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, Geoffry L. Curran, Emily J. Gilles, and Joseph F. Poduslo

Molecular Neurobiology Laboratory

Departments of Neurology, Neuroscience, and Biochemistry/Molecular Biology (KK, TW, GC, EG, JP), Mayo Clinic College of Medicine, Rochester, MN 55905

College of Pharmacy and Pharmaceutical Sciences (KK)

Florida A&M University, Tallahassee, FL 32307
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Corresponding author: Joseph F. Poduslo

Address: Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN55905

Phone: (507) 284 1784
Fax: (507) 284 3383
Email: poduslo.joseph@mayo.edu

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Abbreviations:
AD, Alzheimer’s disease; Aβ40, amyloid beta 40 protein; AIC, Akaike information criterion; APP, amyloid precursor protein; AUC, area under the plasma concentration curve; BBB, blood brain barrier; Gd[N-4ab/Q-4ab]Aβ30, novel magnetic resonance imaging contrast agent with chelated gadolinium; ^125^I-Gd[N-4ab/Q-4ab]Aβ30, novel magnetic resonance imaging contrast agent labeled with ^125^I; BSA, bovine serum albumin; CL, clearance; C_{max}, maximum plasma concentration; DMEM, Dulbecco’s modified eagle medium; IV, intravenous; K_{BP}, rate constant for the transfer from brain to plasma; and K_{EL}, rate for the elimination from plasma; K_{PB}, rate constant for the transfer from plasma to brain; K_{TP}, rate constant for the transfer from the tissue compartment to
plasma; MRI, magnetic resonance imaging; PS, cerebrovascular permeability coefficient-surface area product; PS1, presenilin 1; PUT, putrescine; SC Schwartz criterion; TCA, trichloroacetic acid; T1SE, T1-weighted spin echo; T2SE, T2-weighted spin echo; \( V_p \), residual brain region plasma volume; \( V_{ss} \), steady state volume of distribution; WT, wild type; \( X_B \), amount of extravascular \(^{125}\text{I-Gd}[N-4ab/Q-4ab]A\beta30\) present in the brain; \( X_P \), amount of \(^{125}\text{I-Gd}[N-4ab/Q-4ab]A\beta30\) in plasma; \( X_T \), amount of \(^{125}\text{I-Gd}[N-4ab/Q-4ab]A\beta30\) present in the tissue compartment.

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ABSTRACT

A novel MRI contrast agent based on a derivative of human amyloid β (Aβ) peptide, Gd[N-4ab/Q-4ab]Aβ30, 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 intravenous bolus administration, $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 was rapidly eliminated from the plasma following a 3-exponential disposition, which is saturable at higher concentrations. Nevertheless, the contrast agent exhibited rapid and non-saturable absorption at the BBB. The brain pharmacokinetic profile of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 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 following an intravenous bolus administration of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 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-to-noise ratio during longer MR scans, which may be essential to obtain a high resolution image. In conclusion, this study documents the successful plaque targeting of Gd[N-4ab/Q-4ab]Aβ30 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.
INTRODUCTION

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 Aβ40 and Aβ42, 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 (PET) (Klunk et al., 2003; Klunk et al., 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 PET has lower limits of resolution and can only detect bulk tissue enhancement (Poduslo et al., 2002).

Utilizing the ability of Aβ40 to cross the blood brain barrier (BBB) and accumulate in the brain, our lab and others have developed Aβ40 and its derivatives as MRI probes for imaging amyloid plaques (Wengenack et al., 2000a; Lee et al., 2002; Poduslo et al., 2002; Wadghiri et al., 2003; Poduslo et al., 2004). These probes carry covalently attached gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA), which provides contrast for MR imaging. Previously, we reported the efficacy of putrescine modified
Aβ40 (PUT-Gd-Aβ40) to cross the BBB compared to Gd-Aβ40 without the putrescine modification and to provide contrast enhancement of plaques during MRI of APP, PS1 mouse brains ex vivo following IV injection (Poduslo et al., 2002). However, the utility of PUT-Gd-Aβ40 for diagnostic use in animal models and patients is limited, because the carbodiimide-mediated modification of Aβ40 with putrescine is associated with problems such as crosslinking, aggregate formation, and insolubility (Kandimalla 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]Aβ30, was achieved for its further development as a putative MRI contrast agent (Poduslo et al., 2004). Apart from having chemical purity, the putative neurotoxic domaine found in Aβ40 was truncated to minimize potential cellular toxicity. Furthermore, it is not amyloidogenic like Aβ40 (Giles et al., 2005). Autoradiographic studies conducted on APP/PS1 mouse brain have demonstrated that 125I-Gd[N-4ab/Q-4ab]Aβ30 labels >90% plaques throughout the cortex and hippocampus following IV 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]Aβ30 in both wild type as well as AD transgenic mice (APP/PS1).

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 6-8 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 presenilin 1 (PS1) (Holcomb et al., 1998). The animals were genotyped for the expression of both transgenes by a PCR 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 libidum access to food and water. All procedures performed were in accordance with NIH Guidelines for the Care and Use of Laboratory Animals using protocols approved by the Mayo Institutional Animal Care and Use Committee.

Synthesis of Diamine- and Gd-Substituted Aβ Derivative. Aβ1-30, with the sequence Ahx (Fmoc-6-aminohexanoic acid)-DAEFRHDSGYEVHHQKLWFAEDVGSNKGA, was synthesized as described previously by Poduslo et al. (2004) on an ABI 433 (Foster City, CA) peptide synthesizer using HBTU 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-glutamyl-δ-N-(4-aminobutyl) carbamic acid tert-butyl ester, and aspartic acid residues 7 and 23 were synthesized with N-α-Fmoc-L-aspartyl-g-δ-N-(4-aminobutyl) carbamic acid tert-butyl ester. After completion of the synthesis and final Fmoc deprotection, diethylenetriaminepentaacetic acid anhydride (DTPA) was added to the N-terminal Ahx residue by dissolving 120 mg of the DTPA in 2 ml of DMSO/8 ml DMF and reacting the DTPA solution with the peptide-resin, which had been washed previously with DIEA/DCM. The coupling of DTPA was allowed to proceed overnight at
RT. Completion of the reaction was verified by a negative ninhydrin reaction. The Aβ1-30 peptide was then cleaved from the resin support using 5% crystalline phenol, 5% water, 2.5% triisopropylsilane, and 87.5% TFA for two hours at RT. The peptide was purified by reverse-phase HPLC on a Jupiter C18 column (250 mm x 21.2 mm, Phenomenex Corp) using a gradient system of 0.1% aqueous TFA containing 80% acetonitrile. The calculated mass weight 3390 amu of for Aβ1-30 and 4231 amu for DTPA-[N-4ab/Q-4ab]Aβ30 was confirmed by electrospray ionization mass spectrometry (Sciex API 165).

**Gadolinium chelation and radioiodination of proteins.** The element Gadolinium (Gd) was chelated at equal mole concentration to the DTPA functional group of the Aβ1-30 peptide using Gd(III) chloride hexahydrate (Sigma) in water at RT for one hour (now designated as Gd[N-4ab/Q-4ab]Aβ30). After radioiodination using the chloramine T method (Poduslo and Curran, 1996), the $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 was dialyzed overnight against PBS to remove unbound $^{125}$I and found to be 99% TCA precipitable.

**Pharmacokinetic studies.** Before the beginning of experiment, the femoral vein and the femoral artery of each mouse was catheterized under general anesthesia (isoflurane = 1.5%; oxygen = 4 l/min). After IV administration of 100 µCi of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in the femoral vein, the plasma pharmacokinetics of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 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, centrifuged, and the supernatant was obtained. Following TCA precipitation, the samples were assayed for \(^{125}\)I radioactivity in a gamma counter (Cobra II, Packard). The linearity of \(^{125}\)I-Gd[N-4ab/Q-4ab]A\(\beta\)30 disposition was determined by repeating the experiment with co-administration of 1 or 2 mg of cold Gd[N-4ab/Q-4ab]A\(\beta\)30 with 100 \(\mu\)Ci of \(^{125}\)I-Gd[N-4ab/Q-4ab]A\(\beta\)30.

To determine the brain uptake of \(^{125}\)I-Gd[N-4ab/Q-4ab]A\(\beta\)30, a destructive sampling study design was followed where cohorts of five WT mice and one APP/PS1 were sacrificed at each time point. Following the surgery to catheterize femoral vein and artery, 100 \(\mu\)l of the probe (1 \(\mu\)Ci/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 \(^{131}\)I-BSA (100 \(\mu\)Ci, 100 \(\mu\)l) was injected in the femoral vein of the animals to serve as a measure of residual plasma volume (\(V_p\)). One minute following the \(^{131}\)I-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 \(^{125}\)I and \(^{131}\)I radioactivity. The measured activity was corrected for the background and crossover of \(^{131}\)I activity into the \(^{125}\)I 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 \(^{125}\)I-Gd[N-4ab/Q-4ab]A\(\beta\)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 saturability of \(^{125}\)I-Gd[N-4ab/Q-4ab]A\(\beta\)30 transport at the BBB was
determined by co-administering 0.5, 1, or 2 mg of cold Gd[N-4ab/Q-4ab]Aβ30 with 100 µCi of 125I 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 (isoflurane, 1.5%) and injected with 125I-Gd[N-4ab/Q-4ab]Aβ30 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 125I-Gd[N-4ab/Q-4ab]Aβ30 and then sacrificed after 1, 2, 4, or 8 h. A WT mouse was injected with 1.0 mg 125I-Gd[N-4ab/Q-4ab]Aβ30 and sacrificed after 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 125I-Gd[N-4ab/Q-4ab]Aβ30 and sacrificed after 2 h. At the appropriate time, each animal was given an overdose of sodium pentobarbital (200 mg/kg, IP) and perfused with PBS, followed by neutral-buffered, 10% formalin, and then 10% sucrose, 0.1 M sodium phosphate, pH 7.2. Frozen sections (15 µm) of each brain were cut with a cryostat and then processed with anti-Aβ immunohistochemistry (IH) and emulsion autoradiography for the presence of radiolabeled amyloid deposits.

Briefly, the sections underwent IH for amyloid using a standard anti-Aβ monoclonal mouse antibody (4G8, 1:1000, Signet Laboratories, Dedham, MA) and standard immunoperoxidase methods (Vectastain Elite ABC and DAB kits, Vector Laboratories, Burlingame, CA). Next, the sections were dipped in an autoradiographic emulsion (Type NTB-3, Eastman Kodak, Rochester, NY) for direct comparison of 125I-labeled amyloid
deposits to anti-Aβ IH. The slides were dipped in emulsion, exposed for various
durations, and developed according to the instructions using Kodak Dektol developer
and Fixer (Eastman Kodak, Rochester, NY). The sections were dehydrated with
successive changes of ethanol and xylene and then coverslipped.

Silver grains from sections exposed for 8 weeks were quantitated using
unbiased, stereological techniques. Silver grains were counted over plaques and
adjacent parenchyma in three sections from each animal in the retrosplenial cortex and
CA1 region of the hippocampus using a 10-µm x 10-µm dissector at 400x. The mean
background level of exposed silver grains was also determined for each section. The
background was sampled over the emulsion-coated, blank slide adjacent to the tissue
section in the vicinity of the retrosplenial cortex and hippocampus. The results were
expressed as the mean number of silver grains/100 µm² minus the background.
Statistical analysis was then performed using ANOVA followed by Bonferroni multiple
comparisons (GraphPad 4.0, San Diego, CA).

**Conversion of silver grain densities to radioactivity.** Brain uptake data obtained in
APP/PS1 animals at 60 and 120 min was compared with the microautoradiography data
obtained at the same time points. A proportionality factor relating the number of silver
grains (SGN) to radioactivity (RA) was obtained from the following expression:

**Proportionality factor (RA:SGN)**

Equation 1:

\[
\text{Dose normalized radioactivity in the brain region at a designated time after iv bolus injection} \\
\text{Dose normalized silver grain count in the same brain region and at an identical time point}
\]

A factor to convert SGN to RA was derived by averaging the proportionality factors
obtained at various time points.

Data Analysis. The Gd[N-4ab/Q-4ab]Aβ30 plasma concentration profile following a single intravenous bolus dose of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 was best described by a biexponential disposition function $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$, where $C(t) = ^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 (µCi) / ml of plasma, A and B are the intercepts and $\alpha$ and $\beta$ are the slopes of the biexponential curve. Pharmacokinetic parameters were estimated by nonlinear curve fitting using Gauss-Newton (Levenberg and Hartley) algorithm and weighted least squares, (WinNonlin® Professional, version 4.1, Mountain view, CA). Secondary parameters such as the $C_{\text{max}}$ (maximum plasma concentration), the first ($t_{1/2(\alpha)}$) and second phase ($t_{1/2(\beta)}$) half-lives, the plasma clearance (CL), the steady-state volume of distribution ($V_{ss}$), and area under the plasma concentration curve (AUC) were also calculated using WinNonlin. The mean values of controls and treatments were compared by Student’s t-test using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

The amount of extravascular $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 present in the brain ($X_B$) at various time points was calculated using the following equation system: The residual brain region plasma volume ($V_p$, µl/g) was determined as described previously by Poduslo (1993).

Equation 2: $V_p = \frac{q_p \times 10^3}{C_v \times WR}$

where $q_p$ is the $^{131}$I-BSA content (cpm) of tissue, $C_v$ is the $^{131}$I-BSA concentration (cpm/ml) in plasma, $W$ is the dry weight (g) of the brain region, and $R$ is the wet weight /
dry weight ratio for mice of a defined age group. From the total $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ content ($X_{\text{TB}}, \text{cpm}$) of the brain region, the amount of $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ that enters the brain region extravascular space ($X_{\text{B}}, \text{cpm/g}$) is calculated as

**Equation 3:**

$$X_{\text{B}} = \frac{X_{\text{TB}}}{\text{WR}} - \frac{V_p \cdot C_a}{10^3}$$

where $C_a$ is the final $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ concentration (cpm/ml) in plasma.

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

**Equation 4:**

$$\frac{dX_p}{dt} = (K_{\text{PB}} \times K_{\text{TP}}) \times X_{\text{B}} - (K_{\text{PB}} \oplus K_{\text{PT}} \oplus K_{\text{EL}}) \times X_p$$

**Equation 5:**

$$\frac{dX_T}{dt} = K_{\text{PT}} \times X_p - K_{\text{TP}} \times X_T$$

**Equation 6:**

$$\frac{dX_B}{dt} = K_{\text{PB}} \times X_p - K_{\text{BP}} \times X_B$$

where, $X_p$, amount of $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ in plasma; $X_T$, amount of $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ present in the tissue compartment; $X_B$, amount of extravascular $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ present in the brain;. $K_{\text{PB}}$, rate constant for the transfer from plasma to brain; $K_{\text{TP}}$, rate constant for the transfer from the tissue compartment to plasma; $K_{\text{BP}}$, rate constant for the transfer from brain to plasma; and $K_{\text{EL}}$, rate for the elimination from plasma; Assumptions: all the rates were assumed to be first order.

The rate constants $K_{\text{PB}}$, $K_{\text{TP}}$, $K_{\text{BP}}$, and $K_{\text{EL}}$ were obtained by fitting the above equations simultaneously to the $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ plasma concentration-time and the extravascular $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{A}\beta 30$ in the brain ($X_B$)-time data.
RESULTS

$^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 plasma pharmacokinetics and metabolism. The results obtained from these studies are summarized in Figures 1 & 2 and Tables 1a & 1b. Following IV administration, the $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 concentration in the plasma of WT as well as APP/PS1 mice declined rapidly exhibiting a 3-exponential disposition (Table 1a). Even though, a 2-compartment pharmacokinetic model fitted the plasma data reasonably well (Figure 1), adding another exponential term significantly improved the goodness-of-fit as indicated by the F-test, Akaike information criterion (AIC), and Schwartz criterion (SC). The 3-compartment model parameters such as C$_{\text{max}}$ and A are significantly higher in WT mice compared to APP/PS1 mice, whereas C, and γ are significantly higher in APP/PS1 than in WT mice (Table 1a). However, no significant differences in the plasma pharmacokinetic parameters were observed between WT and APP/PS1 mice when a 2-compartment model was fitted to the data (Table 1b).

A substantial amount of $^{125}$I-Gd[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[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[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[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[N-4ab/Q-4ab]Aβ30 degradation was observed in these tissue slices compared to that in plasma (Figure 2). However, no significant
differences in $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 degradation was observed between APP/PS1 and WT mouse tissues.

To determine if the disposition of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in the peripheral circulation is saturable, the plasma kinetics of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 were studied by co-administering various amounts of unlabeled Gd[N-4ab/Q-4ab]Aβ30 (1 and 2 mg). A 3-compartment pharmacokinetic model fitted to the plasma data resulted in a poor precision in the parameter estimates; most likely due to the saturation of kinetic events described by one or more of the exponential terms in the 3-compartment model. Therefore, a simpler 2-compartment pharmacokinetic model was employed to evaluate the saturability of Gd[N-4ab/Q-4ab]Aβ30 plasma disposition, which gave highly precise parameter estimates (Figure 1, Table 1b). A close examination of the plasma pharmacokinetic parameter values indicated that the co-administration of 1 mg Gd[N-4ab/Q-4ab]Aβ30 with 100 µCi $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 resulted in no significant changes in the plasma pharmacokinetics (Figure 1, Table 1b). However, upon the co-administration of 2 mg Gd[N-4ab/Q-4ab]Aβ30, the clearance (CL) of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 decreased significantly (p<0.01) whereas the AUC (p<0.05) and $C_{\text{max}}$ (p<0.01) increased significantly (Figure 1, Table 1b) compared to the WT mice given labeled reagent alone.

$^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 brain uptake. The results from brain uptake studies are presented in Figures 3-5 and Table 2. The uptake of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 into cortex, hippocampus, and total brain tissue following intravenous administration in 24 week old WT and APP/PS1 mice was adequately described by the three compartment
open model depicted in Figure 3. The parameter values that adequately describe $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 brain kinetics were obtained by fitting the differential equations to the plasma and brain kinetic data, simultaneously. $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 pharmacokinetic profile in the brain of WT mice is characterized by a rapid uptake ($t_{\text{max}} \sim 15 \text{ min}$) from plasma ($K_{PB}, \text{1/min}$), which was estimated as 0.026 ± 0.002 (Figure 4, Table 2) and a significantly lower rate of elimination from brain to plasma ($K_{BP}, \text{1/min}$), which was estimated as 0.006 ± 0.001. The $K_{PB}$ and $K_{BP}$ values in APP/PS1 mice were similar to those seen in WT mice. Elimination of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 from the plasma compartment of WT ($K_{EL} = 0.22 \pm 0.04, \text{1/min}$) as well as APP/PS1 mice (Table 2: $K_{EL} = 0.14 \pm 0.02$) estimated by this method coincided very well with the value estimated by fitting either a 2-compartment (Table 1a: $K_{EL, \text{WT}} = 0.21 \pm 0.04$; $K_{EL, \text{APP/PS1}} = 0.14 \pm 0.01$) or a 3-compartment open model (Table 1b: $K_{EL, \text{WT}} = 0.20 \pm 0.02$; $K_{EL, \text{APP/PS1}} = 0.15 \pm 0.02$) to the plasma data alone.

To verify if the uptake of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 at the BBB is receptor-mediated, various amounts of unlabeled Gd[N-4ab/Q-4ab]Aβ30 were co-administered along with $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in 24 week old WT mice. Upon co-administration of 0.5 mg or 2 mg of Gd[N-4ab/Q-4ab]Aβ30, the extravascular accumulation of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in various brain regions remained unaffected (Figure 5).

**Verification of amyloid plaque targeting of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 by emulsion autoradiography.** A direct verification of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 brain targeting was made by conducting emulsion autoradiography on sections of APP/PS1 mouse brain
obtained at various time points following an IV bolus injection. Similar studies were also
conducted by administering various amounts of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 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 $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 qualitatively depict accumulation of the probe in the brain tissue (Figure 6, A-D). The
autoradiographs clearly demonstrated preferential accumulation of the radiolabeled
probe on the plaques compared to the brain parenchyma. The amount of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 accumulated on the plaques was estimated from similar
autoradiographs by counting the number of exposed silver grains (Figure 6 E). These
data indicate that the maximum number of silver grains were associated with the
plaques one hour after the injection of the probe and decreased significantly in the
following three hours. The elimination rate of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 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 (Figure 7). Furthermore, the accumulation of silver grains on the plaque surface
is directly proportional to the administered dose (Figure 8). It is obvious from both time
course and dose response studies that the accumulation of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 is higher on the hippocampal plaques compared to those in the cortex.

Even though it is clear from the autoradiography studies that $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 preferentially targets amyloid plaques in APP/PS1 transgenic mouse brain,
they do not present direct evidence that gadolinium, which provides contrast for MR
imaging, remained chelated to the DTPA arm of the protein accumulated on the plaque.
Therefore, identical targeting studies were conducted using $^{153}$Gd chelated to the unlabeled protein ($^{153}$Gd[N-4ab/Q-4ab]Aβ30) without the addition of $^{125}$I. The results of the targeting studies showed plaques densely populated with silver grains (Figure 9), which reflect the presence of the probe carrying $^{153}$Gd.
DISCUSSION

Various Aβ derivatives have been developed in our lab as carriers of the MRI contrast agent gadolinium to the plaque surface. If successful, these carriers could help detect 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-4ab/Q-4ab]Aβ30 is such a novel MR contrast agent developed in our lab, which was previously shown to cross the BBB and bind to amyloid plaques in 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, as well as in APP/PS1 mice. The results from these studies provide a comprehensive quantitative estimate of the contrast agent’s capabilities 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-4ab/Q-4ab]Aβ30 available for plaque binding is dependent on the cmax, tmax, and the residence time of the contrast agent in the brain, which in turn is dependent upon the plasma concentration of the contrast agent. Based on the plasma pharmacokinetic profile presented in Figure 1, Gd[N-4ab/Q-4ab]Aβ30 exhibits rapid peripheral elimination, which is saturable only at very high doses. Although, it is believed that lower plasma clearance resulting in high sustained plasma levels of the probe will enhance its BBB permeability, many Aβ40 derivatives developed in our lab as contrast agents exhibit rapid peripheral elimination. It may be that the hepatic metabolism and renal elimination due to 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-Aβ40 (Wengenack et al., 2000a), suggesting that low plasma residence time might be offset by favorable brain kinetics.

Poduslo et al. (2004) demonstrated that permeability surface area product (PS) of Gd[N-4ab/Q-4ab]Aβ30 at the BBB in WT and APP/PS1 mice is as high as that of Aβ40, which is reportedly transported across the BBB via receptor mediated endocytosis (Poduslo et al., 1999; Deane et al., 2003; Kandimalla et al., 2005). The PS value of a molecule is calculated by dividing the amount in the extravascular compartment of the brain following an IV bolus injection by the integral of the amount in the plasma (AUC). Although the plasma AUC of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in mice increased in the presence of unlabeled Gd[N-4ab/Q-4ab]Aβ30 due to saturable peripheral elimination, the amount of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 reaching the extravascular brain tissue remains unchanged, which indicates that its plasma and brain kinetics could be different. Hence, the detailed kinetics of extravascular $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 following IV bolus injection were elucidated in WT as well as APP/PS1 mice.

The kinetics of extravascular $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in the brain tissue are characterized by a rapid absorption phase and an extended elimination phase. This is an ideal kinetic profile for MR imaging, which usually requires longer scan times. Although not amenable to statistical tests due to small sample size, higher accumulation of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 was observed in hippocampus compared to that in cortex despite the presence of greater cortical tissue mass compared to the hippocampal...
tissue. Based on the facts that this difference was observed in both WT as well as 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 $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 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 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 MR contrast agent.

In the above brain pharmacokinetic studies, extravascular accumulation of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 was determined indirectly by subtracting the amount of $^{125}$I-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, a direct verification of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 brain targeting using semi-quantitative techniques like emulsion autoradiography is a necessary prerequisite to conducting expensive and time consuming in vivo MR imaging in WT and APP/PS1 mice. In addition to verifying the extent of amyloid plaque targeting of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30, emulsion autoradiography can also provide an alternative means to obtain the elimination kinetics of the probe from the brain. In the present study, emulsion autoradiography was used to determine: (a) elimination kinetics of the probe from brain parenchyma as well as from plaques; and (b) effect of probe concentration on the extent of plaque targeting.
The results obtained from these studies clearly demonstrated plaque specific targeting of $^{125}$I-Gd[N-4ab/Q-4ab]$\text{A}\beta$30. The extent of targeting was similar to our previously successful MR contrast agent (Wengenack et al., 2000a), putrescine modified $\text{A}\beta$40, which when administered intravenously was shown to provide contrast for imaging plaques in APP/PS1 animals using 7-Telsa MRI system (Poduslo et al., 2002). Additionally, 50 times greater accumulation of the probe on plaques than in the parenchyma was observed and 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 $^{125}$I-Gd[N-4ab/Q-4ab]$\text{A}\beta$30 at the BBB is non-saturable, 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 IV infusion, which can counteract the rapid peripheral elimination of $^{125}$I-Gd[N-4ab/Q-4ab]$\text{A}\beta$30.

In summary, the current study systematically describes plasma and brain pharmacokinetics of $^{125}$I-Gd[N-4ab/Q-4ab]$\text{A}\beta$30, a novel MRI contrast agent to detect amyloid plaques in AD transgenic mouse brain. Emulsion autoradiography studies conducted on the AD mouse brain after IV bolus injection of the contrast agent clearly showed plaque specific accumulation of the contrast agent in the brain, thereby demonstrating the potential of achieving an excellent signal to noise ratio on the MRI scans. Both pharmacokinetic studies and autoradiography studies coincided very well in describing the rapid non-saturable 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 MR in vivo imaging of amyloid plaques in AD transgenic mice.
ACKNOWLEDGEMENTS:

We thank Dr. Dan McCormick and Jane A. Petersen for extending their technical expertise in synthesizing the Aβ40 derivatives; Dr. Karen Duff for the PS1 transgenic mouse line; Dawn Gregor for her excellent technical assistance; and Jennifer Scott for her excellent secretarial assistance.
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permeability, and in vivo targeting to Alzheimer's disease amyloid plaques.

*Biochem* 43:6064-6075.


FOOTNOTES

*Visiting Scientist from: College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307

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LEGENDS FOR FIGURES

**Figure 1.** Plasma pharmacokinetics of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 (100 µCi) in 8-month old WT (n=6) and APP/PS1 (n=6) mice; and the effect of various doses of unlabeled Gd[N-4ab/Q-4ab]Aβ30 co-administered intravenously with $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 (100 µCi). Data are mean ± SD; lines indicate the fit of the two-compartment pharmacokinetic model to the plasma concentration-time data.

**Figure 2.** Degradation of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 in DMEM and various tissues obtained from 24 week old WT mice. Data are mean ± S.D. (n = 5).

**Figure 3.** 3-Compartmental model for blood-brain barrier transport of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30. Abbreviations: $K_{PB}$, rate constant for the transfer from plasma to brain; $K_{BP}$, rate constant for the transfer from brain to plasma; $K_{PT}$, rate constant for the transfer from plasma to tissues; $K_{TP}$, rate constant for the transfer from tissues to plasma; $K_{EL}$, rate for the elimination from plasma. Assumptions: all the rates were assumed to be first order.

**Figure 4.** (a) Plasma and extravascular brain kinetics of $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30. The predicted kinetic profiles were obtained by simultaneous fitting of observed plasma and extravascular brain data. (b) Amount of extravascular $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 present in the cortex, the hippocampus, and the total brain tissue of WT and APP/PS1 mice following IV bolus administration via the femoral vein. Each result represents the mean ± SD of five experiments in WT mice and one replicate in APP/PS1 mice. Each
line indicates the fit of the pharmacokinetic model (Figure 2) to the extravascular $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30-time data.

**Figure 5.** Effect of various doses of unlabeled Gd[N-4ab/Q-4ab]Aβ30 co-administered intravenously with $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30 (100 µCi) on the extravascular accumulation of $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30 measured 15 min following the administration. Each result represents the mean ± SD for three experiments. The differences between $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30 alone and $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30 + various amounts of unlabeled Gd[N-4ab/Q-4ab]Aβ30 were not statistically significant.

**Figure 6.** Typical labeling of an amyloid plaque in hippocampus of 8-month old APP/PS1 transgenic mouse brain following IV injection of 1 mg of $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30 at durations of 1 h (A), 2 h (B), 4 h (C), or 8 h (D) after injection. Sections first underwent immunohistochemistry for Aβ using 4G8 antibody (1:1000) followed by emulsion autoradiography with an exposure time of 8 weeks. (D) Scale bar is 20 µm. (E) Mean silver grain density per 100 µm$^2$ obtained from the brain sections. Two-way ANOVA followed by Bonferroni post-tests were conducted to determine if the differences in the silver grain density due to various concentrations of $^{125}\text{I}$-Gd[N-4ab/Q-4ab]Aβ30 were statistically significant (*p<0.05, **p<0.01).

**Figure 7.** Silver grain density in the brain parenchyma of autoradiographs presented in Figure 6 A-D was converted to radioactivity using Equation 1. The decline of the
radioactivity in the brain parenchyma is well described (cortex, $R^2 = 0.98$; hippocampus, $R^2 = 0.93$) by the rate constant obtained from the brain pharmacokinetic studies.

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

**Figure 9:** Labeling of amyloid plaques in cortex (A) and hippocampus (B) of APP/PS1 transgenic mouse brain following IV injection of $^{153}$Gd[N-4ab/Q-4ab]A$\beta$30. Fixed, frozen sections (15 μm) were cut on a cryostat. Sections first underwent immunohistochemistry for A$\beta$ 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 is 50 μm.
Table 1a. Plasma pharmacokinetic parameter estimates for $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 obtained from a 3-compartment model fit

<table>
<thead>
<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>
</tr>
<tr>
<td>B (μCi/ml)</td>
<td>3.41 ± 0.58</td>
<td>3.68 ± 2.22</td>
</tr>
<tr>
<td>C (μCi/ml)</td>
<td>0.78 ± 0.07</td>
<td>1.53 ± 0.09***</td>
</tr>
<tr>
<td>$\alpha$ half-life (min)</td>
<td>0.48 ± 0.07</td>
<td>0.37 ± 0.18</td>
</tr>
<tr>
<td>$\beta$ half-life (min)</td>
<td>2.80 ± 0.43</td>
<td>1.55 ± 0.60</td>
</tr>
<tr>
<td>$\gamma$ half-life (min)</td>
<td>51.57 ± 7.89</td>
<td>29.4 ± 1.80*</td>
</tr>
<tr>
<td>$K_{EL}$ (1/min)</td>
<td>0.20 ± 0.02</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>AUC (min*μCi/ml)</td>
<td>79.90 ± 4.73</td>
<td>76.75 ± 1.82</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>1.25 ± 0.07</td>
<td>1.30 ± 0.03</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.01
Table 1b. Plasma pharmacokinetic parameter estimates for $^{125}$I-Gd[N-4ab/Q-4ab]Aβ30 obtained from a 2-compartment model fit

<table>
<thead>
<tr>
<th>Parameters</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>
</tr>
</thead>
<tbody>
<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>
</tr>
<tr>
<td>A (µCi/ml)</td>
<td>12.67 ± 1.2</td>
<td>8.78 ± 0.63</td>
<td>12.90 ± 1.43</td>
<td>14.19 ± 2.03</td>
</tr>
<tr>
<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>
</tr>
<tr>
<td>α half-life (min)</td>
<td>0.93 ± 0.15</td>
<td>0.74 ± 0.09</td>
<td>0.75 ± 0.15</td>
<td>0.90 ± 0.26</td>
</tr>
<tr>
<td>β 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>
</tr>
<tr>
<td>$K_{EL}$ (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>
</tr>
<tr>
<td>AUC (min*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>
</tr>
</tbody>
</table>

The data is expressed as mean ± SEM. WT, wild type mice; APP/PS1, Alzheimer’s disease transgenic mice; cold, unlabeled Gd[N-4ab/Q-4ab]Aβ30; *p<0.05, **p<0.01
Table 2. Brain pharmacokinetic parameter estimates for $^{125}\text{I}-\text{Gd}[\text{N-4ab/Q-4ab}]\text{Aβ30}$

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Total Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{EL}$ (1/min)</td>
<td>0.151 ± 0.020</td>
<td>0.150 ± 0.021</td>
<td>0.220 ± 0.036</td>
</tr>
<tr>
<td>$K_{PT}$ (1/min)</td>
<td>0.443 ± 0.053</td>
<td>0.441 ± 0.053</td>
<td>0.480 ± 0.101</td>
</tr>
<tr>
<td>$K_{TP}$ (1/min)</td>
<td>0.053 ± 0.010</td>
<td>0.052 ± 0.010</td>
<td>0.112 ± 0.031</td>
</tr>
<tr>
<td>$K_{PB}$ (1/min)</td>
<td>0.004 ± 0.002</td>
<td>0.004 ± 0.001</td>
<td>0.026 ± 0.002</td>
</tr>
<tr>
<td>$K_{BP}$ (1/min)</td>
<td>0.010 ± 0.009</td>
<td>0.008 ± 0.006</td>
<td>0.006 ± 0.001</td>
</tr>
<tr>
<td>APP/PS1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{EL}$ (1/min)</td>
<td>0.143 ± 0.013</td>
<td>0.142 ± 0.013</td>
<td>0.141 ± 0.019</td>
</tr>
<tr>
<td>$K_{PT}$ (1/min)</td>
<td>0.632 ± 0.105</td>
<td>0.631 ± 0.106</td>
<td>0.660 ± 0.155</td>
</tr>
<tr>
<td>$K_{TP}$ (1/min)</td>
<td>0.180 ± 0.028</td>
<td>0.181 ± 0.028</td>
<td>0.202 ± 0.431</td>
</tr>
<tr>
<td>$K_{PB}$ (1/min)</td>
<td>0.002 ± 0.001</td>
<td>0.003 ± 0.001</td>
<td>0.017 ± 0.002</td>
</tr>
<tr>
<td>$K_{BP}$ (1/min)</td>
<td>0.007 ± 0.001</td>
<td>0.007 ± 0.002</td>
<td>0.008 ± 0.001</td>
</tr>
</tbody>
</table>

The data is expressed as mean ± SEM. WT, wild type mice; APP/PS1, Alzheimer's disease transgenic mice.
Figure 3

\[
\begin{align*}
\text{TISSUE} & \quad \text{PLASMA} \quad \text{BRAIN} \\
K_{\text{TP}} & \quad K_{\text{PB}} \\
K_{\text{TR}} & \quad K_{\text{BP}} \\
K_{\text{EL}} & \quad \text{ELIMINATED}
\end{align*}
\]
Figure 4a

[Graph showing data for Wild Type and APP/PS1 for Plasma and Brain.]

- Wild Type
- Plasma: Observed, Predicted
- Brain: Observed, Predicted

[Graph showing data for Wild Type and APP/PS1 for Plasma and Brain.]

- APP/PS1
- Plasma: Observed, Predicted
- Brain: Observed, Predicted
Figure 5

- $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}]A\beta30}$
- $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}]A\beta30 + 0.5 \text{ mg unlabeled}}$
- $^{125}\text{I-Gd}[N-4\text{ab/Q-4\text{ab}]A\beta30 + 2.0 \text{ mg unlabeled}}$

Brain Extravascular Amount ($\mu$Ci)

- Cortex
- Caudate Putamen
- Hippocampus
- Thalamus
- Brain
- Cerebellum
- Total
Figure 6
Figure 8

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Figure 9