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Detection of Amyloid Plaques Targeted by USPIOs and ARIA Evaluation in a Non-Human Primate Model of Sporadic Cerebral Amyloid Angiopathy (CAA)

A. Rationale:

Neuroscientists currently seek a consistent and accurate method for detecting sporadic cerebral amyloid angiopathy (CAA), which is closely linked to Alzheimer's Disease (AD) pathology (Scholtzova, et al., 2015). Prevailing methods of determining the presence of AD in patients is through cognitive tests, which can be inaccurate (Chan et al., 2019). Previous animal models for AD and CAA, such as mouse models, have had difficulty in examining amyloid related imaging abnormalities (ARIA) that aligns with human pathology.

The usage of transgenic mouse models used for past research on AD has progressed, and the need for a model of CAA and AD more closely related to human pathology has risen (Wadghiri et al., 2003). The use of *Saimiri boliviensis*, commonly known as black-capped squirrel monkeys (SQM), as a non-human primate model is effective, since there is a natural generation of CAA pathology. This can be used in future studies to determine the safety and efficacy of immunotherapeutics and assess the possible long-term consequences of ARIA. Current human and animal data shows that CAA, pathology indicative of AD, is more resistant to clearance than parenchymal amyloid and removal may lead to damaging microhemorrhages (Scholtzova et al., 2013). It has also been shown that CAA may be involved in cerebral edema and/or cerebral microhemorrhages (ARIA-E and/or H) in magnetic resonance imaging (MRI) scans of patients receiving amyloid-β (Aβ) immunotherapy (Scholtzova et al., 2018). However, current tests for CAA are invasive and time-consuming.

Therefore, there is a need for a novel method to detect CAA in AD patients. In previous studies of mouse models with ultra-small paramagnetic iron oxide particles bound to polyethylene glycol and Aβ (USPIO-PEG-Aβ), it has been shown that the particles bind to amyloid plaques, suggesting a potential use for labelling CAA pathology (Belaidi & Bush, 2016). As such, my study focused on *Saimiri boliviensis* as a non-human primate model, with a natural generation of CAA pathology similar to that of humans to determine the ability of the USPIO-PEG-Aβ nanoparticles to target and visualize amyloid deposits.

B. Research Questions, Hypotheses, Expected Outcomes:

a. Research Questions and Objectives:

i. To validate the ability of USPIO-PEG-Aβ40/42 nanoparticles to target and visualize amyloid deposits, correlative to the Alzheimer's disease pathology of sporadic cerebral amyloid angiopathy.

ii. To validate the use of a non-human primate model of squirrel monkeys (*Saimiri* Boliviensis) as a model for determining the safety and efficacy of current immunotherapeutic approaches and studying the pathogenesis and possible long-term consequences of amyloid-related imaging abnormalities.

b. **Hypothesis/Goals/Expected Outcomes:**

i. The correct binding of USPIO-PEG-Aβ40/42 to amyloid deposits of sporadic cerebral amyloid angiopathy that can be used to determine the potential efficacy of different immunotherapies of amyloid-related complications.

ii. The squirrel monkey non-human primate model (*Saimiri* boliviensis) will correctly show labelled amyloid deposits from USPIO-PEG-Aβ40/42, confirmed with immunohistochemistry, validating the usage of squirrel monkeys as a precursor to human clinical trials of potential immunotherapeutics.

C. Research Methods:

a. Procedures:

i. Immunohistochemistry

1. Histological stains will be appropriately chosen according to markers that needed to be tested. Examples include GFAP, which stains for astrocyte locations and 6E10, an anti-amyloid beta antibody. These markers can then be used to compare whether the regions marker on MRI scans were accurate and aligned to the histological markers that showed up after immunohistochemical stains. All sections will be 8 microns thick and cut by my mentor and used for immunohistochemical procedures. Immunohistochemistry will be done to confirm MRI analysis and brain imaging. Different immunohistochemical stains will be utilized for different purposes, such as that of Perl Prussian Blue Stains, Luxol Fast Blue Stains, and Diaminobenzidine-based stains, depending on the affinity for molecules to bind.

ii. *In vivo* MRI Brain Imaging (performed by external laboratory)

1. Initial *in vivo* MRI experiments are designed to test toxicity and utility of the novel MRI compound of bi-functional ultra-small superparamagnetic iron oxide nanoparticles (USPIO) bound to polyethylene glycol and Aβ40/42 (USPIO-PEG-Aβ) to determine Aβ plaques/deposits and to confirm CAA burden in aged SQMs as a vehicular group (Scholtzova et

al., 2015). For direct work with handling the SQMs, I will not have physical contact with the living SQM, instead working with the brain tissue collected by my mentor. For USPIO-PEG-Aβ compound scans, a T2*-weighted image will be acquired using a multigradient echo (MGE) sequence [MGE; TE=2.96ms ES=4ms, 8 echoes, TR=41ms, FA ~13deg., time=45min, 3 repetitions] at spatial isotropic resolution (200μm).

iii. Generation of R2* Maps and Region of Interest Based Analysis

1. R2* maps will be made from 3D MRI images using a software called FireVoxel, developed by the NYU Langone Health faculty of the Department of Radiology, Artem Mikheev and Henry Rusinek. The program allows uploading of 3D DICOM T2*-weighted images and generates an R2* (1/T2*) map based off of the original image that can be color coded or in grayscale. On the MRI scans, T2* maps would show with inverse coloring, flipping analysis that needed to be done (Weissleder et al., 1990). On R2* maps, areas of the brain affected by amyloid plaques show up as bright areas, whereas the inverse scan of T2* show plaques as dark areas. R2* measurements are considered to be linearly related to the iron content in the brain, allowing for accurate analysis of SQM scans.

b. <u>Risk and Safety:</u>

i. This study will involve the usage of potentially hazardous chemicals such as diaminobenzidine (DAB), sodium hydroxide (NaOH), hydrochloric acid (HCl), 100% ethanol, and xylenes. These chemicals can potentially contaminate environmental systems if disposed of incorrectly and may cause irritation and have toxic biological effects. There may potentially also cause permanent damage to those exposed to unsafe levels without regulation. As such, the usage of proper laboratory equipment, such as gloves, nitrile gloves, respirators, and lab coats are to be used in order to minimize the risk of injury due to chemical exposure. Working under a fume hood for volatile substances will help reduce inhalation-related issues. Working under direct lab supervision will also help increase safety.

c. <u>Data Analysis:</u>

i. Using FireVoxel, coregistration of MRI scans received from the MRI facility create R2* maps from 3D MRI images. The program allows uploading of 3D DICOM T2*-weighted images and generates an R2* (1/T2*) map based off of the original image that can be color coded or in grayscale. R2* values will then be

calculated to determine p-values for the effect that USPIO-PEG-Aβ molecules has in increasing the visibility of cerebral amyloid angiopathy in MRI scans done.

ii. Immunohistochemistry will then be compounded with the FireVoxel MRI coregistration analysis in order to confirm the actual presence of associated markers.

iii. Data will be graphed in GraphPad Prism and Excel, analyzed by students

D. References:

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Item #4: Hazardous Chemicals, Activities & Devices:

The handling of all hazardous chemicals will be done under supervision of qualified lab personnel. Potentially hazardous agents will include sodium hydroxide (NaOH), hydrochloric acid (HCl), diaminobenzidine (DAB), and ethanol. Proper training will be undertaken in order to learn techniques for safe operation and handling of necessary chemicals and machinery. Proper equipment will be worn to minimize chemical exposure.

Hydrochloric Acid (HCl) – ACS reagent, 37%

- Product 320331 Sigma-Aldrich
    - Hydrochloric acid is a corrosive chemical that can cause major skin burns. Thus, various

safety measures and precautions will be taken. HCl will be stored by being locked into a tightly closed corrosive-resistant container that is in a well-ventilated place. Appropriate PPE that will be worn will include tightly fitting safety goggles that are tested and approved under appropriate standards for adequate eye and face protection. Nitrile gloves and lab coat will be used for skin protection. Potential spillages will be absorbed, and inhalation will be avoided by working under fume hood. Methods and materials for containment and cleaning up will include: 1) soaking up with inert absorbent material and disposing of as hazardous waste and 2) keeping in a suitable, closed container for disposal. Chemical will ultimately be disposed appropriately to a waste disposal plant.

Ethyl Alcohol – ACS Reagent 200 Proof

- Product 792780, Sigma-Aldrich
    - Ethanol is highly combustible and a major fire hazard in the lab. It also is a skin and eye irritant and will require safety measures and precautions. The chemical will be stored in a tightly closed container in a cool, well-ventilated area, away from all possible sources of ignition and oxidizing agents. All containers will be grounded for electrostatic charge to avoid fire. The chemical will not be stored 23°C. PPE will include eye/face protection (appropriate safety goggles at appropriate government standards for eye protection) and skin protection (nitrile gloves & lab coat). Inhalation of vapor or mist will be avoided by working under fume hood for adequate ventilation and using a respirator if necessary. The chemical will be disposed to an approved waste disposal plant in proper containers.

Sodium Hydroxide (NaOH) Pellets - BioXtra, ≥ 98% Anhydrous

- Product S8045, Sigma-Aldrich
    - Sodium hydroxide is a corrosive chemical that is capable of causing severe skin burns. Thus, various safety measures and precautions will be taken to prevent injuries. The chemical will be stored in a corrosive-resistant container that is tightly closed in a dry and well-ventilated place. Personal Protection Equipment (PPE) will be worn which will include eye and face protection. Appropriate safety goggles that are approved under appropriate government standards for eye protection will be worn along with nitrile gloves and lab coats for skin protection. Respiratory protection will be worn to avoid breathing vapors or dust and samples will be prepared under fume hood for adequate ventilation. Also, skin or eye contact will be avoided. Chemical will be disposed to an approved waste disposal plant for safe removal.

3,3′-Diaminobenzidine ≥ 99% (HPLC)

- Product D8001, Sigma Aldrich
    - 3,3'-Diaminobenzidine (DAB) is an organic compound with the formula $(C_6H_3(NH_2)_2)_2$. DAB is typically used in immunohistochemical stains of nucleic acids and proteins. It is a carcinogenic chemical that is capable of inducing cancer in human tissue. Thus, various safety measures and precautions will be taken to prevent injuries. The chemical will be stored in a corrosive-resistant container that is tightly closed in a dry and well-ventilated place. Personal Protection Equipment (PPE) will be worn which will include eye and face protection. Appropriate safety goggles that are approved under appropriate government standards for eye protection will be worn along with nitrile gloves and lab coats for skin protection. Respiratory protection will be worn to avoid breathing vapors or dust and samples will be prepared under fume hood for adequate ventilation. Also, skin or eye contact will be avoided. Chemical will be disposed to an approved waste disposal plant for safe removal.