Pharmacokinetics and Amyloid Plaque Targeting Ability of a Novel Peptide-Based Magnetic Resonance Contrast Agent in Wild-Type and Alzheimer’s Disease Transgenic Mice

Karunya K. Kandimalla, Thomas M. Wengenack, Geoffrey L. Curran, Emily J. Gilles, and Joseph F. Poduslo

Molecular Neurobiology Laboratory, Departments of Neurology, Neuroscience, and Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, Minnesota (K.K.K., T.M.W., G.L.C., E.J.G., J.F.P.); and College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, Florida (K.K.K.)

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ABSTRACT

A novel magnetic resonance (MR) imaging contrast agent based on a derivative of human amyloid β (Aβ) peptide, Gd(N-4ab/Q-4ab)Aβ 30, was previously shown to cross the blood-brain barrier (BBB) and bind to amyloid plaques in Alzheimer’s disease (AD) transgenic mouse (APP/PS1) brain. We now report extensive plasma and brain pharmacokinetics of this contrast agent in wild-type (WT) and in APP/PS1 mice along with a quantitative summary of various physiological factors that govern its efficacy. Upon i.v. bolus administration, $^{125}$I-Gd(N-4ab/Q-4ab)Aβ 30 was rapidly eliminated from the plasma following a three-exponential disposition, which is saturable at higher concentrations. Nevertheless, the contrast agent exhibited rapid and nonsaturable absorption at the BBB. The brain pharmacokinetic profile of $^{125}$I-Gd(N-4ab/Q-4ab)Aβ 30 showed a rapid absorption phase followed by a slower elimination phase. No significant differences were observed in the plasma or brain kinetics of WT and APP/PS1 animals. Emulsion autoradiography studies conducted on WT and APP/PS1 mouse brain after an i.v. bolus administration of $^{125}$I-Gd(N-4ab/Q-4ab)Aβ 30 in vivo confirmed the brain pharmacokinetic data and also demonstrated the preferential localization of the contrast agent on the plaques for an extended period of time. These attributes of the contrast agent are extremely useful in providing an excellent signal/noise ratio during longer MR scans, which may be essential for obtaining a high resolution image. In conclusion, this study documents the successful plaque targeting of Gd(N-4ab/Q-4ab)Aβ 30 and provides crucial pharmacokinetic information to determine the dose, mode of administration, and scan times for future in vivo MR imaging of amyloid plaques in AD transgenic mice.

Development of amyloid plaques in the extracellular space of the brain parenchyma is considered a primary event in the pathogenesis of Alzheimer’s disease (AD) (Selkoe, 2001). Amyloid plaques consist predominantly of Aβ 40 and Aβ 42, which are produced continuously by cells in the nervous system and peripheral tissues. Currently, there is no definitive diagnosis for AD except clinically by elimination of other neurodegenerative disorders and histologically by post mortem observation of plaques and tangles. Early diagnosis of AD is difficult at present because of the inability to visualize plaques in vivo. In recent years, substantial effort has focused on the development of a pre-mortem diagnosis of AD, which involves detection of the plaques using various imaging techniques such as magnetic resonance imaging (MRI) (Poduslo et al., 2002) and positron emission tomography (Klunk et al., 2003, 2004). MRI used in conjunction with a contrast agent has a theoretical capability of resolving individual plaques and also differentiating plaques from other interfering structures such as blood vessels, myelinated fibers, iron-enriched glial cells, and neuronal cell populations whereas positron emission tomography has lower limits of resolution and can only detect bulk tissue enhancement (Poduslo et al., 2002).

Utilizing the ability of Aβ 40 to cross the blood-brain barrier (BBB) and accumulate in the brain, our laboratory and...
others have developed Aβ 40 and its derivatives as MRI probes for amyloid plaques (Wang et al., 2000a; Lee et al., 2002; Poduslo et al., 2002, 2004; Wadhiri et al., 2003). These probes carry covalently attached gadolinium (Gd) diethylenetriaminepentaacetic acid (DTPA), which provides contrast for MRI. Previously, we reported the efficacy of putrescine (PUT)-modified Aβ 40 (PUT-Gd-Aβ 40) to cross the BBB compared with Gd-Aβ 40 without the putrescine modification and to provide contrast enhancement of plaques during MRI of APP/PS1 mouse brains ex vivo after i.v. injection (Poduslo et al., 2002). However, the utility of PUT-Gd-Aβ 40 for diagnostic use in animal models and patients is limited, because the carbodiimide-mediated modification of Aβ 40 with putrescine is associated with problems such as cross-linking, aggregate formation, and insolubility (Kandimala et al., 2006). To avoid this carbodiimide modification of the peptide and these inherent issues, the complete chemical synthesis of a new probe, Gd-[N-4ab/Q-4ab]Aβ 30, was achieved for its further development as a putative MRI contrast agent (Poduslo et al., 2004). Apart from having chemical purity, the putative neurotoxic domain found in Aβ 40 was truncated to minimize potential cellular toxicity. Furthermore, it is not amyloidogenic like Aβ 40 (Giles et al., 2005). Autoradiographic studies conducted on APP/PS1 mouse brain have demonstrated that 125I-Gd-[N-4ab/Q-4ab]Aβ 30 labels ≥90% plaques throughout the cortex and hippocampus after i.v. injection (Poduslo et al., 2004). The objective of the present study was to evaluate the pharmacokinetics and plaque targeting ability of Gd-[N-4ab/Q-4ab]Aβ 30 in both wild-type and AD transgenic mice (APP/PS1).

Materials and Methods

Subjects. These studies were performed using wild-type mice (B6/SJL) and transgenic mice of the same background strain that express two mutant human proteins associated with familial AD. Wild-type (WT) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 6 to 8 weeks of age. Hemizygous transgenic mice (Tg2576) expressing mutant human amyloid precursor protein (APP695) (Hsiao et al., 1996) were mated with a strain of homozygous transgenic mice (M146L6.2) expressing mutant human PS1 (Holcomb et al., 1998). The animals were genotyped for the expression of both transgenes by a polymerase chain reaction method using a sample of mouse tail DNA. These double transgenic mice have been shown to exhibit an accelerated phenotype with amyloid deposits and behavioral deficits by 12 weeks of age (Holcomb et al., 1998; Wengenack et al., 2000a). The mice were housed in a virus-free barrier facility under a 12-h light/dark cycle with ad libitum access to food and water. All procedures performed were in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1996) using protocols approved by the Mayo Institutional Animal Care and Use Committee.

Synthesis of Diamine- and Gd-Substituted Aβ Derivative. Aβ 1–30, with the sequence Ahx (Fmoc-6-aminohexanoic acid)-DAE-FRHDGSYEVHIIKQLVFAEADVGSNKGA, was synthesized as described previously by Poduslo et al. (2004) on an ABI 433 peptide synthesizer (Applied Biosystems, Foster City, CA) using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate activation and the manufacturer’s suggested synthesis protocols. The starting resin was Ala-NovaSyn TGA (Calbiochem-Novabiochem, San Diego, CA). Glutamic acid residues 3, 11, and 22 were synthesized with N-α-Fmoc-L-asparagyl-γ-N-(4-aminobutylicarboxylic acid tert-butyl ester, and aspartic acid residues 7 and 23 were synthesized with N-α-Fmoc-L-aspartyl-γ-N-(4-aminobutylicarbamoyl) carboxylic acid tert-butyl ester. After completion of the synthesis and final Fmoc deprotection, DTPA anhydride was added to the N-terminal Ahx residue by dissolving 120 mg of the DTPA in 2 ml of dimethyl sulfoxide and 8 ml of dimethylformamide and reacting the DTPA solution with the peptide resin, which had been washed previously with disopropyl etheramine/dichloromethane. The coupling of DTPA was allowed to proceed overnight at RT. Completion of the reaction was verified by a negative ninhydrin reaction. The Aβ 1–30 peptide was then cleaved from the resin support using 5% crystalline phenol, 5% water, 2.5% trisopropylsilane, and 87.5% trifluoroacetic acid for 2 h at RT. The peptide was purified by reverse-phase high-performance liquid chromatography on a Jupiter C18 column (250 mm × 21.2 mm; Phenomenex, Torrance, CA) using a gradient system of 0.1% aqueous trifluoroacetic acid containing 80% acetonitrile. The calculated mass weight of 3390 atomic mass units for Aβ 1–30 and 4231 atomic mass units for DTPA-(N-4ab/Q-4ab)Aβ 30 was confirmed by electrospray ionization mass spectrometry (API 165; Applied Biosystems/MDS Scie (Foster City, CA).

Gadolinium Chelation and Radioiodination of Proteins. The element Gd was chelated at a mole concentration equal to that of the DTPA functional group of the Aβ 1–30 peptide using Gd(III) chloride hexahydrate (Sigma-Aldrich, St. Louis, MO) in water at RT for 1 h (now designated as Gd-[N-4ab/Q-4ab]Aβ 30). After radioiodination using the chlorine T method (Poduslo and Curran, 1996), the 125I-Gd-[N-4ab/Q-4ab]Aβ 30 was dialyzed overnight against PBS to remove unbound 125I and found to be 99% trichloroacetic acid-precipitable.

Pharmacokinetic Studies. Before the beginning of experiment, the femoral vein and the femoral artery of each mouse was catheterized under general anesthesia (1.5% isoflurane and 4 l/min oxygen). After i.v. administration of 100 μCi of 125I-Gd-[N-4ab/Q-4ab]Aβ 30 in the femoral vein, the plasma pharmacokinetics of 125I-Gd-[N-4ab/Q-4ab]Aβ 30 were determined in six WT and six APP/PS1 mice by collecting serial blood samples (20 μl) from the femoral artery over a period of 60 min at time points of 0.25, 1, 3, 5, 10, 15, 30, 45, and 60 min. The blood samples were diluted to a volume of 100 μl using normal saline and centrifuged, and the supernatant was obtained. After trichloroacetic acid precipitation, the samples were assayed for 125I radioactivity in a gamma counter (Cobra II; PerkinElmer Life and Analytical Sciences, Boston, MA). The linearity of 125I-Gd-[N-4ab/Q-4ab]Aβ 30 disposition was determined by repeating the experiment with coadministration of 1 or 2 mg of cold Gd-[N-4ab/Q-4ab]Aβ 30 with 100 μCi of 125I-Gd-[N-4ab/Q-4ab]Aβ 30.

To determine the brain uptake of 125I-Gd-[N-4ab/Q-4ab]Aβ 30, a destructive sampling study design was followed in which cohorts of five WT mice and one APP/PS1 mouse were sacrificed at each time point. After the surgery to catheterize the femoral vein and artery, 100 μl of the probe (1 μCi/ml) was injected into the femoral vein of WT or APP/PS1 mice. At the end of each experiment, which was terminated at 1, 3, 5, 10, 15, 30, 45, 60, 120, 180, 240, or 300 min, an aliquot of 131I-BSA (100 μCi, 100 μl) was injected in the femoral vein of the animals to serve as a measure of residual plasma volume (Vp). One minute after the 131I-BSA injection, the final blood sample was collected, and the animal was sacrificed. The brain of the animal was removed from the cranial cavity, dissected into the anatomical regions (cortex, caudate putamen, hippocampus, thalamus, brain stem, and cerebellum), and then assayed for 131I and 131I radioactivity. The measured activity was corrected for the background and crossover of 131I activity into the 131I channel. The brain regions were lyophilized, and dry weights were determined with a microbalance and converted to wet weights using wet weight/dry weight ratios determined previously.

The permeability of 125I-Gd-[N-4ab/Q-4ab]Aβ 30 at the BBB was determined in a similar fashion. However, the length of the experiment for all permeability studies was kept at 15 min. The satura-

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mined by coadministering 0.5, 1, or 2 mg of cold Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 with 100 \(\mu\)Ci of \(^{125}\)I-labeled compound.

**Labeling of Amyloid Plaques in Vivo.** APP/PS1 transgenic mice (8 months of age) were catheterized in the femoral vein under general anesthesia (1.5% isoflurane) and injected with \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 to determine the time course and dose response of the radiolabeled peptide binding to amyloid plaques detected by emulsion microautoradiography. For the time course experiment, each animal was injected with 1.0 mg of \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 and then sacrificed after 1.25, 2.5, or 5 mg of \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30, and sacrificed for 1 h for comparison. Another APP/PS1 mouse was injected with PBS and sacrificed after 1 h as a negative control. For the dose-response experiment, animals were injected with either 1.25, 2.5, or 5 mg of \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 and sacrificed after 2 h. At the appropriate time, each animal was given an overdose of sodium pentobarbital (200 mg/kg i.p.) and perfused with PBS, followed by neutral-buffered, 10% formalin, and then 10% sucrose and 0.1 M sodium phosphate (pH 7.2). Frozen sections (15 \(\mu\)m) of each brain were cut with a cryostat and then processed with anti-\(\beta\) immunohistochemistry (IH) and emulsion autoradiography for the presence of radioactivity (RA) was obtained from the brain. The differential equation system. The residual brain region volume (\(V_R\)) in microliters per gram) was determined as described previously by Poduslo and Curran (1996):

\[
V_R = \frac{q_p}{C_p} \times \frac{10^3}{\omega R}
\]

where \(q_p\) is the \(^{131}\)I-BSA content (counts per minute) of tissue, \(C_p\) is the \(^{131}\)I-BSA concentration (counts per minute per milliliter) in plasma, \(W\) is the dry weight (g) of the brain region, and \(\omega\) is the wet weight/dry weight ratio for mice of a defined age group. From the total \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 content (\(X_{TP}\) in counts per minute) of the brain region, the amount of \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 that enters the brain region extravascular space (\(X_E\) in counts per minute per gram) is calculated as

\[
X_E = X_{TB} \times C_p \times \frac{V_R}{\omega R}
\]

where \(C_p\) is the final \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 concentration (counts per minute per milliliter) in plasma.

A pharmacokinetic model with one brain compartment (Fig. 3) was investigated to describe \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 uptake in the brain. The differential equations associated with the model are as follows:

\[
\frac{dX_p}{dt} = (K_{BP} + K_{PT}) \times X_B - (K_{PB} + K_{PT} + K_{EL}) \times X_p
\]

\[
\frac{dX_T}{dt} = K_{PB} \times X_T - K_{PT} \times X_B
\]

\[
\frac{dX_B}{dt} = K_{PB} \times X_B - K_{PT} \times X_T
\]

To determine the amount of extravascular \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 present in 8-month-old WT (\(n = 6\)) mice, and the effect of various doses of unlabeled Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 coadministered i.v. with \(^{125}\)I-Gd[\(N-4\alpha b/Q-4\alpha b\)A]\(\beta\) 30 (100 \(\mu\)Ci). Data are mean ± S.D.; lines indicate the fit of the two-compartment pharmacokinetic model to the plasma concentration-time data.
where $X_P$ is the amount of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 present in the tissue compartment; $X_T$ is the amount of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 in plasma. $X_E$ is the amount of extravascular $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 present in the brain. $K_{TP}$ is the rate constant for the transfer from plasma to brain; $K_{TP}$ is the rate constant for the transfer from the tissue compartment to plasma; $K_{EP}$ is the rate constant for the transfer from brain to plasma; and $K_{EL}$ is the rate for the elimination from plasma; all of the rates were assumed to be first-order.

The rate constants $K_{PB}$, $K_{TP}$, $K_{EP}$, and $K_{EL}$ were obtained by fitting the above equations simultaneously to the $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 plasma concentration-time and the extravascular $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 in the brain ($X_E$)-time data.

The results obtained from these studies are summarized in Figs. 1 and 2 and Table 1. After i.v. administration, the $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 concentration in the plasma of WT as well as APP/PS1 mice declined rapidly, exhibiting a three-exponential disposition (Table 1). Even though, a two-compartment pharmacokinetic model fitted the plasma data reasonably well (Fig. 1), adding another exponential term significantly improved the goodness-of-fit as indicated by the $F$ test, Akaike information criterion, and Schwartz criterion. The three-compartment model parameters such as $C_{\text{max}}$ and $A$ are significantly higher in WT mice than in APP/PS1 mice, whereas $C$ and $\gamma$ are significantly higher in APP/PS1 than in WT mice (Table 1). However, no significant differences in the plasma pharmacokinetic parameters were observed between WT and APP/PS1 mice when a two-compartment model was fitted to the data (Table 1).

A substantial amount of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 was found in the liver, kidney, and spleen of both WT and APP/PS1 animals perfused with PBS at the termination of the experiment. The accumulation of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 was higher in the kidney than in the liver or spleen. However, no significant differences in the accumulation of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 in these organs was observed between WT and APP/PS1 animals (data not shown). The kinetics of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 degradation was further elucidated in vitro in slices of liver, kidney, and spleen of WT and APP/PS1 mice. Substantial $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 degradation was observed in these tissue slices compared with that in plasma (Fig. 2). However, no significant differences in $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 degradation were observed between APP/PS1 and WT mouse tissues.

To determine whether the disposition of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 in the peripheral circulation is saturable, the plasma kinetics of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 was studied by coadministering various amounts of unlabeled Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 (1 and 2 mg). A three-compartment pharmacokinetic model fitted to the plasma data resulted in poor agreement with the data.

### Results

**$^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 Plasma Pharmacokinetics and Metabolism.** The results obtained from these studies are summarized in Figs. 1 and 2 and Table 1. After i.v. administration, the $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 concentration in the plasma of WT as well as APP/PS1 mice declined rapidly, exhibiting a three-exponential disposition (Table 1). Even though, a two-compartment pharmacokinetic model fitted the plasma data reasonably well (Fig. 1), adding another exponential term significantly improved the goodness-of-fit as indicated by the $F$ test, Akaike information criterion, and Schwartz criterion. The three-compartment model parameters such as $C_{\text{max}}$ and $A$ are significantly higher in WT mice than in APP/PS1 mice, whereas $C$ and $\gamma$ are significantly higher in APP/PS1 than in WT mice (Table 1). However, no significant differences in the plasma pharmacokinetic parameters were observed between WT and APP/PS1 mice when a two-compartment model was fitted to the data (Table 1).

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To determine whether the disposition of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 in the peripheral circulation is saturable, the plasma kinetics of $^{125}$I-Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 was studied by coadministering various amounts of unlabeled Gd$_{\text{N-4ab/Q-4ab}}$Aβ30 (1 and 2 mg). A three-compartment pharmacokinetic model fitted to the plasma data resulted in poor agreement with the data.

#### Table 1

<table>
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<tr>
<th>Parameters</th>
<th>WT, 100 μCi</th>
<th>APP/PS1, 100 μCi</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μCi/ml)</td>
<td>16.12 ± 1.17</td>
<td>11.83 ± 1.30*</td>
</tr>
<tr>
<td>$A$ (μCi/ml)</td>
<td>11.93 ± 1.13</td>
<td>6.61 ± 1.94*</td>
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<tr>
<td>$B$ (μCi/ml)</td>
<td>3.41 ± 0.58</td>
<td>3.68 ± 2.22</td>
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<tr>
<td>$C$ (μCi/ml)</td>
<td>0.78 ± 0.07</td>
<td>1.53 ± 0.09***</td>
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<tr>
<td>$\alpha$ half-life (min)</td>
<td>0.48 ± 0.07</td>
<td>0.37 ± 0.18</td>
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<td>$\beta$ half-life (min)</td>
<td>2.80 ± 0.43</td>
<td>1.55 ± 0.60</td>
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<tr>
<td>$\gamma$ half-life (min)</td>
<td>51.57 ± 7.89</td>
<td>29.4 ± 1.80*</td>
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<tr>
<td>$K_{TP}$ (1/min)</td>
<td>0.20 ± 0.02</td>
<td>0.15 ± 0.02</td>
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<tr>
<td>AUC (min $\times$ μCi/ml)</td>
<td>79.90 ± 4.73</td>
<td>76.75 ± 1.82</td>
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<tr>
<td>CL (ml/min)</td>
<td>1.25 ± 0.07</td>
<td>1.30 ± 0.03</td>
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<table>
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<tr>
<th>Parameter</th>
<th>WT, 100 μCi</th>
<th>APP/PS1, 100 μCi</th>
<th>WT, 100 μCi + 1 mg cold</th>
<th>WT, 100 μCi + 2 mg cold</th>
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<tr>
<td>$C_{\text{max}}$ (μCi/ml)</td>
<td>13.77 ± 1.21</td>
<td>10.55 ± 0.66</td>
<td>15.07 ± 1.48</td>
<td>18.00 ± 2.12**</td>
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<td>$A$ (μCi/ml)</td>
<td>12.67 ± 1.2</td>
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<td>12.90 ± 1.43</td>
<td>14.19 ± 2.03</td>
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<td>$B$ (μCi/ml)</td>
<td>1.10 ± 0.27</td>
<td>1.77 ± 0.16</td>
<td>2.16 ± 0.36</td>
<td>3.81 ± 0.82</td>
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<td>$\alpha$ half-life (min)</td>
<td>0.93 ± 0.15</td>
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<td>0.75 ± 0.15</td>
<td>0.90 ± 0.26</td>
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<td>$\beta$ half-life (min)</td>
<td>30.16 ± 12.16</td>
<td>25.39 ± 3.46</td>
<td>23.99 ± 5.79</td>
<td>18.16 ± 4.64</td>
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<td>$K_{TP}$ (1/min)</td>
<td>0.21 ± 0.04</td>
<td>0.14 ± 0.01</td>
<td>0.17 ± 0.03</td>
<td>0.15 ± 0.03</td>
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<tr>
<td>AUC (min $\times$ μCi/ml)</td>
<td>64.87 ± 12.20</td>
<td>74.12 ± 5.80</td>
<td>88.83 ± 11.58</td>
<td>118.60 ± 14.98*</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>1.54 ± 0.29</td>
<td>1.35 ± 0.10</td>
<td>1.12 ± 0.14</td>
<td>0.84 ± 0.10**</td>
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</table>

WT, wild type mice; APP/PS1, Alzheimer’s disease transgenic mice; cold, unlabeled Gd$_{\text{N-4ab/Q-4ab}}$Aβ30.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.
precision in the parameter estimates, most probably due to the saturation of kinetic events described by one or more of the exponential terms in the three-compartment model. Therefore, a simpler two-compartment pharmacokinetic model was used to evaluate the saturability of Gd[N-4ab/Q-4ab]Aβ 30 plasma disposition, which gave highly precise parameter estimates (Fig. 1; Table 1). A close examination of the plasma pharmacokinetic parameter values indicated that the coadministration of 1 mg of Gd[N-4ab/Q-4ab]Aβ 30 with 100 µCi of \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30 resulted in no significant changes in the plasma pharmacokinetics (Fig. 1; Table 1). However, upon the coadministration of 2 mg of Gd[N-4ab/Q-4ab]Aβ 30, the clearance (CL) of \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30 decreased significantly \((p < 0.01)\), whereas the AUC \((p < 0.05)\) and \(C_{\text{max}}\) \((p < 0.01)\) increased significantly (Fig. 1; Table 1) compared with that in the WT mice given labeled reagent alone.

\(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30 Brain Uptake. The results from brain uptake studies are presented in Figs. 3 to 5 and Table 2. The uptake of \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30 into cortex, hippocampus, and total brain tissue after i.v. adminis-

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**Fig. 3.** 3-Compartmental model for blood-brain barrier transport of \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30.

**Fig. 4.** A, plasma and extravascular brain kinetics of \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30. The predicted kinetic profiles were obtained by simultaneous fitting of observed plasma and extravascular brain data. B, amount of extravascular \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30 present in the cortex, the hippocampus, and the total brain tissue of WT and APP/PS1 mice after i.v. bolus administration via the femoral vein. Each result represents the mean ± S.D. of five experiments in WT mice and one replicate in APP/PS1 mice. Each line indicates the fit of the pharmacokinetic model (Fig. 2) to the extravascular \(^{125}\text{I}-\text{Gd}[N-4ab/Q-4ab]\)Aβ 30-time data.
Verification of Amyloid Plaque Targeting of $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ by Emulsion Autoradiography.

Direct verification of $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ brain targeting was made by performing emulsion autoradiography on sections of APP/PS1 mouse brain obtained at various time points after an i.v. bolus injection. Similar studies were also performed by administering various amounts of $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ to determine the effect of dose on the extent of plaque targeting.

The representative autoradiographs obtained from the hippocampal region of 8-month-old APP/PS1 transgenic mice injected with $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ qualitatively depict accumulation of the probe in the brain tissue (Fig. 6, A–D). The autoradiographs clearly demonstrated preferential accumulation of the radiolabeled probe on the plaques compared with the brain parenchyma. The amount of $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ accumulated on the plaques was estimated from similar autoradiographs by counting the number of exposed silver grains (Fig. 6E). These data indicate that the maximum number of silver grains was associated with the plaques 1 h after the injection of the probe and decreased significantly in the following 3 h. The elimination rate of $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ from the brain parenchyma obtained from the pharmacokinetic experiments coincided well with the rate obtained via the silver grain count resulting from the emulsion autoradiography studies (Fig. 7). Furthermore, accumulation of silver grains on the plaque surface is directly proportional to the administered dose (Fig. 8). It is obvious from both time course and dose-response studies that the accumulation of $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ is higher on the hippocampal plaques than on in the cortex.

Even though it is clear from the autoradiography studies that $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}}]\beta 30$ preferentially targets amyloid plaques in APP/PS1 transgenic mouse brain, they do not present direct evidence that gadolinium, which provides con-
contrast for MRI, remained chelated to the DTPA arm of the protein accumulated on the plaque. Therefore, identical targeting studies were performed using $^{153}$Gd chelated to the unlabeled protein ($^{153}$Gd[$N$-$4$ab/$Q$-$4$ab]/$H_{9252}$30) without the addition of $^{125}$I. The results of the targeting studies showed plaques densely populated with silver grains (Fig. 9), which reflect the presence of the probe carrying $^{153}$Gd.

**Discussion**

Various Aβ derivatives have been developed in our laboratory as carriers of the MRI contrast agent gadolinium to the plaque surface. If successful, these carriers could help identify amyloid plaques present in APP/PS1 mouse brain and eventually in humans, which is critical for the early detection of Alzheimer’s disease. Gd[$N$-$4$ab/$Q$-$4$ab]/Aβ 30 is such a novel MRI contrast agent developed in our laboratory, which was previously shown to cross the BBB and bind to amyloid plaques in the APP/PS1 mouse brain (Poduslo et al., 2004). Therefore, extensive plasma and brain pharmacokinetic studies and emulsion autoradiography studies were conducted on this contrast agent in WT and APP/PS1 mice. The results from these studies provide a comprehensive quantitative estimate of the capabilities of the contrast agent to target amyloid plaques and also outline physiological conditions under which the potential of this contrast agent could be realized.

The amount of Gd[$N$-$4$ab/$Q$-$4$ab]/Aβ 30 available for plaque binding is dependent on the $C_{\text{max}}$, $t_{\text{max}}$, and the residence time of the contrast agent in the brain, which in turn is dependent on the plasma concentration of the contrast agent. Based on the plasma pharmacokinetic profile presented in Fig. 1, Gd[$N$-$4$ab/$Q$-$4$ab]/Aβ 30 exhibits rapid peripheral elimination, which is saturable only at very high doses. Although it is believed that a lower plasma clearance resulting in high sustained plasma levels of the probe will enhance BBB permeability, many Aβ 40 derivatives developed in our laboratory as contrast agents exhibit rapid peripheral elimination. It may be that the hepatic metabolism and renal elimination caused by cationic charge density, a common structural feature shared by many of these proteins, is responsible for the rapid systemic elimination. Previous studies have demonstrated that imaging of amyloid plaques in AD transgenic mouse brain was possible even with contrast agents with low plasma residence time, such as PUT-Aβ 40 (Wengenack et al., 2000a), suggesting that low plasma residence time might be offset by favorable brain kinetics.

Poduslo et al. (2004) demonstrated that the permeability surface area product of Gd[$N$-$4$ab/$Q$-$4$ab]/Aβ 30 at the BBB in WT and APP/PS1 mice is as high as that of Aβ 40, which is reportedly transported across the BBB via receptor-mediated endocytosis (Poduslo et al., 1999; Deane et al., 2003, 2004; Kandimalla et al., 2005). The permeability surface area product value of a molecule is calculated by dividing the amount in the extravascular compartment of the brain after an i.v. bolus injection by the integral of the amount in the plasma (AUC). Although the plasma AUC of $^{125}$I-Gd[$N$-$4$ab/$Q$-$4$ab]/Aβ 30 could be used as a measure of BBB permeability, this measure is not directly comparable to the AUC at the BBB due to the different pharmacokinetic properties of the probe at the BBB.
4ab]Aβ 30 in mice increased in the presence of unlabeled Gd[N-4ab/Q-4ab]Aβ 30 because of saturable peripheral elimination, the amount of 125I-Gd[N-4ab/Q-4ab]Aβ 30 reaching the extravascular brain tissue remained unchanged, which indicates that its plasma and brain kinetics could be different. Hence, the detailed kinetics of extravascular 125I-Gd[N-4ab/Q-4ab]Aβ 30 after an i.v. bolus injection were elucidated in WT and APP/PS1 mice.

The kinetics of extravascular 125I-Gd[N-4ab/Q-4ab]Aβ 30 in the brain tissue is characterized by a rapid absorption phase and an extended elimination phase. This is an ideal kinetic profile for MRI, which usually requires longer scan times. Although not amenable to statistical tests because of the small sample size, higher accumulation of 125I-Gd[N-4ab/Q-4ab]Aβ 30 was observed in hippocampus than in cortex despite the presence of greater cortical tissue mass compared with that of hippocampal tissue. Based on the facts that this difference was observed in both WT and APP/PS1 mice and that there was no significant difference in the plaque burden between these two regions in 6-month-old APP/PS1 animals (Wengenack et al., 2000b), the observed differences in 125I-Gd[N-4ab/Q-4ab]Aβ 30 uptake could be mostly due to differences in the blood flow or capillary density between the hippocampus and the cortex regions. Despite some differences in the plasma pharmacokinetic parameters, no major differences were observed in the brain kinetics between WT

Fig. 8. Typical labeling of an amyloid plaque in the hippocampus of an 8-month-old APP/PS1 transgenic mouse brain after i.v. injection of 1.25 mg (A), 2.5 mg (B), or 5 mg (C) of 125I-Gd[N-4ab/Q-4ab]Aβ 30. Animals were perfused 2 h after injection. Frozen sections (15 μm) were cut on a cryostat. Sections first underwent immunohistochemistry for Aβ using 4G8 antibody (1:1000) followed by emulsion autoradiography with an exposure time of 8 weeks. (C) Scale bar, 20 μm. Two-way ANOVA followed by Bonferroni post-tests were performed to determine whether the differences in the silver grain density due to various concentrations of 125I-Gd[N-4ab/Q-4ab]Aβ 30 are statistically significant (*, p < 0.05).

Fig. 9. Labeling of amyloid plaques in cortex (A) and hippocampus (B) of APP/PS1 transgenic mouse brain after i.v. injection of 125I-Gd[N-4ab/Q-4ab]Aβ 30. Fixed, frozen sections (15 μm) were cut on a cryostat. Sections first underwent immunohistochemistry for Aβ using 4G8 antibody (1:1000) followed by emulsion autoradiography with an exposure time of 3 months. A, retrosplenial cortex. B, CA1 subfield of the hippocampus. Scale bar, 50 μm.
and APP/PS1 mice, which justifies the use of WT mice as cheaper alternatives to APP/PS1 mice in the further development of Gd-N-[4ab/Q-4ab]Aβ30 as an MRI contrast agent.

In the above brain pharmacokinetic studies, extravascular accumulation of 125I-Gd-[N-4ab/Q-4ab]Aβ30 was determined indirectly by subtracting the amount of 125I-Gd-[N-4ab/Q-4ab]Aβ30 present in the brain vasculature from the amount present in the total brain tissue. Although very convenient, this method could yield misleading results, particularly if the probe has significant accumulation in the BBB endothelial cells. Therefore, direct verification of 125I-Gd-[N-4ab/Q-4ab]Aβ30 brain targeting using a semiquantitative technique such as emulsion autoradiography is a necessary prerequisite to conducting expensive and time-consuming in vivo MRI in WT and APP/PS1 mice. In addition to verifying the extent of amyloid plaque targeting of 125I-Gd-[N-4ab/Q-4ab]Aβ30, emulsion autoradiography can also provide an alternative means for obtaining the elimination kinetics of the probe from the brain. In the present study, emulsion autoradiography was used to determine 1) elimination kinetics of the probe from brain parenchyma as well as from plaques and 2) the effect of probe concentration on the extent of plaque targeting.

The results obtained from these studies clearly demonstrated plaque-specific targeting of 125I-Gd-[N-4ab/Q-4ab]Aβ30. The extent of targeting was similar to that of our previously successful MRI contrast agent (Wengenack et al., 2000a), putrescine-modified Aβ40, which, when administered i.v., was shown to provide contrast for imaging plaques in APP/PS1 animals using a 7-T MRI system (Poduslo et al., 2002). In addition, 50 times greater accumulation of the probe on plaques than in the parenchyma was observed, which could significantly aid in the detection of plaques against the background. Slow removal of the probe from plaques, which is even more evident on the hippocampal plaques, allows for longer MRI scan times. Confirming the pharmacokinetic observations that the uptake of 125I-Gd-[N-4ab/Q-4ab]Aβ30 at the BBB is nonsaturable, the silver grain density on the plaques present in both cortical and hippocampal regions increased linearly with the administered dose. This important observation will provide increased rationale for changing the mode of administration to continuous i.v. infusion, which can counteract the rapid peripheral elimination of 125I-Gd-[N-4ab/Q-4ab]Aβ30.

In summary, the current study systematically describes plasma and brain pharmacokinetics of 125I-Gd-[N-4ab/Q-4ab]Aβ30, a novel MRI contrast agent to detect amyloid plaques in AD transgenic mouse brain. Emulsion autoradiography studies conducted on the AD mouse brain after i.v. bolus injection of the contrast agent clearly showed plaque specific accumulation of the contrast agent in the brain, thereby demonstrating the potential for achieving an excellent signal/noise ratio on the MRI scans. Both pharmacokinetic studies and autoradiography studies coincided very well in describing the rapid nonsaturable uptake into and slow elimination of the probe from the brain. This information will be immensely helpful in determining the dose, mode of administration, and scan times for future in vivo MRI of amyloid plaques in AD transgenic mice.

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Address correspondence to: Dr. Joseph F. Poduslo, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905. E-mail: poduslo.joseph@mayo.edu.