Targeting Rat Anti-Mouse Transferrin Receptor Monoclonal Antibodies through Blood-Brain Barrier in Mouse

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ABSTRACT

Drug targeting through the brain capillary endothelium, which forms the blood-brain barrier (BBB) in vivo, may be achieved with peptidomimetic monoclonal antibodies that target peptide transcytosis systems on the BBB in vivo. Murine monoclonal antibodies to the rat transferrin receptor, such as the OX26 monoclonal antibody, are targeted through the BBB on the transferrin receptor in the rat. However, the present studies show the OX26 monoclonal antibody is not an effective brain delivery vector in mice. The emergence of transgenic mouse models creates a need for brain drug-targeting vectors for this species. Two rat monoclonal antibodies, 8D3 and RI7-217, to the mouse transferrin receptor were evaluated in the present studies. Both the RI7-217 and the 8D3 antibody had comparable permeability-surface area products at the mouse BBB in vivo. However, owing to a higher plasma area under the concentration curve, the mouse brain uptake of the 8D3 antibody was higher, 3.1 ± 0.4% of injected dose (ID)/g compared with the brain uptake of the RI7 antibody, 1.6 ± 0.2% ID/g, at 60 min after i.v. injection. Conversely, the mouse brain uptake of the OX26 antibody, which does not recognize the mouse transferrin receptor, was negligible, 0.06 ± 0.01% ID/g. The RI7-127 antibody was more selective for brain because this antibody was not measurably taken up by liver. The capillary depletion technique demonstrated transcytosis of the RI7-217 antibody through the mouse BBB in vivo. The brain uptake of the 8D3 antibody was saturable, consistent with a receptor-mediated transport process. In conclusion, these studies indicate rat monoclonal antibodies to the mouse transferrin receptor may be used for brain drug-targeting studies in mice such as transgenic mouse models.

Drug targeting through the brain capillary endothelium, which forms the blood-brain barrier (BBB) in vivo, may be achieved with peptidomimetic monoclonal antibodies that target peptide transcytosis systems on the BBB in vivo. Murine monoclonal antibodies to the rat transferrin receptor, such as the OX26 monoclonal antibody, are targeted through the BBB on the transferrin receptor in the rat. However, the present studies show the OX26 monoclonal antibody is not an effective brain delivery vector in mice. The emergence of transgenic mouse models creates a need for brain drug-targeting vectors for this species. Two rat monoclonal antibodies, 8D3 and RI7-217, to the mouse transferrin receptor were evaluated in the present studies. Both the RI7-217 and the 8D3 antibody had comparable permeability-surface area products at the mouse BBB in vivo. However, owing to a higher plasma area under the concentration curve, the mouse brain uptake of the 8D3 antibody was higher, 3.1 ± 0.4% of injected dose (ID)/g compared with the brain uptake of the RI7 antibody, 1.6 ± 0.2% ID/g, at 60 min after i.v. injection. Conversely, the mouse brain uptake of the OX26 antibody, which does not recognize the mouse transferrin receptor, was negligible, 0.06 ± 0.01% ID/g. The RI7-127 antibody was more selective for brain because this antibody was not measurably taken up by liver. The capillary depletion technique demonstrated transcytosis of the RI7-217 antibody through the mouse BBB in vivo. The brain uptake of the 8D3 antibody was saturable, consistent with a receptor-mediated transport process. In conclusion, these studies indicate rat monoclonal antibodies to the mouse transferrin receptor may be used for brain drug-targeting studies in mice such as transgenic mouse models.

ABBREVIATIONS: BBB, blood-brain barrier; mAb, monoclonal antibody; TR, transferrin receptor; TCA, trichloroacetic acid; AUC, plasma area under the concentration curve; MRT, mean residence time; PS, permeability-surface area; ID, injected dose.
antibody is also a rat IgG2a to the mouse TfR, and immunocytochemistry shows specific binding of the 8D3 mAb to capillary endothelium in mouse brain (Kissel et al., 1998).

Experimental Procedures

Materials. 125I-Na was obtained from Amersham (Arlington Heights, IL) and chloramine-T was supplied by MCA Reagents (Cincinnati, OH). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). The ammonium sulfate precipitated 8D3 rat mAb to the mouse TfR was provided by Dr. Britta Engelhardt (W.G. Kerckhoff Institut, Bad Nauheim, Germany), and was purified by protein G affinity chromatography as described previously (Kang and Pardridge, 1994). The RI7-217 (designated RI7) rat hybridoma protein G affinity chromatography as described previously (Kang and Pardridge, 1994). The antibody was obtained under Material Transfer Agreement with a derivative-free nonlinear regression analysis (PARBMDP, Computing Facilities). The area under the plasma concentration (AUC) was calculated as follows:

\[
\text{AUC} = \frac{D(AUMC)}{AUC_0} - 60 \text{ min}
\]

where \( D_{(60 \text{ min})} \) is the terminal plasma concentration and \( AUC_0 \) is the plasma volume of each organ, which has been determined with the 125I-rat IgG2a in mice previously (Skaalos and Partridge, 1995). In the calculation of the PS product, it is assumed there is no efflux of radioactivity from brain during the first 60 min after i.v. administration, as demonstrated previously (Pardridge et al., 1991). The organ uptake of all three mAbs was expressed as percentage of injected dose (ID)/g of brain, and was determined as follows:

\[
\% \text{ID/g} = \text{PS} \times \text{AUC}_0 - 60 \text{ min}
\]

In a separate series of experiments, the organ uptake and pharmacokinetics were examined after the administration of 125I-8D3 cojected with 0, 1, or 4 mg/kg unlabeled 8D3 mAb. In these experiments, the specific activity of the labeled antibody was 13 μCi/μg.

Capillary Depletion Analysis. The anesthetized mice were administered 50 μl of PBS containing 0.1% BSA and 5 μCi of 125I-RI7 mAb into the jugular vein. Arterial blood and brain were collected at 0.5, 1, 6, and 24 h after injection of the isotope solution. After centrifugation of the blood samples, plasma was counted for total radioactivity and was assayed for TCA precipitation. The brain tissue was homogenized in ice-cold physiological buffer for capillary depletion analysis, as described previously (Triguero et al., 1990). The total brain homogenate was fractionated into the capillary pellet and the postcapillary supernatant by the capillary depletion technique. Vd values for the homogenate and the capillary pellet were calculated as follows:

\[
\text{Vd} = \frac{(\text{cpm/g brain})/C_p(t)}{}
\]

where \( C_p(t) \) is the counts per minute per microliter of plasma at brain sampling time (t). For the postcapillary supernatant, the Vd was calculated by subtracting the Vd of capillary pellet from the Vd of homogenate. Statistical analysis was performed by Student’s t test.

Results

The plasma profiles for the three monoclonal antibodies are shown in Fig. 1. The plasma concentration for the three antibodies are shown in Fig. 1 (left), and the plasma TCA precipitability of the 125I-mAbs is shown in Fig. 1 (right). All three antibodies are relatively stable based on the high TCA precipitability during the experimental time period.

The plasma profiles in Fig. 1 were subjected to a pharmacokinetic analysis and these parameters are shown in Table 1.
The plasma clearance of the RI7 mAb > the 8D3 mAb >> the OX26 mAb. The systemic volume of distribution ($V_{ss}$) of the OX26 mAb, 50 ± 2 ml/kg, approximates the plasma volume in mice (Table 1), whereas the $V_m$ of the 8D3 or RI7 mAb is ~3-fold higher (Table 1).

The organ uptake of the three mAbs in mice at 60 min after i.v. injection was measured and these data are shown in Table 2 for brain, liver, kidney, and heart. The organ Vd is shown for the three mAbs for the four organs in comparison with the plasma Vd for each of the four organs. The Vd was determined from the organ uptake of $^{125}$I-labeled rat IgG2B isotype control antibody, which is restricted to the plasma volume in mice (Skarlatos and Pardridge, 1995). There is virtually no brain uptake of the OX26 mAb by mouse brain because the brain Vd of this antibody is nearly identical with the brain plasma volume (Table 2). The brain uptake of the OX26 mAb is 0.06 ± 0.01% ID/g (Table 2). Conversely, the brain uptake of either the 8D3 or the RI7 mAb is high, 1.6 to 3.1% ID/g (Table 2). The 8D3 mAb is significantly transported into liver and kidney, whereas the RI7 mAb is not measurably taken up by these organs, and is selective for brain (Table 2).

To demonstrate that the RI7 mAb was effectively transcytosed through the BBB in vivo, a capillary depletion analysis was performed. The uptake of the $^{125}$I-R17 mAb was measured in mouse brain at 0.5, 1, 6, and 24 h after i.v. injection. The Vd was measured in the total brain homogenate, the capillary pellet, and the postvascular supernatant (Fig. 2). These data show that the RI7 mAb was effectively transcytosed through the BBB in vivo because the Vd of the postvascular supernatant increased with time and approximated 1000 μl/g at 24 h after i.v. injection (Fig. 2). The RI7 mAb was relatively stable in plasma as the plasma TCA precipitability at 0.5, 1, 6, and 24 h after i.v. injection was 96 ± 1, 93 ± 1, 86 ± 3, and 80 ± 2%, respectively (mean ± S.E., n = three mice per point).

The saturation of the organ uptake of the labeled 8D3 antibody is shown in Table 3. The brain uptake, percentage of ID/g, is reduced 6-fold and the BBB PS product is reduced 95% by a 4-ml/kg dose of unlabeled 8D3 antibody (Table 3). This dose increases the plasma AUC ~3-fold. In addition to brain, the uptake of the labeled 8D3 antibody by heart, lung, and spleen is inhibited by the 4 mg/kg-dose of unlabeled antibody, whereas the uptake systems in liver and kidney are less inhibited (Table 3).

**Discussion**

The results of these investigations are consistent with the following conclusions. First, the OX26 mouse mAb to the rat TfR is not significantly taken up by mouse brain in vivo. Second, both the 8D3 and the RI7 rat mAbs to the mouse TfR are effectively transported into brain in vivo. Third, the organ selectivity for the RI7 and 8D3 mAbs are different because the 8D3 mAb is taken up by liver, and to a lesser extent, kidney, whereas the RI7 mAb is not taken up by these organs and is selective for brain (Table 2). Fourth, the brain uptake of the 8D3 mAb is saturable (Table 3), consistent with a receptor-mediated process.

These data show clearly that the murine OX26 mAb to the rat TfR cannot be used for brain drug delivery studies in mouse models because the uptake of this murine mAb by mouse brain is not different from the brain uptake of a brain plasma marker (Table 2). Conversely, the brain Vd of either the 8D3 or the RI7 rat mAb to the mouse TfR is ~10-fold higher than the brain Vd of the OX26 mAb (Table 2). The brain uptake, expressed as %ID/g brain, for either the 8D3 or RI7 mAb is nearly 50-fold greater than the brain uptake of the OX26 mAb (Table 2). The data in Table 2 allow for comparison of the uptake by mouse brain of either the 8D3 or RI7 mAb, and the brain uptake of the OX26 mAb in rats. The BBB PS product for the OX26 mAb in the rat at 60 min after i.v. injection, 1.6 ± 0.1 μl/min/g (Bickel et al., 1993), is not significantly different from the BBB PS products for either the 8D3 or RI7 mAb in mouse brain at this time period (Fig. 3). In contrast, the brain uptake (%ID/g), for either the 8D3 or the RI7 mAbs in mouse brain, 1.6 to 3.1% ID/g (Table 2), is up to 12-fold greater than the brain uptake of the OX26 Mab.

**Table 1**

Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$^{125}$I-8D3 mAb</th>
<th>$^{125}$I-R17 mAb</th>
<th>$^{125}$I-OX 26 mAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (%ID/ml)</td>
<td>36.3 ± 1.9</td>
<td>27.9 ± 3.4</td>
<td>77.0 ± 2.4</td>
</tr>
<tr>
<td>k (min⁻¹)</td>
<td>0.0019 ± 0.0001</td>
<td>0.010 ± 0.001</td>
<td>0.0013 ± 0.0002</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>363 ± 26</td>
<td>69 ± 4</td>
<td>542 ± 57</td>
</tr>
<tr>
<td>AUC (μl/min/ml)</td>
<td>2059 ± 117</td>
<td>1245 ± 133</td>
<td>4447 ± 156</td>
</tr>
<tr>
<td>V₀ (ml/kg)</td>
<td>126 ± 6</td>
<td>137 ± 17</td>
<td>50 ± 2</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>0.24 ± 0.03</td>
<td>1.4 ± 0.1</td>
<td>0.066 ± 0.010</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>524 ± 37</td>
<td>99 ± 6</td>
<td>782 ± 83</td>
</tr>
</tbody>
</table>

**Table 2**

Organ uptake of $^{125}$I-8D3 mAb, $^{125}$I-R17 mAb, and $^{125}$I-OX 26 mAb by BALB/c mice at 60 min after i.v. injection

<table>
<thead>
<tr>
<th>Organ</th>
<th>$^{125}$I-8D3</th>
<th>$^{125}$I-R17</th>
<th>$^{125}$I-OX 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vd (μl/g)</td>
<td>102 ± 8</td>
<td>107 ± 17</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>liver</td>
<td>58</td>
<td>62 ± 9</td>
<td>162 ± 19</td>
</tr>
<tr>
<td>kidney</td>
<td>62</td>
<td>110 ± 65</td>
<td>136 ± 8</td>
</tr>
<tr>
<td>heart</td>
<td>94</td>
<td>159 ± 12</td>
<td>87 ± 8</td>
</tr>
<tr>
<td>PS (μl/min/g)</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.046 ± 0.006</td>
</tr>
<tr>
<td>liver</td>
<td>8.3 ± 1.8</td>
<td>0</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>kidney</td>
<td>2.4 ± 0.1</td>
<td>0</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td>heart</td>
<td>0.59 ± 0.08</td>
<td>0.80 ± 0.10</td>
<td>0</td>
</tr>
<tr>
<td>%ID/g</td>
<td>3.1 ± 0.4</td>
<td>1.6 ± 0.2</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>brain</td>
<td>16.9 ± 6.1</td>
<td>0</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>kidney</td>
<td>4.9 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>heart</td>
<td>1.2 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0</td>
</tr>
</tbody>
</table>
The RI7 and 8D3 mAbs have comparable BBB PS products (Tables 2 and 3); the higher PS product for the 8D3 antibody in the experiments reported in Table 3 is about twice the PS product recorded in the experiments reported in Table 2. The specific activity of the 125I-8D3 mAb used in the experiments reported in Table 3 is 10-fold higher than the specific activity of the 125I-8D3 mAb used in the experiments reported in Table 2 (see Experimental Procedures). Therefore, the dose of 8D3 mAb (in micrograms) was 10-fold higher in the experiments reported in Table 2. The PS product was reduced in these experiments due to the saturation of the BBB transport of the anti-TfR mAbs (Table 3). Because the BBB PS products for the 8D3 and RI7 antibodies are comparable, the higher brain uptake of the 8D3 mAb (Fig. 3) is due solely to the higher plasma AUC of the 8D3 mAb compared with the RI7 antibody (Table 1). The higher plasma AUC for the 8D3 mAb is surprising because this mAb is targeted to the liver, whereas the RI7 mAb is not taken up by liver (Table 2). The plasma AUC should be inversely related to the hepatic clearance of the mAb. The absence of significant hepatic uptake of the RI7 mAb in mice is also unexpected given the high level of transferrin receptor in liver (Rudolph et al., 1988). The selective uptake of the RI7 mAb by brain, but not liver, is not due to organ-specific gene expression because there is a single mouse TfR gene (Stearne et al., 1985). The RI7 mAb may recognize an epitope that is fully expressed at the BBB TfR, and perhaps other organs such as bone marrow or spleen, but is masked in part at the TfR in mouse liver. Alternatively, TCA-precipitable metabolites of the RI7 mAb may be exported from the liver to blood. The BBB PS product for the RI7 mAb may actually be about twice the value shown in Fig. 3 because the RI7 hybridoma secretes mAb with endogenous light chain (see Experimental Procedures), similar to the OX26 hybridoma (Yoshikawa and Pardridge, 1992). The endogenous light chain would combine with the RI7 heavy chain and this mixed mAb is presumed to have a much reduced affinity for the TfR. Genetically engineered forms of the OX26 mAb have been recently expressed from cloned OX26 light and heavy chain genes, which eliminates the problem of endogenous light chain secretion (Li et al., 1999), and similar genetically engineered forms of either the RI7 or 8D3 mAb could be produced.

The OX26 mAb undergoes receptor-mediated transcytosis through the BBB in rat brain and this has been demonstrated by capillary depletion analysis, thaw mount autoradiography, and immunogold electron microscopy (Bickel et al., 1994; Skarlatos and Pardridge, 1995; Broadwell et al., 1996). The anti-TfR mAbs undergo receptor-mediated transcytosis through the BBB similar to transferrin (Fishman et al., 1987; Skarlatos and Pardridge, 1995). The RI7 mAb may undergo receptor-mediated transcytosis through the mouse BBB in vivo based on the capillary depletion studies shown in Fig. 2. The Vd of the postvascular supernatant for the RI7 mAb is only ~40% of the corresponding value for the OX26 mAb (Table 1). This suggests a very early and rapid phase of clearance of the 8D3 and RI7 mAbs occurs immediately after i.v. administration.

Table 2 (see Experimental Procedures) indicates that the relative uptake of the RI7 mAb at 24 h after i.v. injection of the isotope solution was performed at 0.5, 1, 6, and 24 h after i.v. injection of the isotope solution. Data are means ± S.E. (n = 3).

by rat brain in vivo, 0.26% ID/g (Bickel et al., 1993). This higher uptake in mouse brain, normalized per gram organ weight, is directly proportional to the pharmacokinetic parameter, i.e., the plasma AUC. For example, the plasma AUC for the 8D3 mAb (Table 1) is 12-fold higher in mice, 2059 ± 117% ID · min/ml (Table 1) compared with the 60-min AUC value for the OX26 mAb in rats, 168 ± 76% ID · min/ml (Fig. 3). The plasma AUC for the RI7 mAb is 7-fold higher in mice relative to the plasma AUC of the OX26 mAb in rats. The plasma AUC is inversely proportional to the approximately 10-fold lower body weight of mice relative to rats because the blood volume (ml) in mice is 10-fold lower than in rats. Therefore, the higher brain uptake of the mAb in mice, expressed as %ID/g brain, is proportional to the higher plasma AUC in this species, and this is expected based on the much lower body weight of mice relative to rats. The pharmacokinetic estimates are semiquantitative because the experimental time was only 60 min (Fig. 1), but the plasma t1/2 of all three mAbs is longer (Table 1). In addition, the measurements at the early time points of plasma clearance are restricted to 0.25 min (see Experimental Procedures). However, the initial plasma concentration (A, percentage of ID/ml, Table 1) for the 8D3 or RI7 mAb is only ~40% of the corresponding value for the OX26 mAb (Table 1). This suggests a very early and rapid phase of clearance of the 8D3 and RI7 mAbs occurs immediately after i.v. administration.

Fig. 2. Vd values of 125I-RI7 mAb in brain homogenate, capillary pellet, and postcapillary supernatant. Capillary depletion analysis of the brain tissue was performed at 0.5, 1, 6, and 24 h after i.v. injection of the isotope solution. Data are means ± S.E. (n = 3).

The RI7 and 8D3 mAbs have comparable BBB PS products (Table 2). The PS product for the RI7 mAb is lower body weight of mice relative to rats. The pharmacokinetic parameter, i.e., the plasma AUC, for the 8D3 mAb (Table 1) is 12-fold higher in mice, 2059 ± 117% ID · min/ml (Table 1) compared with the 60-min AUC value for the OX26 mAb in rats, 168 ± 76% ID · min/ml (Fig. 3). The plasma AUC for the RI7 mAb is 7-fold higher in mice relative to the plasma AUC of the OX26 mAb in rats. The plasma AUC is inversely proportional to the approximately 10-fold lower body weight of mice relative to rats because the blood volume (ml) in mice is 10-fold lower than in rats. Therefore, the higher brain uptake of the mAb in mice, expressed as %ID/g brain, is proportional to the higher plasma AUC in this species, and this is expected based on the much lower body weight of mice relative to rats. The pharmacokinetic estimates are semiquantitative because the experimental time was only 60 min (Fig. 1), but the plasma t1/2 of all three mAbs is longer (Table 1). In addition, the measurements at the early time points of plasma clearance are restricted to 0.25 min (see Experimental Procedures). However, the initial plasma concentration (A, percentage of ID/ml, Table 1) for the 8D3 or RI7 mAb is only ~40% of the corresponding value for the OX26 mAb (Table 1). This suggests a very early and rapid phase of clearance of the 8D3 and RI7 mAbs occurs immediately after i.v. administration.

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In summary, these studies compare the mouse brain uptake of three different anti-TfR mAbs and show that the OX26 mAb used for rat brain transport studies cannot be used in mice. Either the 8D3 or the RI7 mAb may be used in mouse brain uptake studies, and each mAb has particular advantages. The 8D3 mAb has a higher plasma AUC and higher brain uptake and percentage ID/g compared with the RI7 mAb (Fig. 3), but the RI7 mAb is selective for brain with no measureable uptake by liver or kidney (Table 2). The availability of these rat mAbs to the mouse TfR will make possible future studies of brain drug delivery in the mouse, including transgenic mouse models.

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References

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TABLE 3
Dose-dependent organ uptake of 125I-8D3

<table>
<thead>
<tr>
<th>mg/kg</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Lung</th>
<th>Spleen</th>
<th>AUC(0–60 min)</th>
<th>PS(brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (tracer)</td>
<td>mean (± S.E.) (n = 5)</td>
<td>3.07 (0.21)</td>
<td>11.75 (0.57)</td>
<td>3.23 (1.19)</td>
<td>2.24 (0.54)</td>
<td>12.61 (2.62)</td>
<td>99.00 (13.51)</td>
<td>931.60 (57.26)</td>
</tr>
<tr>
<td>1</td>
<td>mean (± S.E.) (n = 3)</td>
<td>2.46 (0.26)</td>
<td>17.29 (2.96)</td>
<td>6.40 (0.42)</td>
<td>0.83 (0.17)</td>
<td>5.93 (0.69)</td>
<td>20.58 (1.11)</td>
<td>2826.67 (210.37)</td>
</tr>
<tr>
<td>4</td>
<td>mean (± S.E.) (n = 5)</td>
<td>0.50 (0.00)</td>
<td>8.35 (1.16)</td>
<td>4.25 (0.71)</td>
<td>0.69 (0.36)</td>
<td>3.94 (1.00)</td>
<td>20.67 (1.11)</td>
<td>2826.67 (210.37)</td>
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</table>

Fig. 3. Comparison of the BBB PS product, AUC, and brain uptake, percentage ID/g brain, at 60 min after i.v. injection for the RI7-217, 8D3, and OX26 mAbs in the mouse, and for the OX26 mAb in the rat. The rat OX26 mAb data are from Bickel et al. (1995).


