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Fractional synthesis and clearance rates for amyloid β

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To the Editor

We are writing regarding the 2006 publication of a new stable isotope labeling kinetics (SILK) protocol for assessing the fractional synthesis and clearance rates of amyloid β (A β) in the brain¹. This protocol has been used to demonstrate the relatively accelerated rate of turnover of A β peptides in the brain¹ and to assess the effect of drugs on A β production². However, we are concerned that aspects of the protocol have not been fully appreciated, raising questions about the interpretation of findings from the protocol.

Our main concern is the estimation of fractional clearance rates for A β . In the Alzheimer's disease literature, clearance usually refers to degradation or removal of A β by proteolytic enzymes, microglia and blood-brain barrier transport. Several studies have assumed that clearance rates measured by the SILK protocol reflect these factors^{1–3}, but we suggest this is not the case. Understanding the distinction between clearance of A β and the phenomenon measured by the SILK protocol requires knowledge of details of the protocol. In brief, the protocol¹ consists of a production phase, during which ¹³C₆-labeled leucine (¹³C₆-leucine) is infused intravenously and the proportion of synthesized and secreted A β labeled with ¹³C₆-leucine at amino acid 17 is measured, and a clearance phase, during which the proportion of A β labeled with ¹³C₆-leucine is monitored for some hours after cessation of the peripheral infusion. Cerebrospinal fluid (CSF) is sampled with a catheter inserted between the third and fourth lumbar vertebrae, with hourly sampling for 36 h. During peripheral ¹³C₆-leucine infusion, approximately 10% of leucine available for protein synthesis is ¹³C₆ labeled. The rate at which the proportion of A β labeled with ¹³C₆-leucine approaches the saturation proportion of 10% provides an estimate of the synthesis rate of A β ¹. Interpretation of changes in this proportion after the production phase is less clear. The key observation in regard to our above concern is that the protocol does not measure the absolute amount of newly synthesized A β but rather the relative proportion of A β labeled with ¹³C₆-leucine at amino acid 17 (ref. 1). Because the processes of degradation or removal of A β by proteolytic enzymes, microglia and blood-brain barrier transport do not distinguish between labeled and unlabeled A β , they do not influence the relative proportion of these molecules in fluids leaving the parenchyma (Fig. 1). The SILK protocol measures

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proportions, and therefore findings by the SILK protocol do not reflect the influence of parenchymal clearance on A β levels.

What does the clearance rate obtained by the SILK protocol measure? We have implemented computer models to characterize this aspect of the protocol. (Supplementary Methods). As originally described¹, clearance rates are estimated using data from hours 24 through 36 of the protocol. By hour 24 of the protocol, neuronal APP amyloid precursor protein and secreted will have turned over one or more times since the end of the infusion, and the proportion of newly secreted A β originating within the brain that is labeled will approach zero. Nonetheless, a measurable fraction of labeled A β remains within the substantial volume of CSF contained in the subarachnoid space at this time. This fraction declines logarithmically (as described in the original protocol¹). Logarithmic decline is consistent with expectation for a process of dilution by bulk flow and turnover of CSF. For example, a person with a CSF volume of 150 ml and production of CSF of 20 ml h⁻¹ at the choroid plexus and other brain sites will clear CSF labeled A β at a rate of 20/150 (13.33%) per hour (Supplementary Fig. 1). That is, rates measured at the clearance phase of the SILK protocol reflect CSF turnover. We conclude that there is little reason to extend CSF sampling beyond the production phase of the protocol and that time and resources could be saved, and study subject burden avoided, by ending the protocol at hour 15.

Our second concern is that fractional synthesis rate estimates by the protocol are underestimates of the actual synthesis rates. This is because the mixture of labeled and unlabeled A β molecules in fluids leaving the brain is diluted by a large volume of CSF containing only unlabeled A β before being sampled at the lumbar spine (Fig. 1). Dilution slows the rate at which the proportion of A β that is labeled approaches 10% saturation. We have demonstrated by computer simulation that this downward bias may be substantial (Supplementary Results). Moreover, the downward bias is a function of CSF volume, such that persons with larger CSF volume have a larger downward bias (Supplementary Fig. 1). This is relevant to the recent observation that synthesis rate estimates by the SILK protocol are comparable in individuals with sporadic Alzheimer's disease and age-match controls³. On the basis of the greater volume of circulating CSF in people with Alzheimer's disease, one would expect slower observed rates in affected individuals and faster observed rates in controls if synthesis rates were comparable in these two groups (Supplementary Fig. 2). The lack of an observed difference³ may simply reflect small sample size and type II error. Alternatively, if this finding holds in larger samples, it would be consistent with higher neuronal A β synthesis rates in sporadic Alzheimer's disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Bateman RJ et al. Nat. Med. 12, 856–861 (2006). [PubMed: 16799555]
2. Bateman RJ et al. Ann. Neurol. 66, 48–54 (2009). [PubMed: 19360898]
3. Mawuenyega KG et al. Science 330, 1774 (2010). [PubMed: 21148344]

Figure 1.

During the infusion of $^{13}\text{C}_6$ -labeled leucine, the fraction of labeled Aβ secreted by neurons increases from 0% to a theoretical maximum of 10% at the neuronal fractional synthesis rate. Within the brain, secreted molecules are cleared by processes that do not discriminate labeled from unlabeled molecules and do not influence the fraction that is labeled. Labeled molecules leaving the brain are diluted by a large volume of CSF containing unlabeled Aβ before being sampled from the lumbar subarachnoid space.