

## ***In vitro* uptake evaluation of a <sup>18</sup>F-Labeled Sulforhodamine 101 in CNS cells**

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**Background:** We have previously reported the synthesis and biological evaluation of a sulfonamide derivative of Sulforhodamine 101 (SR101), namely SR101 N-(3-[<sup>18</sup>F]-Fluoropropyl)sulfonamide ([<sup>18</sup>F]2B-SRF101), designed as a new positron emission tomography (PET) agent for detecting astrocytosis in early stages of Alzheimer's disease (AD). The fluorescent dye SR101 is an astroglial marker and has been used for the detection of astrocytes in the neocortex of rodents in numerous work. We have confirmed 2B-SRF101's ability to detect astrocytes in culture similarly than SR101, using fluorescence microscope images. *In vivo* biological assessment of [<sup>18</sup>F]2B-SRF101 using micro-PET/CT revealed a higher uptake in cortex and hippocampus of 10-month-old triple-transgenic (3xTg) mice compared with the control group (1). However, the cellular specificity of this radiotracer in the CNS needs to be elucidated, especially considering that SR101 uptake was reported also in other CNS cells (2).

**Aims:** In this work we aimed to elucidate the cellular specificity of 2B-SRF101 in neurons and astrocytes using isolated mice cortex/hippocampus cells.

**Methods:** Enriched astrocytes cultures were prepared from cortices of P0-P2 3xTg or C57 control mice. Neuronal primary cultures were obtained from C57 embryos. Fluorescence confocal images were acquired after 1 min SR101 or 2B-SRF101 (10 μM) incubation in live cells. Cell uptake was determined after 10, 20 and 40 min incubation of confluent cells with [<sup>18</sup>F]2B-SRF101 (90 μCi) using a Gamma counter.

**Results:** Astrocyte specific uptake was observed for SR101 and 2B-SRF101 in cells derived from both 3xTg and non-Tg mice, with a preferential cytoplasmic distribution, without showing specific uptake in healthy neurons in culture. This result was also observed in internalization assays with [<sup>18</sup>F]2B-SRF101 in which radiotracer uptake was higher in astrocytes than in neuronal cultures in the three time points evaluated.

**Conclusion:** In this work we brought evidence of astrocytic preference of both SR101 and 2B-SRF101, validating [<sup>18</sup>F]2B-SRF101 as a promising candidate tracer for astrocytosis detection.

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<sup>1</sup> Kreimerman I, et al. *Front Neuroscience*, 13: 734; 2019.

<sup>2</sup> Hill R, et al. *Nat Methods*, 11: 1081-1082; 2014.

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