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Peripheral Amyloid- β Levels Regulate Amyloid- β Clearance from the Central Nervous System

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Abstract

Amyloid- β (A β) is cleared from the brain by both proteolytic digestion and transport across the blood-brain-barrier into the peripheral circulatory system. To investigate the role peripheral A β levels play in regulating A β brain clearance, we measured the clearance of [¹²⁵I]-A β _{1–40} injected into the brains of liver-ligated rats that allowed peripheral A β levels to be maintained at elevated levels for approximately one hour with/without a single peripheral bolus of unlabeled A β _{1–40}. We found that elevating peripheral A β levels significantly decreased [¹²⁵I]-A β _{1–40} brain clearance, thus supporting the hypothesis that peripheral A β levels regulate A β clearance from the central nervous system.

Keywords

Alzheimer's disease; blood-brain-barrier; hepatic clearance; liver; passive immunization; peripheral sink

INTRODUCTION

Molecules that bind amyloid- β (A β) in plasma are sometimes referred to as A β sinks [1,2] and have been shown to alter A β levels in the brain [3–5]. How these A β -sequestering strategies work is not well understood [1]. One hypothesis is that by reducing the amount of bio-available A β in plasma, the dynamic equilibrium between A β in the periphery versus the central nervous system (CNS) shifts to favor increased clearance from the brain [2]. Consistent with this idea are data showing that peripherally injected soluble lipoprotein

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receptors and A β antibodies reduce free A β levels in plasma and correspondingly reduce A β burden in the CNS [3,5]. However, there are also data that show increased plasma A β concentrations do not alter [125 I]-A β_{1-40} efflux from the brain [6].

Addressing this issue directly is challenging from the standpoint of measuring clearance of known amounts of A β in the CNS while at the same time limiting peripheral A β disposal, a process that occurs within minutes. In this regard, several reports indicate the liver plays an important role in peripheral A β clearance [7–9]. We therefore reasoned that if liver-mediated clearance were transiently disrupted, it might be possible to hold or “clamp” peripheral A β levels long enough to determine the effects of elevating plasma A β on the clearance of [125 I]-A β_{1-40} injected into the brain.

MATERIAL AND METHODS

Surgical procedures

Experiments were approved by the VAPSHCS institutional animal care and use committee (IACUC). Sprague Dawley male rats were randomly assigned a sham surgery or liver ligation. Following isoflurane anesthesia both jugular veins were cannulated and the portal vein and hepatic artery (celiac axis) were ligated, while control animals received loose ligatures that did not restrict blood flow. A 2 μ Ci bolus of human [125 I]-A β_{1-40} (Amersham, Piscataway, NJ) supplemented with 10 μ g of unlabeled human A β_{1-40} (Bachem, Torrance, CA) in 0.1% BSA/10 μ -units/ml $^{-1}$ heparin/0.9% saline was injected into the right jugular vein followed by 200 μ l of heparin/saline solution. Blood (200 μ l) was collected from the left jugular vein. Plasma proteins were precipitated with 20% Trichloroacetic acid to determine both intact and degraded [125 I]-A β_{1-40} levels using a gamma counter (CobraII, Packard Bell). In brain clearance experiments (see below), animals undergoing liver ligations also received [125 I]-A β_{1-40} /[14 C]-inulin brain injections where animals were divided in two groups, one receiving and one not receiving a bolus injection of 10 μ g of unlabeled A β_{1-40} in 200 μ l of saline via jugular catheter over 30 seconds followed by 200 μ l of saline to ensure complete delivery of the bolus.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using statistical moment analysis [10]. The area under the concentration versus time curve (AUC) was calculated for each plasma concentration (Cp) versus time (t) using the trapezoidal method. The terminal slope for each data set was estimated by log-linear regression after visually identifying the terminal portion of each curve. The mean residence time (MRT) was calculated as AUMC/AUC with AUMC as the area of the Cp*t versus t curve. Whole body clearance (Cl) was calculated as dose/AUC.

Determining A β Brain Clearance

Measuring [125 I]-A β_{1-40} clearance from the brain followed an established brain efflux index (BEI) method [11]. Briefly, Sprague Dawley male rats (200–250 grams) were anesthetized with isoflurane and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA.) The brain injectate (1 micro liter) comprised 0.02 μ Ci [125 I]-A β_{1-40} (Phoenix Pharmaceuticals, Burlingame, CA) and 0.01 μ Ci [14 C]-inulin (PerkinElmer, Shelton, CT), used as a reference tracer molecule to account for non-specific diffusion or disruption of the blood brain barrier (BBB), dissolved in 122 mM NaCl, 25 mM NaHCO $_3$, 3 mM KCl, 1.4 mM CaCl $_2$, 1.2 mM MgSO $_4$, 0.4 K $_2$ mM HPO $_4$, 10 mM D-glucose and 10 mM HEPES, pH 7.4. Each animal received a 1 μ l injection into both the ipsilateral and contra-lateral hemispheres of parietal cortex (0.2 mm anterior and 5.5 mm lateral to the bregma at a depth of 4.5 mm) using a 5 μ l microsyringe mounted in an ultramicro-syringe pump system

(World Precision Instruments, Sarasota, FL). Prior to this, we verified that no radiolabeled compounds diffused into the non-injected hemisphere by injecting only one hemisphere and then counting the radiolabel in the injected and non-injected hemispheres. No radiolabel was detected in the non-injected hemisphere. By convention, the right (ipsilateral) cortex was injected 70 minutes before the contra-lateral cortex. Immediately following the contra-lateral injection, the animals were sacrificed and both hemispheres were excised. A multi-channel scintillation counter (Tri-Carb 2300TR; Packard Instruments) measured [^{125}I] counts at 0–45 kilo electron volts (keV) and [^{14}C] counts at 46–156 keV. The BEI(%) is defined as the relative percentage of Test drug (T, [^{125}I]-A β_{1-40}) in the brain compared to the Reference drug (R, [^{14}C]-inulin) in the brain after 70 minutes and is described by the formula: $\text{BEI}(\%) = [1 - ((T_{70}/R_{70}) / (T_0/R_0))] \times 100$ with T_0 and R_0 representing the total amounts of test and reference compounds injected into the brain. Decreased BEI(%) indicated impaired A β clearance from the brain. Statistical comparisons were performed using analysis of variance (ANOVA).

RESULTS

We predicted that blocking liver-mediated A β disposal would permit a single peripheral injection of A β_{1-40} to elevate circulating plasma A β_{1-40} levels long enough to test the hypothesis that altering the A β concentration gradient between the CNS and the periphery changes A β clearance from the brain.

First, to test the utility of this approach, we measured peripheral clearance of [^{125}I]-A β_{1-40} injected directly into the blood stream of anesthetized rats whose liver-mediated A β disposal was occluded by ligating the portal vein and celiac artery. Figure 1 shows that liver ligation, compared to sham surgery, caused an immediate and significant reduction in the A β_{1-40} clearance rate from plasma. The mean clearance rate was calculated as 22.8 ml/kg/min $\pm$ 6.30 for liver ligated animals and 1.27 ml/kg/min $\pm$ 0.36 for liver intact animals ($p < 0.001$). Liver ligation also produced a significant increase in the mean residence time (mean MRT = 88.3 min $\pm$ 16.6 with liver ligated; mean MRT = 41.0 min $\pm$ 6.9 with liver intact; $p < 0.01$).

Having shown that liver ligation markedly retards peripheral A β_{1-40} clearance, we then examined the effect of elevating plasma A β levels on A β clearance from the CNS under liver-ligated conditions. To accomplish this we used the BEI method [11] to measure the specific clearance of [^{125}I]-A β_{1-40} injected into the parietal cortex of rats. Figure 2 shows that with the liver ligated, a single bolus injection of unlabeled A β_{1-40} (10 μg) into the peripheral blood supply caused a statistically significant 40.7% decrease in the amount of [^{125}I]-A β_{1-40} cleared from the brain compared to liver-ligated, untreated control animals ($p < 0.01$).

DISCUSSION

A β is generated by many cell types throughout the body (including circulating platelets and leukocytes [12]), and traffics both in and out of the brain via receptor-mediated processes [2,13]. The biological significance of this dynamic relationship between CNS and peripheral A β pools has been underscored by data showing that peripheral A β -binding compounds reduce A β in the brain [3,4,14]. Current evidence supports a number of different, but not necessarily mutually exclusive, potential mechanisms that may help explain how these manipulations enhance A β clearance from the brain [1]. For example, peripherally injecting A β -binding antibodies reduces free plasma A β in a mouse model of AD [3], suggesting that increasing the A β concentration gradient between the brain and the periphery thermodynamically drives A β out of the brain. However, in these chronic treatment strategies other potential processes may also influence A β brain clearance, including

increased CNS amyloid phagocytosis or altered oligomeric A β stability [1]. Further-more, investigators failed to detect a change in CNS [125 I]-A β_{1-40} clearance following peripheral A β bolus injections [6]. This finding, which contrasts with the current report, may be explained by methodological differences. We measured clearance of [125 I]-A β_{1-40} injected directly into the cortical parenchyma whereas in the previous study [6], [125 I]-A β_{1-40} was delivered to the CNS indirectly by injecting radiolabeled A β_{1-40} into the periphery. The latter approach would make it challenging to distinguish between the clearance of [125 I]-A β_{1-40} accumulated in luminal BBB domains versus that which had entered the CNS parenchyma.

In the current study, we examined CNS A β clearance under acute, short-term conditions where the effects of increased A β plasma levels would, in principle, be more easily addressed. To accomplish this it was also necessary to simultaneously retard peripheral A β disposal. With the liver occluded from the circulatory system, we found that elevating plasma A β levels significantly reduced that rate at which [125 I]-A β_{1-40} was cleared from the CNS. In addition to addressing the role peripheral A β levels play in regulating CNS A β clearance, these data also support and extend previous findings [7–9] by illustrating the substantial contribution of the liver to peripheral A β clearance. To the extent the peripheral circulatory system can act as an A β sink, these data demonstrate that the liver is the primary drain.

In keeping with the majority of previously published studies that have examined *in vivo* A β clearance from the CNS, we focused on A β_{1-40} because it is the most abundant A β species in plasma, and since both A β_{40} and A β_{42} share the same transporter at the BBB [15], A β_{1-40} is likely to be representative of the family of circulating A β peptides. Importantly, A β_{1-40} is much less likely than A β_{1-42} to form oligomers/aggregates that would alter functional circulating A β concentrations in ways that would complicate interpreting these findings in terms of concentration gradients [1].

These results do not address the specific mechanism(s) by which peripheral A β concentrations influence A β clearance from the brain. One possibility is that elevated A β concentrations reduce cerebral blood flow [16] or promote degradation of receptors involved in A β efflux from the brain [14]. Nonetheless, these data are consistent with the idea that central-peripheral A β concentration gradients regulate A β brain clearance. Moreover, these results highlight the potential significance of taking advantage of both the liver's greater pharmacological accessibility (compared to the brain) and its dominating capacity to regulate the peripheral A β pool in order to devise new strategies to combat AD.

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Fig. 1.

The mean retention time of [^{125}I]- $\text{A}\beta_{1-40}$ injected directly into the circulating blood stream of rats receiving surgical ligation of the portal vein and celiac artery ($N = 5$) was significantly reduced ($p < 0.001$) compared to non-ligated controls ($N = 3$). Error bars represent standard error of the mean (SEM).

Fig. 2.

Brain efflux index BEI(%) indicates specific clearance of [125 I]-A β_{1-40} injected into parietal cortex. BEI(%) was significantly reduced ($p < 0.01$) in liver-ligated animals receiving concomitant peripheral injections of unlabeled A β_{1-40} (closed histogram, $N = 5$) compared to liver-ligated untreated controls (Con, open histogram, $N = 5$). Error bars represent standard error of the mean (SEM).