BioClotAD

Development of a neuroimaging biomarker of the pro-coagulant state in Alzheimer's disease: The BioclotAD Project

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Aims, research questions and working hypothesis

Alzheimer's disease (AD) is the most common form of dementia[1]. Its multifactorial nature includes an early haemostatic dysregulation, inducing a pro-thrombotic milieu. This leads to increased fibrin levels and degradation-resistant clots in the brains of AD patients, which exacerbate hypoperfusion, neurodegeneration and blood-brain barrier (BBB) disruption[2]. These phenomena occur early in the course of AD but not in all patients. Early detection of such abnormalities would be invaluable to identify patients likely to benefit from anticoagulant therapies[3]. Therefore, the aim of this project is to develop an imaging biomarker, based on a fibrin binding probe (FBP)[4], to non-invasively identify the pro-coagulant state in AD.

Means/methods implemented by the consortium

To optimize and test FBP, we employed extensive *in vitro*, *ex vivo*, and *in vivo* assays in AD animal models, targeting both vascular and parenchymal fibrin accumulations.
First, we labelled FBP with the long half-life radioisotope ^{89}Zr to track cerebral occlusions using PET imaging over five consecutive days, identifying appropriate time points for imaging. After establishing the biodistribution period, we implemented a pre-targeting strategy using click chemistry paired with the short half-life radionuclide ^{68}Ga to improve the clinical translational potential. This approach enabled the detection of vascular fibrin deposits in an AD mouse model via PET imaging.
To detect the fibrin deposits in the brain parenchyma, we conjugated FBP to a ligand of the transferrin receptor (TfR) to facilitate BBB crossing[5]. The fibrin-binding affinity of this conjugate was tested via *in vitro* ELISA assays and *in vivo* PET imaging. In parallel, we evaluated the specific binding of an anti-fibrin monoclonal mouse antibody (1101) and its TfR-conjugated version (1101-Tfr) using ELISA. The *in vivo* binding of these probes to cerebral fibrin was further assessed in an AD mouse model through PET imaging, *ex vivo* biodistribution analyses, and autoradiographic studies.

What are the outcomes of the project?

(milestones achieved, key results obtained)

The BioClotAD project has yielded several key results. For vascular fibrin detection, we demonstrated that the ^{89}Zr-FBP was effective in a surgical model of thrombosis, showing higher brain uptake in AD mice compared to wild-type (WT) controls 24 hours post-injection. Signal intensity decreased over time in AD mice but remained unchanged in WT littermates, confirming the lack of specific binding in healthy animals. Additionally, studies the pre-targeting approach using the ^{68}Ga-based radiotracer successfully accumulated in the damaged artery of a thrombosis model, and exhibit significantly higher brain uptake in AD mice compared to WT controls.

For detecting parenchymal fibrin, while the FBP-TfR was successfully synthesized, *in vivo* PET studies did not reveal significant differences in brain retention between AD and WT mice. However, the synthesis and testing of 1101 and 1101-TfR proved successful. PET imaging showed enhanced brain penetration of 1101-TfR compared to 1101 alone in WT mice after 2 hours. Notably, after 72 hours, a higher brain-to-blood ratio of 1101-TfR was observed in AD mice compared to WT mice and those treated with monospecific 1101 alone.

Significance and impact of the work on the fiel

The BioClotAD project provides the first feasible neuroimaging strategies to non-invasively detect the pro-thrombotic environment associated with AD. These biomarkers offer the potential for early identification of patients who could benefit from anticoagulant therapies, allowing for more personalized treatment strategies.

Next steps and future challenges

In the near term, we will confirm the binding efficacy of FBP and 1101 probes (and their respective TfR conjugates) on brain tissue from AD and WT mice by immunofluorescence. Following successful validation, we will extend these tests to human brain tissue from AD patients and healthy controls to ensure the probes' ability to bind microthrombi in human brains. Concurrently, we aim to expand our imaging protocols to include SPECT, a more accessible imaging modality, to broaden the potential clinical application of our biomarkers.
Looking ahead, our current focus has been on AD models with consistently abnormal clotting profiles. The next phase will involve testing our biomarkers in more heterogeneous AD models where only some subjects develop clotting issues at various stages. This approach will help evaluate the biomarkers' diagnostic potential in a more diverse and representative population. We would also like to assess whether these biomarkers can monitor treatment response. Ultimately, we aim to confirm the safety and efficacy of the biomarkers before moving to clinical trials to improve personalized care for AD patients experiencing clotting abnormalities.

References

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