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Evidence that a synthetic amyloid-ß oligomer-binding peptide (ABP) targets amyloid-ß deposits in transgenic mouse brain and human Alzheimer's disease brain

Balu Chakravarthy *, Shingo Ito, Trevor Atkinson, Chantal Gaudet, Michel Ménard, Leslie Brown, James Whitfield

Human Health Therapeutics, National Research Council of Canada, Ottawa, Ontario, Canada

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ABSTRACT

The synthetic ~5 kDa ABP (amyloid-ß binding peptide) consists of a region of the 228 kDa human pericentrioloar material-1 (PCM-1) protein that selectively and avidly binds *in vitro* $A\beta_{1-42}$ oligomers, believed to be key co-drivers of Alzheimer's disease (AD), but not monomers (Chakravarthy et al., (2013) [3]). ABP also prevents $A\beta_{1-42}$ from triggering the apoptotic death of cultured human SHSY5Y neuroblasts, likely by sequestering Aß oligomers, suggesting that it might be a potential AD therapeutic. Here we support this possibility by showing that ABP also recognizes and binds $A\beta_{1-42}$ aggregates in sections of cortices and hippocampi from brains of AD transgenic mice and human AD patients. More importantly, ABP targets $A\beta_{1-42}$ aggregates when microinjected into the hippocampi of the brains of live AD transgenic mice.

1. Introduction

The ~5 kDa amyloid-ß (Aß) binding peptide, ABP, is based on a region of the 228 kDa pericentriolar material-1 (PCM-1) protein [3]. The PCM-1 protein is involved in several centrosomal functions that include cell cycle transit, cilium formation, neuron migration and dendrite patterning [1,6,9,13,14]. The development of ABP was prompted by our discovery of two novel PCM-1 activities which are tandemly encoded in the KDKTPKSKSKKRNSTQLKSRVKNI sequence in the 1291–1323 region of the human PCM-1 protein (GenBank: AAA60120.1). The first of two previously unknown PCM-1 activities (encoded in the protein's KRNSTQLKSRVKNI sequence) is the ability to inhibit PKC activity [2]. The second activity, encoded in the protein's KDKTPKSKSK sequence, is the ability to selectively and avidly bind $A\beta_{1-42}$ oligomers but not $A\beta_{1-42}$ monomers or $A\beta_{1-40}$ the other major Aβ peptide which does not form oligomers as effectively as $A\beta_{1-42}$ [3]. However, we do not know how either inhibiting PKCs or binding $A\beta_{1-42}$ oligomers might contribute to PCM-1's several functions *in vivo*.

Regardless of the as yet unknown role of the KDKTPKSKSK sequence in PCM-1's functions, an $A\beta_{1-42}$ oligomer–binding peptide containing it could be a therapeutic for treating Alzheimer's disease (AD) that might prevent $A\beta_{1-42}$ oligomers from accessing their target proteins [3]. These $A\beta_{1-42}$ oligomers are known to accumulate in the brains of human AD patients and Tg-AD mice (transgenic AD-model mice) and are believed to be one of the two principal co-drivers of AD pathology [7,11,12,15]. Therefore we synthesized the ~5 kDa ABP peptide bearing the KDKTPKSKSK sequence that selectively binds $A\beta_{1-42}$ oligomers, but not $A\beta_{1-42}$ monomers or the poorly aggregating $A\beta_{1-40}$ *in vitro* [3].

While ABP binds "pure" synthetic $A\beta_{1-42}$ oligomers in ELISA and Western blot assays, can it target the $A\beta_{1-42}$ aggregates that accumulate in the complex surroundings of human and Tg-AD murine AD brains? Here we show that ABP does bind $A\beta_{1-42}$ aggregates in sections of cerebral cortices and hippocampi from AD-transgenic mice and when microinjected into the hippocampi of these mice. Most importantly we will show that ABP also binds to similar $A\beta_{1-42}$ aggregates in cerebral cortices and hippocampi in human post-mortem brains from AD patients [4].

2. Materials and methods

2.1. Materials

N-terminal FITC-amyloid-binding peptide (FITC-ABP) was synthesized by CanPeptide (Pointe-Claire, QC, Canada). Alexa

* Corresponding author. Address: Human Health Therapeutics, National Research Council, 1200 Montreal Rd., M-54, Ottawa, ON K1A 0R6, Canada. Fax: +1 613 990 0899.
E-mail address: Balu.Chakravarthy@nrc-cnrc.gc.ca (B. Chakravarthy).

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488–6E10 monoclonal anti-Aβ antibody was obtained from Covance Research Products Inc. (Emeryville, CA).

Double transgenic AD mice (B6.Cg-Tg) harboring PSEN1dE9 and APP_{Swe} transgenes that accumulate oligomeric $Aß_{1-42}$ aggregates and their corresponding wild type strain C57BL6 that do not were obtained from Jackson Laboratory and maintained at NRC. All animal studies were approved by NRC Institute's Animal Care Committee.

2.2. Human brain tissues (cognitively normal and AD patients)

Frozen post-mortem human brain hippocampal and frontal cortex tissues from aged (63–91 years), but cognitively normal (normal), and AD patients (73–93 years) were obtained from brain banks maintained at Douglas Hospital Research Centre Brain Bank, McGill University, Montreal QC, Canada and at the Maritime Brain and Tissue Bank (MBTB), Dalhousie University, Halifax, NS, Canada. Further information on these human tissues may be found in Chakavarthy et al. [4].

Procurement and handling of all human samples were approved by National Research Council's Ethics Committee.

2.3. Immunohistological studies

Frozen hemi-brains from mice and human hippocampal and cortical tissues were embedded in OCT and 10-μm sections were prepared using a Jung CM 3000 cryostat and stored at −80 °C until use. Tissue sections were thawed and OCT peeled from sections with a razor blade and then incubated with Dako protein blocking reagent for 30 min at room temperature. Blocking agent was removed and sections were gently washed in TBS. Synthetic FITC-ABP (1:250 dilution of 5.0 μg/μl solution) or Alexa 488-6E10 (1:500 dilution) in antibody diluent was added and incubated for 1 h at room temperature. Sections were then washed twice with TBS, rinsed in Milli Q water, excess rinse solution removed and sections were cover-slipped with Dako Fluorescent Mounting Media.

2.4. Microinjection studies

The *in vivo* microinjection studies were performed using the intracerebral microinjection technique as described before [10] and followed the standard operating procedure approved by the Institute's Animal Care Committee. Briefly, mice were anesthetized with isoflurane and placed in a stereotaxic frame (David Kopf, Tujunga, CA, USA) set to the coordinates of the hippocampus in the mouse brain. A small hole was made 2.0 mm lateral, −2.3 mm anterior to the bregma, and a fine injection needle fitted on a 5.0 μL microsyringe (Hamilton, Reno, NE, USA) was advanced to a depth of 2.5 mm. One minute after the needle was inserted, 2 μl of FITC-ABP solution (5 μg, 2.5 mg/ml) in ECF buffer was administered over a period of 4 min (0.5 μL/min). The needle was kept in place for a subsequent 4 min to minimize any backflow and then withdrawn gently. Mice were monitored until they had fully recovered from anesthesia. Thirty min after microinjection, mouse brain was removed and split into two hemi-brains and immediately snap frozen on powdered dry ice for at least 30 min and then stored in −80 °C freezer until use. Cryostat sections (10-μm) were prepared as described above, sections were cover-slipped with Dako Fluorescent Mounting Media containing nuclear stain DAPI. In some cases the same sections were also stained with Aß antibody Alexa 488–6E10 to determine the co-localization of 6E10 antibody and FITC-ABP.

3. Results

We have shown that the ~5 kDa ABP, with its KTFKTRKASAQASLASKDKTPKSKSKKRNSTQLKSRVKNI sequence corresponding to 1275–1314 region of the human PCM-1 (GenBank: AAA60120.1) selectively binds oligomeric aggregates of human $Aß_{1-42}$ *in vitro* [3]. Also, 6E10-binding $Aβ_{1-42}$ is co-precipitable from murine Tg-AD tissue with anti-PCM-1 antibody suggesting *in vivo* interaction of PCM-1 with endogenous Aß [3]. Therefore, we expected that ABP would also target the $Aβ_{1-42}$ aggregates in the cortices and hippocampi from the brains of late-stage AD patients. We, like many others, have previously shown with the Aß-specific monoclonal antibody 6E10 that brains from AD patients contain substantially more $Aβ_{1-42}$ aggregates than brains from age-matched cognitively normal humans [4]. Thus, as can be seen in Fig. 1A, Aß-selective 6E10 antibody exhibited substantially higher binding in the cortices and hippocampi of AD patients compared to non-AD control patients. Similarly, FITC-ABP also bound what appeared to be amyloid deposits in human brain cortices and hippocampi (Fig. 1B), and as seen with 6E10 antibody, there was higher binding in tissues from AD patients compared to non-AD patients. That FITC-ABP indeed bound amyloid deposits in these tissues was confirmed by the co-localization of FITC-ABP and Alexa 488–6E10 signals in the overlay studies (data not shown) as described for AD transgenic mice below.

We next asked whether ABP would target similar $Aβ_{1-42}$-containing aggregates in the cortices and hippocampi from the brains of double transgenic B6.Cg-AD-Tg mice that accumulate human $Aß_{1-42}$ [5]. As can be seen in Fig. 2A, FITC-ABP bound what looked like amyloid deposits (shown by arrows) in the hippocampus of the Tg-mouse brain, but no such spots were visible in wild-type (Wt) mouse brain which does not produce human Aß. To confirm that these ABP-binding spots on Tg-mouse brain were Aβ aggregates we probed the Tg-mouse brain sections with both FITC-ABP and Alexa 488-labeled Aß-specific 6E10 antibody. As shown in Fig. 2B, the aggregates in the cortex and hippocampus of the Tg-AD mouse were stainable with both FITC-ABP and 6E10 antibody. The merging of signals (yellow) from FITC-ABP (green) and 6E10 (red) antibody strongly indicated their co-localization. Thus, ABP did indeed target $Aβ_{1-42}$-containing aggregates in the memory-encoding hippocampi of $Aβ_{1-42}$-accumulating Tg-AD mice as was seen with human AD brain (Fig. 1).

We next asked whether ABP would target these aggregates in a living brain as seen in ex-vivo tissue sections. To answer this question we microinjected FITC-ABP into the hippocampi of wild-type and Tg-AD mice as described in the Section 2. When injected into the AD Tg hippocampus (ipsilateral), ABP bound the Aβ aggregates, but there was no staining of aggregates in the non-injected contralateral hippocampus (Fig. 3A). Nor were there any ABP targets when the peptide was microinjected into the hippocampi of the wild-type mice (Fig. 3B). To confirm that the micro-injected FITC-ABP did bind Aß deposits, in some studies we immunostained the brain sections with Aß-specific 6E10 antibody. As shown in Fig. 3C, we found that a significant fraction of the amyloid deposits was stained with both FITC-ABP (green) and anti-Aβ 6E10 (red) as indicated by the merging of these two signals (yellow).

4. Discussion

ABP's KDKTPKSKSK region corresponding to the 1275–1314 region of the multifunction PCM-1 can selectively bind $Aβ_{1-42}$ oligomers that in association with hyperphosphorylated tau oligomers are believed to co-drive the development of AD pathology [7,11,12,15]. Obviously any agent such as the small ABP peptide that can selectively and avidly bind $Aβ_{1-42}$ oligomers might be able

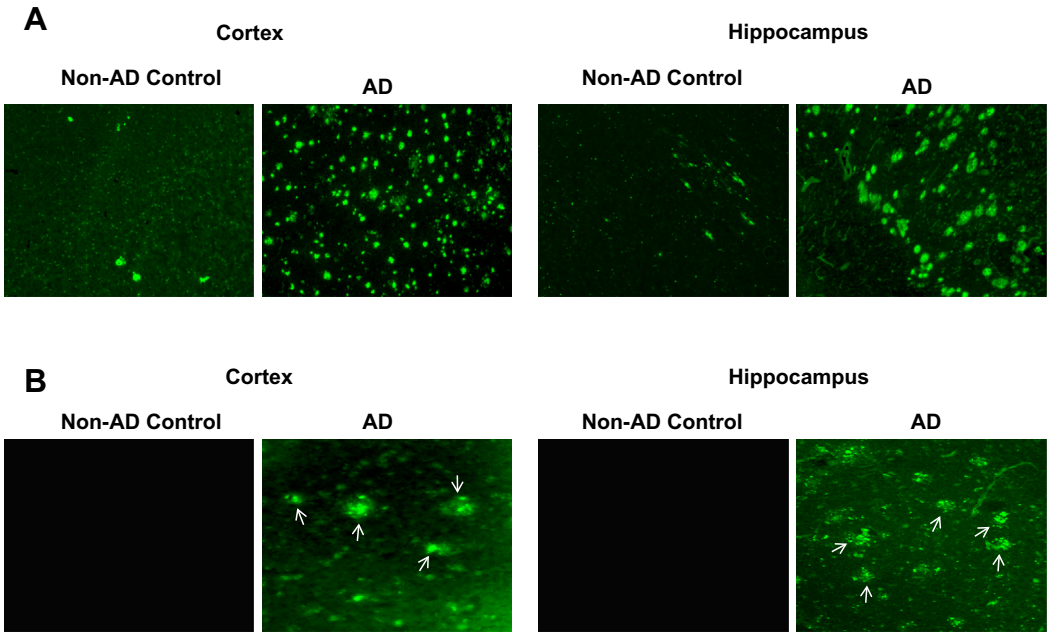

Fig. 1. FITC-ABP binds ß-amyloid aggregates which may accumulate at low levels in the brains of cognitively normal senior humans but at much higher levels in human AD cortices and hippocampi. Cryosections (~10 μm) of brains from cognitively normal human (non-AD Control) and AD patients were incubated with either (A) Aß-specific antibody 6E10 (Alexa-488 labeled) or (B) FITC-ABP as described in Section 2. Bound Alexa488–6E10 antibody or FITC-ABP was visualized with a fluorescence microscope. Arrows in B indicate FITC-ABP bound to amyloid deposits in the brain.

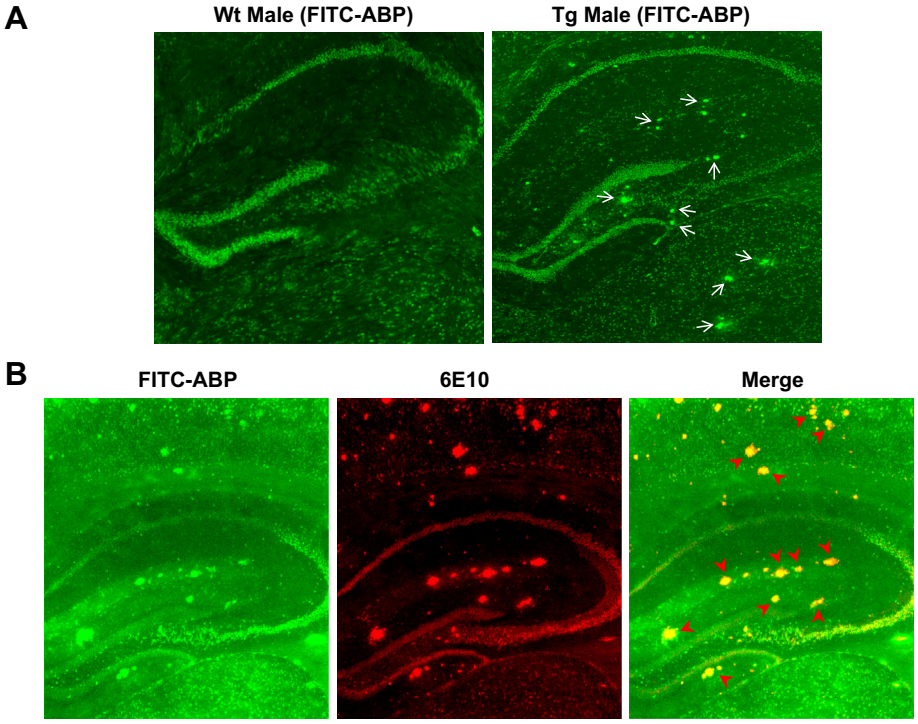

Fig. 2. FITC-ABP co-localizes with amyloid-ß-specific antibody that recognizes amyloid deposits in AD-model transgenic mice. Cryosections (~10 μm) of brains from wild type (Wt) and transgenic mice (Tg) expressing human $A\beta_{1-42}$ were incubated with either (A) FITC-ABP alone (green) or (B) simultaneously with FITC-ABP (green) and Alexa-488 6E10 anti-amyloid ß antibody (red); or first with FITC-ABP followed by Alexa-488 6E10 as described in the Section 2. The binding was visualized with a fluorescence microscope. Co-localization of FITC-ABP and amyloid-ß antibody (B) was indicated by the merging of fluorescence signals from both the molecules (yellow, arrow heads). Arrows in A indicate amyloid deposits.

to stop or at least delay the development of AD if it can cross the blood–brain-barrier and reach key AD-linked disease centers such as the entorhinal cortex and the hippocampus in the early stages of AD development.

Here we have shown that ABP when microinjected into the hippocampi of living Tg-AD mouse brains targets $A\beta_{1-42}$-containing aggregates that are stainable by anti-Aß antibody. ABP also binds to similar Aβ-containing aggregates in samples of human

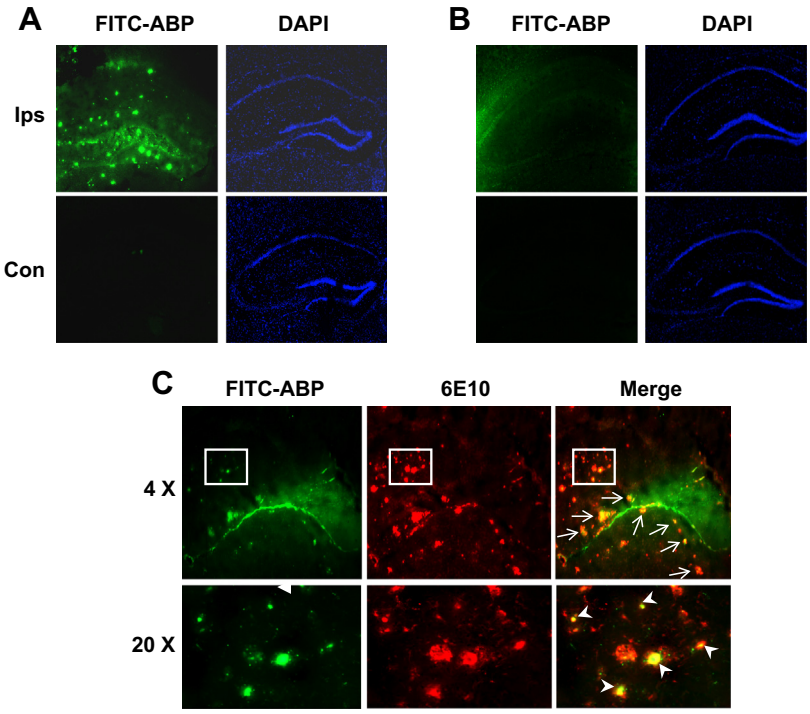

Fig. 3. Intracerebrally microinjected FITC-ABP binds to amyloid β deposits in the brains of living AD-model transgenic mice. FITC-ABP was introduced into the hippocampal region of (A) AD-model transgenic and (B) wild-type mice via intracerebral microinjection (ipsilateral side, Ips) as described in the Section 2. The contralateral (Con) hemisphere of the brain did not receive FITC-ABP. Thirty min after injection, brains were removed, hippocampal sections prepared and FITC-ABP binding was determined with a fluorescence microscope as described in the Section 2. In some experiments (C) hippocampal sections were also incubated *in vitro* with amyloid-ß-specific Alexa-488 6E10 antibody. Co-localization of FITC-ABP (green) and amyloid-ß antibody (red) was indicated by the merging of fluorescence signals from both the molecules (yellow; arrows and arrow heads). The region in each box was magnified to 20×.

end-stage AD brains. However, it is difficult to say from these studies that the Aβs in these aggregates are $A\beta_{1-42}$ oligomers like those we demonstrated to bind ABP *in vitro* [3]. They most likely are because of ABP's strong preference for the oligomers in "pure" preparations *in vitro*; the lack of any detectable binding of ABP to "amyloid-like" deposits in living or sectioned wild-type murine hippocampi (or cortices) not expressing human $A\beta_{1-42}$ and in the cortices and hippocampi of non-AD human brains. Moreover, it is likely that these aggregates in the brain tissue contain $A\beta_{1-42}$ oligomers because, as Cohen et al. [8] have shown, $A\beta_{1-42}$ monomers first form fibrils which in turn attract oligomers-forming monomers. The oligomers then use these first fibrils as nuclei for their build-up. As a result of this nucleation process Aß oligomers co-exist with amyloid-ß plaques. Clearly, some way must be found to isolate the components of these aggregates intact from brain tissues to directly confirm the identities of ABP's *in vivo and in vitro* Aß targets.

The possible ability of ABP to treat AD was suggested by our report that it significantly reduced the toxic actions of both $A\beta_{1-42}$ and its functional proxy $A\beta_{25-35}$ on the cultured human SH-SY5Y neuroblasts [3]. But to assess the AD-therapeutic value of ABP more realistically, i.e., *in vivo*, we must find ways to deliver this peptide into the brain by either direct microinjection into the entorhinal cortices or hippocampi of Tg-AD mice or via a vascular route by linking it to transporters that cross the blood–brain-barrier and then determine whether ABP can prevent or perhaps even reverse the AD-like symptoms in these Tg-AD animals.

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