Cellular specificity assessment and longitudinal PET study in a transgenic mice model of a ^{18}F-labelled Sulforhodamine 101 in astrocytosis processes in Alzheimer´s Disease

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Purpose/Background *

Alzheimer's disease (AD) is a neurodegenerative disorder of the Central Nervous System (CNS), characterized neuropathologically by the presence of amyloid plaques (AP) and neurofibrillary tangles. As the pathology progresses, numerous reactive astrocytes are arranged around the AP and neurofibrillary tangles. PET molecular imaging is a promising modality for the early detection and staging of AD. [^{11}C]L-deprenyl-D2 ([^{11}C]DED) has been used to evaluate astrocytosis, being a selective irreversible inhibitor of MAO-B. We have reported the synthesis and biological evaluation of a sulfonamide derivative of Sulforhodamine 101 (SR101), namely SR101 N-(3-[^{18}F]-Fluoropropyl)sulfonamide ([^{18}F]2B-SRF101). The fluorescent dye SR101 is an astroglial marker and was used for the detection of astrocytes in the neocortex of rodents. We have confirmed 2B-SRF101's ability to stain astrocytes in culture similarly than SR101. A preliminary *in vivo* assessment of [^{18}F]2B-SRF101 using micro-PET/CT revealed a higher uptake in cortex and hippocampus of 9 to 10 months old triple-transgenic (3xTg) mice compared with the control non-Tg group (C57BL6J). Besides, the cellular specificity of this radiotracer in the CNS needs to be elucidated.

In this work we aimed to: i) elucidate the cellular specificity of 2B-SRF101 in neurons and astrocytes using isolated mice cortex/hippocampus cells, ii) perform a longitudinal biological assessment by PET/CT images of [^{18}F]2B-SRF101, comparing with [^{11}C] DED.

Methods *

In order to elucidate the cellular specificity of the radiotracer, enriched astrocytic cultures were prepared from cortices of newborn (P0-P2) 3xTg or C57BL6J control mice. Neuronal primary cultures were obtained from C57BL6J embryos (E15-17). Live cell fluorescence confocal images were acquired after incubation of cell cultures with SR101 or 2B-SRF101. In addition, cell uptake was determined up to 40 min of incubation time, of both astrocytic or neuronal cultures with [^{18}F]2B-SRF101 using a Gamma counter.

PET/CT scans were acquired in a preclinical PET-MRI3T and SPECT/CT (Mediso nanoScan®). Studies were performed after 15 min of i.v. injection in B6x129 F2 and 3xTg mouse model of AD at different animal ages (3, 6, 9, 12 and 15 months old), respectively. The

following volumes of interest (VOIs) of different brain regions were selected for image evaluation: striatum, cortex, hippocampus, hypothalamus, thalamus, amygdala, olfactory bulb, and midbrain. The cerebellum was used as a reference, and the target to cerebellum ratio was calculated for each VOI (SUVR).

Results *

Astrocyte specific uptake was observed for SR101 and 2B-SRF101 in cultures derived from both 3xTg and B6x129 F2 mice, without showing specific uptake in healthy neurons in culture. This result was also observed in internalization assays with [^{18}F]2B-SRF101 in which radiotracer uptake was higher in astrocytes than in neuronal cultures in the three time points evaluated.

In PET/CT studies of 3 month old animals, [^{18}F]2B-SRF101 and [^{11}C]DED uptake between both groups was compared. No significant difference was found in any of the analyzed regions and frames ($p > 0.05$). In the case of 6 months old animals, the following significant differences were observed ($p < 0.05$): [^{18}F]2B-SRF101 uptake in the amygdala, being higher in the control group (CG); [^{18}F]2B-SRF101 uptake in the hypothalamus, being higher in the CG. The opposite takes place in the hippocampus, in the 30 to 45 min frame; [^{11}C]DED uptake in the hippocampus and cortex, being higher in the CG than in the 3xTg mice, only in the 15 to 30 min frame. Brain analysis in the 9 to 15 months old animals is being completed.

Conclusion *

We brought evidence of astrocytic preference of both SR101 and 2B-SRF101 compared to neurons, validating [^{18}F]2B-SRF101 as a promising candidate tracer for astrocytosis detection. They could be useful tools, enabling a better assessment of the AD pathology in a multitracer approach.

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