2014

Pilot Data on Brain-to-Blood Efflux of B-Amyloid Peptides in Man

Steve Meaney
Dublin Institute of Technology, steve.meaney@dit.ie

Maura Heverin
Karolinska Institute

Ingemar Bjorkhem,
Karolinska Institute

Dorotea Religa
Karolinska Institute

John Wahren
Karolinska Institute

See next page for additional authors

Authors
Steve Meaney; Maura Heverin; Ingemar Bjorkhem,; Dorotea Religa; John Wahren; and Ulf Diczfalusy

2014

Pilot data on brain-to-blood efflux of b-amyloid peptides in man

Steve Meaney

Maura Heverin

Ingemar Bjorkhem

Dorotea Religa

John Wahren

See next page for additional authors

Authors
Steve Meaney, Maura Heverin, Ingemar Bjorkhem, Dorotea Religa, John Wahren, and Ulf Diczfalusy

Pilot data on brain-to-blood efflux of β-amyloid peptides in man

Heverin, M.[1], Religa, D.[1], Wahren, J.[2], Diczfalusy, U.[1], Björkhem, I.[1], **Meaney, S.**[3]

1 Department of Laboratory Medicine, Karolinska University Hospital - Huddinge, Sweden; 2 Department of Surgical Sciences, Karolinska University Hospital - Solna, Sweden; 3 School of Biological Sciences, College of Sciences and Health, Dublin Institute of Technology – Kevin Street, Dublin 8

Background to the study

- Alzheimer's disease (AD) is the most common cause of dementia and affects nearly 40,000 individuals in Ireland.
- The β-amyloid peptide (Aβ) plays a key role in the pathogenesis of the AD and the presence of Aβ plaques in the brain is diagnostic.
- The hypothesis posits that Aβ deposition is a critical factor in the disease process and that production and clearance of Aβ are key drivers of the disease[1].
- Flux of Aβ from the brain is believed to contribute to the overall level of Aβ within in brain[2] and antibody mediated brain-to-blood efflux has been observed in animal models[3].
- Clearance of from the blood is believed to be mainly via the liver, kidney and spleen[4].
- Data from human studies indicate that the about 6% of the Aβ pool present in the cerebrospinal fluid is cleared per hour[5].
- There are no data available on the magnitude of the cerebral output of Aβ peptides in man or the hepatic uptake.
- The aim of this work was to investigate if the concentration Aβ peptides is different in jugular venous plasma and arterial plasma and so estimate direct values for both brain-to-blood Aβ efflux and hepatic clearance in man.

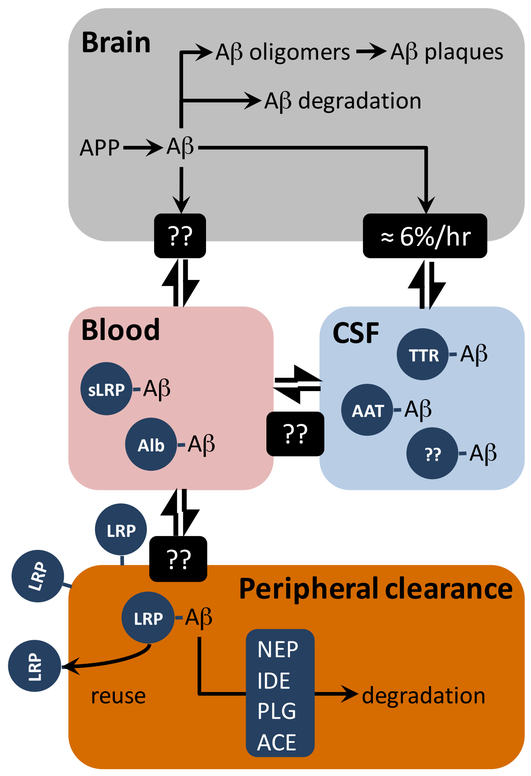

Figure 1. Model of Aβ fluxes in man. Aβ produced in the brain passes into the blood, either directly across the blood-brain barrier or via the cerebrospinal fluid (CSF). It is carried in a complex with numerous different proteins. The liver, kidney and spleen can take up and metabolise Aβ via various proteases. LRP1=low density lipoprotein receptor related protein 1; Alb=albumin; TTR=transthyretin; AAT=α1-antiprotease; NEP= nephyrilisin; IDE=insulin degrading enzyme; PLG=plasmin; ACE=angiotensin converting enzyme

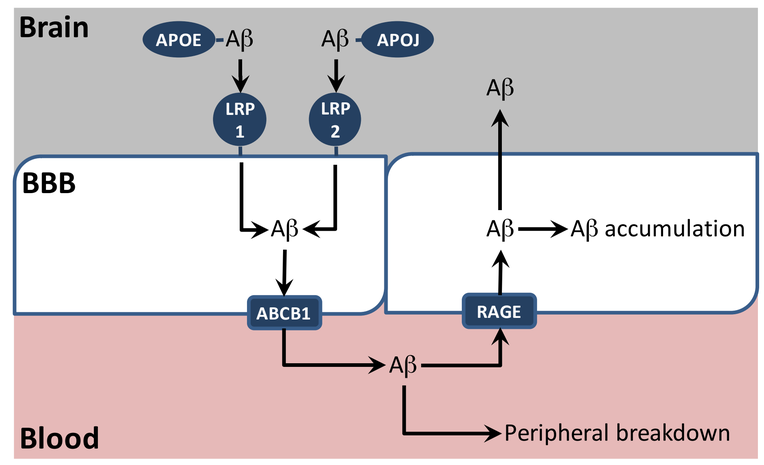

Figure 2. Mechanism of transport of Aβ across the blood-brain barrier. Aβ produced in the brain becomes bound to apolipoproteins within the brain interstitial fluid. Brain microvascular endothelial cells can take up APOE-Aβ and APOJ-Aβ complexes via mechanisms dependent on LRP1 or LRP2, respectively. Intracellular Aβ may then be effluxed into the circulation via a process involving ABCB1 where the Aβ may either be degreaded peripherally or become subjected to RAGE dependent uptake. This latter process leads to the re-entry of Aβ into the brain and is effectively a futile cycle. APOE=apolipoprotein E; APOJ=apolipoprotein J; LRP1=low density lipoprotein receptor related protein 1; LRP2=low density lipoprotein receptor related protein 2; ABCB1=ATP binding cassette transporter B1; RAGE=receptor for advanced glycation endproducts.

Experimental Methods

- Blood samples were obtained as described[6].

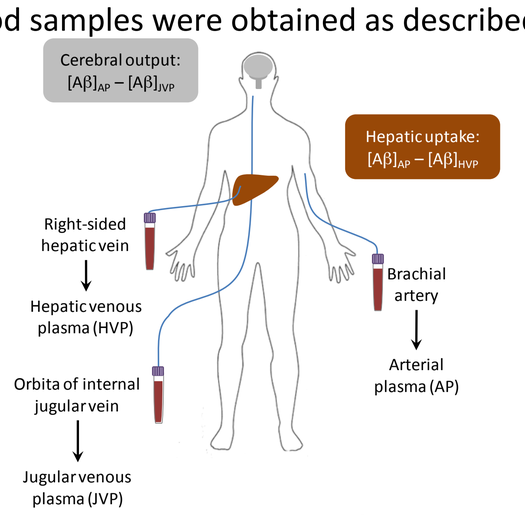

Figure 3. Sampling strategy for organ specific arterial and venous plasma. Blood samples were taken simultaneously from catheters inserted percutaneously and positioned as above.

- **Plasma samples**: These were available in connection with a previous study on brain sterol fluxes[6]. Briefly, ten healthy males, mean age 29 years (range, 21–38 years) were recruited for this study and blood samples were taken after an overnight fast. Plasma was frozen at -80°C until required for analysis.
- **Ethics**: All experiments involving human volunteers were reviewed and approved by the ethics committees at the Huddinge Hospital and the Karolinska Hospital. Participants gave informed consent to participate in the study
- **ELISA for Aβ**: Specific antibodies against $Aβ_{x-40}$ and $Aβ_{x-42}$ were used as primary antibodies. The reporter antibody was horseradish-peroxidase-linked anti-rabbit IgG and colour was developed with o-phenylenediamine. The detection limit for synthetic $Aβ_{x-40}$ and $Aβ_{x-42}$ was 1 pM. All samples were analyzed in the linear range of the ELISA.

Data Analysis: The concentration of $Aβ_{x-40}$ and $Aβ_{x-42}$ in the arterial and venous plasma was compared using a non-parametric approach. The significance level was set at 0.05. The molar concentrations of $Aβ_{x-40}$ and $Aβ_{x-42}$ were calculated. The percent extraction of individual Aβ species was calculated according to:

$$\text{Percent extraction} = [(C_a\text{-}C_v)/C_a]\ x\ 100$$

Negative values were considered to represent a net output. Daily fluxes were estimated according to:

$$\text{Daily extraction} = (C_a\text{-}C_v)\ x\ \text{organ plasma flow}$$

For the purposes of this calculation the cerebral and hepatic plasma flow were set at 650L.d^{-1} and 1000 L/ d^{-1} respectively.

Results

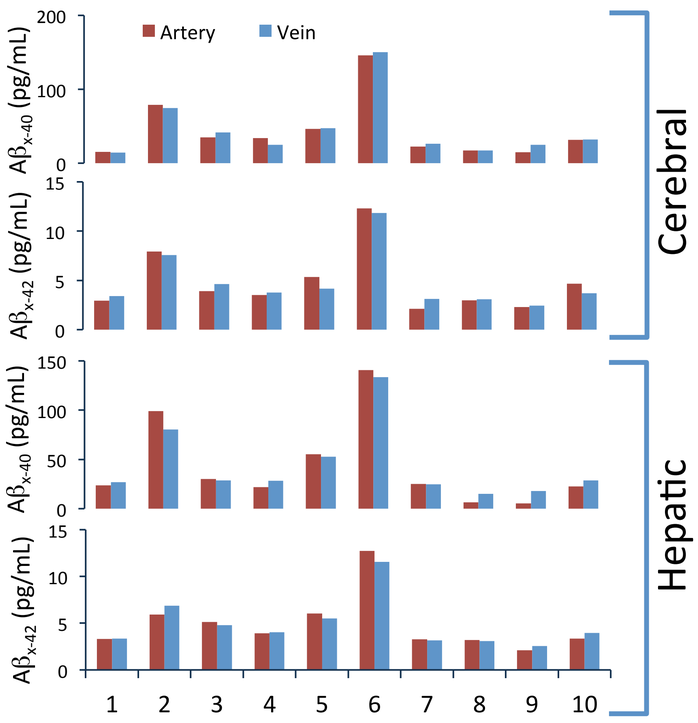

Figure 4. Paired absolute values of $Ab_{x-40/42}$ in cerebral and hepatic plasma. In the cerebral circulation, greater concentration in the vein is consistent with output while the opposite applies in the hepatic circulation. Each number corresponds to an individual participant.

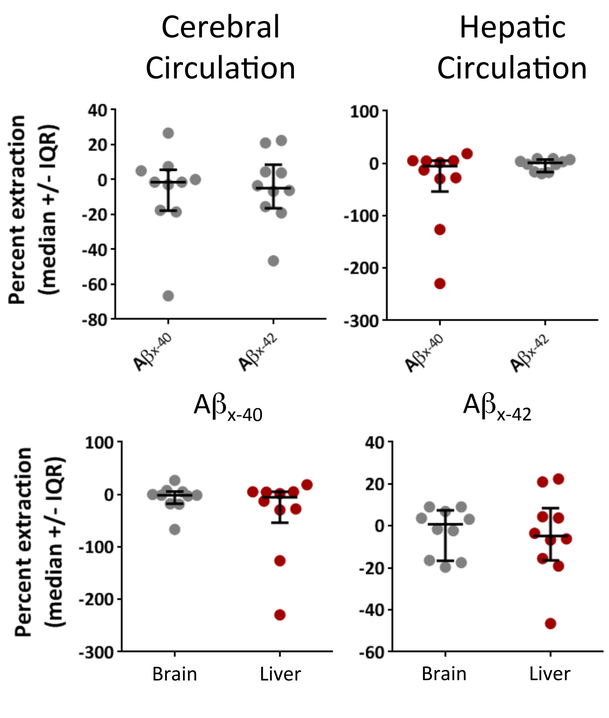

Figure 5. Absolute values of $Ab_{x-40/42}$ in cerebral and hepatic plasma and inter oRgan fluxes. In the cerebral circulation a negative percent extraction is equivalent to an output while in the hepatic circulation the opposite applies. No statistically significant difference were found using the Wilcoxon matched-pairs signed rank test.

Discussion

- This is the first attempt to directly quantify the brain-to-blood passage of Aβ in man. The daily cerebral output of $Aβ_{x-40}$ was estimated to be ≈ 1ng.d^{-1} and that of of $Aβ_{x-41}$ was estimated to be ≈ 3ng.d^{-1}.
- Although the data was not statistically significant the values are in reasonable agreement with data from a transgenic rat model of ≈ 1.6ng.d^{-1} for $Aβ_{x-40}$.
- There are two main limitations to this work:
  i) The main limitation in the current study is the small number of samples available which has affected the power of the study.
  ii) A further limitation is that the material analysed was collected in connection with a previous study on brain sterol homeostasis.
- Given the paucity of the data available we considered it prudent to commence these investigations on a pilot basis and use the data to design larger studies.
- Based on the data available in connection with this study we estimate that a sample size of 40 would be required to have an 80% power to detect a difference in percent extraction of 13.5%.
- While this is an ambitious number of participants for a relatively invasive procedure we believe that the data generated would be very valuable for the field

References

1. Hardy, J, (2009) J Neurochemistry, 110:1129-34; 2. Deane, R *et al* (2009) CNS Neurol Disord Drug Targets, 8:16-30; DeMattos, R. (2002) Science, 295:2264-67;
4. Kandimalla, K (2005) J Phaemacol Exp Ther, 323:1370-78; 5. Huang, Y et al (2012), Arch Neurol, 69:1591-7; 6. Meaney, S et al (2007) J Lipid Res, 48:994-51