Aminosyn II Effectively Blocks Renal Uptake of 18F-labeled Anti-Tac Disulfide-stabilized Fv

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ABSTRACT

Because intact IgG has limitations as a tumor-imaging agent, radiolabeled Fv fragments are being evaluated. Due to the high renal accumulation of Fv fragments, methods to block renal uptake are being sought. This study evaluated how well Aminosyn II, a Food and Drug Administration-approved 15% amino acid solution, would block the renal accumulation of 18F anti-Tac disulfide-stabilized Fv (dsFv) fragments (small fragments with high renal uptake). The anti-Tac dsFv is directed against the α subunit of the interleukin 2 receptor. It was labeled at specific activities of 1.1-2.7 mCi/mg using 14C-succinimidyl 4-[18F]fluoromethyl benzoate. Four adult baboons were injected i.v. with 0.7-1.9 mCi and 150 μg of dsFv. Each baboon was preinjected with Aminosyn II i.v. and, on a separate occasion, with a control solution. Thirty min before injection of 18F-labeled anti-Tac dsFv, a bolus of either solution was given, followed by a constant infusion of 13.3 ml/kg/h. Quantitative positron emission tomography imaging was performed. The amino acid levels in serum were measured serially. The baseline levels of lysine (and other amino acids) in plasma were not significantly different in either the Aminosyn II or control infusion group and did not change during the control infusion. In the Aminosyn II group, lysine levels in plasma 5 min before anti-Tac dsFv infusion were 5-15 times higher than the baseline value and continued to rise during the infusion. The areas under the curve in blood of the 18F-labeled anti-Tac dsFv, from time of injection to end of imaging, expressed as percentage injected dose (%ID), were 28.9 ± 4.0% ID × h/1iter (mean ± SD) for the control group and 32.09 ± 11.15% ID × h/ileter for the Aminosyn II group (P = 0.54). The peak concentration of 18F-labeled anti-Tac dsFv in the kidney of the controls was 24.53 ± 4.34% ID, and the value in the Aminosyn II group was 5.39 ± 1.89% ID, representing a mean decrease of 78.5%. The times to reach 90% of the peak levels of 18F in the kidney were 5.6 ± 3.0 min for the Aminosyn II group and 33.8 ± 4.8 min for the control group. The amounts excreted in urine by 90 min were 47.7 ± 8.55%ID and 78.5 ± 12.8%ID (P = 0.01) for the controls and Aminosyn II group, respectively. In conclusion, Aminosyn II effectively blocks the renal accumulation of 18F-labeled anti-Tac dsFv. Use of Aminosyn II should allow much higher tracer administration for the same radiation exposure to the target organ (kidney).

INTRODUCTION

The use of antibody fragments is being evaluated as a way to overcome some of the major limitations of using intact radiolabeled antibodies for targeting tumors, namely, slow pharmacokinetics, poor penetration into tissues (1, 2), and immunogenicity (3). Molecular engineering has provided new fragment reagents that may improve tumor:non tumor ratios and decrease immunogenicity. These genetically engineered variable-region fragments (Fv) consist of portions of the heavy-light-chain variable domains that maintain the antigen-binding specificity of an intact antibody (1, 2, 4-12). Several preclinical trials have used radiolabeled single-chain Fv, in which the Vh and VL are linked by covalent bonds through a spacer arm (4, 13); alternatively, dsFv,2 in which the chains are linked by a disulfide bond, have been used (14, 15). Because of their smaller size (M, ~25,000), the kinetics of localization of Fv are much faster than intact IgG, their distribution is more uniform (1, 2), and they are expected to be less immunogenic (16).

A major drawback of Fv fragments for imaging and for radioimmunotherapy is their high renal uptake (1, 7-12). This decreases imaging sensitivity in the abdomen and results in too high a radiation dose to the kidneys. In recent work with anti-Tac dsFv, an antibody directed against the α subunit of the interleukin 2 receptor (17), we have seen as much as 70%ID of dsFv rapidly localize in mouse kidneys (10, 12).

Recently, several investigators have shown that injecting lysine can block renal accumulation of Fab fragments in the kidney (18-21). Our study of lysine showed that it effectively blocked the renal accumulation of anti-Tac dsFv labeled with either 125I or 99mTc (10). In that study, we showed that Aminosyn II, a commercially available amino acid solution similar to that used by Hammond et al. (22), could also block renal uptake. This study differs from these others in several ways: (a) we used baboons rather than mice to more closely approximate the potential effectiveness in humans of Aminosyn II on 18F-labeled anti-Tac dsFv renal uptake; (b) we measured the changes in serum amino acid levels after Aminosyn II infusion; and (c) we performed paired studies in the same animal to take advantage of the quantitative aspects of PET imaging, therefore minimizing the effect of variability between animal groups when nonpaired studies are performed; and (d) we performed bolus injections and infusions of Aminosyn II rather than bolus injections alone.

MATERIALS AND METHODS

MoAbs. We used a disulfide-bonded Fv fragment of anti-Tac murine MoAb (15). Anti-Tac is an IgG2a that recognizes the interleukin 2 receptor α subunit (23). Production of anti-Tac dsFv has been described previously (15). The products were >98% pure, as determined by size-exclusion HPLC with an UV detector. The anti-Tac dsFv was originally provided in PBS, but the buffer was exchanged for borate by dialysis prior to labeling.

Radiolabeling. Anti-Tac dsFv was labeled with 18F (109.8-min half-life). The 18F labeling method was performed as described previously (12, 24). Briefly, N-succinimidyl 4-[18F]fluoromethyl) benzoate was prepared and purified by reverse-phase HPLC using 30% acetonitrile-water. This reagent (74-148 MBq) was then dissolved in ~5 μl of acetonitrile, and the anti-Tac dsFv (25-100 μg) was added in 100 μl borate buffer (0.1 M, pH 8.9). After reacting at room temperature for 10 min, 18F-labeled anti-Tac dsFv was purified using a Beckman SEC 2000 size-exclusion HPLC column with PBS (0.05 M NaH2PO4, 0.05 M Na2HPO4, and 0.15 M NaCl, pH 6.9). The specific activities of 18F-labeled anti-Tac dsFv at the time of injection ranged from 457.0 to 473.6 MBq/mg (4.7 to 14.9 mCi/mg; 118 to 373 Ci/mmol), and >98% of the 18F was bound to anti-Tac dsFv, as determined by instant TLC and size-exclusion HPLC (Waters 510; Millipore Corp., Milford, MA) using a TSK 2000 column. The column was eluted with 0.067 PBS-0.1 mM KCl buffer (pH 6.8), with a

2 The abbreviations used are: dsFv, disulfide-stabilized Fv; %ID, percentage injected dose; MoAb, monoclonal antibody; HPLC, high-performance liquid chromatography; PET, positron emission tomography; ROI, region of interest; Gm, osmol; AUC, area under the curve; LMWP, low molecular weight protein.
flow rate of 0.5 ml/min. The HPLC had an on-line NaI gamma detector (y RAM; IN/US Systems, Inc., Pine Brook, NJ). The dsFv contained <2.5 endotoxin units per kg of body weight. At the time of injection, the doses contained 0.7–1.9 mCi of radioactivity and 150 μg of antibody (Table 1).

**Animal Models.** Four baboons, three female and one male, ages 16 or 17 years, were obtained from Southwest Research Foundation (San Antonio, TX). All animals appeared free of illness or pathogens. The serum creatinine level consisted of a known fraction of the infusion rate [bolus = infusion rate/dose, it is, therefore, directly related to radiation exposure. In some instances, the serum creatinine level was adjusted as necessary. They also received supplemental O2. Respiratory rate, end-tidal PCO₂, electrocardiography, and body temperature were monitored continuously throughout the study.

**PET Studies.** While they were anesthetized, the baboons were positioned on a GE Advance PET scanner (Milwaukee, WI), which acquires 35 slices simultaneously with an interslice separation of 4.25 mm and a reconstructed resolution of 6.5 mm. Positioning was assisted by a transmission scan and an ⁶⁸Ga/⁶⁷Ge pin source was performed for attenuation correction. The postinjection field of view (15 cm). A preinjection, 8-min transmission scan using a 5-MHz transducer. The resulting data in the ROI was then expressed as a fraction of the injected dose per g (cc were corrected for decay and for counting efficiency of the scanner for ISF and reconstructed using a Manning filter in a 128 X 128 matrix. The images were then corrected for attenuation and for a total of 29 scan frames collected over 3 h. The images were acquired in a 256 x 256 word mode matrix. The images were then corrected for attenuation and reconstructed using a Hanning filter in a 128 x 256 matrix. The images were corrected for decay and for counting efficiency of the scanner for ¹⁸F and expressed in nCi/cc.

The images were transferred into our VAX computer system and analyzed by use of locally developed computer software. A ROI was drawn to encompass each kidney, the entire spleen, and portions of liver, lung, bone marrow, and the aorta. To calculate the blood time-activity curve from the imaging data, a small ROI was placed within the aorta in various slices. Because the aorta is small, partial volume effects were significant and required correction based on the known PET scanner resolution and the size of the aorta, which was determined with an ultrasound device (Diasonics Spectra, Milpitas, CA) using the physical half-life to extrapolate from the last measured data point. Because the residence time represents the integral of the activity in a given organ normalized by the administered dose, it is, therefore, directly related to radiation exposure. In some instances, the integral was determined from decay-corrected data and, therefore, was referred to as decay-corrected residence time.

**Aminosyn II and Control Fluid Infusion.** Aminosyn II (Abbott Laboratories, North Chicago, IL) was injected i.v. to block the renal uptake of anti-Tac dsFv. Aminosyn II is a 15% amino acid solution that contains various amino acids including 108 mm lysine and 88 mm arginine. Aminosyn II is approved for human parenteral nutrition by the Food and Drug Administration of the United States. Aminosyn II was diluted with sterile water to decrease the osmolarity of the solution to 800 mOsm/liter and, thus, allow administration through a peripheral vein. We attempted to obtain steady-state levels of amino acids, in particular lysine, by administering a bolus of Aminosyn II followed by a constant infusion, which was continued throughout the PET scan. The infusion rate of Aminosyn II was based on each baboon’s body weight. The rate of 13.3 ml/kg/h was based on what was considered to be a safe i.v. dose to administer for at least 3 h. The bolus of Aminosyn II, given over 5 min, consisted of a known fraction of the infusion rate [bolus = infusion rate/ (0.693/0.1333 h)]. The selection of this volume for the bolus was based on a report of a 7.9-min elimination half-life of lysine (28). We expected that the bolus, together with the infusion rate, would establish an equilibrium level of lysine. All four animals were used in a nonsaltine control study, in which ¹⁸F anti-Tac dsFv was given with normal saline to which 50% hypertonic glucose had been added, creating an 800 mOsm/ml solution. These control studies were performed using a bolus-and-infusion technique that was identical to that described for the Aminosyn II in three animals; one baboon received only a slow infusion of normal saline. The order of infusion of Aminosyn II or saline/glucose was alternated between animals.

The serum amino acid levels were monitored in six of eight studies by drawing baseline venous blood samples prior to injection; immediately after the 5-min bolus of fluid; 25 min into the fluid infusion (immediately before ¹⁸F anti-Tac dsFv); and ~60, 120, and 180 min after anti-Tac dsFv infusion. Infrequently, samples at individual time points were not obtained because of technical problems. In two animals undergoing prolonged Aminosyn II infusion (see below), additional samples were obtained (Fig. 2). In two studies, plasma levels of amino acid were not obtained because of technical reasons. The amino acids analyzed include lysine, arginine, glutamic acid, leucine, hydroxyproline, and asparagine (Corning Clinical Labs, Baltimore, MD; Protein Chemistry Laboratory, University of Texas Medical Branch Cancer Center, Galveston, TX).

Steady-state amino acid levels in plasma were not reached by the end of the 3-h infusion. Because the amino acid levels continued to rise during the 3-h

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Table 1 Baboon dosing data

<table>
<thead>
<tr>
<th>Baboon no.</th>
<th>Reagent</th>
<th>Date of study</th>
<th>Weight (kg)</th>
<th>Bolus (ml)</th>
<th>Infusion rate (ml/h)</th>
<th>Dose injected (mCi)</th>
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<tr>
<td>C164</td>
<td>Aminosyn II</td>
<td>5/19/96</td>
<td>15.7</td>
<td>40</td>
<td>209</td>
<td>1.9</td>
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<tr>
<td></td>
<td>Normal saline†</td>
<td>2/17/95</td>
<td>15.8</td>
<td>N/A</td>
<td>N/A</td>
<td>1.72</td>
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<tr>
<td>C87</td>
<td>Aminosyn II</td>
<td>3/15/96</td>
<td>19.4</td>
<td>50</td>
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<td>0.92</td>
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<tr>
<td></td>
<td>Glucose</td>
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<td>19.9</td>
<td>51</td>
<td>265</td>
<td>0.79</td>
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<tr>
<td>C470</td>
<td>Aminosyn II (prolonged)</td>
<td>2/21/97</td>
<td>21.6</td>
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<td>2.24</td>
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<tr>
<td></td>
<td>Glucose</td>
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<td>18.7</td>
<td>48</td>
<td>249</td>
<td>0.98</td>
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<tr>
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<td>Aminosyn II</td>
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<td>17.2</td>
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<td>229</td>
<td>1.24</td>
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<tr>
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<td>Aminosyn II (prolonged)</td>
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<td>16.0</td>
<td>41</td>
<td>213</td>
<td>1.78</td>
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<tr>
<td></td>
<td>Glucose</td>
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<td>0.85</td>
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<tr>
<td></td>
<td></td>
<td>7/12/96</td>
<td>13.5</td>
<td>35</td>
<td>180</td>
<td>0.7</td>
</tr>
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</table>

† i.v. bolus of Aminosyn II (800 mOsm/liter), followed by 30-min infusion of the same solution prior to anti-Tac dsFv injection.
‡ Maintenance infusion of saline.
§ N/A, not available (study not performed).
‰ i.v. bolus of glucose/normal saline (800 mOsm/liter) followed by 30-min infusion of the same solution prior to anti-Tac dsFv injection.
* i.v. bolus of Aminosyn II (800 mOsm/liter), followed by a 2-h infusion of the same solution prior to anti-Tac dsFv.
infusion, a study was performed in baboons C878 and C470 in which the bolus and amino acid infusion of Aminosyn II were repeated, but the anti-Tac dsFv was injected 120 min into the Aminosyn II infusion rather than at 30 min. The infusion of Aminosyn II was continued until the end of the PET study, which allowed us to determine whether the higher serum amino acid level would result in additional renal blockage of the 18F-labeled anti-Tac dsFv.

At 25 min into the Aminosyn II infusion, the 18F-labeled anti-Tac dsFv (diluted in 9 ml of normal saline) was injected i.v. over 1 min using a Harvard infusion pump (South Natick, MA). At the start of the anti-Tac dsFv infusion, the PET scanner was started, and serial i.v. sampling was performed to generate an 18F-labeled anti-Tac dsFv blood and plasma time-activity curve; in one baboon in which venous access failed, intra-arterial sampling was performed. Samples were obtained at ~0.5, 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min after anti-Tac dsFv injection. In addition, the integrity of the 18F-labeled anti-Tac dsFv in serum samples obtained at 3–30 min was determined using our size-exclusion HPLC (as described above). The urinary excretion of radioactivity was determined by collecting cumulative urine samples through the bladder catheter at 30-min intervals for up to 3 h.

Immune Response. Serum from all baboons were tested for evidence of immune response to anti-Tac dsFv. In brief, 100 μl of serum were incubated with 0.3 μg of radiolabeled anti-Tac dsFv or 0.05 μg of 125I-labeled B6.2. After a 30-min incubation at room temperature, the samples were refrigerated and passed through a size-exclusion HPLC, as described above. The retention times of the samples were compared to the retention time of the radiolabeled anti-Tac dsFv or B6.2 run in PBS. If an immune response were present, complexes would be formed.

Statistics. To evaluate statistical significance between the Aminosyn II and control groups, paired t tests were performed.

RESULTS

The blood clearance of 18F-labeled anti-Tac dsFv was fast. At 1.5 min after i.v. injection of the tracer, there was 6.10 ± 0.30%ID/100 ml of blood in the control group and 7.60 ± 1.66%ID/100 ml in the Aminosyn II group (P = 0.127). By 173 min, this activity had decreased to 0.337 ± 0.10%ID/100 ml and 0.32 ± 0.12%ID/100 ml of blood for the control and Aminosyn II group, respectively. The AUCs of blood (decay corrected) from the time of 18F-labeled anti-Tac dsFv injection to the end of imaging (3 h), determined from an ROI over the abdominal aorta, were 28.94 ± 4.05%ID × h/liter for the control animals and 32.09 ± 11.15%ID × h/liter for the Aminosyn II group (P = 0.54). The ratio of the blood activity determined from the ROI from the aorta was a mean of 1.2 ± 0.2 times higher (after correction for partial volume) than that determined from the gamma counter. HPLC analysis of serum from patients infused with glucose (control) or Aminosyn II showed almost exclusively intact 18F dsFv in serum from immediately after injection of the radiolabeled dsFv and up to 30 min; at later times, the radioactivity concentration was too low to obtain reliable HPLC (data not shown).

The peak concentration of 18F-labeled anti-Tac dsFv measured in the right kidneys of the control group was 24.53 ± 4.34%ID, whereas the Aminosyn II group showed 5.39 ± 1.89%ID (P = <0.001; Fig. 1). Only data from the right kidneys are shown because they had less contamination from adjacent structures than the left kidneys, which abutted the spleen. In spite of the adjacent spleen, the left kidney ROI had activity similar to that in the right. The Aminosyn II infusion decreased the peak concentration in the kidneys to 21.5 ± 3.6% (mean ± SD) of that in the control baboons (Fig. 1). The times to 90% peak tracer accumulation in the kidneys were 33.8 ± 4.8 min in control baboons and 5.6 ± 3.0 min in the Aminosyn II group.

The decay-corrected residence time in the right kidneys of animals receiving Aminosyn II was significantly shorter than that of the controls (P = 0.002; Table 2). The decay-corrected residence times during the prolonged and regular Aminosyn II infusions were similar.

The peak concentrations of 18F measured in blood, liver, spleen, and lung were similar for both the control group and the Aminosyn II group. In contrast, the concentration in the kidney was significantly different. The highest concentration of activity was in the kidney (Table 3).

To obtain dosimetry estimates, the residence time (effective) of tracer for entire organs was calculated. The residence times in liver,
spleen, lung, and bone marrow were similar for both the control infusion and the Aminosyn II infusion (Table 3). The residence time in total kidney tissue of the Aminosyn II group was lower than that of the control. This finding is similar to that presented above for the right kidney, except that it is not decay corrected and it is based on the total weight of both kidneys rather than that of the right kidney only.

Whole-body retention of $^{18}$F was determined from urine excretion values (Fig. 2). The whole-body retention in the Aminosyn II group was less than that in the control group and showed significant differences up to 90 min ($P = 0.01$). The mean effective whole-body half-life of $^{18}$F was $52 \pm 7$ min for the Aminosyn II infusion and $71 \pm 15$ min for the control group.

Lysine and arginine were the main amino acids of interest (29, 30), but because both showed similar kinetics and because lysine appears to be more effective in blocking renal uptake of certain proteins than arginine (31), only the data for lysine will be presented. The lysine levels in baseline plasma prior to infusion were not significantly different during the Aminosyn II or glucose control infusion, with $192 \pm 38 \mu$mol/liter and $183 \pm 35 \mu$mol/liter, respectively (Fig. 3; $P = 0.769$). During the Aminosyn II infusion, the lysine levels continued to rise after the $^{18}$F-labeled anti-Tac dsFv injection and did not reach equilibrium, even after 180 min of infusion. The increase in lysine levels varied from animal to animal (Fig. 3). Similar patterns were also seen for the other amino acids included in the infusion, arginine, glutamic acid, methionine, and leucine (data not shown). In two baboons receiving both Aminosyn II and the control infusion, the levels of hydroxyproline and asparagine changed very little. These amino acids were not present in the Aminosyn II infusate (data not shown). The lysine levels in the two baboons receiving the prolonged infusion (120 min) of Aminosyn II prior to the $^{18}$F-labeled anti-Tac dsFv were higher than those seen in animals receiving the shorter infusion (Fig. 3).

**Immune Response to anti-Tac dsFv.** *In vitro* HPLC immunogenicity assay showed that none of the baboons had an immune response against the B6.2 murine MoAb, which three had received 10 years previously, or against the anti-Tac dsFv at the time of their control or Aminosyn II infusions. In addition, HPLC of the serum after i.v. injection of the anti-Tac dsFv revealed no evidence of antimouse antibodies in 8 of the 10 animals. Baboon C474 developed an immune response after two exposures to the anti-Tac dsFv, as revealed by *in vitro* testing of serum. Baboon C164 also developed an immune response after two previous injections of the anti-Tac dsFv; this was detected during a third injection, in which we observed altered pharmacokinetics, with longer blood pool retention of radioactivity that circulated as a high molecular weight complex. This also resulted in a very prolonged whole-body retention and lower renal uptake. Both animals were excluded from further studies. The other two baboons did not develop an immune response prior to their third study (prolonged Aminosyn II infusion). Dosimetry estimates were obtained from the data and are provided in the “Appendix.”

**DISCUSSION**

Recent preclinical studies have shown that the high renal uptake of radiolabeled Fab after i.v. injection can be effectively blocked by concurrently administering lysine or other amino acid-containing solutions (18, 19). Similar results have been observed with anti-Tac dsFv fragments in rodents (10). Few attempts at applying these methods in clinical trials have been reported. Behr et al. (32) showed that administration of an Food and Drug Administration-approved amino acid solution could minimally block renal accumulation of radiolabeled Fab. We speculated that this minimal blockage was due to the low concentration and low rates of infusion used. In our study, we built on previous work by using a large-animal model that should be more representative of the situation expected in humans than a mouse model. In addition, we used Aminosyn II, a commercially available amino acid solution, and infused it at high rates.

Our Aminosyn II infusion was effective in reducing renal accumulation of anti-Tac dsFv to $\sim$22% of the control values. We also demonstrated that there was no adverse effect on other normal organ or tissue distribution, including that in the blood. This block in renal uptake translates into a $\sim$4-fold decrease in renal radiation dose for $^{18}$F-labeled anti-Tac dsFv. This dose-sparing effect is likely to be much higher when radiometal isotopes are used for labeling because these tend to be retained in the tissues, such as the kidney, and have much lower rates of egress following catabolism (7, 33).

A novel aspect of our work was the correlation of circulating amino
acid levels with kidney uptake. During the initial Aminosyn II infusion, lysine levels were seven times higher than those observed during the control infusion. A surprising result was that the prolonged infusion of Aminosyn II, which resulted in 8 times higher peak lysine levels as well as a larger cumulative lysine levels (AUC), did not result in lower anti-Tac dsFv concentrations in the kidney. This suggests that the mechanisms for blocking renal uptake were already saturated by the lower amino acid levels (lysine and arginine). Although we focused on lysine, arginine levels were also increased and may have contributed significantly to renal blocking of the dsFv. This would be in line with prior animal work suggesting that doses of lysine above a certain amount saturate renal uptake of lysine and are not more effective at blocking uptake of anti-Tac dsFv (10).

Although we did not directly compare the effectiveness of a single bolus of Aminosyn II to an infusion, it is unlikely that a single bolus would be adequate because the half-life of anti-Tac dsFv is longer than that of lysine. This would be consistent with our previous work in mice (10).

High renal uptake is a common problem when dealing with LMWPs, including enzymes, tissue proteins, immunoprotein fragments, and proteohormones, where 30–60% of an administered dose can be found in the kidneys (31). Renal uptake of LMWP depends on filtration rate, which, in turn, mostly depends on molecular size. Once filtered, LMWP generally undergo endocytosis at the proximal tubule with subsequent degradation in the lysosomes (34). Wochner et al. (35) have shown that glomerular filtration is important in the clearance of IgG fragments but is not important in the clearance of intact antibody. Arend et al. (36) reported that the main mechanisms for catabolism of Fab fragments are removal from the circulation by glomerular filtration and subsequent reabsorption and degradation in the proximal tubule. The mechanism by which L-lysine or arginine blocks renal uptake of anti-Tac dsFv is most likely a charge-related effect, in which the anti-Tac dsFv is prevented from binding to the proximal renal tubule. This is consistent with the increased urinary excretion and decreased whole-body retention of the dsFv with the Aminosyn II infusion.

An additional advantage of this study over other preclinical studies is that by using PET we were able to quantitatively follow the kinetics of anti-Tac dsFv in a single animal during both Aminosyn II and control infusions. Therefore, this allowed us to make paired measurements and reduced the variability inherent in performing nonpaired studies.

It is important to note that, although some of these animals had received intact IgG and Fab fragments several years previously and had developed antimusme antibodies, they did not have antimusme antibodies at the time of the anti-Tac dsFv infusions analyzed in this study. Our studies also suggest that, in spite of their small size, Fv may still be immunogenic.

In conclusion, we have demonstrated that, by using a bolus followed by an infusion of Aminosyn II, an FDA-approved amino acid solution for parenteral nutrition, high serum levels of amino acids are obtained that significantly block renal uptake of anti-Tac dsFv. This should result in improved dosimetry and less interference with diagnostic imaging.

ACKNOWLEDGMENTS

We thank Dr. Peter Choyke for performing ultrasound of the aorta; Wendy Lithicun, Wendy Sandsoval, Susan Fluck, and George Elliott for expert technical assistance with the PET studies; Deby Drumm for expert technical assistance with HPLC; and the Protein Chemistry Laboratory, Cancer Center Core Facility, University of Texas Medical Branch at Galveston (Galveston, TX), for amino acid analysis.

**REFERENCES**


**APPENDIX**

<table>
<thead>
<tr>
<th>Control group (g)</th>
<th>Aminosyn group (g)</th>
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<tr>
<td>Kidney 116</td>
<td>98</td>
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<tr>
<td>Spleen 44</td>
<td>37</td>
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<tr>
<td>Liver 384</td>
<td>393</td>
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<tr>
<td>Lung 187</td>
<td>191</td>
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<tr>
<td>Bone marrow 294</td>
<td>301</td>
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<td>Total body 16,600</td>
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**Table A2 14F anti-Tac residence times, extrapolated to human organs**

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<tr>
<th>Control group (h)</th>
<th>Aminosyn group (h)</th>
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<tr>
<td>Kidney 0.435</td>
<td>0.132</td>
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<tr>
<td>Spleen 0.048</td>
<td>0.064</td>
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<tr>
<td>Liver 0.110</td>
<td>0.120</td>
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<tr>
<td>Lung 0.112</td>
<td>0.201</td>
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<tr>
<td>Bone marrow 0.066</td>
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<tr>
<td>Total body (remainder) 0.937</td>
<td>0.663</td>
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<tr>
<td>$T_{1/2}$ Effective control (h) 1.184</td>
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</tr>
<tr>
<td>$T_{1/2}$ Effective Aminosyn (h) 0.8676</td>
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**Table A3 Radiation dose estimates 14F anti-Tac dsFv**

<table>
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<th>Target organ</th>
<th>Control infusion (rad/mCi)</th>
<th>Aminosyn II (rad/mCi)</th>
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</thead>
<tbody>
<tr>
<td>Adrenals</td>
<td>0.056</td>
<td>0.038</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.030</td>
<td>0.325</td>
</tr>
<tr>
<td>Liver</td>
<td>0.072</td>
<td>0.070</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.085</td>
<td>0.141</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.049</td>
<td>0.038</td>
</tr>
<tr>
<td>Red marrow</td>
<td>0.042</td>
<td>0.041</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.226</td>
<td>0.269</td>
</tr>
<tr>
<td>Urinary bladder wall</td>
<td>0.488</td>
<td>0.911</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.050</td>
<td>0.074</td>
</tr>
<tr>
<td>Total body</td>
<td>0.030</td>
<td>0.029</td>
</tr>
<tr>
<td>Effective dose equivalent 0.139</td>
<td>0.135</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Data from the computer program MIRDose3 (37).


Aminosyn II Effectively Blocks Renal Uptake of $^{18}$F-labeled Anti-Tac Disulfide-stabilized Fv

Jorge A. Carrasquillo, Lixin Lang, Millie Whatley, et al.


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