L-Lysine Effectively Blocks Renal Uptake of $^{125}$I- or $^{99m}$Tc-labeled Anti-Tac Disulfide-stabilized Fv Fragment

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ABSTRACT

In this study, we investigated the ability of L-lysine to block renal uptake of $^{125}$I- or $^{99m}$Tc-labeled Fv fragments. Anti-Tac disulfide-stabilized Fv fragment (dsFv) was derived from a murine monoclonal antibody that recognizes the $\alpha$ subunit of the interleukin-2 receptor (IL-2$\alpha$). The $^{125}$I- or $^{99m}$Tc-labeled dsFv was injected i.v. into non-tumor-bearing nude mice or into nude mice bearing SP2/Tac (IL-2$\alpha$ positive) and SP2/0 (IL-2$\beta$ negative) tumors. We then evaluated the pharmacokinetics of L-[3H]lysine and the effect of L-lysine dose, timing of administration, and route of delivery on catabolism and biodistribution of i.v. dsFv. Peak renal uptake of i.v. or i.p. injected L-[3H]lysine occurred within 5 and 15 min, respectively. The kidney uptake of L-lysine exhibited a dose-response effect. When L-lysine was coinfused or injected shortly before dsFv, renal uptake of dsFv was blocked to <5% of the control, but longer intervals were less effective. Aminosyn II and Travasol 10% (parenteral amino acid solutions) also blocked renal uptake of radiolabeled dsFv. Administration of L-lysine did not alter the blood kinetics and slightly increased the tumor uptake of dsFv, but it did prevent catabolism in the kidney and resulted in lower amounts of catabolites in the serum and urine. In conclusion, we have shown that a blocking dose of lysine, injected with or immediately before the injection of radiolabeled dsFv, is most effective in blocking the renal uptake of dsFv. This is consistent with the rapid uptake of L-[3H]lysine by the kidney and is further substantiated by the relative ineffectiveness of lysine injected immediately after the radiolabeled dsFv injection.

INTRODUCTION

The use of radiolabeled MoAbs for radioimmunodetection and radioimmunotherapy has been extensively evaluated since the initial clinical report of Mach et al. (1) and others (2–4). The major limitations of intact immunoglobulins for targeting tumor are their poor penetration into tissues (5, 6) and the development of human anti-mouse immune response (7). Fab fragments have been used as an alternative, in particular when they are used for imaging, because of their improved kinetics and their lower immunogenicity (8–10). The use of molecular engineering techniques has provided new reagents that may further improve the tumor:non-tumor ratios and decrease immunogenicity. Genetically engineered variable-region fragments (Fv fragments) consist of portions of the heavy- and light-chain variable domains (V_H and V_L, respectively) that maintain the antigen-binding specificity of an intact antibody (11–14). Several preclinical trials have used radiolabeled scFv in which the V_H and V_L are linked by covalent bonds through a spacer arm (6, 15–19); alternatively, dsFv, in which the chains are linked by a disulfide bond, has been used (14, 20). Because of their smaller size (~25 kDa), Fvs have much faster kinetics than intact IgG, their distribution is more uniform (5, 6), and they are expected to be less immunogenic (7).

Although radiolabeled Fvs have shown favorable tumor:non-tumor ratios, they have also shown undesirable elevations in renal uptake. This high renal uptake decreases imaging sensitivity in the abdomen and results in unfavorable dosimetry. In recent preclinical work with anti-Tac dsFv, an antibody directed against the $\alpha$ subunit of IL-2$\alpha$ (21), we have seen from 50% to 70% of the ID rapidly localize in mouse kidneys (22). This high renal uptake has been confirmed with directly iodinated dsFv and dsFv labeled with active esters containing either $^{18}$F or $^{99m}$Tc (22, 23). The radioactivity uptake in the kidney is particularly striking when $^{111}$In or $^{177}$Lu is used for labeling Fab or Fv fragments because their catabolic products are not as rapidly excreted (17, 24–26).

Hammond et al. (27) showed that patients receiving L-lysine infusions had lower levels of $^{111}$In somatostatin analogue in the kidneys than did controls. Similarly, L-lysine blocked renal uptake of a $^{18}$F-labeled somatostatin analogue (28). In addition, preclinical work with L-lysine has shown good blockage of renal uptake of radiolabeled Fab in nude mice models (24–26). In this study, we evaluated whether L-lysine would effectively block the renal uptake of dsFv labeled with $^{125}$I or $^{99m}$Tc. In addition, we evaluated the pharmacokinetics of L-lysine, the timing of its administration with respect to radiolabeled dsFv, and its effect on catabolite production.

MATERIALS AND METHODS

MoAbs. We used a disulfide-bonded Fv fragment of anti-Tac murine MoAb (20). Anti-Tac is an IgG2a that recognizes the IL-2$\alpha$ receptor (21). Production of anti-Tac dsFv has been described previously (20). Briefly, the $V_H$ and $V_L$ domains were expressed in separate Escherichia coli cultures. The proteins were recovered as cytosolic inclusion bodies and refolded. The refolded anti-Tac dsFv was purified by ion-exchange and size-exclusion chromatography. The product was >98% pure as determined by size-exclusion HPLC with a UV detector.

Radiolabeling. The anti-Tac dsFv was labeled with $^{125}$I using the Iodo-Gen method as described previously (29). Briefly, 100 $\mu$g of dsFv in 80 $\mu$l of PBS, pH 7.2, and approximately 1 mCi of sodium iodide-125 were added in a conical polypropylene vial coated with 10 mCi of iodogen. After incubation for 10 min at room temperature, the radiolabeled product was purified using a PD-10 column (Pharmacia Biotech AB, Uppsala, Sweden). The specific activity of the $^{125}$I-labeled anti-Tac dsFv was about 40–50 mCi/mg. The radiochemical purity of $^{125}$I-labeled anti-Tac dsFv was >98% as confirmed by instant TLC and size-exclusion HPLC.

The anti-Tac dsFv was also labeled with $^{99m}$Tc using MAG3 chelate. Benzoyl MAG3 was synthesized according to the method of Fritzsche et al. (30) and radiolabeled with $^{99m}$Tc using the method of Visser et al. (31) and Yoo et al. (32). In brief, 150 $\mu$l of 1.0 $\mu$m sodium carbonate (pH 11.7), 25 $\mu$l of benzoyl-MAG3 (1 mg/ml in 9:1 acetonitrile:water), 200 $\mu$l of $^{99m}$Tc pertechnetate (up to 10 mCi), and 100 $\mu$l of stannous chloride monohydrate (1 mg/ml) were added in a 5-ml glass vial. The vial was placed in a boiling water bath for 10 min. The reaction solution was then cooled in an ice-water bath and its pH was adjusted to between 5.7 and 6.3 by the addition of 270 $\mu$l of 1.0 N sulfuric acid. The radioactivity uptake in the kidney was further substantiated by the relative ineffectiveness of L-lysine injected immediately after the radiolabeled dsFv injection.

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2 The abbreviations used are: MoAb, monoclonal antibody; dsFv, disulfide-stabilized Fv; scFv, single-chain Fv; LWMP, low molecular weight protein; ID, injected dose; IL-2$\alpha$, $\alpha$ subunit of the interleukin-2 receptor; MAG3, mercaptopropanyl-glucuronic acid; GFR, glomerular filtration rate; HPLC, high-performance liquid chromatography.
dimethylaminopropyl)-carbodiimide were added. The esterification reaction was performed at room temperature for 30 min with gentle shaking. The reaction solution was diluted to 8 ml with distilled water, and the product, 99mTc-2,3,5,6-tetrafluorophenyl ester of MAG3, was purified by a Sep-Pak C18 column (Waters, Milford, MA). The diluted reaction mixture was loaded into the column. The column was sequentially eluted with 20 ml of distilled water, 30 ml of 20% ethyl alcohol in 0.01 M sodium phosphate at pH 6.7, 0.5 ml of ethyl ether, and finally with acetonitrile. The eluate with acetonitrile was collected in 1-ml fractions. The radioactive fractions were mixed with 10 ml of 0.1 M 4-morpholinoethanesulfonic acid (Fishier Scientific, Fair Lawn, NJ), pH 5.6. The organic solvent was then evaporated off with a stream of nitrogen gas to the final volume of 10–20 µl. To this solution, 10 µl of N,N-dimethylformamide, 6 µl of 1 M sodium bicarbonate, and 50 µl of dsFv (1.25 mg/ml of PBS, pH 7.4) were added. The conjugation reaction was performed on an ice bath for 30 min. The final product, 99mTc-MAG3-dsFv, was purified with a size-exclusion PD-10 column (Pharmacia Biotech AB, Uppsala, Sweden). The purity of the final product was >98% as determined by size-exclusion HPLC using a TSK G2000SW column (TosoHaas, Philadelphia, PA; 0.067 m sodium PBS-0.1 M KCl, pH 6.8, 0.5 ml/min) and an on-line radioactivity detector. In most studies, we used i.v. coinjections of the 125I- and 99mTc-labeled dsFv. In that case, after labeling each preparation, they were mixed in the desired concentration and injected i.v. in a final volume of 200 µl.

**Immunoreactivity.** Immunoreactivity was determined using a modification of the cell binding assay of Lindmo et al. (33). Aliquots of the radiolabeled dsFv (0.7–1.0 ng/100 µl) were incubated for 2 h at 4°C with 1.1 X 10^4 to the IL-2Ra-positive SP2/Tac cell line and the IL-2Ra-negative SP2/0 cell line. The percentage of the cell binding assay was evaluated relative to that of the radiolabeled dsFv.

Groups of mice (n = 5) were injected i.p. with 10 mg of L-lysine. Ten µCi of 99mTc-DTPA were then injected via the tail vein at 5, 15, and 60 min after the L-lysine injection. Serial timed tail vein sampling was then performed in each mouse at approximately 2, 5, 10, 15, 30, and 60 minutes, using calibrated 10-µl micropipets. The area underneath the blood curve was then calculated by trapezoidal integration (Sigma plot; Jandel Scientific, San Rafael, CA).

**Timing of L-Lysine Administration.** The timing of L-lysine administration relative to that of the radiolabeled dsFv was evaluated. Groups of mice (n = 5) were injected i.p. with 50 mg of L-lysine in 200 µl of 0.1 M phosphate buffer. The mice were then injected i.v. with a total of 1–2 µg of anti-Tac dsFv labeled with 1.5–2 µCi of 125I and 4–5 µCi of 99mTc in 200 µl of PBS with 1% human serum albumin. The dsFv was injected at various times, including 10 sec and 5 min before and 2, 15, 30, 60, and 120 min after L-lysine injection. In addition, a control group received the radiolabeled dsFv without i.p. L-lysine. The mice were euthanized 15 min after i.v. injection of the radiolabeled dsFv.

**Effect of L-Lysine on GFR.** To determine if L-lysine affected the GFR, we used 99mTc-DTPA, a commercially available reagent for measuring GFR. Groups of mice (n = 4) were injected i.p. with 1 or 10 mg of L-lysine. Ten µCi of 99mTc-DTPA were then injected via the tail vein at 5, 15, and 60 min after the L-lysine injection. Serial timed tail vein sampling was then performed in each mouse at approximately 2, 5, 10, 15, 30, and 60 minutes, using calibrated 10-µl micropipets. The area underneath the blood curve was then calculated by trapezoidal integration (Sigma plot; Jandel Scientific, San Rafael, CA).

**Effect of L-Lysine on Catabolite Formation.** We evaluated the effect of L-lysine and Aminosyn II on the appearance of catabolites in the serum and urine after i.v. injection of radiolabeled dsFv. Groups of mice (n = 5) were injected i.v. with 1–2 µg of anti-Tac dsFv labeled with 1.5–2 µCi of 125I and 4–5 µCi of 99mTc in 200 µl of PBS, followed 15 min later by i.p. injection of 50 mg of L-lysine. Mice were sacrificed at 15, 45, and 90 min after dsFv injection. The uptake of 125I- or 99mTc-labeled anti-Tac dsFv was measured by liquid scintillation counting.

**Administration of L-Lysine after dsFv Injection.** Groups of mice (n = 5) were injected i.v. with 1–2 µg of anti-Tac dsFv labeled with 1.5–2 µCi of 125I and 4–5 µCi of 99mTc in 200 µl of PBS, followed 15 min later by i.p. injection of 50 mg of L-lysine. Mice were sacrificed at 15, 45, and 90 min after dsFv injection. The uptake of 125I- or 99mTc in the kidneys, blood, and liver was determined. Data were compared to the distribution data from the ID.

**Effect of L-Lysine on Catabolite Formation.** We evaluated the effect of L-lysine and Aminosyn II on the appearance of catabolites in the serum and urine after i.v. injection of radiolabeled dsFv. Groups of mice (n = 5) were injected i.v. with 1–2 µg of anti-Tac dsFv labeled with 1.5–2 µCi of 125I and 4–5 µCi of 99mTc and with a total of 1–2 µg in 200 µl of PBS that was mixed with either 50 mg of L-lysine or 0.2 ml of Aminosyn II. The animals were euthanized 15 min after i.v. injection of dsFv, and urine and serum samples were obtained and analyzed by size-exclusion HPLC. An aliquot of the 125I-dsFv in Aminosyn II was analyzed by HPLC before injection. All HPLC runs were performed using a TSK G2000SW column eluted with 0.067 M sodium PBS-0.1 M KCl, pH 6.8.
(0.5 ml/min), equipped with an on-line NaI gamma detector (γ RAM, IN/US Systems, Inc., NJ). In addition, the % ID/g of blood was also determined to allow calculation of the total amount of radioactivity as intact dsFv or as metabolites.

To determine if dsFv remained intact or had undergone catabolism, in a separate experiment, groups of mice (n = 3) were injected via the tail vein with 10 μCi and 3 μg of 125I-labeled anti-Tac dsFv in 200 μl of PBS. Serum, kidney, and urine samples were obtained 15, 45, and 360 min after the injection of dsFv. The kidneys were removed immediately after euthanasia and frozen on dry ice. They were then allowed to thaw at room temperature, and this cycle was repeated twice more. The kidneys were then placed in 4-fold excess of ice-chilled PBS and homogenized using a tissue homogenizer (Tissue Tearor, Scientific Equipment, Bartlesville, OK) at 30,000 rpm for 2 min. The kidney homogenates were centrifuged at 20,000 × g (J2-21; Beckman, Palo Alto, CA) for 60 min. The total activity in the homogenized kidney was determined by counting in a dose calibrator. To determine recovery in the supernatant and cell pellet, these fractions were also counted in the dose calibrator. Serum, urine, and kidney supernatants were then analyzed using a size-exclusion HPLC system. The total activity in the homogenized kidney was determined by counting in a dose calibrator. The recoveries in the supernatants from all of the samples were >80%.

Biodistribution in Tumor-bearing Mice. Once the optimum L-lysine dose that blocked renal uptake was determined, this dose was used in conjunction with a full biodistribution study to determine the effects of L-lysine on other organs, including tumor. Groups of mice (n = 5) containing SP20 and SP2/Tac xenografts were given i.v. injections of 1-2 μg of anti-Tac dsFv radiolabeled with 1.5-2 μCi of 125I and 4-5 μCi of 99mTc. Mice were euthanized at 15, 45, and 90 min, and their organs were removed, weighed, and counted. To determine the whole-body retention, the carcasses were also counted. Data were expressed as both % ID/g of tissue and tumor:normal tissue ratios.

RESULTS

Lysine Pharmacokinetics in Blood and Kidney. A comparison of the i.v.- or i.p.-administered L-[3H]lysine showed similar time-activity curves and maximal uptake of L-lysine in blood or kidney for a given dose level. Nevertheless, differences in the time to peak renal levels were noted (Fig. 1, A and B). Peak renal uptake of 3H from i.v.-injected L-lysine occurred quickly (<5 min). Peritoneal egress of L-[3H]lysine was also prompt and resulted in peak kidney uptake occurring by 15 min; at least half of the maximum kidney uptake was seen by 2 min after i.p. injection. Whereas changes in renal uptake (% ID/g) of L-lysine were observed between 1 and 10 to 50 mg, few differences in blood retention were noted either at 15 or 30 min. Although uptake in the kidneys showed dose-dependent changes with doses of 1 or 10 mg, doses of >10 mg showed similar kidney uptake. Doses ranging from 1 to 50 mg resulted in similar blood concentrations (% ID/g).

Effect of L-Lysine on GFR. The area underneath the 99mTc-DTPA blood time-activity curve for the groups of animals receiving the various doses of L-lysine or PBS control was not significantly different (ANOVA, P = 0.11).

Timing of L-Lysine Administration. Intraperitoneal L-lysine was most effective in blocking renal uptake when given immediately before the radiolabeled dsFv (Fig. 2). When given 2 min before dsFv, L-lysine decreased the renal uptake of 125I-labeled dsFv to 5.4% of the control, and the 99mTc was blocked to 8.6% of the control. The effectiveness of blockage decreased to 24.5% of the control 125I-labeled dsFv and to 45.3% of the control 99mTc-labeled dsFv when dsFv was injected at 30 min after the L-lysine. The blockage of renal uptake of radiolabeled dsFv was not as effective when the dsFv preceded the L-lysine administration by 10 sec and resulted in 25.8% of the control concentrating in the kidney. When dsFv preceded the L-lysine by 5 min, the blockage was even less effective, resulting in 58.8% of the control uptake in the kidney.

Effect of L-Lysine Dose. Coinjection of L-lysine with 99mTc- and 125I-labeled anti-Tac dsFv blocked the renal uptake of both radiolabeled dsFvs in a dose-dependent fashion. L-lysine doses of 10 and 50 mg blocked renal uptake of 99mTc-labeled dsFv by 75-97% and 125I-labeled dsFv by 80-97%, respectively (Fig. 3). Aminosyn II and 10% Travasol partially blocked (30-50%) the renal uptake of dsFv. The HPLC analyses of the 125I-labeled dsFv in Aminosyn II and 10% Travasol showed an additional low molecular weight peak that was not present in the original material before incubation with Aminosyn II and was likely due to a reducing agent present in the amino acid solutions. This smaller molecular weight fragment was approximately 11% of the original dsFv peak. This breakdown of the 125I-labeled dsFv was also reproduced when the dsFv was incubated in sodium bisulfite, 600 μg/ml. The HPLC of the urine showed a similar profile to that seen in the injected material, with approximately 14% of the 125I in the smaller molecular weight fragment and 86% as dsFv.

Administration of L-Lysine after dsFv Injection. When radiolabeled dsFv was injected alone, the kidney uptake of the radionuclides...
L-lysine blocks renal uptake of dsFv

Fig. 2. The importance of the timing of the L-lysine injection on renal uptake of radiolabeled dsFv was evaluated. L-lysine (50 mg) was injected i.p. into the non-tumor-bearing mice at various times before (−) or after (+) i.v. injection of dsFv labeled with 99mTc (●) or 125I (▪). The +10 s and +5 min studies were only performed with 99mTc. Control non-tumor-bearing mice did not receive L-lysine. The concentration of radiolabeled dsFv in the kidneys was determined 15 minutes after its injection. Columns, mean; bars, SD (n = 5).

Fig. 3. The effect of L-lysine dose on renal uptake of 125I (●) or 99mTc (▪) dsFv was evaluated. Doses of L-lysine ranging from 0 to 50 mg were coinjected i.v. with a mixture of 125I- and 99mTc-labeled dsFv. Mice (n = 5) were sacrificed 15 min after injection, and the concentration of the radiolabeled dsFv in the kidney was plotted (mean).

15 min postinjection was extremely high (Fig. 4). When L-lysine was injected 15 min after dsFv (when most had accumulated in the kidneys) and mice were sacrificed 30 min and 75 min later (i.e., 45 and 90 minutes after dsFv), significant differences were seen in kidney uptake between the L-lysine-treated group and the no-lysine control (P < 0.003). The delayed L-lysine-injection group had lower concentrations in the kidney of both 125I and 99mTc than the control group (<30.0 and 41.3% for 125I and 50.3 and 59.5% for 99mTc in the test and control groups, respectively, at 45 and 90 min). The amount of uptake of 125I- and 99mTc-labeled dsFv were somewhat different from each other but paralleled each other in both the no-lysine and delayed L-lysine injection groups.

Catabolites of dsFv in the Circulation, Kidney, and Urine. The HPLC analysis of the serum and urine obtained 15 min after 125I- and 99mTc-labeled dsFv injection showed that blocking with L-lysine or Aminosyn II decreased the fraction of activity appearing as low molecular weight 125I catabolites (Table 1). In addition, the fraction of the 125I activity appearing in the urine as dsFv markedly increased to over 92%, compared to a mean of 44% for the no-lysine control. The fraction of 99mTc bound to dsFv in the urine also showed a similar pattern to that of the 125I, with larger amounts of 99mTc appearing as dsFv-bound in the L-lysine-treated group compared to the no-lysine control. In contrast, in the blood, the fractions in the 99mTc-dsFv were similar for both the amino acid-treated and the control group. When the radioactivity in the different fractions was normalized using the total amount of radioactivity remaining in the circulation, the percentage of the ID remaining as dsFv in the circulation was similar for 125I and 99mTc, and the main difference was the amount of catabolites generated that appear in the blood (data not shown).

Serial HPLC measurements were made at 15, 45, and 360 minutes after 125I-dsFv injection without lysine co-infusion (Table 2). At 15 min, slightly over half (54%) of the activity retained in the circulation was intact dsFv, but the fraction of intact dsFv rapidly decreased, with only a minimal amount of intact dsFv present at 6 h. A similar pattern was seen for the 125I-dsFv in the urine. The % ID values of 125I bound to the different products in the kidney are shown in Table 3.

Effect of L-Lysine on Biodistribution of dsFv in Tumor-bearing Mice. Table 4 compares the distribution of 99mTc in animals receiving L-lysine and in those not receiving lysine. As expected, the major difference was in the blocked 99mTc uptake by the kidney in the L-lysine group compared to the control group: 9.4% ID/g versus 144.8% ID/g, respectively, at 15 min postinjection. The concentration in blood was not significantly different between the two groups. The uptake of 99mTc by tumor was higher in the L-lysine group than in the
presented as % radioactivity in the eluted fraction ± SD.

As with WmXc, the most significant differences were in the no-lysine groups receiving "Tc-labeled dsFv. Other tissues did not show significant differences between L-lysine and no-lysine controls showed significant differences in various tissues (Table 5). As with 125I-labeled and 99mTc-labeled dsFv were injected i.v. alone or co-infused with L-lysine or Aminosyn in non-tumor-bearing nude mice. Data are presented as % radioactivity in the eluted fraction ± SD.

The comparison of 125I distribution in animals receiving L-lysine and in the no-lysine controls showed significant differences in various tissues (Table 5). As with 99mTc, the most significant differences were in the renal uptake that was considerably blocked by L-lysine (6.03%) at 15 min after dsFv injection. There were significant differences in 125I uptake by the blood that were related to the catabolites of 125I-labeled dsFv, which returned to the circulation as non-protein-bound 125I. In addition, uptake by several other organs, including the stomach, intestine, and muscles, was also different and most likely related to the elevated 125I catabolites in the blood. Uptake of 125I by tumor was higher in the L-lysine group than in the control group, as was the case for 99mTc.

Animals receiving L-lysine had much higher tumor:tissue ratios for both 125I-labeled and 99mTc-labeled dsFv than did the no-lysine controls (Figs. 5, A and B and 6, A and B).

The whole-body retention, determined by adding the radioactivity in all organs and the carcass, showed that when L-lysine was given, the retention levels of 125I and 99mTc were almost superimposable to each other and were much lower than in the no-lysine control. This resulted in whole-body retention differences >40% of the ID by 15 min after dsFv administration. In addition, when the no-lysine controls were compared, the whole-body retention levels of 125I were greater than those of 99mTc.

Table 1 HPLC analysis of the serum and urine of 125I-labeled and 99mTc-labeled dsFv in non-tumor-bearing nude mice.

Table 2 HPLC analysis of metabolites following i.v. injection of 125I-labeled anti-Tac dsFv in non-tumor-bearing mice.

Table 3 HPLC analyses of radioactivity in the kidney following i.v. injection of 125I-labeled dsFv in non-tumor-bearing nude mice.

Table 4 Biodistribution of 99mTc-MAG3-labeled anti-Tac dsFv with 50 mg of L-lysine or without lysine coinjection.

DISCUSSION

The use of intact radiolabeled MoAbs has shown some limitations related to their size, including slow penetration into tumors, nonhomogenous tumor distribution (5, 6), high blood pool background, and high incidence of immune response (7). As a result, smaller fragments have been used with improved targeting and less immunogenicity. Unfortunately, smaller molecules such as Fab or Fv show high renal concentration (17, 22, 24, 26), which is potentially problematic in terms of imaging and therapy. Our previous studies using 18F-,
Table 5 Biodistribution of $^{125}$I-labeled anti-Tac dsFv with 50 mg of L-lysine or without lysine co-injection

<table>
<thead>
<tr>
<th>Organ</th>
<th>15 min With L-lysine</th>
<th>15 min Without lysine</th>
<th>45 min With L-lysine</th>
<th>45 min Without lysine</th>
<th>90 min With L-lysine</th>
<th>90 min Without lysine</th>
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<tr>
<td>Blood</td>
<td>7.6 ± 2.8</td>
<td>10.1 ± 2.1</td>
<td>5.0 ± 2.9</td>
<td>8.1 ± 1.4</td>
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<td>Liver</td>
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<td>3.4 ± 0.7</td>
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<td>Kidney</td>
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<td>113.2 ± 24.6</td>
<td>10.8 ± 3.5</td>
<td>16.1 ± 14.3</td>
<td>11.7 ± 2.1</td>
<td>20.0 ± 6.9</td>
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<td>Intestine</td>
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<td>2.0 ± 0.2</td>
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<td>Stomach</td>
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<td>5.4 ± 0.6</td>
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<td>Lung</td>
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<td>2.7 ± 0.4</td>
<td>1.9 ± 0.7</td>
<td>2.7 ± 0.4</td>
<td>1.7 ± 0.7</td>
<td>2.3 ± 0.8</td>
</tr>
</tbody>
</table>

The concentration in the different organs at 15, 45, or 90 min after dsFv injection was expressed as the %ID/gram ± SD; n = 5.

$^a\quad P < 0.05$ compared with no lysine.

$^b\quad P < 0.01$ compared with no lysine.

99mTc-, and $^{125}$I-labeled anti-Tac dsFv resulted in very high concentration in the kidneys (22), in the range seen in this study. The high renal uptake is a common problem when dealing with LMWPs, including enzymes, tissue proteins, immunoprotein fragments, and proteohormones, where 30–60% of the administered dose can be found in the kidney (35). Renal uptake of LMWPs is dependent on filtration, which is in turn mostly dependent on molecular size. Once filtered, LMWPs generally undergo endocytosis at the proximal tubule and then undergo degradation in the lysosomes (36). Wochner et al. (37) have shown that glomerular filtration is important in the clearance of IgG fragments but is not important in the clearance of intact antibody. Arend and Silverblatt (38) reported that the main mechanism for catabolism of Fab fragments is removal from the circulation by glomerular filtration and subsequent reabsorption and degradation in the proximal tubule.

In the kidney, LMWPs can be blocked with inhibitors of tubular absorption (acetate and cyanide), but these are not relevant methods in clinical studies. Preclinical studies by several investigators have shown that L-lysine and arginine can block renal uptake of various LMWPs (35, 39, 40), including lysozyme, $\beta_2$-microglobulin, growth hormone, insulin, and Fab fragments (24–26). Clinical studies have also shown effectiveness in blocking albumin and various LMWPs, including $\beta_2$-microglobulin, $\kappa$ and $\lambda$ chain, aprotinin, Fab, and somatostatin analogue (27, 28, 35, 39–41).

Although a few preclinical studies have evaluated the use of L-lysine to block renal uptake of antibody fragments or LMWPs, they have not evaluated the pharmacokinetics of L-lysine in the kidney. Our study shows the rapid pharmacokinetics and dose-dependent changes in L-lysine concentration in the kidney. The 1-mg L-lysine dose showed lower concentrations in the kidney than the 10-mg dose, although the % ID/g in blood was similar for the various L-lysine doses. This suggested that the higher doses exhibited Michaelis-Menten kinetics in the kidney, saturating the mechanism of release of L-lysine or a catabolite from tubular cells.

In contrast to previous studies that used arbitrary dosing schedules (24, 25), we evaluated the importance of the timing of the L-lysine injection. We found that the most effective regimen was an i.v. co-infusion of the L-lysine and dsFv. As did DePalatis et al. (26), we found i.p. administration of L-lysine given immediately before radiolabeled dsFv to be extremely effective in blocking renal uptake. The blocking effect of preadministration of L-lysine dissipated the longer it preceded or followed the radiolabeled dsFv injection. Because the filtration of dsFv by the kidney occurs very quickly, delaying L-lysine i.p. injection even 10 sec or 5 min after radiolabeled dsFv caused the renal uptake to increase significantly.

The mechanism by which L-lysine blocks renal uptake of dsFv is most likely a charge-related effect, in which the dsFv is prevented from being filtered by the kidney.
from binding to the proximal renal tubular cells and therefore does not undergo endocytosis (36, 39). Although the total dosage of L-lysine was important in blocking renal uptake of radiolabeled dsFv, our studies showed that 2 min after i.p. injection of L-lysine, its concentration in the kidney was less than it was at 15 min. Therefore, better blocking of dsFv was seen at earlier times, when the lower L-lysine concentration was present in the kidney.

Nonetheless, administration of L-lysine after dsFv had been concentrated in the kidney improved the net clearance of the tracer from the kidney. This is most likely due to prevention of further renal uptake of dsFv that is in the circulation or that returns to the circulation from the extravascular space. This would suggest that repeat administration of L-lysine after the initial L-lysine block would be beneficial.

As shown previously (25) for Fab and confirmed in this study for $^{125}$I-labeled dsFv, it is the intact dsFv, and not a radiolabeled catabolite, that is endocytosed by the renal tubular cells, as shown in our kidney homogenates. This is also consistent with the presence of predominantly intact radiolabeled dsFv in the urine after L-lysine injection versus radiolabeled catabolites in urine in the absence of L-lysine. In the absence of L-lysine, the dsFv accumulated in the kidney is catabolized and removed from tubular cells back into the circulation, as demonstrated by the decrease in renal radioactivity and the increase in catabolites in the serum. In the presence of L-lysine, the retention of $^{125}$I and $^{99m}$Tc in the whole body was similar because little activity accumulated in the kidney. In contrast, the animals injected with dsFv without lysine retained $^{125}$I longer than $^{99m}$Tc. This is consistent with catabolism of these reagents in the kidney and the slower clearance of the iodide catabolites than of the $^{99m}$Tc catabolites. The increase in $^{125}$I catabolite formation and its longer whole-body retention is the likely explanation for the less favorable tumor: tissue ratios for $^{125}$I-dsFv compared to $^{99m}$Tc-dsFv. Furthermore, our study showed that L-lysine injection has no detrimental effect on tumor uptake.

The two commercially approved parenteral amino acid preparations we evaluated proved effective in blocking renal uptake of radiolabeled dsFv. In this mouse model, we were limited to volumes of 200–400 μl that contained 3.2–6.3 mg of L-lysine and total amino acid loads of 30–60 mg. When compared to pure L-lysine, the effect of these doses of Aminosyn containing only 6.3 mg of L-lysine were comparable to 10 mg of pure L-lysine in displacing dsFv. This is likely related to a favorable blocking effect of some of the other amino acids present in the solution. The HPLC of serum and urine from these mice showed, in addition to dsFv and very low molecular weight catabolites, a smaller fragment consistent with single chains. This was also seen in the injected radiolabeled dsFv when it was mixed in the Aminosyn and was shown to be secondary to the reducing agents present in the commercial preparations. This is unlikely to be a problem in vivo because the radiolabeled dsFv can be administered through a separate i.v. line.

The catabolite studies extend our previous work by showing that most activity in the kidney at 15 min is present as intact dsFv. This suggests that dsFv is catabolized in the kidney and that the catabolic products are rapidly secreted into the blood. This can explain why improved tumor: tissue ratios were seen when renal uptake was blocked with amino acids.

Because L-lysine blocks both iodinated dsFv (labeled in the tyrosine) and $^{99m}$Tc-labeled dsFv (labeled in the L-lysine), the blockage is not one induced by competitive inhibition of some metabolic product. Our studies showed that L-lysine doses that block renal uptake did not significantly change GFR. The effect of L-lysine on GFR has been controversial, with some studies showing increased GFR, no effect on GFR, or nephrotoxicity (42–44). Clinically, doses of 60 g L-lysine have been administered in normal volunteers with an increase in GFR but without effect on absolute proximal reabsorption rate (41).

In summary, we have shown that the timing of the injection of the blocking dose of L-lysine relative to the injection of the radiolabeled dsFv is critical. We have also shown for the first time that lysine is capable of significantly blocking the renal uptake of low molecular weight radiolabeled dsFv.

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Fig. 6. The SP2/Tac tumor: normal tissue ratio of $^{99m}$Tc in mice receiving $^{99m}$Tc-labeled dsFv was plotted at 15 (B), 45 (W), and 90 (□) min after injection. Mice (n = 5) received co-infusion of $^{125}$I- and $^{99m}$Tc-labeled dsFv without lysine (A) or with 50 mg of L-lysine (B). *, P < 0.01 and **, P < 0.05 compared with no-lysine group; columns, mean; bars, SD.
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L-Lysine Effectively Blocks Renal Uptake of $^{125I}$- or $^{99mTc}$-labeled Anti-Tac Disulfide-stabilized Fv Fragment

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