicity. A modification of the cytotoxicity assay was used to determine the kinetics of intoxication by DAB$_{486}$IL-2. The assay was performed as described above, except that cells were incubated with 300 ng/ml DAB$_{486}$IL-2 for 1, 2, 3, 4, or 6 h prior to radiolabeling and harvesting rather than for the standard 18 h. In addition, cells were labeled for 60 min rather than for the standard 90 min.

Pharmacokinetic studies. For the rat study, four animals were anesthetized with pentobarbital and incisions were made in neck areas to the right and left of midline. Then, 250 μg DAB$_{486}$IL-2 (approximately 1 mg/kg) in 0.65 ml 0.01 M TRIS (pH 8.0)/0.15 M NaCl (TRIS-buffered saline) was injected into the exposed external right jugular vein. Blood samples (0.2 ml) were drawn from the exposed left external jugular at 1, 5, 15, 30, 45, 60, 90, and 120 min after administration. For determination of the effects of anti-DT antibodies on the clearance of DAB$_{486}$IL-2, a parallel study was performed in rats previously immunized with DT.

For the monkey study, two animals (one female, one male) were anesthetized with ketamine and catheters were implanted in both femoral veins. DAB$_{486}$IL-2 (0.25 mg/kg) was injected as an i.v. bolus into the right femoral vein in approximately 2 ml TRIS-buffered saline. Blood samples (1.5 ml) were drawn from the left femoral vein at 1, 5, 15, 30, 60, 120, 180, and 240 min after injection.

All blood samples were allowed to clot briefly on ice and were centrifuged within 15 min of collection. Serum samples were stored frozen (–20°C) until later analysis using the cytotoxicity assay described above. Previous studies have demonstrated that between 95% and 100% of DAB$_{486}$IL-2 is recoverable in the serum fraction and that the bioactivity of the molecule remains stable in serum for up to 2 h at 37°C (data not shown).

Tissue distribution of [^{35}S]-DAB$_{486}$IL-2 in rats. A single i.v. bolus injection of 5.7 μg [^{35}S]-DAB$_{486}$IL-2 was given to rats. At 5, 15, 30, 90, and 360 min postinjection, animals (four time point) were killed using CO_2 and the following organs were removed: liver, stomach, kidney, adrenal, lung, thymus, lymph node, and spleen. Samples from each tissue (approximately 100 mg) were weighed and homogenized in 0.5 ml PBS in a glass tissue grinder. A 100 μl aliquoit of each sample was added to a scintillation vial with 200 μl perchloric acid and 100 μl hydrogen peroxide to solubilize and decolorize the material, and the vials were incubated overnight at 75°C. After the vials had cooled, 5 ml scintillation fluid was aded and the radioactivity was measured.

The radioactive label in plasma was measured by adding 50 μl plasma to 0.5 ml of a solution containing 10 mM ethylenediamine tetraacetic acid (EDTA), 2% sodium dodecyl sulfate (SDS), and 50 mM Na_2CO_3 in a 7-ml scintillation vial. Then, 5 ml Ecolume scintillation fluid was added to each vial and the total radioactivity was determined. A second 50 μl aliquoit of plasma was added to 1 ml 5% trichloroacetic acid (TCA). The resulting protein precipitate was collected by centrifugation and solubilized as described above, and the amount of radioactivity associated with larger protein fragments was measured.

Urinary excretion of [^{35}S]-DAB$_{486}$IL-2 in rats. A dose of 5.7 μg [^{35}S]-DAB$_{486}$IL-2 was given as an i.v. bolus to four rats at study initiation. The animals were then housed individually in Nalgene metabolic cages for an 8-day period. At twice-daily intervals, urine and feces were collected. The total volume of urine from each pool was measured, a 100 μl sample from each pool was added directly to scintillation fluid, and radioactivity was determined. A second 100 μl sample from each pool was added to 1 ml 5% TCA: 50 μl fetal calf serum was then added and the resulting

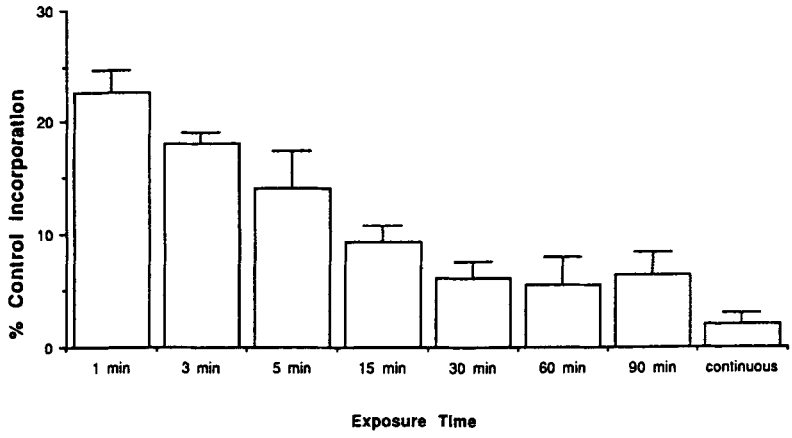

Fig. 1. Exposure time required for inhibition of protein synthesis induced by $DAB_{486}IL\text{-}2$. C91/PL cells were treated with $DAB_{486}IL\text{-}2$ at a dose of 300 ng/ml. At various time points the cells were washed and fresh media were added. The cells were then incubated for a total of 18 h and incorporation of [^{14}C]-leucine was measured. Values shown represent the mean ± SD from two experiments

protein precipitate was collected by centrifugation. The precipitate was solubilized with 0.5 ml of a solution containing 0.01 M EDTA, 2% SDS, and 0.05 M $NaCO_3$. The solubilized mixture was added to 5 ml scintillation fluid, and the radioactivity was measured. In addition, representative samples of fecal matter (approximately 50 mg) were mixed with 100 µl perchloric acid and 200 µl hydrogen peroxide to solubilize and decolorize the material, and the samples were then incubated overnight at 75°C. The mixture was cooled, scintillation fluid was added, and the radioactivity was determined.

Results

In Vitro cytotoxicity of $DAB_{486}IL\text{-}2$

It has previously been demonstrated that $DAB_{486}IL\text{-}2$ is specifically cytotoxic only to IL-2 receptor-bearing cells [1]. The C91/PL cell line constitutively expresses approximately 1,700 high-affinity IL-2 receptors/cell and is sensitive to $DAB_{486}IL\text{-}2$ in the range of 1–1,000 ng/ml. For determination of the time required for $DAB_{486}IL\text{-}2$ to bind irreversibly to cell-surface receptors, C91/PL cells were treated with a fixed amount of $DAB_{486}IL\text{-}2$ (300 ng/ml) for varying times and then washed free of drug. Fresh media was added and cells were incubated for a total of 18 h before [^{14}C]-leucine incorporation was measured. As shown in Fig. 1, incubation with $DAB_{486}IL\text{-}2$ for as little as 1 min reduced protein synthesis in these cells by >75%, with maximal inhibition of protein synthesis being achieved by a 30-min exposure.

In a separate study, the time required to achieve $DAB_{486}IL\text{-}2$ binding, internalization, and inhibition of protein synthesis was examined. C91/PL cells were treated continuously with a single dose of $DAB_{486}IL\text{-}2$ (300 ng/ml), and [^{14}C]-leucine incorporation was examined at various time points. As shown in Fig. 2, some inhibition of protein synthesis in these cells could be observed as early as 1 h after $DAB_{486}IL\text{-}2$ addition, with maximal inhibition being observed by 4–6 h.

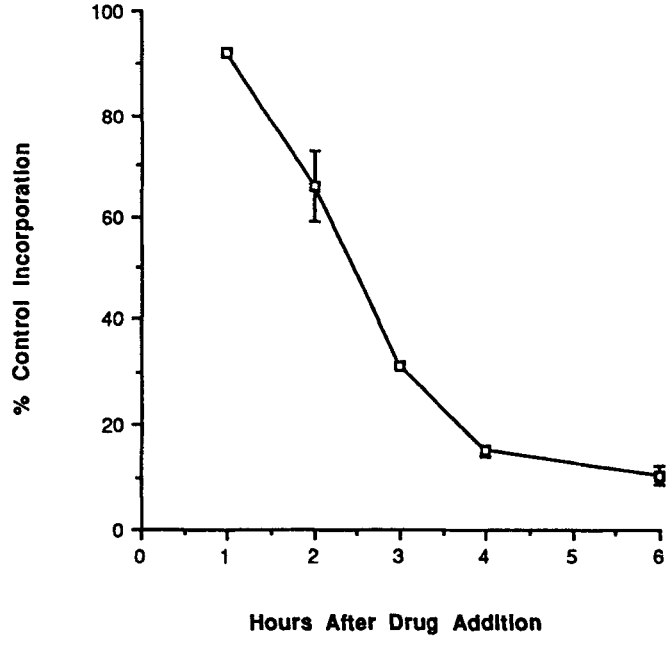

Fig. 2. Time course for inhibition of protein synthesis by $DAB_{486}IL\text{-}2$. C91/PL cells were treated continuously with 300 ng/ml $DAB_{486}IL\text{-}2$ and [^{14}C]-leucine incorporation was measured at various time points. Values shown represent the mean ± SD from two experiments

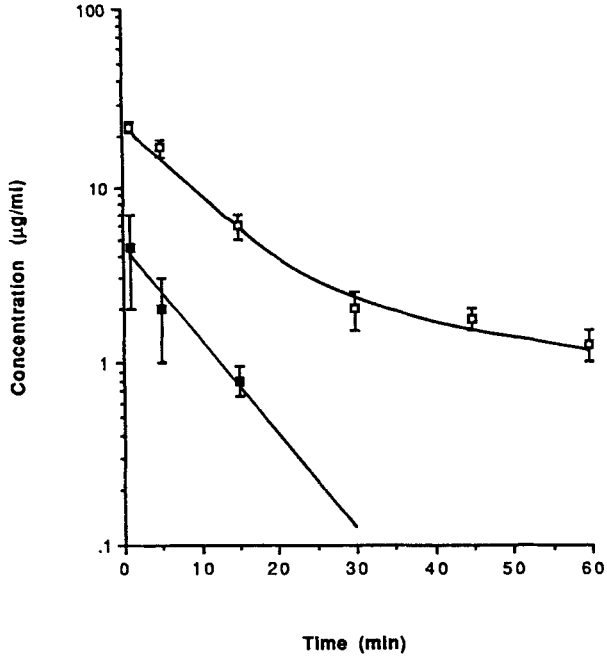

Fig. 3. Clearance of $DAB_{486}IL\text{-}2$ in naive (□) and preimmune (■) rats. Animals were given 250 µg (approximately 1 mg/kg) $DAB_{486}IL\text{-}2$ by an i.v. bolus injection. Biologically active $DAB_{486}IL\text{-}2$ was measured in serum samples taken at various time points

Table 1. Pharmacokinetic parameters associated with clearance of $DAB_{486}IL$-2 in naive and preimmunized rats

Parameter	Naive rats	Preimmune rats
$t_{1/2}\alpha$(min)	5.9± 0.6	5.4± 0.1
$t_{1/2}\beta$(min)	21.7± 2.9	–
Vd(ml)	9.6± 0.9	120.0±37.2
Cxt (μg ml^{-1} min)	287.7±15.6	37.4±26.5
Clp (ml kg^{-1} min)	3.6± 0.1	69.1±21.2
Approximate dose (μg/kg)	1,000	1,000
Weight (kg)	244.5±17.7	242.5± 3.5

$t_{1/2}\alpha$, half-life alpha phase; $t_{1/2}\beta$, half-life beta phase; Vd, volume of distribution; Cxt, total exposure to drug or area under the curve; Clp, clearance rate

Pharmacokinetics of $DAB_{486}IL$-2 in rats

The serum clearance of $DAB_{486}IL$-2 following i.v. bolus administration was examined over a 120-min period in both naive and preimmune rats (Fig. 3). Despite identical doses of drug, serum concentrations of bioactive $DAB_{486}IL$-2 in naive rats were approximately 10-fold those measured in preimmune rats. After 15 min, the level of drug in the serum of preimmune animals was below the detection level of the assay. The bioactivity in serum for naive animals closely fit (r >0.9) an open, two-compartment model for clearance; an initial rapid clearance ($t_{1/2}\alpha$, 5.9 min; Table 1) was followed by a more prolonged terminal phase ($t_{1/2}\beta$, 21.7 min). Although the calculated initial $t_{1/2}$ value for preimmune rats was identical to that of naive rats (Table 1), a second clearance phase was not observed in the former animals. In addition, the apparent volume of distribution (Vd) in preimmune animals was 12-fold that observed in naive rats (120 vs 9.6 ml) and the total drug exposure (Cxt) was 7 times lower (37.4 vs 287.7 μg ml^{-1} min), suggesting the immediate sequestration of $DAB_{486}IL$-2, possibly into antibody-drug complexes.

Tissue distribution of [^{35}S]-$DAB_{486}IL$-2 in rats

The tissue distribution of [^{35}S]-$DAB_{486}IL$-2 in the rat was determined over a 360-min period (Table 2). Drug was primarily distributed to the liver (16.7% of the injected dose/g tissue) at levels 15-fold those found in the kidney (0.98%). Small amounts of drug were found in spleen (0.48%), lung (0.31%), adrenal (0.07%), stomach (0.06%), and thymus (0.02%) tissues. Over the 360-min time course of this experiment, the concentration of total radioactivity in the liver and in the kidney decreased by approximately 50%. The concentration of total radioactivity in lung (0.31%–0.64%) and thymus (0.02%–0.14%) tissues appeared to increase over this period.

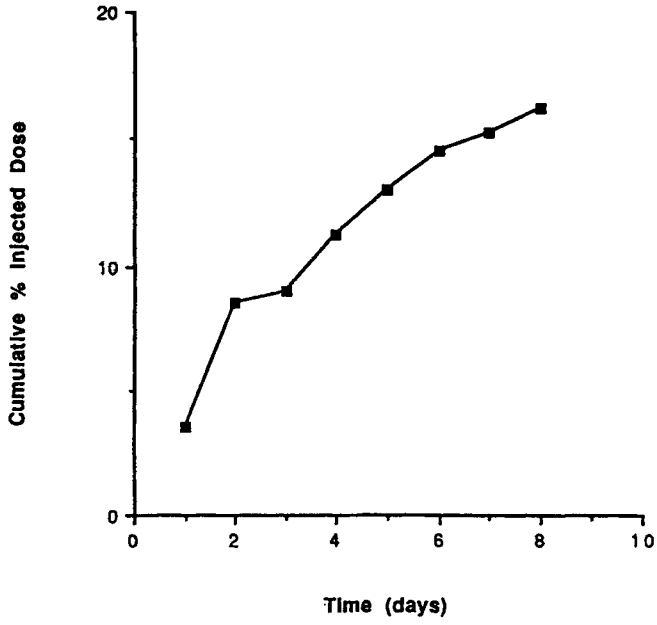

Fig. 4. Urinary excretion of radioactivity after an i.v. bolus injection of [^{35}S]-$DAB_{486}IL$-2 in the rat. Over 80% of the radioactivity excreted was present as TCA-soluble material

In the plasma, an initial rapid clearance of radioactive material over the first 15 min was observed, followed by a slow rise in radioactive counts after 30 min; approximately 10% of the total injected dose subsequently reappeared. Greater than 90% of total radioactivity in the plasma was TCA-precipitable at all time points. Therefore, significant metabolic degradation of [^{35}S]-$DAB_{486}IL$-2 did not appear to occur in the plasma over the duration of this study.

Urinary excretion of [^{35}S]-$DAB_{486}IL$-2 in rats

Approximately 16% of the total injected dose of [^{35}S]-$DAB_{486}IL$-2 was excreted in urine over 8 days, as shown in Fig. 4. Most of the radiolabel (8.5%) was excreted within the first 48 h, with 1%–2% of the injected dose

Table 2. Distribution of [^{35}S]-$DAB_{486}IL$-2 in various tissues and plasma in rats

Tissue	5 min	15 min	30 min	90 min	360 min
Plasma	17.00±1.10	10.10±0.20	5.40±0.20	7.70±0.50	9.50±0.90
Liver	16.70±0.82	16.20±2.99	10.39±1.41	7.84±0.76	8.78±1.74
Kidney	0.98±0.12	1.04±0.07	0.56±0.05	0.52±0.03	0.59±0.11
Spleen	0.48±0.08	0.45±0	0.38±0.06	0.26±0.04	0.31±0.04
Lung	0.31±0.05	0.26±0.05	0.18±0.02	0.38±0.09	0.64±0.11
Adrenal	0.07±0	0.08±0.01	0.06±0	0.04±0	0.04±0
Stomach	0.06±0.01	0.14±0.04	0.31±0.10	0.50±0.12	0.61±0.07
Thymus	0.02±0	0.02±0	0.05±0.02	0.07±0	0.14±0.03

Values are expressed as a percentage of the injected dose/organ or of the total plasma volume

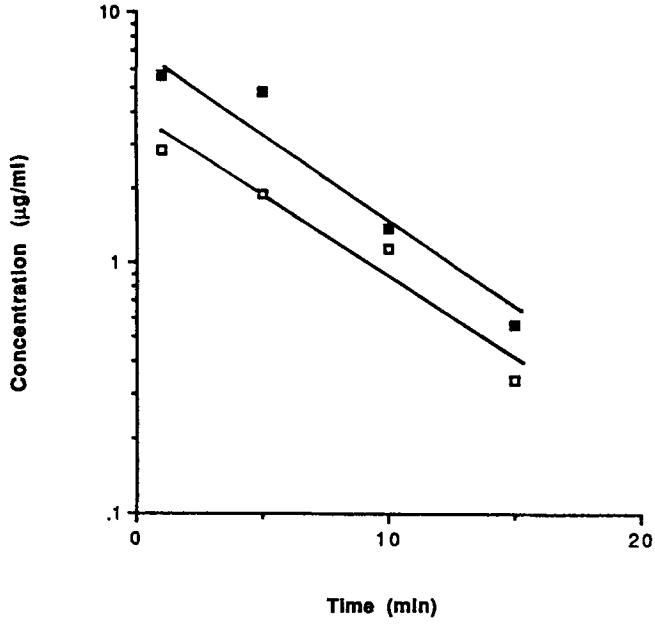

Fig. 5. Clearance of $DAB_{486}IL$-2 in monkeys 8–33 (□) and 8–35 (■). Animals were given 0.25 mg/kg $DAB_{486}IL$-2 by an i.v. bolus injection. Biologically active $DAB_{486}IL$-2 was measured in serum samples taken at various time points

being excreted daily thereafter. Greater than 80% of the radioactivity excreted in the urine was present as TCA-soluble material, suggesting that the radiolabeled material measured in urine is composed of metabolic degradation products. No radioactive material was detected in the feces.

Pharmacokinetics of $DAB_{486}IL$-2 in the monkey

The serum clearance of $DAB_{486}IL$-2 following i.v. bolus administration was examined over a 240-min period in two monkeys (Fig. 5). At the dose delivered, which was approximately one-fourth of that given to rats, serum drug levels were not detectable beyond 15 min after administration. The clearance of $DAB_{486}IL$-2 in the monkey following bolus administration fit a one-compartment model, with the serum half-life being 4.6 min (Table 3). The clearance rate of drug (Clp) in the monkey study (6.8 and 10 ml kg^{-1} min) was 2- to 3-fold that observed for the rat model (3.6 ml kg^{-1} min). Also, the total exposure to the drug (Cxt), when adjusted for the different doses given, was 3 times lower in the monkey than in the rat. Thus, considerable variation in the pharmacokinetics of $DAB_{486}IL$-2 is apparent in the two species studied.

Discussion

$DAB_{486}IL$-2 is selectively cytotoxic only to those eukaryotic cell lines that bear high-affinity IL-2 receptors [1, 17], suggesting that $DAB_{486}IL$-2 is an attractive candidate for the treatment of IL-2 receptor-positive malignancies. In vitro studies [5, 8] have demonstrated that cells from patients with adult T-cell leukemia that constitutively express IL-2 receptors are sensitive to the cytotoxic action of

Table 3. Pharmacokinetic parameters associated with clearance of $DAB_{486}IL$-2 in monkeys

Parameter	Animal 8–33	Animal 8–35
Sex	Male	Female
$t_{1/2}$(min)	4.6	4.6
Vd (ml)	273	167
Cxt (µg ml^{-1} min)	25	36.8
Clp (ml kg^{-1} min)	10	6.8
Dose (µg/kg)	250	250

$t_{1/2}$, half-life; Vd, volume of distribution; Cxt, total exposure to drug or area under the curve; Clp, clearance rate

$DAB_{486}IL$-2. In this report, the interaction of $DAB_{486}IL$-2 with the C91/PL HTLV-I-transformed cell line, which constitutively expresses high numbers of IL-2 receptors (approximately 1,700 high-affinity sites/cell), has been further characterized. Binding of $DAB_{486}IL$-2 to IL-2 receptors on the surface of C91/PL cells occurs rapidly. A 30-min drug exposure is required for nearly maximal inhibition of protein synthesis; however, incubation with $DAB_{486}IL$-2 for as little as 1 min prior to drug removal by washing inhibited [^{14}C]-leucine incorporation in these cells by 78%. When the kinetics of inhibition of protein synthesis was examined (e.g., the time required for binding, internalization, and inactivation of elongation factor-2), inhibition of [^{14}C]-leucine incorporation in C91/PL cells could be observed as early as 1 h after $DAB_{486}IL$-2 addition, with a maximal effect on protein synthesis being achieved by 4–6 h with continuous exposure to $DAB_{486}IL$-2.

The circulating half-life of $DAB_{486}IL$-2 following i.v. bolus administration for both the rat and the monkey is approximately 5 min. However, the overall clearance rate for the two species differed, with drug being cleared from circulation 2–3 times faster in the monkey. Since the primate data did not fit the two-compartment model described for the rodent data, it is possible that an inability to detect drug levels beyond 15 min in the monkey (due to assay sensitivity) resulted in an incomplete pharmacokinetic assessment. Early clinical studies of $DAB_{486}IL$-2 indicate that serum clearance of the drug is similar to that predicted for the two animal species studied (e.g., approximately 5 min).

The presence of high levels of neutralizing antibodies to DT significantly influenced the clearance of bioactive $DAB_{486}IL$-2 in the rat: the second clearance phase was abolished and the apparent volume of distribution was increased 12-fold, suggesting rapid antibody-antigen complex formation. Antibodies may either promote the clearance of $DAB_{486}IL$-2 from circulation or mask detection of the in vitro biological activity of the molecule. If antibodies mask detection by the formation or complexes, such complexes may actually prolong the circulation of the drug if the interaction is reversible. Adriamycin, for example, can reversibly complex with serum proteins, which serve as a circulating "silent reservoir" for the drug [3]. Indeed, clearance studies with [^{35}S]-$DAB_{486}IL$-2 in preimmune rats have shown a persistence of radiolabeled material in circulation paralleling that in naive rats. Thus, clinical implica-

tions for the development of antibodies to $DAB_{486}IL\text{-}2$ cannot be predicted at this time. The development of antibodies to other biologicals such as the interferons [4], and some murine monoclonal antibodies [19] has not resulted in adverse clinical effects despite the induction of changes in pharmacokinetic profiles.

In rats, the primary site of distribution outside the vasculature appears to be the liver (16.7% injected dose/organ), with levels 15–60 times lower being found in kidney, spleen, and lung tissues. The tissue distribution of $DAB_{486}IL\text{-}2$ appears to be similar to that observed for IL-2 alone [11]. Unexpectedly, the concentration of DAB_{486} IL-2 in the lung increased 2-fold (from 0.31% to 0.64%) over the 360-min study, which may be attributable to continued uptake by alveolar macrophages. The slow accumulation of small amounts of radioactive material in the thymus (0.02%–0.06%) is potentially due to specific uptake by a small number of IL-2 receptor-positive T-cells [2]. Persistence of radioactive material in the liver would suggest that labeled protein is metabolized by hepatic cells. Urinary excretion of only metabolic degradation products has been observed and would support this hypothesis. The liver may also serve as a reservoir for redistribution of [^{35}S]-labeled material into the plasma compartment. Such a shift may account for the observed rise in subsequent plasma radioactive counts (90 and 360 min) in the rat distribution study. If the same pattern of tissue distribution and excretion for $DAB_{486}IL\text{-}2$ holds true for humans, these studies suggest that toxic effects would be manifested first in the liver and kidney.

Paolozzi et al. [8] have shown that primary cultures of HTLV-I-infected tumor cells require 75 to 240 min continuous exposure to $DAB_{486}IL\text{-}2$ for the occurrence of a maximal decrease in cell viability. The discrepancy between this observation and the present data may be attributable to differences in experimental technique. Alternatively, the discrepancy could be the result of differences in high-affinity receptor numbers, in the efficiency of internalization, or in the rate of metabolism in primary cultured cells vs HTLV-I-infected cell lines such as C91/PL. Rapid, irreversible binding of $DAB_{486}IL\text{-}2$ to the high-affinity IL-2 receptor suggests that $DAB_{486}IL\text{-}2$ can result in tumor cell cytotoxicity despite a short circulating half-life following i.v. bolus administration. However, the rapid clearance of $DAB_{486}IL\text{-}2$ in monkeys, and preliminarily in humans, suggests that the achievement of *optimal* therapeutic efficacy may require administration of the drug by infusion. Furthermore, since the toxicity of $DAB_{486}IL\text{-}2$ may partly be associated with peak serum concentrations, infusion may enhance the therapeutic index by minimizing the drug's nonspecific cytotoxicity, which occurs at concentrations of >5,000 ng/ml. Studies are currently being conducted with $DAB_{486}IL\text{-}2$ to examine alternate routes of administration and infusion conditions so as to achieve maximal therapeutic benefit with minimal toxicity.

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