ÉCOLE DOCTORALE
Chimie & Sciences du Vivant

SOUCHES AMYLOÏDES ET MALADIE D'ALZHEIMER : RÔLES DANS LA PROPAGATION DE LA PATHOLOGIE AMYLOÏDE ET L'INTERACTION AMYLOÏDE/TAU

AMYLOID STRAINS AND ALZHEIMER'S DISEASE: ROLES IN THE PROPAGATION OF AMYLOID PATHOLOGY AND AMYLOID/TAU INTERACTION

Etablissement **Université Grenoble Alpes**

École doctorale **CSV- Chimie et Sciences du Vivant**

Spécialité **Neurosciences - Neurobiologie**

Unité de recherche **Grenoble Institut des Neurosciences**

Encadrement de la thèse **Alain BUISSON**

Financement du 01-10-2024 au 01-10-2027 *origine* **ministère de l'enseignement supérieur et de la recherche**

Début de la thèse le **1 octobre 2024**

Date limite de candidature (à 23h59) **30 avril 2024**

Mots clés - Keywords

Maladie d'Alzheimer, peptides beta-amyloides, synaptotoxicité, stratégies thérapeutiques, électrophysiologies, imagerie cellulaire

Alzheimer's disease, amyloid beta peptides, synaptotoxicity, therapeutic strategies, electrophysiology, cellular imagery

Description de la problématique de recherche - Project description

La maladie d'Alzheimer (MA) se caractérise par l'accumulation cérébrale de peptides amyloïdes ß agrégés (Aβ, plaques amyloïdes) et d'agrégats anormaux de protéines tau (1,2). Les peptides Aß natifs sont constamment produits dans le cerveau humain, où ils restent généralement dans un état soluble avec une conformation α-hélique. Cependant, les peptides Aß peuvent adopter des conformations alternatives mal repliées et sujettes à l'agrégation, riches en feuillets ß qui favorisent leur association en oligomères, en structures fibrillaires et en amyloïde. Des variantes structurelles distinctes, également appelées souches d'Aβ, portant des mutations ponctuelles dans la séquence du peptide Ab ont été identifiées. Elles peuvent expliquer la variabilité de la pathologie chez les patients atteints de la maladie d'Alzheimer et entre eux. Par exemple, des études récentes ont démontré que les patients atteints de formes rapidement progressives de la MA présentent des structures moléculaires distinctes de A□ par rapport aux patients atteints de la MA normalement progressive (3). L'impact de ces variantes structurelles sur l'évolution de la pathologie de la MA reste toutefois partiellement inconnu. L'objectif de cette proposition est de caractériser les mécanismes moléculaires par lesquels des variantes sélectionnées d'Aβ favorisent et amplifient la pathologie amyloïde et tau cérébrale. À cette fin, nous générerons des variants Aβ spécifiques et utiliserons des modèles de souris cellulaires et in vivo pour évaluer l'impact des variants Aβ sur la pathologie de la maladie d'Alzheimer (Fig. 1). Nous vérifierons si les variants Aβ :
i. induisent différentes pathologies cellulaires (LTP, synaptotoxicité et pathologie tau).
ii. transfèrent leurs caractéristiques biophysiques et pathologiques à des modèles de souris.

Alzheimer's disease (AD) is characterized by the cerebral accumulation of aggregated amyloid ß peptides (Aβ, amyloid plaques) and abnormal tau protein aggregates (1,2). Native Aß peptides are constantly produced in the human brain where they usually remain in a soluble state with a α-helical conformation. However Aß peptides can adopt alternative misfolded aggregation-prone conformations rich in ß-sheets that favour their association into oligomers, fibrillar structures and amyloid. Distinct structural variants, also called strains of Aβ, bearing point mutation in the Ab peptide sequence have been identified. They may explain the variability of the pathology within and among the AD patients. For example, recent studies demonstrated that patients with rapidly progressive forms of AD exhibit distinct molecular structures of A□ when compared with those with normally progressive AD (3). The impact of these structural variants on AD-pathology evolution is however still partly unknown.
The aim of this proposal is to characterize the molecular mechanisms by which selected variants of Aβ promote and amplify cerebral amyloid and tau pathology. For this purpose, we will generate specific Aβ variants and use cellular and in vivo mouse models to evaluate

how Aβ variants impact AD pathology (Fig 1). We will test whether Aβ variants:
i. induce different cellular pathology (LTP, synaptotoxicity, and tau pathology).
ii. transfer their biophysical and pathologic characteristics to mouse models of AD after intracerebral inoculation and induce different long-term amyloid accumulation and spreading patterns.
iii. conserve their biological specificities after self-replication in mouse models.

Thématique / Domaine / Contexte

We selected amyloid strains that exhibit specific oligomers profiles (Fig 2): i. Human (H) A□1-42 produces an amyloid strain rich in oligomers or fibrils depending of the re-suspension protocol; ii. H A□ 1-42 with the icelandic mutation A2T generates amyloid strain less prone to aggregation that forms dimers, tetramers but no dodecamers ((4); unpublished results); iii. H A□ 1-42 with the Osaka mutation called E22D produces a peptide highly resistant to degradation with an enhanced oligomerization and fibrillization profile (5); iv. H Aβ 1-42 with the mutation F19S/L34P that remains as a monomer in solution (6).
We evaluated the impact of the selected Aβ variants on synaptic density by transfecting specific APP in primary neuronal cultures. We showed that APPwt, APPswe and APPOsaka expression induced a drastic reduction of the synaptic density but no effect was observed when neurons were transfected with APPIcelandic (Fig. 3).
Further, we analysed the influence of oligomers of Aβ1-42 Osaka and Iceland (at 100 nM) on synaptic plasticity (LTP). While the peptide bearing the Osaka deletion abolishes LTP, Aβ1-42 with the Icelandic mutation does not alter the LTP (Fig 4) validating the differential effect of the selected strains on AD pathologies.
Finally, in two preliminary studies, APP/PS1 mouse models were inoculated in the CA1 region of the hippocampus with control, AD brains (Fig 5a,b), Aβ1-42 oligomers or PBS (Fig 5c). Amyloid load was evaluated at 1 to 4 months post-inoculation. We observed that an amplification of amyloid pathology in the mice that received the AD brains (Fig. 5a, b) or the Aβ1-42 oligomers (Fig 5c).
These results validate the impact of oligomers on spreading and evolution of AD pathology.

Our project relies on a procedure that we implemented to produce recombinant ABeta peptides in order to generate Ab peptides with specific disease-associated amino acid mutations. This procedure is based on the expression of plasmids containing A□1–42 cDNA in Escherichia coli and allowed us to produce 4 strains of A□ peptides (H A□1-42, H A□1-42 with the Osaka (A□E22D), Icelandic (A□A2T) and F19S/L34P mutations)) bearing subtle modifications in their sequences. Our preliminary results and other publications leave no doubt on the ability of the selected A beta peptides to generate different oligomerization profiles (5).

This project involves a strong collaboration with the research group led by Dr Dhenain in Paris. Experiments will be performed in the GIN and complementary approaches will be performed in Paris in Dr Dhenain Group.

Objectifs

We will 1) characterize the structural profiles of the selected Aβ variant 2) study their impacts on AD pathologies with a specific interest on APP processing; synaptic morphology and plasticity and tau pathology in primary neuronal cultures and acute brain slices exposed to A□ variants. All the methods to perform these studies are mastered by Partner 1 (6). Next, we will evaluate whether intrahippocampal inoculation in APPswe/PS1dE9 mice of the selected Aβ strain promotes differences in Aβ misfolding, spreading and synaptotoxicity in this mice model of AD. We will also study amyloid load and spreading and behavioural outcomes in mice 4 months after injection of the selected strains. Partner 2 (Dhenain’s team) already has a large experience in Aβ inoculation in mice and in the evaluation of their impacts (7,8).

Méthode

This project involves technical approaches ranging from molecular biology, and cellular biology. We will be using neuronal cultures approaches to develop humain neuronal cultures from iPSCs. By transfecting these neurons, we will manipulate the expression of the major identified molecular actors of Alzheimer's disease physiopathology.

Résultats attendus - Expected results

There is currently no effective treatment for AD making the societal demand for a cure even stronger . The induction of AD lesions by inoculation of Aβ strains is revolutionizing our vision of AD physiopathology (11,12). The fact that humans who have been accidentally contaminated with amyloid peptides developed a cerebral amyloidosis provided the first demonstration that AD can be caused by peripheral contaminations (13). These discoveries bring new concepts on mechanisms associated with AD such as seeding, spreading and A□ strain effects. The strain effect is still poorly understood and the influence of selected amyloid strains on pathology evolution including on the induction of amyloid load and tau pathologies will bring important insights on the pathologic cascades involved in the spreading of AD. Further understanding of the concept of amyloid strains will greatly influence future treatments against AD and promotes the development of personalized medicine for AD patients.

Références bibliographiques

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Précisions sur l'encadrement - Details on the thesis supervision

L'encadrement et le suivi de formation et d'avancement des recherches du doctorant se fera au cours de réunions hebdomadaires avec son encadrant. Des réunions bi-hebdomadaires avec l'ensemble de l'équipe seront organisées afin de participer à la trajectoire de recherche de l'ensemble de l'équipe.

Conditions scientifiques matérielles et financières du projet de recherche

L'intégration au sein de l'équipe Inserm dirigée par A. Buisson permettra d'assurer le cout environné de la thèse. Ce projet a été sélectionné et financé par une association de patients Alzheimer.

Ouverture Internationale

Les travaux réalisés au cours de cette thèse seront présentés à des congrès internationaux.

Objectifs de valorisation des travaux de recherche du doctorant : diffusion, publication et confidentialité, droit à la propriété intellectuelle,...

Les travaux réalisés seront valorisés par des publications dans des revues internationales à comité de lecture.

Collaborations envisagées

Collaboration avec l'équipe du Dr Dhenain est un élément important de ce projet.

Profil et compétences recherchées - Profile and skills required

le candidat devra avoir une formation dans le domaine des neurosciences avec un intérêt particulier pour les processus neurovégétatifs. Elle/il sera formé(e) aux techniques d'études basées sur des approches de biologie moléculaires et cellulaires de la physio-pathologie neurale dans le cadre de la maladie d'Alzheimer. Une formation à l'électrophysiologie sera également possible dans le cadre de la thèse.

The candidate will have a background in neuroscience, with a particular interest in neurovegetative processes. He/she will be trained in molecular and cellular biology approaches to neural pathophysiology in Alzheimer's disease. Training in electrophysiology will also be possible as part of the thesis.

Dernière mise à jour le 13 mars 2024