Synthesis of [\(^{18}\text{F}\)]2B-SRF101: A Sulfonamide Derivative of the Fluorescent Dye Sulforhodamine 101

Ingrid Kreimerman\(^1\), Williams Porcal\(^1,2\), Patricia Oliver\(^1\), Eduardo Savio\(^1,2,*\) and Henry Engler\(^1\)

\(^1\) Uruguayan Centre of Molecular Imaging (CUDIM) Radiopharmacy Department, Montevideo, Uruguay; \(^2\) Facultad de Química, Universidad de la República - Departamento de Química Orgánica Montevideo, Uruguay
Cell Culture Studies with SR101 and 2B-SRF101

Methods

Astrocyte Primary Cultures

Enriched astrocyte cultures were prepared from cortices of 1 day old Sprague Dawley rat pups according the methods described by Saneto and De Vellis with minor modifications [1]. Animal protocol was approved by the Ethical Committee of “Instituto de Investigaciones Biológicas Clemente Estable”, in agreement with national regulations (CNEA, Uruguay). Briefly, animals were decapitated, skull retired and meninges removed carefully. Cortical tissue was chopped and dissociated with 0.5% trypsin-EDTA for 25 min at 37 °C. Trypsinization was stopped with DMEM-HEPES containing 10% FBS. 50 μg/mL DNaseI was added and mechanical disaggregation performed to obtain a suspension that was passed through an 80-μm mesh and then spun 10 min at 800 g. The pellet obtained was re-suspended in DMEM-HEPES containing 10% FBS + 100 IU/ml penicillin + 100 μg/mL streptomycin, and then plated in a 25 cm² tissue culture flask. Media was changed every other day until confluence. Then, closed culture bottles were agitated 48 h at 250 rpm and back to incubation. A week later, cells were seeded on 35 mm Petri dishes at 2.8 x 10⁴ cells/cm² and used 5 -7 days later.

Sulforhodamine Staining

Confluent enriched astrocyte cultures were rinsed with phosphate saline solution (10 mM, pH 7.2) containing 20 mM glucose and then incubated 1 min in a culture oven with either: i- 10 μM SR101 from a 10 mg/ml stock dissolved in saline solution; ii- 10 μM of SR101 from a 10 mg/ml stock dissolved in saline solution containing 10% ethanol; iii- 10 μM 2B-SRF101 that was prepared from a 10 mg/ml dissolved in saline solution containing 20% ethanol and finally diluted in 10% ethanol; iv- 10 μM SR101 prepared from a 10 mg/ml stock dissolved in DMSO and diluted in saline solution containing 0.5% DMSO or; v- 10 μM 2B-SRF101 obtained by diluting a 10 mg/ml stock dissolved in DMSO and then in saline solution plus 0.5% DMSO. After 1 min, cells were rinsed in warm glucosated PBS and immediately imaged in either an Epifluorescence IX71 Olympus microscope attached with a DPP70 Olympus camera or a FV300 Olympus Confocal microscope to obtain better spatial resolution. All images were obtained with the same acquisition parameters by using the DPP controller. Evaluation of fluorescence intensity was made in all images obtained by using Image J (NIH). Each experimental condition was performed by triplicate and 5 fields per plate were obtained.

Results

A brief incubation of living astrocytes with commercial SR101 dissolved in saline solution was enough to stain nearly all the cells present in a confluent culture. Labelling was preferentially located in mitochondria that were distributed throughout the whole cell body. All cells from the same culture batch incubated with SR101 (dissolved in saline solution containing 10% ethanol), were recognized. The labelling of mitochondria remained unaffected but was less intense compared to the compound...
solved in the saline solution. 2B-SRF101 identified the astrocytes and its mitochondria. The effect of ethanol in the labelling intensity was higher according to the levels of fluorescence obtained in images acquired in same conditions (Figure 1). Dissolution of both stocks of SR101 and 2B-SRF101 in DMSO caused a significant decrease in the intensity of fluorescence, however, it allowed that 2B-SRF101 labelled the whole astrocyte body (Figure 2). This novel compound might be used not only to recognize astrocytes but also to evaluate the mitochondrial changes associated with astrocyte neurotoxicity.

**Figure 1.** Labelling of astrocytes with SR101 and 2B-SRF101 dissolved in NaCl 0.9% and NaCl 0.9%- 10% ethanol.

**Figure 2.** Labelling of astrocytes with SR101 and 2B-SRF101 dissolved in DMSO.

**Conclusions**

The novel compound detected the astrocytes and the mitochondriae in cell cultures with higher affinity than the SR101.

**References**

[1] Olivera S, Fernandez A, Latini A, Rosillo JC, Casanova G, Wajner M, et al. Astrocytic proliferation and mitochondrial dysfunction induced by accumulated glutaric acidemia I (GAI) metabolites: Possible implications for GAI pathogenesis. Neurobiol. Dis 2008; 32(3): 528-34.