

Supplemental Materials and Methods

Quantitative analyses

Kinetic compartment models

The general configuration of compartment modeling is a three-tissue compartment model with 6 rate constants where C_B is the compartment for specific binding (1, 2). A common assumption is that the compartments for free (C_F) and non-specific (C_{NS}) binding equilibrate rapidly and form one compartment representing non-displaceable radiotracer concentration (C_{ND}). In this way the model can be simplified into a 2-tissue compartment model with 4 rate constants. K_1 (mL/cm³/min) and k_2 (1/min) represents the influx and efflux of radiotracer through the blood brain barrier by diffusion. The rate constants k_3 (1/min) and k_4 (1/min) correspond to the transfer of radioligand between C_{ND} and C_B . This model gives the following expressions of differential equations where $C_T(t)$ is the radioactivity concentration in brain and $C_P(t)$ is the concentration in plasma:

$$dC_{ND}(t) / dt = K_1 \cdot C_P(t) - (k_2 + k_3) \cdot C_{ND}(t) + k_4 \cdot C_B(t) \quad \text{Eq. 1}$$

$$dC_B(t) / dt = k_3 \cdot C_{ND}(t) - k_4 \cdot C_B(t) \quad \text{Eq. 2}$$

$$C_T(t) = C_{ND}(t) + C_B(t) \quad \text{Eq. 3}$$

In case that all the three compartments in brain (C_F , C_{NS} , and C_B) can be assumed to equilibrate rapidly, thus effectively forming one single compartment (C_T), the model can be further simplified into a one-tissue compartment model (1T-CM). If this assumption is valid then K_1 is still the influx rate constant for diffusion of radioligand over the blood-brain-barrier with the same numerical value as in case of the 2T-CM. The efflux ratio is described by the rate constant k_2^* and its relationship to the rate constants in the 2T-CM is given by Equation 4.

$$k_2^* = k_2 / (1 + k_3 / k_4) \quad \text{Eq. 4}$$

As a first step in the analysis, a non-constrained two-tissue compartment model was fitted to the whole gray matter TAC with parameters for the four rate constants K_1 , k_2 , k_3 , k_4 , as well as terms for blood volume and shift between plasma input and brain TACs. The blood volume and shift values were then fixed for all subsequent compartment analyses.

Calculation of V_T and DVR

A single PET examination, using high specific activity of a radiotracer, is not sufficient to estimate classical receptor binding parameters such as receptor density (B_{max}) and affinity (K_d). Nevertheless the ratio B_{max}/K_d can be directly estimated from a single measurement and is often referred to as the binding potential (BP) and can be related to the ratio k_3/k_4 in the kinetic analysis. BP_{ND} has been used to denote BP when calculated on the basis of non-displaceable binding, i.e. in case of 2T-CM or reference region methods, and equals C_B/C_{ND} at equilibrium conditions.

The total distribution volume (V_T) was calculated from the 1T-CM and 2T-CM analyses using the following equations, respectively:

$$V_T = K_1 / k_2^* \quad \text{Eq. 5}$$

$$V_T = (K_1 / k_2) \cdot (1 + k_3 / k_4) \quad \text{Eq. 6}$$

Using the V_T for each ROI (V_{TROI}) and that of the reference region (V_{TRef}), the distribution volume ratio was calculated using the following equation:

$$DVR = V_{TROI} / V_{TRef} \quad \text{Eq. 7}$$

Peak time (pseudo equilibrium) Ratio and Late time Ratio (quasi steady state) approaches

The ratio between the TAC of a target region to that of a reference region at a certain time point during the PET experiment can be used as a simplified way to estimate DVR. Two alternatives have been used to identify a specific time point for ratio calculation. One alternative is based on the fact that the curve for specific binding of most established reversible neuroreceptor radioligands reaches a peak during time of PET data acquisition. Theoretically this peak has been suggested to represent equilibrium conditions *in vivo* (1). In this study of [^{18}F]AZD4694, the kinetics of the radioligand warrants the calculation of standard uptake volume ratio (SUVR) around the peak time equilibrium as described by Olsson et al (3). The peak time of specific binding was calculated by subtracting the TAC of the reference region i.e. cerebellum from TAC of the target region and defined as $dC_B/dt = 0$ (1, 3). To correct for possible difference in delivery the cerebellum TAC was corrected by multiplying it with the R parameter of the given ROI obtained from the SRTM fit. This correction was only performed to establish the time for peak equilibrium and not to calculate the actual SUVRs.

Alternatively SUVR was calculated at a late time after injection (Late Ratio). This late time, or quasi steady state, ratio approach has been used to estimate DVR for other amyloid-beta radioligands and has been validated against compartment modeling methods (4). For comparative purposes [^{18}F]AZD4694 was evaluated using this late time ratio method with data from 51 to 63 minutes.

Non-linear reference region models

BP_{ND} was obtained from the ratio k_3/k_4 and DVR as $BP_{ND}+1$. The simplified reference tissue model (SRTM) was fitted for each target region to obtain the parameters R, k_2 and BP_{ND} (5), and DVR was calculated as $BP_{ND}+1$.

Linear graphical methods

Data were also analyzed with the linear graphical method proposed by Logan and coworkers (6). Using the metabolite corrected arterial blood curve as input function the last 10 points of the Logan plot were fitted to obtain the total distribution volume (V_T) (33 to 93 minutes post injection). Using the cerebellum as reference region (RefLogan), the last 11 points of the linear plot were fitted to obtain distribution volume ratio (DVR) (27 to 93 minutes post injection) (7). The RefLogan approach was further examined with regard to changing the measurement duration (t_{end}) or using alternate values for starting time (t^*) when fitting the regression line on the plot.

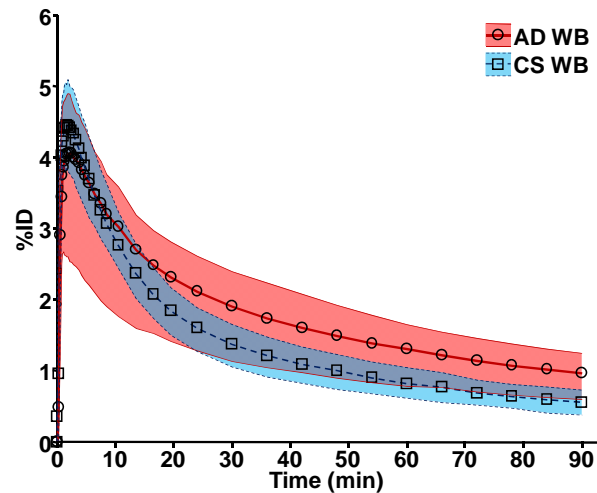
Group differences in specific binding

Group differences between AD patients and CSs in binding parameters were assessed by calculating effect size (ES) or Z-score (ZS) values.

Supplemental Results

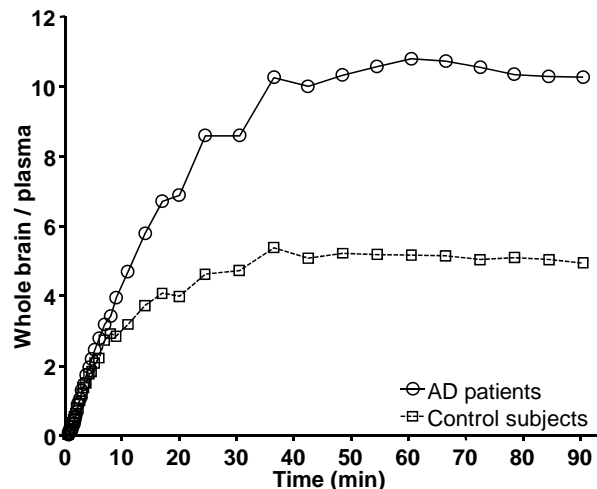
Whole brain uptake

The TAC in whole brain increased rapidly, reaching a peak after about 90 seconds when ~4% of injected radioactivity was in brain both in AD patients and control subjects (CS) (Supplemental Fig. 1). Peak uptake was more variable in AD patients with lowest peak values around 2.5%. After the initial peak the whole brain radioactivity declined rapidly with faster washout in CSs, leaving ~1% of injected radioactivity in brain after 90 minutes.



Supplemental FIGURE 1. Mean and standard deviation of percent injected radioactivity in whole brain for control subjects and AD patients.

The ratio of radioactivity in brain and metabolite corrected plasma reached an essentially stable, horizontal level approx. 40 minutes after injection in both AD patients and CSs (Supplemental Fig. 2).



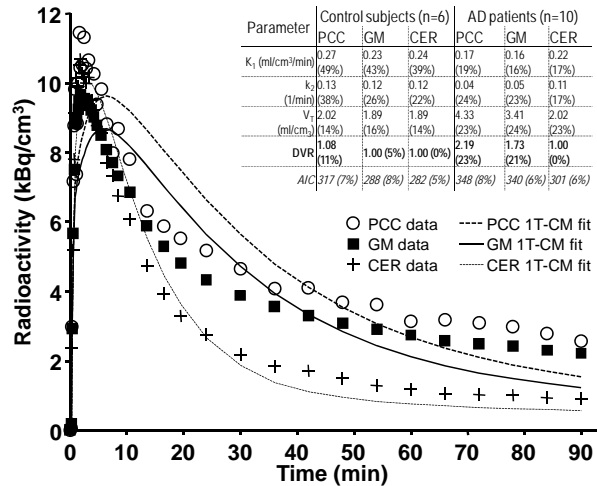
Supplemental FIGURE 2. Group-wise inter-individual mean of the ratio of whole brain radioactivity and metabolite corrected plasma radioactivity of $[^{18}\text{F}]\text{AZD4694}$ vs. time for AD patients (n=10) and control subjects (n=6).

Non-linear models

The one-tissue compartment model (1T-CM) could not successfully describe the regional TACs, including cerebellum (Supplemental Fig. 3). The regional AIC values were accordingly lower for 2T-CM (AIC score for GM in AD patients = 161, in CS = 157) than for 1T-CM (AIC score for GM in AD = 340, in controls = 288) for all TACs and subjects (results for se-

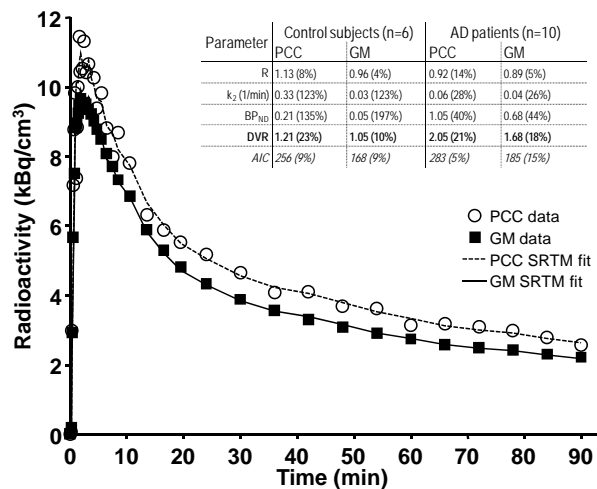
lected ROIs with 1T-CM in insert table in Supplemental Fig. 3). F-tests comparing the one- and two-tissue compartment model indicated that the latter was significantly preferred for all target ROIs and the cerebellum ($p < 0.05$ for all TACs and subjects, data not shown). Rate constants and other outcome measures obtained with 1T-CM can be seen in the insert table in Supplemental Fig. 3 for selected regions.

Supplemental FIGURE 3. Results of one-tissue compartment model (1T-CM) fitting to time-activity curves (TACs) for selected regions of a single AD patient (AD4). Measured TACs are shown using unconnected markers. Three regions are shown: two target regions (posterior cingulate cortex: PCC, and total gray matter: GM) and the reference region (cerebellum: CER). Fitted model curves are shown using continuous lines with no markers.



The data could also be described by compartment tissue model (SRTM) using the cerebellum as reference region (Supplemental Fig. 4). The R-values were lower in PCC in the AD patients compared to the control subjects using SRTM (AD: 0.92, CS: 1.13). Rate constants and other outcome measures obtained with SRTM can be seen in the insert table in Supplemental Fig. 4 for selected regions.

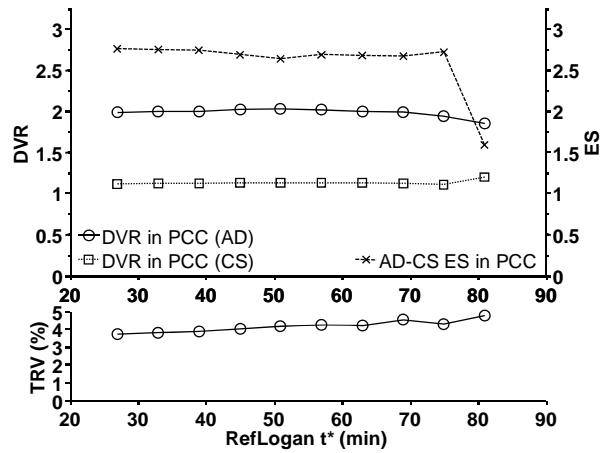
Supplemental FIGURE 4. Results of simplified reference tissue model (SRTM) for selected regions of a single AD patient (AD4). Measured TACs are shown using unconnected markers. Two target regions are shown: posterior cingulate cortex: PCC and total gray matter: GM. Fitted model curves are shown using continuous lines with no markers.



Stability of reference Logan

Varying the start time (t^*) of fitting the RefLogan plot in the range of 27 to 75 minutes did not have a substantial impact on the estimated DVR values, the effect size or test-retest variability (Supplemental Fig. 5).

Supplemental FIGURE 5. Demonstration of the stability of RefLogan with fixed t_{end} and using alternate values for starting time (t^*). Stability is characterized by showing: the group-wise inter-individual mean of distribution volume ratio (DVR) estimates in posterior cingulate cortex (PCC) for AD patients and control subjects (CS); the DVR effect size (ES) between AD patients ($n=10$) and control subjects ($n=6$) in PCC; test-retest variability (TRV) in total gray matter.



Group differences in specific binding

The ESs were overall in the range of 2.5-3 for most methods (Supplemental Table 1). The ZSs were above 7 with some values even above 10.

| Supplemental TABLE 1. Effect sizes | | | | | | | |
|------------------------------------|-----------|------|------|------|-------|------|-------|
| Model | Parameter | PCC | | PFC | | GM | |
| | | ES | ZS | ES | ZS | ES | ZS |
| 2T-CM | DVR | 2.58 | 7.20 | 2.53 | 8.99 | 2.58 | 10.73 |
| RefLogan | DVR | 2.76 | 7.56 | 2.60 | 9.17 | 2.65 | 10.79 |
| Peak Ratio | SUVR | 3.14 | 7.15 | 2.89 | 12.07 | 2.80 | 12.41 |
| Late Ratio | SUVR | 2.68 | 7.57 | 2.49 | 7.85 | 2.54 | 9.31 |

Data shown are effect sizes (ES) and Z-scores (ZS) based on DVR (SUVR) estimates from all AD patients ($n=10$) and control subjects ($n=6$). Selected regions include: posterior cingulate cortex (PCC), prefrontal cortex (PFC) and total gray matter (GM)

References

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