Targeting of Transferrin Receptors in Nude Mice Bearing A431 and LS174T Xenografts with $[^{18}F]$Holo-Transferrin: Permeability and Receptor Dependence

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The goal of this study was to investigate whether $[^{18}F]$-labeled transferrin (Tf), which has a molecular weight (M<sub>t</sub>) of ~79,000, binds to Tf receptor sites in tumors in a specific manner within the time frame commensurate with the half-life of $[^{18}F]$ (109.7 min). We have previously shown that $[^{18}F]$holo-Tf ($[^{18}F]$Tf) maintains all properties of native Tf in vitro and that it can specifically target liver Tf receptor sites in vivo. **Methods:** The distribution of $[^{18}F]$Tf, using $[^{18}F]$albumin (Alb) or $[^{14}C]$Alb as a control, was studied over a 6-h period in nude mice bearing LS174T and A431 xenografts of a high- and low-permeability tumor, respectively. **Results:** Measurements of Tf receptor concentration in the tumor extracts suggest similar binding capacities. In vivo, liver uptake values were higher for $[^{18}F]$Tf than for both $[^{18}F]$Alb and $[^{14}C]$Alb throughout the study, indicating specific binding. In contrast, tumor Tf uptake values remained below those of the Alb tracers, and tumor-to-blood ratios of $[^{18}F]$Tf in each xenograft increased in parallel with those of the Alb tracers. The permeabilities of $[^{14}C]$Alb and $[^{18}F]$Tf in LS174T were calculated to be 1.29 ± 0.49 and 1.03 ± 0.38 µL/min/g (mean ± SD), respectively, whereas the permeabilities of the two tracers in A431 were 0.79 ± 0.24 and 0.44 ± 0.04 µL/min/g. Pharmacokinetic modeling of the data using these permeabilities and the high plasma and extracellular concentrations of endogenous Tf showed that the observed uptake values in the two xenografts are consistent with a non-receptor-mediated distribution. In the liver, the absence of permeability barriers yields specific $[^{18}F]$Tf binding to receptors compared with the $[^{14}C]$Alb control, within 5 min after injection. **Conclusion:** Receptor-mediated accumulation of $[^{18}F]$Tf in tumor xenografts is impaired by rate-determining permeability and competition from endogenous Tf and is not achieved in a time frame of 6 h.

**Key Words:** transferrin; $[^{18}F]$; neoplasms; pharmacokinetics; liver


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resolution is lower because these nuclides emit higher energy positron particles. Another approach is to use lower molecular weight proteins to reduce the effect of tumor permeability barriers. Previous work from our laboratory has shown that specific xenograft binding of \(^{18}\text{F}\)-labeled proteins with molecular weight (M\(_{r}\)) \(\sim 25,000\) is achievable before 6 h (10).

As the trend toward using smaller fragments continues, significant efforts are being devoted to better defining which ligands can successfully be used for specific targeting of tumor sites using an \(^{18}\text{F}\) label, an issue in which the molecular weight of the protein used will be of critical importance. Some studies have reported specific targeting within 6 h from injection of radiolabeled Fab fragments (M\(_{r}\) \(\sim 50,000\)) (11) and F(ab\(^\prime\))\(_{2}\) fragments (M\(_{r}\) \(\sim 100,000\)) (12).

This study was aimed at investigating the potential of \(^{18}\text{F}\)-labeled transferrin (TF) M\(_{r}\) \(\sim 79,000\) as a radiopharmaceutical for targeting tumor TF receptors (TF-Rs). Radiolabeled TF appears to be an ideal candidate for monitoring tumor growth because tumor metabolism relies heavily on the availability of iron (13). The principal mechanism by which all cells are able to accumulate iron is through receptor-mediated endocytosis of iron-loaded holo-Tf (14,15). The high concentration of Tf in serum (10–20 \(\mu\)mol), the high affinity of holo-Tf for the TF-R (\(K_{d} = \sim 2\) nmol) and the rate at which Tf cycling inside the cell is maintained (half-time = \(\sim 10\) min) allow for a constant influx of iron in cells, where it is expended for various metabolic purposes (16,17). Quantitative in vivo determination of TF-R turnover would provide information on tumor iron requirement.

We have previously reported that \(^{18}\text{F}\)TF, labeled according to our scheme (18,19), maintains all in vitro properties of native TF (20). Using PET, we were able to define the approach to equilibrium by \(^{18}\text{F}\)TF and, using \(^{18}\text{F}\)albumin (Alb) as a control, to show the specificity of this interaction for the TF-R in the baboon liver (21). Because permeability to the liver is high, this is the ideal case for studying receptor-dependent targeting. The current challenge is to achieve receptor specific binding in a less permeable target. We studied the distribution of \(^{18}\text{F}\)TF in mice bearing LS174T and A431 xenografts to determine whether the distribution is TF-R mediated or permeability dependent. To assess TF-R binding specificity, we used \(^{14}\text{C}\)Alb or \(^{18}\text{F}\)Alb as a control. We used the LS174T xenograft because of its high uptake of minibodies (22) and, as a result, potentially high permeability. We also used the A431 xenograft, which has previously been used in our laboratory (10). Finally, we modeled these results to better define the receptor and permeability dependence of xenograft targeting of molecules at short times after injection.

**Materials and Methods**

**Radiolabeling**

Human holo-TF and baboon Alb were purchased from Sigma Chemical Co. (St. Louis, MO). These two tracers were used for a previous baboon liver study (21). The labeling procedure is described in detail in a previous article (20). Human \(^{14}\text{C}\)Alb was purchased from American Radiolabeled Chemicals (St. Louis, MO; 1.49 MBq).

**Nude Mouse Xenograft Models**

A431 cells were originally obtained from G. Todaro (National Cancer Institute, National Institutes of Health [NIH]). This is a human epidermoid carcinoma cell line that has been shown to yield subcutaneous tumors with high efficiency in immunologically incompetent mice (23). Cells were grown in a humidified atmosphere containing 5% CO\(_2\) and 95% air at 37°C in Dulbecco’s modified Eagle medium with 4.5 g/L glucose, supplemented with 10% fetal bovine serum (GibcoBRL; Life Technologies, Grand Island, NY). LS174T cells were obtained from J. Greiner (National Cancer Institute, NIH) and grown in the same incubator in Eagle’s minimal essential medium containing 10% fetal bovine serum and nonessential amino acids. These cells are derived from a human colon adenocarcinoma (24). Both cell lines were grown until confluent (approximately 1 wk) and passaged (1 to 4–1 to 6) after trypsinization. Before being implanted in nude mice, cells were trypsinized, washed in culture medium and resuspended in phosphate-buffered saline at a density of \(2 \times 10^7\)/mL. Approximately 100 \(\mu\)L of the cell suspensions were injected subcutaneously in the flank of 4- to 6-wk-old BALB/c nu/nu athymic nude mice (weight 17–23 g). One set of biodistribution studies (experiment A) was performed with A431 xenografts alone, whereas in another study (experiment B), A431 and LS174T cells were injected simultaneously in opposite flanks. Xenografts were allowed to grow for 10–14 d. Final tumor weight was between 0.5 and 1 g at the time of the biodistribution experiments.

**Biodistribution Studies**

**Experiment A.** Initial experiments were performed on mice bearing only the A431 xenograft. The time course of tissue and tumor distribution of \(^{18}\text{F}\)TF or \(^{18}\text{F}\)Alb was determined after injection of \(\sim 1.85\) MBq labeled protein in the tail vein in separate studies. Animals were studied under a protocol approved by the NIH Animal Care and Use Committee. We performed five studies with \(^{18}\text{F}\)TF and three with \(^{18}\text{F}\)Alb. In each biodistribution study, a minimum of four animals per time point was used. The animals were killed 30, 60, 180 and 360 min after injection. Blood, heart, lung, spleen, kidney, muscle, femur and xenograft samples were weighed, and radioactivity was determined using a Packard 5600 gamma counter (Packard Instrument Co., Meriden, CT). Dilutions of the labeled proteins were counted simultaneously for accurate determination of the injected dose. The relative amount of radioactivity in the organs was calculated and expressed as percentage of injected dose per gram of tissue (%ID/g) normalized to a 20-g mouse. The datasets from all experiments with \(^{18}\text{F}\)TF and \(^{18}\text{F}\)Alb were combined before further analysis. Thus the data presented for each time point were obtained from a minimum of 12 animals per time point.

**Experiment B.** In a separate study, mice bearing both A431 and LS174T xenografts were used. Tumor distribution of \(^{18}\text{F}\)TF and \(^{14}\text{C}\)Alb was determined after simultaneous injection of \(\sim 1.85\) MBq \(^{18}\text{F}\)- and 7.4 MBq \(^{14}\text{C}\)-labeled protein in the tail vein. Five animals were used for each time point. The animals were killed 5, 15, 30, 60, 180 and 360 min after injection. The \(^{18}\text{F}\) radioactivity was determined as for the previous experiments. After allowing 3 d for radioactive decay (\(\sim 39\) half-lives), the specimens were solubilized in 1 mL Solvable (Packard) overnight at 50°C. Twenty
milliliters of scintillation fluid (Acquasol; Packard) were then added to each vial. [14C]Alb radioactivity in the samples was then quantitatively determined in a Packard beta counter that had been calibrated with quenched 14C standards. Uptake values (%ID/g) for [14C]Alb were determined as for the 18F data.

Tracer Quality Control

Tracer stability for all proteins was assessed by sodium dodecyl sulfate, polyacrylamide gel electrophoresis (SDS-PAGE) of plasma samples taken from the animals at different time points after injection. After electrophoresis, the gels were dried onto filter paper and immediately autoradiographed by exposing overnight on an imaging plate and visualized using a BAS-1500 Phosphorimager (Fuji Medical Systems, Inc., Stamford, CT) for determination of 18F radioactivity. Gels containing [14C]Alb were later exposed for 3 wk on Biomax MR autoradiographic film (Eastman Kodak, Rochester, NY). In preliminary experiments, [18F]Tf stability was also determined by silica gel thin-layer chromatography (TLC) on similar samples as described (27) to rule out the presence of low-molecular-weight metabolites.

Ribonucleic Acid Analysis

Northern blot analysis was used to determine the level of Tf-R expression in the xenografts. Total ribonucleic acid (RNA) was extracted from A431 and LS174T xenografts and cells grown in culture using RNAzol B (TEL-TEST Inc., Friendswood, TX) and following the manufacturer's suggested procedure. Fifteen micrograms of total RNA were denatured by heating at 65°C for 15 min and loaded onto a 1% agarose formaldehyde gel (25). The samples were electrophoresed for 2 h at 100 V and subsequently transferred to a nylon membrane using a Turboblotter apparatus (Schleicher and Schuell, Keene, NH). After ultraviolet crosslinking, the membrane was hybridized with 32P-labeled complementary DNA (cDNA) probes. Human Tf-R and β-actin cDNA plasmids were purchased from the American Type Culture Collection (Rockville, MD). After hybridization, the membranes were washed twice in two times standard saline citrate with 0.1% SDS at 70°C, exposed overnight on imaging plates and analyzed using a BAS-1500 Phosphorimager. The Tf-R signal was normalized for that of β-actin, which served as a control of the total amount of RNA loaded.

Transferrin Binding Assays

The presence of Tf binding sites was assessed in A431 and LS174T xenografts. 125I-labeled human diferric-Tf was purchased from DuPont-NEN (Boston, MA). Membrane preparations were prepared freshly for each experiment as follows. Five hundred-milligram samples were minced and homogenized in 20 mmol Tris, 1 mmol ethylenediaminetetraacetic acid ([EDTA] pH 7.4) containing protease inhibitors (Compleat; Boehringer-Mannheim, Indianapolis, IN) with a Polytron homogenizer (Kinematica, Littau-Lucerne, Switzerland) on ice. The suspension was subsequently centrifuged at 12,000 rpm (18,000g) in a Beckman JA-20 rotor (Beckman, Brea, CA). The supernatant was discarded, and the pellet was resuspended in 20 mmol Tris, 1 mmol EDTA and protease inhibitors at a final concentration of 1–2 mg protein/mL of suspension. Protein concentration was determined with a Pierce Coomassie-Plus protein assay kit (Pierce Chemical Co., Rockford, IL). Aliquots of the membrane suspensions were incubated with known amounts of [125I]Tf for 1 h while rotating at 4°C. Bound counts were subsequently separated by a 10-min centrifugation at 14,000 rpm in an Eppendorf centrifuge (Brinkmann Instruments Co., Westbury, NY). Concentrations of Tf-R were calculated using a Macintosh computer (Apple, Cupertino, CA) and Kaleidograph software (Abelbeck Software, Version 3.0; distributed by Synergy Software, Reading, PA) by fitting the experimental data to a model with a single class of saturable receptors. The values are expressed as nmol/kg starting tissue weight.

Pharmacokinetic Modeling of Blood Distribution Data

Uptake and loss of a tracer macromolecule from a tissue with continuous capillaries can be described by the following pharmacokinetic equation (25):

\[
dt \phi C_t = k C_p - L C_t,
\]

where \( \phi \) is the interstitial volume fraction (mL interstitial volume/g tissue), \( k \) is the permeability from plasma to the interstitial volume (μL/min/g) and \( L \) is the loss rate from tissue (e.g., by lymph flow) (μL/min/g). The total (free plus bound) and free concentrations of tracer referred to the tissue interstitium are given by \( C_t \) and \( C_f \), respectively, and \( C_p \) is the plasma concentration.

The plasma concentration was calculated by dividing the blood %ID/g values by 1 – hematocrit, and a hematocrit value of 0.48 was used (27). A biexponential fit to the experimental data then served as the plasma input curve. Tissue blood space was estimated from the tissue-to-blood ratio of [18F]Tf at 5 min (2.8% and 2.3% for A431 and LS174T, respectively), assuming negligible extravasation of tracer into the tissue at this early time point. For the liver, the tissue-to-blood ratio of [14C]Alb was used because the 5-min uptake of [18F]Tf was already significantly higher than that of [14C]Alb (paired t test \( P < 0.001 \)), indicating that tissue binding was occurring already. This value was calculated to be 21.5%, which is consistent with reported values (27). Uptake values for each organ were recalculated by subtracting the contribution of blood radioactivity to the overall %ID/g values. Permeabilities were calculated for the 15-, 30- and 60-min time points by dividing the blood-subtracted tissue concentration by the integrated plasma tracer concentration at each time point. A plasma density of 1 g/mL was assumed. The average of the 15-, 30- and 60-min values in each organ for each tracer was used as the permeability rate.

Receptor-ligand binding can be described by the following equation, which relates the concentration of free to bound tracer:

\[
C_b = \frac{K_B C_l}{1 + K_B C_l}.
\]

However, in the presence of a high endogenous concentration of the macromolecule in plasma, \( C_{p,\text{endog}} \) (which presumably is in equilibrium with the free interstitial concentration), the relationship is given by the following equation:

\[
C_b = \frac{K_B C_l}{1 + K_B \frac{k}{L} C_{p,\text{endog}}} = \frac{B_0 C_l}{L \frac{C_{p,\text{endog}}}{C_{p,\text{endog}}}},
\]

where \( K_a \) is the affinity of the ligand for receptor, and \( B_0 \) is the concentration of receptor referred to interstitial volume. The dissociation constant \( (1/K_a) \) for Tf and Tf-R is 2 nmol (/S).

Pharmacokinetic data of [14C]Alb, the nonbinding control, were analyzed to obtain estimates of \( \phi \) and \( L \) for the two tumors. Because these two parameters are highly coupled, we constrained \( \phi \) in a range similar to other tumors (28) and found a value of \( L \) that fit the data. \( \phi \) values of 0.2 and 0.25 mL/g for A431 and LS174T.
RESULTS

Blood Distribution Studies

Uptake values for experiments A and B are reported in Table 1. Blood radioactivity decreased over 6 h with similar rates for all three tracers. SDS-PAGE and TLC analysis (not shown) of blood samples taken at different times showed no evidence of circulating metabolites in the blood. All blood radioactivity co-migrated with the starting material, although the total amount of radioactivity decreased with time. In experiment A, %ID/g values of [18F]Tf and [18F]Alb in the A431 xenografts increased with similar rates over the 6 h. In experiment B, accumulation of [18F]Tf and [124I]Alb in the A431 and LS174T xenografts increased with time, and the LS174T xenograft showed higher absolute uptake values after the 5-min time point. The highest absolute [18F]Tf uptake values were obtained at 6 h in both xenografts (3.51 ± 0.83 %ID/g for A431 and 6.05 ± 1.90 %ID/g for LS174T, mean ± SD). The same was observed for [124I]Alb uptake (4.79 ± 0.95 %ID/g in A431 and 8.17 ± 2.53 %ID/g in LS174T at 6 h). In both sets of experiments, the only organ showing a striking difference in absolute uptake values between [18F]Tf and either of the Alb compounds is the liver. In this organ, Tf is taken up very rapidly compared with Alb (experiment B at 5 min 18.24 ± 11.4 %ID/g for [18F]Tf and 11.58 ± 1.57 %ID/g for [124I]Alb).

Figure 1 shows the time course of blood-subtracted radioactivity for the two tracers in liver, A431 and LS174T xenografts in experiment B. In the liver, values for [18F]Tf were statistically higher than those for [124I]Alb (paired t test P < 0.001) at all time points except 360 min. However, the bound liver [18F]Tf fraction (blood-subtracted bound [18F]Tf/blood [18F]Tf concentration) varied only slightly (from 0.14 ± 0.03 at 5 min to 0.10 ± 0.02 at 360 min). In both xenografts, there was a parallel increase of radioactivity for both tracers and no apparent preferential retention of [18F]Tf compared with [124I]Alb, as [124I]Alb values are slightly higher at all time points.

Binding Experiments and Ribonucleic Acid Analysis

To rule out the possibility of receptor downregulation after transplantation as a cause for low specific retention of [18F]Tf in the xenografts, the presence of Tf-R was determined by two independent methods. The concentration of messenger RNA (mRNA) for Tf-R was measured in both A431 and LS174T xenografts and compared with the concentration present in cells grown in culture (Fig. 2). Northern blot analysis showed the presence of the 4.9-kilobase mRNA for Tf-R in all samples tested. When normalized for the expression of β-actin mRNA, the amount of Tf-R mRNA was found to be very similar in A431 cells and xenografts and slightly higher (~20%) in both LS174T cells and xenografts. Binding capacity also was directly measured on tissue extracts using [125I]Tf (Table 2). The two xenografts have very similar levels of Tf binding sites.

Pharmacokinetic Modeling

The data from experiment B were used to calculate permeabilities of [18F]Tf and [124I]Alb in the two xenografts (Table 2). The permeability of [124I]Alb was higher in both xenografts (1.29 μL/min/g in LS174T and 0.79 μL/min/g in A431) than that of [18F]Tf (1.03 μL/min/g in LS174T and 0.44 μL/min/g in A431). The more permeable tumor, LS174T, had less discrimination in permeability between the two different molecular weight proteins (Alb permeability was only 30% higher than that of Tf compared with 60% higher for A431). The [18F]Tf values were used to simulate the expected dependence of tumor [18F]Tf uptake on total receptor concentration (nmol receptor/kg tissue) based on the model (Fig. 3). The distribution predicted by the model is in agreement with the observed low tumor uptake values and the levels of Tf binding sites measured in the tumor extracts (Table 2).

DISCUSSION

The main goal of this study was to determine whether [18F]Tf can be used to trace Tf-Rs in tumor xenografts in a short time frame (<6 h). Based on current knowledge of the biodistribution of proteins, tumor permeability and competition from endogenous Tf are the two most likely variables that prevent us from showing specific binding in the xenografts. From the data we present, it appears that measurements of specific [18F]Tf uptake can only be shown in the liver. The liver constitutes an optimal model for proving specific Tf-R targeting of [18F]Tf. Hepatocytes contain most of the Tf-R found in the liver (29). Tf-R binding sites on hepatocytes have been reported in the range of 40,000–165,000 sites per cell (30), or, assuming 10⁶ cells/g tissue, 65–270 nmol receptor/kg tissue (Table 2). The sinusoids of the liver and the elevated levels of Tf-R expression allow barrier-free diffusion of the Tf compound to these specific binding sites. The liver shows higher retention values for [18F]Tf compared with [124I]Alb (Table 1), which is consistent with the fact that the liver expresses high amounts of Tf-R and that the Alb compounds only trace extracellular space. The absolute uptake values for [18F]Tf decrease with time in parallel with blood radioactivity, and, by the 360-min time point, [18F]Tf values are not significantly different from those of [124I]Alb or [128I]Alb. The blood-subtracted data in experiment B (Fig. 1) show that the difference in uptake between [18F]Tf and [124I]Alb decreases with time. By 360 min, blood-subtracted liver [18F]Tf uptake is not significantly higher than [124I]Alb. However, the bound liver [18F]Tf fraction (blood-subtracted bound [18F]Tf/blood [18F]Tf concentration) decreases only slightly (from 0.14 ± 0.03 at 5 min to 0.10 ± 0.02 at 360 min). The fact that liver-bound [18F]Tf does not increase with time suggests that the bound protein is continuously being replaced by newly available [18F]Tf present in the
### TABLE 1

**Uptake Values in Biodistribution Experiments**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>30 min</th>
<th>60 min</th>
<th>180 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
<td>Alb</td>
<td>Ti</td>
<td>Alb</td>
</tr>
<tr>
<td>Blood</td>
<td>33.33 ± 5.84</td>
<td>30.22 ± 6.63</td>
<td>28.41 ± 3.38</td>
<td>29.10 ± 5.55</td>
</tr>
<tr>
<td>Liver</td>
<td>14.30 ± 4.64</td>
<td>4.89 ± 0.96</td>
<td>11.08 ± 3.10</td>
<td>5.54 ± 1.85</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.45 ± 1.22</td>
<td>4.03 ± 0.88</td>
<td>5.12 ± 0.94</td>
<td>4.04 ± 0.94</td>
</tr>
<tr>
<td>A431</td>
<td>2.80 ± 0.94</td>
<td>2.51 ± 0.85</td>
<td>3.51 ± 0.84</td>
<td>3.85 ± 1.49</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.97 ± 0.28</td>
<td>1.32 ± 1.00</td>
<td>1.29 ± 0.55</td>
<td>1.43 ± 0.57</td>
</tr>
<tr>
<td>Kidney</td>
<td>9.04 ± 2.53</td>
<td>6.84 ± 1.86</td>
<td>8.34 ± 2.41</td>
<td>7.70 ± 1.51</td>
</tr>
<tr>
<td>Femur</td>
<td>2.73 ± 0.47</td>
<td>1.95 ± 0.44</td>
<td>3.11 ± 0.84</td>
<td>2.52 ± 0.75</td>
</tr>
<tr>
<td>Heart</td>
<td>8.24 ± 1.93</td>
<td>6.90 ± 1.80</td>
<td>8.21 ± 2.00</td>
<td>7.16 ± 1.34</td>
</tr>
<tr>
<td>Lung</td>
<td>11.25 ± 2.78</td>
<td>10.03 ± 3.65</td>
<td>9.16 ± 1.15</td>
<td>9.98 ± 2.08</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>5 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
<td>Alb</td>
</tr>
<tr>
<td>Blood</td>
<td>51.93 ± 5.26</td>
<td>55.52 ± 12.09</td>
</tr>
<tr>
<td>Liver</td>
<td>18.24 ± 1.14</td>
<td>11.58 ± 1.57</td>
</tr>
<tr>
<td>Spleen</td>
<td>8.85 ± 1.41</td>
<td>10.47 ± 1.74</td>
</tr>
<tr>
<td>A431</td>
<td>1.51 ± 0.59</td>
<td>2.14 ± 0.63</td>
</tr>
<tr>
<td>LS174T</td>
<td>1.21 ± 0.45</td>
<td>1.50 ± 0.37</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.54 ± 0.20</td>
<td>1.14 ± 0.42</td>
</tr>
</tbody>
</table>

%ID/g = percentage of injected dose per gram of tissue; Ti = transferrin; Alb = albumin.

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**Experiment B**

%ID/g values for [18F]Ti and [14C]Alb (mean ± SD, n = 5)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>5 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ti</td>
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<tr>
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</tr>
<tr>
<td>LS174T</td>
<td>1.21 ± 0.45</td>
<td>1.50 ± 0.37</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.54 ± 0.20</td>
<td>1.14 ± 0.42</td>
</tr>
</tbody>
</table>

%ID/g = percentage of injected dose per gram of tissue; Ti = transferrin; Alb = albumin.
Concentration/permeability

Liver

If-A

concentration (nmol/kg tissue)

65–270 ± 216 ± 26

Permeability Tf

(µL/min/g tissue)

>13.3 ± 0.04

Permeability Alb

(µL/min/g tissue)

— ± 0.38

n = 3

Mean ± SD.

Recalculated from reference 30.

Tf-R = transferrin receptor; Alb = albumin.

blood, consistent with the ability of this compound to reproduce the cycling kinetics described for Tf (15). Thus, [18F]Tf appears at equilibrium between tissue-associated and blood radioactivity, with perhaps a slight reduction in the ability to bind Tf-Rs over time.

Our results are consistent with the presence of a large liver binding and cycling capacity for Tf, given the fact that tracer amounts of [18F]Tf (20–55 nmol at time of injection) compete with the endogenous Tf (10–20 µmol) (31) for receptor binding and that considerable amounts of the tracer are retained. We have obtained similar results in baboons using dynamic PET imaging (21). In the baboon, we showed that the liver has a binding compartment for [18F]Tf that rapidly reaches equilibrium, with elevated flux rates exchanging approximately 0.1 nmol of total Tf/min/mL tissue between blood and liver.

In the xenografts, there is no specific uptake of [18F]Tf in the time frame studied. Uptake of [18F]Tf slowly increases with time in parallel with [18F]Alb and [14C]Alb (Table 1 and Fig. 1). We know that adequate levels of Tf binding sites are

TABLE 2

Tf-R Binding Capacity of Tissue Membrane Preparations and Calculated Permeabilities from Experiment B

<table>
<thead>
<tr>
<th>Concentration/permeability</th>
<th>Liver*</th>
<th>A431†</th>
<th>LS 174T†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Tf-R concentration</td>
<td>65–270</td>
<td>216 ± 24</td>
<td>226 ± 16</td>
</tr>
<tr>
<td>(nmol/kg tissue)</td>
<td></td>
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<tr>
<td>Permeability Tf</td>
<td>&gt;13.3</td>
<td>0.44 ± 0.04</td>
<td>1.03 ± 0.38</td>
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<td>(µL/min/g tissue)</td>
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<tr>
<td>Permeability Alb</td>
<td>—</td>
<td>0.79 ± 0.24</td>
<td>1.29 ± 0.49</td>
</tr>
<tr>
<td>(µL/min/g tissue)</td>
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*n = 3.
†Mean ± SD.
‡Recalculated from reference 30.

Tf-R = transferrin receptor; Alb = albumin.

FIGURE 1. Time course of blood-subtracted 18F radioactivity in experiment B for liver (A), A431 (B) and LS174T (C) (n = 5; error bars = SD).

FIGURE 2. Autoradiograph. (A) Northern blot analysis of total RNA extracted from A431 and LS174T cells grown in culture and as xenografts. Blot was hybridized with Tf-R and β-actin cDNA probes. (B) Quantitation (histogram) of autoradiograph shows slightly higher relative amounts of Tf-R mRNA, when normalized to amount of β-actin mRNA, in LS174T cells and xenografts.
of proteins from plasma to tumor interstitial space is much lower. Our interpretation of the work by Baxter et al. (33) is that permeability in the human colon cancer xenograft used in their study of Fab molecules, which have a slightly lower molecular weight than Tf, is approximately 50 times lower than in the liver. The models of transport described by Sung et al. (26,34) indicate that tumor uptake of monoclonal antibodies at early times is dominated by capillary permeability and plasma kinetics and is not highly influenced by antigen binding. The use of the nonspecific Alb compounds allowed us to evaluate the contribution of tumor binding because differences in distribution between [18F]Tf and Alb should be a measure of Tf-R targeting. However, the tumor concentrations of the Alb tracers were always higher than those of [18F]Tf, a reflection of the higher capillary permeability of Alb (Table 2) and the low degree of specific binding of [18F]Tf. This is in marked contrast to the data obtained from the liver, in which permeability, cycling rates and expression of Tf-R sites are sufficient to result in specific binding of [18F]Tf. The model simulations (Fig. 3) indicate that, given the permeability of [18F]Tf in the two xenografts and the concentration of endogenous Tf in plasma and interstitial space, the observed uptake values of [18F]Tf in the two xenografts are consistent with the measured Tf-R concentrations in tissue extracts, and these values differ little from what would be observed in the presence of no receptors. The LS174T showed higher permeability (Table 2) than the A431 xenograft for both proteins. However, the permeability values in this xenograft were still not adequate to provide sufficient tracer delivery to the tumor extracellular space.

Before we can generalize the conclusions of these experiments to the biodistribution properties of all radiopharmaceuticals with Mn ~79,000, we must consider the effect of the endogenous concentrations of Tf. Figure 4 shows a model simulation of the dependence of the distribution of [18F]Tf on binding capacity ($\phi \times B_d$) using the same measured permeability values in the two xenografts (Table 2) and affinity constant of Tf, but assuming a zero concentration of endogenous Tf. It appears that under these theoretical conditions, specific targeting of [18F]Tf may be obtainable given the Tf-R concentrations measured in the tumor extracts (Table 2; also compare binding capacity dependence in Fig. 3 with that in Fig. 4).

Our data also show that commonly used xenograft models such as A431 and LS174T have very different permeabilities to high-molecular-weight proteins, which affect considerably the biodistribution of these tracers. For example, when comparing the tumor uptake of [14C]Alb as a function of time (Table 1, experiment B), ratios of LS174T to A431 are nearly 2 at 180 min. This is attributed to the higher permeability and not to specific retention. Therefore, the permeability rates of these models must be considered when performing comparisons between different tumors, as overall uptake will depend not only on characteristics of the
our data show that $^{18}$F]Tf has preferential binding to the liver compared with Alb and reaches equilibrium consistent with the cycling properties of Tf. This double-tracer approach does not allow the evaluation of Tf-R expression in the A431 and LS174T xenografts because no preferential binding of $^{18}$F]Tf is observed compared with the nonspecific control $^{18}$F]Alb, and tracer distribution appears controlled by permeability rather than receptor expression. Other approaches will be necessary to image Tf-R expression in tumors.

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REFERENCES