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Amyloid-β efflux from the CNS into the plasma

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Abstract

Objective—The aim of this study was to measure the flux of amyloid-β (Aβ) across the human cerebral capillary bed in order to determine if transport into the blood is a significant mechanism of clearance for Aβ produced in the central nervous system (CNS).

Methods—Time-matched blood samples were simultaneously collected from a cerebral vein (including the sigmoid sinus, inferior petrosal sinus, and the internal jugular vein), femoral vein, and radial artery of patients undergoing Inferior Petrosal Sinus Sampling (IPSS). For each plasma sample, Aβ concentration was assessed by three assays and the venous to arterial Aβ concentration ratios were determined.

Results—Aβ concentration was increased by ~7.5% in venous blood leaving the CNS capillary bed compared to arterial blood, indicating efflux from the CNS into the peripheral blood ($p < 0.0001$). There was no difference in peripheral venous Aβ concentration compared to arterial blood concentration.

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Conflict of interest: Ms. Roberts, Dr. Elbert, Dr. Patterson, Ms. Sigurdson, Ms. Connors, Mr. Ovod, Ms. Munsell, Dr. Mawuenyega, Dr. Miller-Thomas, Dr. Moran, and Dr. Cross declare no conflicts of interest.

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Interpretation—Our results are consistent with clearance of CNS-derived Aβ into the venous blood supply with no increase from a peripheral capillary bed. Modeling these results suggests that direct transport of Aβ across the blood-brain barrier accounts for ~25% of Aβ clearance, and reabsorption of cerebrospinal fluid Aβ accounts for ~25% of the total CNS Aβ clearance in humans.

INTRODUCTION

Abnormal metabolism of the protein amyloid-β (Aβ) is hypothesized to initiate a pathological cascade leading to Alzheimer's disease (AD). Amyloid plaques, one of the pathologic hallmarks of Alzheimer's disease, are composed primarily of aggregates of Aβ, which is generated by the cleavage of amyloid precursor protein (APP) by beta-secretase and gamma-secretase.[1] Aβ production occurs primarily by neurons in the central nervous system (CNS), but also in peripheral tissues. In autosomal dominant Alzheimer's disease, mutations causing overproduction of $A\beta_{42}$ raise soluble levels and induce aggregation and plaque formation leading to early onset of Alzheimer's disease.[2, 3] Additionally, individuals with Down's syndrome, who have three copies of APP and higher levels of Aβ, all develop AD.[4] Taken together with recent data demonstrating altered clearance of CNS $A\beta_{42}$ in sporadic Alzheimer's disease[5], the balance between production and clearance of Aβ may contribute to amyloid pathology and the development of AD.

After generation in the brain, soluble Aβ enters the extracellular fluid and can be cleared by a variety of mechanisms including phagocytosis by glia, proteolytic degradation, transport to the cerebrospinal fluid (CSF) with subsequent reabsorption into the venous blood, and direct transport across the blood-brain barrier (BBB) into the venous blood (Figure 1).[6–8] Concentrations of Aβ in the CSF, which is in direct communication with the brain interstitial fluid (ISF), are useful AD biomarkers, with low $A\beta_{42}$ concentrations when amyloid plaque is present.[9] Although transport from the CNS into the venous blood is thought to be a significant mechanism for clearing CNS-derived Aβ[10], plasma Aβ has not shown utility as an AD biomarker.[11, 12] While clearance of CNS Aβ into the peripheral plasma has been demonstrated in animal models,[13, 14] the contribution of centrally produced Aβ to peripheral blood Aβ concentration in humans is unknown. Additionally, the relative importance of different clearance mechanisms remains unclear.[15]

Removal of Aβ peptides from the brain using immunotherapies such as Aβ-targeted monoclonal antibodies has emerged as a prominent therapeutic strategy.[16, 17] Additionally, mechanistic work on the bidirectional transport of Aβ across the BBB via transporters such as RAGE and LRP has opened up the possibility of modulating Aβ BBB transport as a therapeutic strategy.[10, 18–20] By elucidating the contribution of the various natural mechanisms of Aβ efflux, this work will provide insight into the physiology of Aβ clearance and facilitate the design of improved therapies directed at removing Aβ from the brain.

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SUBJECTS AND METHODS

Participants

This study recruited patients undergoing venous cerebral blood sampling for medical reasons. All participants were being evaluated for autonomous pituitary microadenomas and were scheduled to undergo Inferior Petrosal Sinus Sampling (IPSS), a procedure requiring moderate sedation, fluoroscopically-guided placement of catheters into the jugular veins and inferior petrosal sinuses bilaterally, and timed sample collection for measurement of ACTH levels.[21] Fourteen participants were enrolled in this study. Three withdrew due to lack of radial artery access, eleven completed the protocol, and ten produced enough sample for analysis. Participants were over 18 years of age (27 – 53 years) and included 1 male and 10 females. Patients were confirmed by medical history review to have no cognitive impairment or diagnosis of dementia. The study was performed with written informed consent of the participants and the approval of the Washington University School of Medicine Institutional Review Board.

Sample Collection

In order to measure the flux of Aβ across the brain, we used time-matched arterial, peripheral venous, and central venous blood samples. The study protocol involved placement of an arterial line in the radial artery and sampling of blood from the sigmoid sinus and internal jugular bulb, in addition to sampling from routine IPSS locations (common femoral vein and inferior petrosal sinuses) under moderate sedation with IV Fentanyl and Versed (Figure 1). All samples were drawn from catheters. The total volume of blood collected during the study was 144 ml (54 ml for research and 90 ml for clinical) over 20 minutes. The amount of blood drawn for research at was 5 ml at the internal jugular bulb (IJ), 5 ml at the sigmoid sinus (SS), 2 ml × 3 at the left inferior petrosal sinus (IPSL), 2 ml × 3 at the right inferior petrosal sinus (IPSR), 5 ml × 2 and 2 ml × 3 at the peripheral vein (PV), and 5 ml × 2 and 2 ml × 3 at the radial artery. Four sets of samples were drawn before administration of corticotrophin-releasing hormone (CRH) and one set was drawn 10 minutes after administration. At each time point, an arterial, peripheral venous, and central venous sample were simultaneously collected in tubes containing 50 μl 0.4 M EDTA and 50 μl 1x protease inhibitor cocktail (Roche). Samples were placed immediately on ice, plasma was separated by centrifugation, 10 μl ^{15}N-labeled Aβ internal standard (rPeptide, consisting of 0.01 ng/μl $A\beta_{40}$ and 0.001 ng/μl $A\beta_{42}$ stored in 4:1 0.1% NH_4OH:MeCN at −80 °C) was added to a 0.5 ml aliquot of plasma, and aliquots were frozen in polypropylene tubes and stored at -80 °C. Due to low sample volume, patient 7 was not analyzed by IP/MS and patient 1 was not analyzed by xMAP.

ELISA

The ELISA protocol was run as previously described,[22] with the following modifications: 2.5 μg/ml HJ5.1 antibody (anti-$A\beta_{13-28}$) was used to coat Costar 3690 plates and 0.1 μg/ml biotinylated 6E10 (anti-$A\beta_{1-17}$, Covance) antibody was used for detection.

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xMAP

$A\beta_{40}$ and $A\beta_{42}$ isoforms were analyzed in plasma using the INNO-BIA plasma Aβ Forms kit (Innogenetics) according to the manufacturer's instructions. Briefly, $A\beta_{40}$ and $A\beta_{42}$ were simultaneously identified and quantified using a dual laser flow cytometry based immunoassay. Capture antibodies 21F12 (anti-$A\beta_{42}$) and 2G3 (anti-$A\beta_{40}$) were covalently coupled to separate sets of microsphere beads embedded with different ratios of red dyes. The emission signature following excitation at 635 nm was used to identify the analyte as either $A\beta_{40}$ or $A\beta_{42}$. Biotinylated 3D6 was used as the detector antibody. After incubation with phycoerythrine-labeled streptavidin, assays were analyzed using the Luminex 100 IS instrument (Luminex) and mean fluorescence intensity after excitation at 532 nm was used for quantification.

Immunoprecipitation/Mass Spectrometry (IP/MS)

CNBr-activated Sepharose beads were conjugated to HJ5.1 antibody and pre-cleaned with formic acid. Plasma samples containing 450 μl plasma with 9 μl ^{15}N-labeled Aβ internal standard (rPeptide, consisting of 0.01 ng/μl $A\beta_{40}$ and 0.001 ng/μl $A\beta_{42}$ stored in 4:1 0.1% ammonium hydroxide to acetonitrile at -80 °C) were treated with 2.5 μl 1x complete protease inhibitor (Roche), 55 μl 5 M urea, 2.5 μl 5% Tween-20 in PBS, and 30 μl 50% antibody bead slurry in PBS with 0.02% azide. Immunoprecipitation was carried out for 2 hours at 25 °C with end over end rotation. After washing the beads with 0.5 M urea and 25 mM ammonium bicarbonate, samples were eluted with neat formic acid, digested with Lys-C, and desalted using C08 TopTips (Glygen). Samples were dried using a Centrivap Concentrator (Labconco), resuspended in 25 μl of 10% acetonitrile, 0.1% formic acid in water, and transferred to autosampler vials. Using a 4 μl injection volume, samples were run on a nanoACQUITY UPLC system coupled to a Xevo TQ-S mass spectrometer (Waters). Peptides were separated on a reverse-phase column (1.7 μm BEH130 C18 100 μm × 100 mm) operated at a flow rate of 500 nl/min with a gradient mixture of solvents and analyzed by targeted mass spectrometry on the mid-domain $A\beta_{17-28}$ peptide (LVFFAEDVGSNK).[23] The ratio of endogenous Aβ (^{14}N) to ^{15}N uniformly-labeled internal standard Aβ was calculated using MassLynx V4.1 software and quantified according to a standard curve.

Aβ Standards Preparation

Recombinant Aβ (rPeptide) was purchased and stored at 0.6 mg/ml in formic acid at -80 °C. Stock Aβ was diluted through a series of buffers before use and standards produced at final concentrations of 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml, 78 pg/ml, and 0 pg/ml. In order to reproduce plasma matrix effects on samples, 50 μl preparations of Aβ standards for IP/MS were spiked into 400 μl of Aβ depleted plasma. Plasma depletion was performed by immunoprecipitation with HJ5.1 antibody beads and measured to be more than 95% depleted of Aβ by ELISA.

Statistical Analyses

Venous Aβ concentrations were divided by time-matched arterial Aβ concentrations to calculate the V/A ratio. Data was not normalized and all data points were included in the analysis. Data are presented as mean (95% CI). Venous to arterial Aβ ratios from

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experimental sampling locations were compared by unpaired two-tailed t-tests with alpha equal to 0.05. Assays were compared by one-way ANOVA and Bonferroni's multiple comparison test. All statistical analysis was performed using GraphPad Prism version 5.0 for Mac OS X (GraphPad Software).

RESULTS

Plasma Aβ concentrations were measured by 3 assays: IP/MS, ELISA, and xMAP. xMAP measured the $A\beta_{40}$ and $A\beta_{42}$ isoforms separately, while IP/MS and ELISA targeted a mid-domain peptide common to both isoforms and thus measured total Aβ. Total Aβ measurement by xMAP was taken as the sum of $A\beta_{40}$ and $A\beta_{42}$ isoforms, which compose the majority of Aβ. By IP/MS, the average plasma [Aβ] was 365 pg/ml (352–377 95% CI). With ELISA, the average plasma [Aβ] was 173 pg/ml (157–189 95% CI). Using xMAP, the average plasma [$A\beta_{40}$] was 201 pg/ml (194–208 95% CI), [$A\beta_{42}$] was 41 pg/ml (40–43 95% CI), and total [Aβ] was 243 pg/ml (235–251 95% CI).

In order to normalize the different Aβ concentrations among the assays, and to compare the arterial-venous concentration differences, the venous/arterial (V/A) ratio was utilized to calculate the change in Aβ concentration across the brain capillary bed. A ratio greater than 1 corresponds to a net increase in [Aβ] after flow through the capillary bed (brain efflux), a ratio less than 1 corresponds to a net decrease in [Aβ] (brain influx), and a ratio equal to 1 indicates no net flux.

The mean Aβ concentrations for the different central venous sampling sites were not significantly different by one-way ANOVA for any of the assays used, so due to the limited sample size (n=11), all central venous sampling site V/A ratios (IJ, SS, IPSL, and IPSR) were grouped together and compared to peripheral V/A ratio measurements. The V/A ratios for Aβ by IP/MS and ELISA were 1.087 (1.029 – 1.146 95% CI) and 1.111 (1.033 – 1.188 95% CI) respectively for blood from central veins, indicating efflux from the CNS into the plasma (Figure 2). The $A\beta_{40}$ V/A ratio for central venous samples by xMAP was 1.067 (1.018 to 1.115 95% CI), also indicating efflux from the CNS capillary bed. The $A\beta_{42}$ V/A ratio for central venous samples was 1.031 (0.9928 to 1.070 95% CI), which was not significant, but trended to efflux. In contrast, the Aβ V/A ratio for blood that flowed through a peripheral capillary bed was not different from 1 by any assay (IP/MS 0.969 $p=0.13$, ELISA 1.045 $p=0.25$, $xMAP_{40}$ 1.003 $p=0.92$, $xMAP_{42}$ 0.9906 $p=0.59$), indicating no net efflux or influx of peripheral amyloid-β.

Despite the differences in baseline Aβ concentration, the V/A ratios were consistent between the IP/MS, ELISA, and xMAP assays with no significant difference in their means ($p > 0.05$) by Bonferroni's multiple comparison test. Thus, we combined all plasma total Aβ concentration measures to increase the power of the study (Figure 3). The mean V/A ratio for central venous samples was 1.075 (1.046–1.104 95% CI), indicating that flow across the brain capillary bed results in an increase of venous plasma Aβ concentration by 7.5% (4.6–10.4% 95% CI). In contrast, the mean V/A ratio for peripheral venous samples was 1.003 (0.9754–1.030 95% CI).

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While the V/A ratio was consistent among the three assays used, the absolute changes in Aβ concentration (V-A) varied with the average plasma Aβ concentration measured by that assay. Assay-dependency of absolute measures of plasma Aβ concentration is widely reported in the literature.[24] Therefore, we utilized the average concentrations by all three assays as estimates for modeling. In this study, the average plasma concentration of total Aβ peptides as measured by IP/MS, ELISA, and xMAP was 264 pg/ml. The average V-A difference across the brain was 13.6 pg/ml. Other values used for modeling are listed in Table 1.

The plasma V-A difference across the brain multiplied by cerebral blood flow rate yields the rate of efflux of Aβ peptides out of the brain.

$$\mathrm{V-A\ difference} \times \mathrm{Q_{cerebral}}{=}13.6\ \mathrm{pg/ml} \times 715\ \mathrm{ml/min}{=}9.7\ \mathrm{ng/min}$$

Based on this approximation, all processes that result in transfer of Aβ peptides from the brain into the central veins sum to a rate of mass transfer of 9.7 ng/min.

Total turnover of Aβ peptides in the brain

Using stable isotope labeling kinetics (SILK), we recently reported a fractional turnover rate of 0.14 h^{-1} for total irreversible loss of soluble Aβ in the CNS of normal controls.[2] This rate multiplied by the pool size of Aβ peptides in the brain yields the rate at which Aβ peptides are removed from the brain.

The average total brain pools of $A\beta_{40}$ and $A\beta_{42}$ in aged normal controls have been measured using formic acid extraction to be 2 pmol/g wet tissue and 2 pmol/g wet tissue respectively.[25] Of these total pools, soluble forms accounted for 50% of $A\beta_{40}$ and 23% of $A\beta_{42}$.[25] The total combined concentration of soluble Aβ is thus calculated to be 6410 pg/g of wet tissue. This value is in the range of normal water-soluble brain Aβ concentrations reported in the literature.[26] Using the sex-weighted average brain mass for our population of 10 females and 1 male (1211 g)[27] and assuming homogeneity:

$$[\mathrm{A}\beta] \times \mathrm{brain\ mass}{=}6410\ \mathrm{pg/g} \times 1211\ \mathrm{g}{=}7760\ \mathrm{ng}$$

estimates the soluble Aβ peptide pool size in the normal brain. The turnover rate of Aβ peptides is then:

$$7760\ \mathrm{ng} \times 0.14\ \mathrm{h}^{-1}{=}18.1\ \mathrm{ng/min}$$

This is higher than the amount transferred to the cerebral venous blood (9.7 ng/min), suggesting that transport of Aβ across the blood brain barrier and via CSF reabsorption into the venous blood may account for approximately 50% of the total clearance rate.

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Mass transfer by CSF reabsorption

Although CSF was not collected in this study, literature values can be used to estimate the typical contribution of CSF reabsorption to our measured efflux values. At steady state, the rate at which Aβ peptides in the ISF become Aβ peptides in the CSF is balanced by the rate at which Aβ peptides in the CSF are reabsorbed into the venous circulation. The CSF reabsorption rate multiplied by the CSF concentration of Aβ peptides yields the rate of transfer of Aβ peptides from the brain into the blood assuming no accumulation or significant degradation of Aβ in the CSF.[28] Using the same IP/MS technique described in this study, the average CSF total Aβ concentration from twelve normal controls (ages 29 – 72) by IP/MS was measured to be 12.4 ng/ml.[2]

$$[\mathrm{A}\beta]_{\mathrm{CSF}} \times \mathrm{CSF\ reabsorption\ rate}=12.4\,\mathrm{ng/ml} \times 0.4\,\mathrm{ml/min}=5\,\mathrm{ng/min}$$

This indicates that mechanisms of efflux into the venous blood other than CSF reabsorption such as direct transport across the BBB result in a transfer of 9.7 – 5 = 4.7 ng/min of Aβ peptides from the brain into the blood. Thus direct transport across the BBB represents ~25% of total CNS Aβ clearance, while CSF reabsorption accounts for ~25% of CNS Aβ clearance.

Plasma turnover rate

While the rate of human plasma Aβ turnover has not been reported, we utilized our efflux measurement to estimate a minimum plasma Aβ turnover rate at steady state. The plasma pool size can be estimated as the total blood volume times the average measured plasma Aβ concentration.

$$\mathrm{Plasma\ pool\ size\ A}\beta\mathrm{\ peptides}=\mathrm{plasma\ volume}\times\mathrm{plasma\ concentration\ of\ A}\beta\mathrm{\ peptides}=870\,\mathrm{ng}$$

Using this estimate for the plasma pool size of Aβ peptides, the minimum value for the fractional turnover rate of plasma Aβ may then also be estimated.

$$\mathrm{Minimum\ plasma\ A}\beta\mathrm{\ fractional\ turnover\ rate}=\mathrm{Plasma\ A}\beta\mathrm{\ appearance\ rate/pool\ size}=9.7\,\mathrm{ng/min}/870\,\mathrm{ng}=0.67\,\mathrm{h}^{-1}$$

DISCUSSION

In order to quantify *in vivo* brain transport of Aβ across the BBB in humans, we measured Aβ concentrations in time-matched arterial and venous samples, then calculated the V/A ratio, which indicates the net transport across a capillary bed. To our knowledge, no studies have previously addressed this in humans and these results are likely to be helpful for modeling amyloidosis, risk and causes of AD, and diagnostic and therapeutic approaches for AD.

A major strength of this study is the direct sampling from human cerebral veins and a time-matched artery for direct comparison in paired assay measurements. For arterial measurements, we assumed the Aβ concentration to be uniform in arterial blood due to

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mixing and used the radial artery as a surrogate for the blood entering the cerebral capillary bed. Within the heart, deceleration of blood during diastole destabilizes fluid flow inducing intracardiac vortices and transient turbulence.[29] The turbulence serves to mix the blood leaving the heart, resulting in homogeneous arterial blood.[30] Thus, arterial homogeneity is a common assumption in arteriovenous difference studies.[31] As this was a cerebral venous study, we did not have access to both radial and carotid arterial blood to verify this assumption.

Central venous measurements were made using catheterization of veins directly draining the brain. Thus, the V/A ratios calculated from cerebral sampling sites inform how the brain vascular bed changes the blood concentration of Aβ, while V/A ratios calculated from a peripheral venous sampling site serve as a control. While all blood samples were obtained from catheters, we do not anticipate that this sampling method affected the measured Aβ concentrations compared to venous puncture sampling.[32] Additionally, we do not believe that central venous catheterization significantly altered the Aβ measurements by disrupting the BBB as the blood was collected upstream from the tip of the catheter.

While the novel sampling method utilized in this study afforded more accurate measures of CNS to plasma Aβ flux, this data has several potential limitations. Eleven patients undergoing IPSS procedures were recruited for this study in order to access cerebral venous blood for the investigation of brain Aβ clearance mechanisms in young non-AD people. The sample collection procedure limited the ability to recruit patients and obtain samples. The participants were being evaluated for pituitary microadenomas and received CRH during sample collection. The effect of a pituitary microadenoma on brain Aβ flux is unknown. While acute and chronic overexpression of CRH have been shown to increase levels of soluble Aβ and accelerate amyloid pathology in transgenic mouse models of AD,[35, 36] we do not anticipate that the administration of CRH used in this experiment would alter normal brain Aβ flux over the time course of our sample collections (10 minutes). Further, samples collected before and after CRH administration showed no significant change in Aβ levels ($p=0.81$).

In order to overcome assay-related variability, three different assays were used to measure Aβ levels. Both assays measuring total Aβ demonstrated significant efflux from the CNS into the plasma. The fact that $A\beta_{40}$ as measured by xMAP demonstrated clear efflux, while $A\beta_{42}$ did not, may be due to the inherent difficulty in measuring $A\beta_{42}$ levels in plasma, which were 5 fold lower than $A\beta_{40}$. Another possibility is that $A\beta_{42}$ is less efficiently effluxed out of the CNS. The combined data obtained in this study was consistent with a 7.5% increase in plasma total Aβ concentration caused by net efflux of CNS-derived Aβ into the venous blood. In contrast, the V/A ratio of Aβ measured in the peripheral femoral vein was not significantly different from 1, indicating that there is no net efflux or influx of Aβ across the leg capillary bed. Although the intensive nature of the procedure limited the number of participants, multiple measures and replication by three independent assays provides evidence that there is net Aβ efflux from the brain to the plasma in humans.

Efflux measurements were not consistent between individuals or within individuals over time indicating that the movement of Aβ between the CNS and the periphery may be a

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dynamic process. Given the temporal variation in CSF Aβ levels[37] and the large rapid changes in cerebral blood flow that can occur over seconds in response to neural activity,[28, 38] it is not surprising that Aβ efflux from the CNS to the plasma may also be highly variable. Additionally, recent evidence has demonstrated that sleep and anesthesia drive large convective increases in ISF-CSF exchange that facilitate brain metabolite clearance, thus illustrating the dependence of at least one mechanism of CNS efflux on the sleep-wake status of the patient.[39] Given that the patients in this study were conscious during sample collection, the CNS to plasma efflux measured might underestimate the rates of efflux during sleep.

Prior studies have found inconsistent relationships between plasma Aβ levels and AD.[40] Additionally, there is no correlation between Aβ concentrations in matched CSF and plasma samples from patients with Alzheimer's disease[41] or APP transgenic mice.[42] Based on our data, Aβ cleared from the CNS raises plasma concentrations by only 7.5% and this increase can only be observed when measured in central veins before significant dilution and peripheral clearance occurs. Although soluble Aβ is approximately 50 times more concentrated in the brain and CSF than the plasma, the small CSF volume and large plasma volume results in only a small peripheral concentration increase.[43] Thus, despite brain efflux of Aβ into the plasma, plasma Aβ concentrations may not accurately reflect CNS AD pathology due to the effects of dilution and variation in blood Aβ clearance rates.

Brain derived Aβ is cleared from the CNS by several mechanisms including direct transport across the blood-brain-barrier (BBB), movement into the CSF and eventual reabsorption into the venous circulation, and phagocytic and enzymatic degradation within the CNS (Figure 1).[6] Of these four mechanisms for brain Aβ clearance, both transport across the BBB and reabsorption into the venous circulation via CSF would cause an increase in the Aβ concentration of blood leaving the brain compared to blood entering the brain. Additionally, previous work has demonstrated that both of these Aβ transport processes are disrupted in transgenic mouse models of AD.[44]

Because little is known about human Aβ brain to blood transport, we used our data to generate estimates of the relative importance of several clearance mechanisms. Our estimates of total brain amyloid-β clearance rates suggest that transport of Aβ to the blood by CSF and direct BBB transport may account for 50% of the total clearance of CNS amyloid-β. Of the Aβ cleared from the CNS into the venous blood, we estimate that half is transported directly across the BBB. However, this calculation is dependent on several assumptions regarding CSF reabsorption. CSF Aβ levels measured via lumbar catheter rather than at the point of reabsorption were used for modeling. The existence of a rostrocaudal gradient of Aβ in the CSF is debated. Tarnaris et al. compared $Aβ_{42}$ levels in cisternal and lumbar CSF and found a small rostrocaudal gradient.[45] In contrast, work by the Blennow group utilizing serial sampling measurements of 10 ml CSF did not demonstrate a spinal gradient for $Aβ_{42}$.[46, 47] Based on these studies, if a gradient does exist from the site of reabsorption to the lumbar sampling site, the effect will likely be small and result in slight underestimation of the CSF clearance by our theoretical calculations. Additionally, this calculation assumes that all of the CSF is reabsorbed into the cerebral venous blood and recent studies suggest that alternative routes via the optic and olfactory nerves, cribiform

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plate, nasal submucosa, and cervical lymphatics are significant routes of CSF reabsorption.[48] CSF reabsorption can also occur in the spinal cord, particularly if superior sagittal sinus compliance is decreased.[28] CSF reabsorbed by this route would not contribute to the elevation in Aβ measured in cerebral venous blood. Thus this modeling estimate provides the minimum contribution expected from direct transport across the BBB, which may represent up to 50% of total clearance of CNS Aβ.

These measurements provide the first direct evidence of CNS to plasma Aβ clearance in humans and are important for understanding the physiologic processes regulating Aβ metabolism. Further understanding of brain clearance mechanisms of Aβ will provide insights into Alzheimer's disease and potentially aid in diagnostic and therapeutic development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002 Jul 19; 297(5580):353–6. [PubMed: 12130773]
2. Potter R, Patterson BW, Elbert DL, et al. Increased in vivo amyloid-beta42 production, exchange, and loss in presenilin mutation carriers. Science translational medicine. 2013 Jun 12.5(189): 189ra77.
3. Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature medicine. 1996 Aug; 2(8):864–70.

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4. Wisniewski KE, Dalton AJ, McLachlan C, Wen GY, Wisniewski HM. Alzheimer's disease in Down's syndrome: clinicopathologic studies. Neurology. 1985 Jul; 35(7):957–61. [PubMed: 3159974]
5. Mawuenyega KG, Sigurdson W, Ovod V, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science. 2010 Dec 24.330(6012):1774. [PubMed: 21148344]
6. Wang YJ, Zhou HD, Zhou XF. Clearance of amyloid-beta in Alzheimer's disease: progress problems and perspectives. Drug discovery today. 2006 Oct; 11(19–20):931–8. [PubMed: 16997144]
7. Zlokovic BV. Clearing amyloid through the blood-brain barrier. Journal of neurochemistry. 2004 May; 89(4):807–11. [PubMed: 15140180]
8. Saido T, Leissring MA. Proteolytic degradation of amyloid beta-protein. Cold Spring Harbor perspectives in medicine. 2012 Jun.2(6):a006379. [PubMed: 22675659]
9. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nature reviews Neurology. 2010 Mar; 6(3):131–44.
10. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. CNS & neurological disorders drug targets. 2009 Mar; 8(1):16–30. [PubMed: 19275634]
11. Lopez OL, Kuller LH, Mehta PD, et al. Plasma amyloid levels and the risk of AD in normal subjects in the Cardiovascular Health Study. Neurology. 2008 May 6; 70(19):1664–71. [PubMed: 18401021]
12. Mehta PD, Pirttila T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1–40 and 1–42 in Alzheimer disease. Archives of neurology. 2000 Jan; 57(1):100–5. [PubMed: 10634455]
13. Maness LM, Banks WA, Podlisny MB, Selkoe DJ, Kastin AJ. Passage of human amyloid beta-protein 1–40 across the murine blood-brain barrier. Life sciences. 1994; 55(21):1643–50. [PubMed: 7968239]
14. Ghersi-Egea JF, Gorevic PD, Ghiso J, Frangione B, Patlak CS, Fenstermacher JD. Fate of cerebrospinal fluid-borne amyloid beta-peptide: rapid clearance into blood and appreciable accumulation by cerebral arteries. Journal of neurochemistry. 1996 Aug; 67(2):880–3. [PubMed: 8764620]
15. Zlokovic BV, Yamada S, Holtzman D, Ghiso J, Frangione B. Clearance of amyloid beta-peptide from brain: transport or metabolism? Nature medicine. 2000 Jul.6(7):718.
16. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America. 2001 Jul 17; 98(15):8850–5. [PubMed: 11438712]
17. Demattos RB, Lu J, Tang Y, et al. A plaque-specific antibody clears existing beta-amyloid plaques in Alzheimer's disease mice. Neuron. 2012 Dec 6; 76(5):908–20. [PubMed: 23217740]
18. Deane R, Du Yan S, Submamaryan RK, et al. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nature medicine. 2003 Jul; 9(7):907–13.
19. Deane R, Wu Z, Sagare A, et al. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. Neuron. 2004 Aug 5; 43(3):333–44. [PubMed: 15294142]
20. Deane R, Wu Z, Zlokovic BV. RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. Stroke; a journal of cerebral circulation. 2004 Nov; 35(11 Suppl 1):2628–31.
21. Oldfield EH, Doppman JL, Nieman LK, et al. Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing's syndrome. The New England journal of medicine. 1991 Sep 26; 325(13):897–905. [PubMed: 1652686]
22. Bateman RJ, Munsell LY, Chen X, Holtzman DM, Yarasheski KE. Stable isotope labeling tandem mass spectrometry (SILT) to quantify protein production and clearance rates. Journal of the American Society for Mass Spectrometry. 2007 Jun; 18(6):997–1006. [PubMed: 17383190]
23. Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. Nature medicine. 2006 Jul; 12(7):856–61.

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24. Okereke OI, Xia W, Irizarry MC, et al. Performance characteristics of plasma amyloid-beta 40 and 42 assays. Journal of Alzheimer's disease : JAD. 2009; 16(2):277–85.
25. Wang J, Dickson DW, Trojanowski JQ, Lee VM. The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. Experimental neurology. 1999 Aug; 158(2):328–37. [PubMed: 10415140]
26. McDonald JM, Cairns NJ, Taylor-Reinwald L, Holtzman D, Walsh DM. The levels of water-soluble and triton-soluble Abeta are increased in Alzheimer's disease brain. Brain research. 2012 Apr 23.1450:138–47. [PubMed: 22440675]
27. Hartmann P, Ramseier A, Gudat F, Mihatsch MJ, Polasek W. Normal weight of the brain in adults in relation to age, sex, body height and weight. Der Pathologe. 1994 Jun; 15(3):165–70. [PubMed: 8072950]
28. Martin BA, Reymond P, Novy J, Baledent O, Stergiopulos N. A coupled hydrodynamic model of the cardiovascular and cerebrospinal fluid system. American journal of physiology Heart and circulatory physiology. 2012 Apr 1; 302(7):H1492–509. [PubMed: 22268106]
29. Sengupta PP, Pedrizetti G, Narula J. Multiplanar visualization of blood flow using echocardiographic particle imaging velocimetry. JACC Cardiovascular imaging. 2012 May; 5(5): 566–9. [PubMed: 22595166]
30. Seo JH, Mittal R. Effect of diastolic flow patterns on the function of the left ventricle. Physics of Fluids (1994-present). 2013; 25(11):–.
31. Eikelis N, Lambert G, Wiesner G, et al. Extra-adipocyte leptin release in human obesity and its relation to sympathoadrenal function. American journal of physiology Endocrinology and metabolism. 2004 May; 286(5):E744–52. [PubMed: 14722031]
32. Ortells-Abuye N, Busquets-Puigdevall T, Diaz-Bergara M, Paguina-Marcos M, Sanchez-Perez I. A cross-sectional study to compare two blood collection methods: direct venous puncture and peripheral venous catheter. BMJ open. 2014; 4(2):e004250.
33. Schillinger F, Schillinger D, Montagnac R, Milcent T. Post catheterisation vein stenosis in haemodialysis: comparative angiographic study of 50 subclavian and 50 internal jugular accesses. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association – European Renal Association. 1991; 6(10):722–4.
34. Staniloae CS, Mody KP, Sanghvi K, et al. Histopathologic changes of the radial artery wall secondary to transradial catheterization. Vascular health and risk management. 2009; 5(3):527–32. [PubMed: 19590587]
35. Kang JE, Cirrito JR, Dong H, Csernansky JG, Holtzman DM. Acute stress increases interstitial fluid amyloid-beta via corticotropin-releasing factor and neuronal activity. Proceedings of the National Academy of Sciences of the United States of America. 2007 Jun 19; 104(25):10673–8. [PubMed: 17551018]
36. Dong H, Murphy KM, Meng L, et al. Corticotrophin releasing factor accelerates neuropathology and cognitive decline in a mouse model of Alzheimer's disease. Journal of Alzheimer's disease : JAD. 2012; 28(3):579–92.
37. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology. 2007 Feb 27; 68(9):666–9. [PubMed: 17325273]
38. Fox PT, Raichle ME. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proceedings of the National Academy of Sciences of the United States of America. 1986 Feb; 83(4):1140–4. [PubMed: 3485282]
39. Xie L, Kang H, Xu Q, et al. Sleep drives metabolite clearance from the adult brain. Science. 2013 Oct 18; 342(6156):373–7. [PubMed: 24136970]
40. Hansson O, Zetterberg H, Vanmechelen E, et al. Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. Neurobiology of aging. 2010 Mar; 31(3):357–67. [PubMed: 18486992]
41. Mehta PD, Pirttila T, Patrick BA, Barshatzky M, Mehta SP. Amyloid beta protein 1–40 and 1–42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease. Neuroscience letters. 2001 May 18; 304(1–2):102–6. [PubMed: 11335065]

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42. DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. Science. 2002 Mar 22; 295(5563):2264–7. [PubMed: 11910111]

43. Das R, Nachbar RB, Edelstein-Keshet L, et al. Modeling effect of a gamma-secretase inhibitor on amyloid-beta dynamics reveals significant role of an amyloid clearance mechanism. Bulletin of mathematical biology. 2011 Jan; 73(1):230–47. [PubMed: 20411345]

44. Banks WA, Kumar VB, Farr SA, Nakaoke R, Robinson SM, Morley JE. Impairments in brain-to-blood transport of amyloid-beta and reabsorption of cerebrospinal fluid in an animal model of Alzheimer's disease are reversed by antisense directed against amyloid-beta protein precursor. Journal of Alzheimer's disease : JAD. 2011; 23(4):599–605.

45. Tarnaris A, Toma AK, Chapman MD, et al. Rostrocaudal dynamics of CSF biomarkers. Neurochemical research. 2011 Mar; 36(3):528–32. [PubMed: 21191652]

46. Bjerke M, Portelius E, Minthon L, et al. Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. International journal of Alzheimer's disease. 2010; 2010

47. Vanderstichele H, Bibl M, Engelborghs S, et al. Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: a consensus paper from the Alzheimer's Biomarkers Standardization Initiative. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2012 Jan; 8(1):65–73.

48. Johanson CE, Duncan JA 3rd, Klinge PM, Brinker T, Stopa EG, Silverberg GD. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. Cerebrospinal fluid research. 2008; 5:10. [PubMed: 18479516]

49. Hurley PJ. Red cell and plasma volumes in normal adults. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 1975 Jan; 16(1):46–52.

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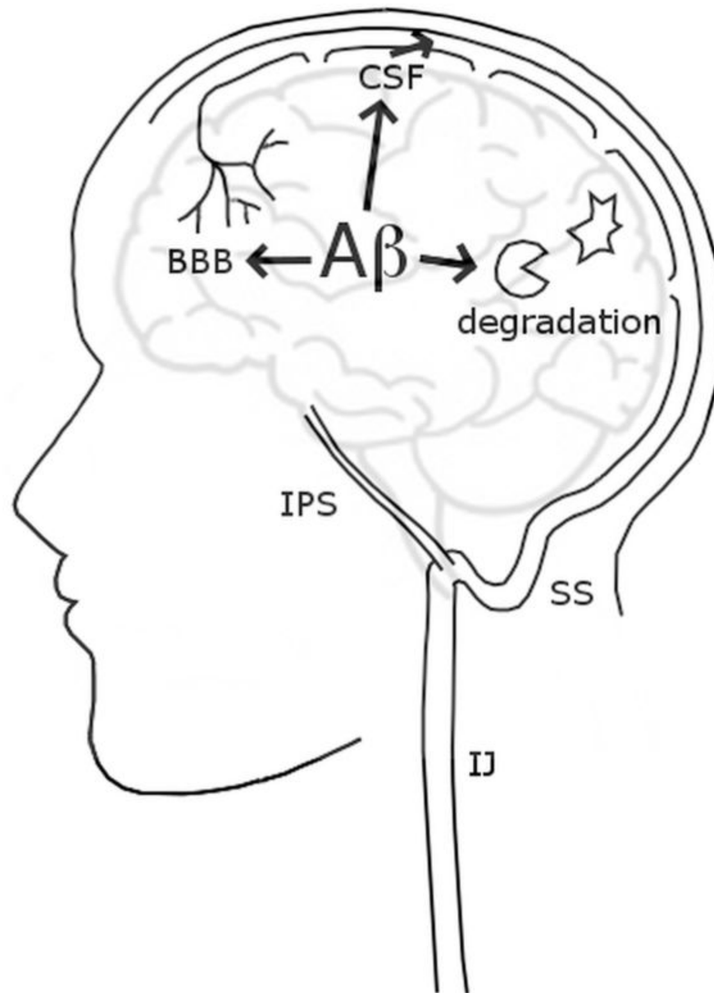

Figure 1.

A diagram of the central venous system including sampling sites for central Aβ measurements. CNS Aβ is removed by a variety of mechanisms including direct transport across the BBB into the venous blood, transport to the CSF with subsequent reabsorption into the venous blood, and degradation by phagocytes and enzymes. IPS= Inferior Petrosal Sinus, SS= Sigmoid Sinus, IJ= Internal Jugular

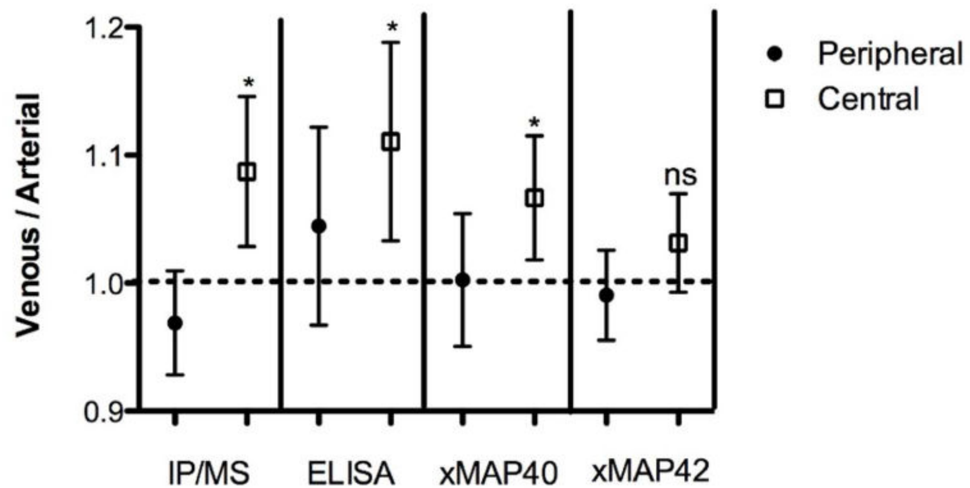

Figure 2.

Ratios of venous to arterial plasma Aβ concentrations by 3 assays: IP/MS, ELISA, and xMAP. Central indicates veins draining the brain capillary bed and includes measurements from the internal jugular vein (IJ), sigmoid sinus (SS), left inferior petrosal sinus (IPSL), and right inferior petrosal sinus (IPSR). Peripheral represents measurements from the peripheral femoral vein (PV). *$p < 0.01$, ns = not significant. Error bars denote 95% confidence intervals.

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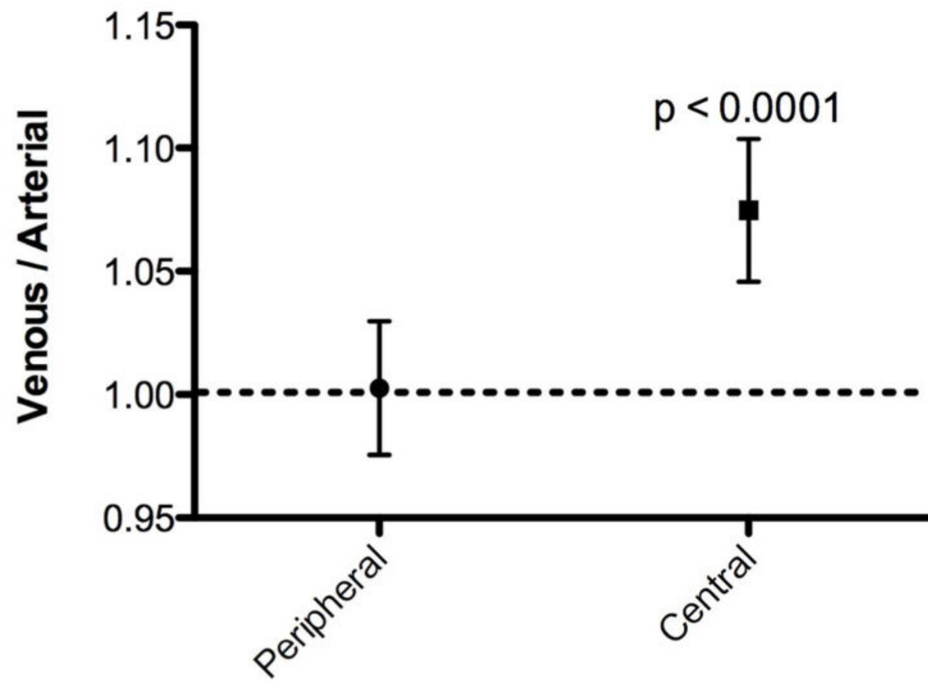

Figure 3.

Combined assay results. Central includes measurements from the internal jugular (IJ), sigmoid sinus (SS), left inferior petrosal sinus (IPSL), and right inferior petrosal sinus (IPSR). Peripheral represents measurements from the femoral vein (PV). Error bars denote 95% confidence intervals.

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Table 1

Values Used for Modeling

Quantity	Estimate
CSF concentration of Aβ peptides ($[A\beta]_{CSF}$)	12.4 ng/ml[2]
CSF reabsorption rate	0.4 ml/min[28]
Cerebral blood flow rate ($Q_{cerebral}$)	715 ml/min[28]
Adult plasma volume	3.3 L[49]
Plasma concentration of Aβ peptides	264 pg/ml
V-A difference across the brain	13.6 pg/ml