3rd. International Conference of Molecular Imaging - ICMI 2015

Development and evaluation of potential ^{11}C and ^{18}F radiotracers for molecular imaging of neurodegenerative diseases using Positron Emission Tomography

Soledad Fernandez[1,2], Javier Giglio[1], Laura Reyes[1], Patricia Oliver[1], Mercedes González[3], Henry W. Engler[1], Ana Rey[2], Hugo Cerecetto[3,4]

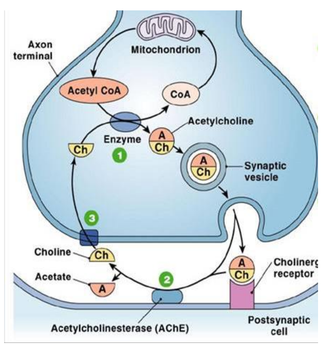
1. Centro Uruguayo de Imagenología Molecular (CUDIM), Montevideo, Uruguay. 2. Facultad de Química, Cátedra de Radioquímica, Montevideo, Uruguay. 3. Facultad de Ciencias, Grupo de Química Medicinal, Montevideo, Uruguay. 4. Facultad de Ciencias, Centro de Investigaciones Nucleares, Montevideo, Uruguay.

INTRODUCTION

Alzheimer's disease (AD) is a progressive multifactorial neurodegenerative disorder that causes dementia in late adult life. It is characterized by the presence of intracellular neurofibrillary tangles and extracellular amyloidal protein deposits. Whereas the neuropathological features of Alzheimer's disease are known, the intricacies of the physiopathology have not been clearly defined.

The Uruguayan Centre of Molecular Imaging (CUDIM), the Laboratory of Radiochemistry and the group of Medicinal Chemistry from the University in Uruguay are collaborating in the development of new radiopharmaceuticals for diagnosis of AD.

CHOLINERGIC HYPOTHESIS



Cholinergic deficit in brains of patients with AD → ¿CAUSE? → Result of brain damage

Treatment strategies:

- Ach precursors (choline)
- Postsynaptic agonists Ach
- Acetylcholinesterase (AChE) inhibitors

Utility PET for imaging of cholinergic function → Determining protocol for treatment of patients with AD

AChE inhibitors in PET allow :

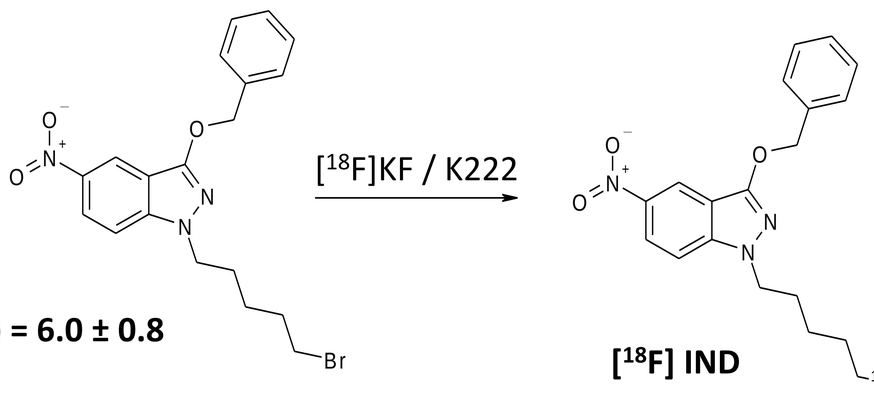
Evaluation of the pharmacokinetics of the drug.

Quantification of binding to the enzyme as a method for determining the therapeutic efficacy.

Measuring the concentration of brain acetylcholinesterase.

LABELING WITH ^{18}F

A new AChE inhibitor [3-(benzyloxy)-1-(5-bromopentil)-5-nitro-1*H*-indazole], IND, was evaluated in vitro for its anti-cholinesterase activity and was selected for labeling. The labeling was performed by substitution of the Br atom in the IND by ^{18}F in order to obtain [^{18}F]IND. The precursor dissolved in DMSO was treated with K^{18}F in presence of Kryptofix-222. The labeling and purification was performed in the nucleophilic synthesis module Tracerlab FXFN GE. The best results were obtained using 5 mg of the precursor at 160 ° C for 10 minutes



AChE (IC_{50}, µM) = 6.0 ± 0.8

[^{18}F] IND

The [^{18}F] IND was obtained with a yield of 25.0 ± 5.5 % (n = 5), not decay corrected.

Analytical control system

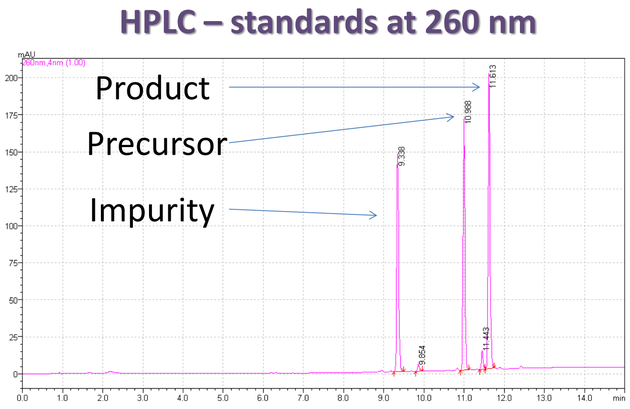
Colum: Agilent Zorbax Eclipse Plus C18, 4.6x150mm, 5µm

Mobile phase: A- Formic acid 0,1% (V/V) in H2O; B- Formic acid 0,08% (V/V) in MeCN

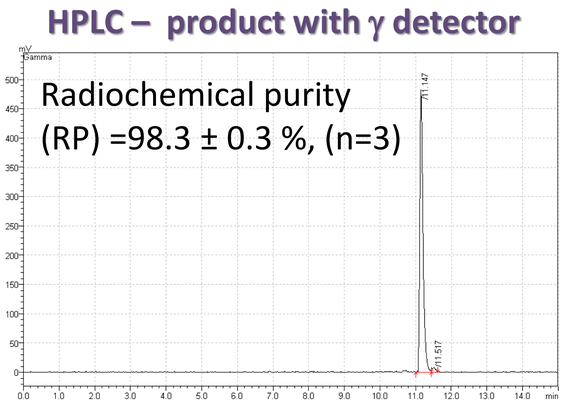
Gradient: 0 min: 10% B; 0 a 10 min: 10 a 100% B; 10 a 15 min: 100% B

Flow: 2,0 mL/min.

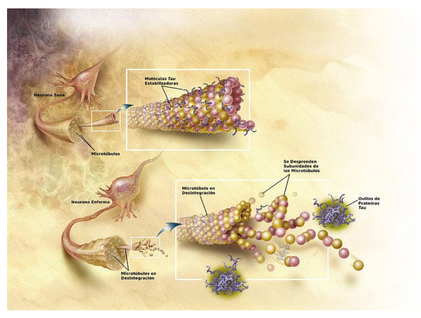
HPLC – standards at 260 nm



HPLC – product with γ detector



HYPOTESIS OF TAU PROTEIN



Tangles are produced by hyperphosphorylation of tau protein, which loses its ability to bind tubulin.

Isolated kinases responsible tau hyperphosphorylation in vivo

TPKI → GSK-3 Glycogen synthase kinase

TPKII → CDK5 Cyclin dependent kinase 5

Overexpressed in the brains of patients with AD. Responsible for over 95% of the hyperphosphorylation of tau

GSK-3 INHIBITORS

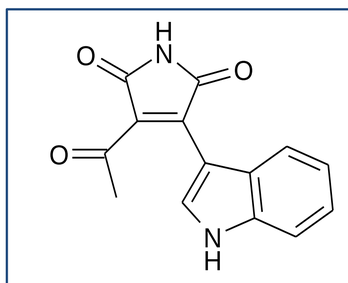
Increased phosphorylation of tau

Increased formation and toxicity of β-amyloid peptide

Overexpression of GSK-3

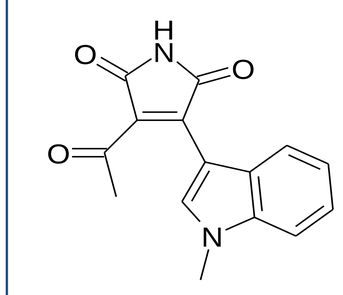
Decrease in long term potentiation processes (LTP): memory and learning.

Increased activation of microglia. Neuronal death.



VP3.16 (37) Reversible

IC_{50} = 4,47 ± 0,35 µM



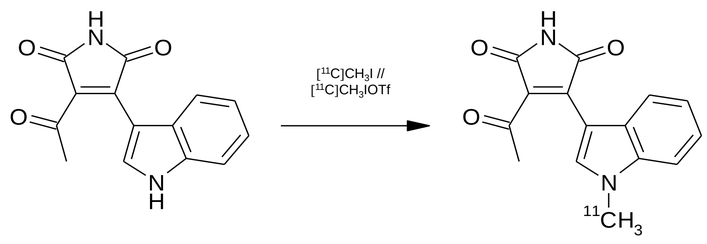
VP3.36 (38) Reversible

IC_{50} = 0,89 ± 0,19 µM

Pérezl D.I., et al. J. Med. Chem. 2011, 54, 4042–4056.

LABELING WITH ^{11}C

The GSK3 inhibitor 3-acetyl-4-(1*H*-indol-3-yl)pyrrolidine-2,5-dione (VP3,16) was labelled in a Tracerlab FXC$_{pro}$ using CH_3I in basic milieu using DMF as solvent.

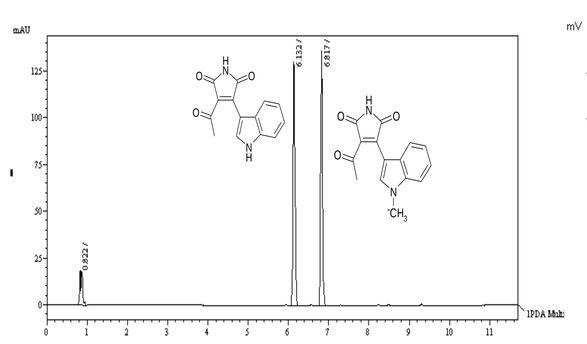


VP3.16 (MAR) → VP3.36 (^{11}C-MAR)

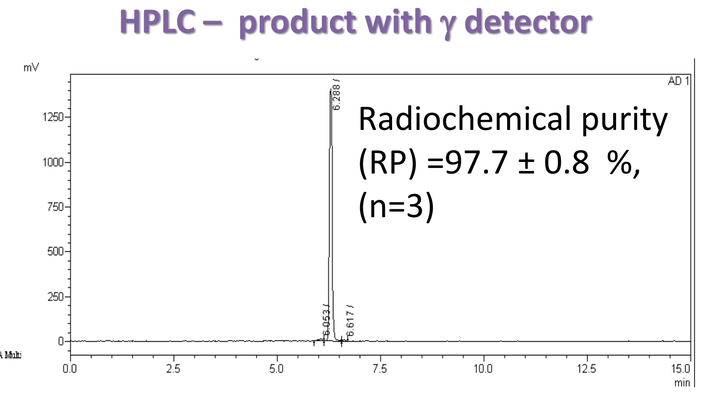
[^{11}C] VP3.36 was obtained with a yield of 16.2 ± 4.2 % (n = 5), not decay corrected

Analytical control system (Same conditions as for ^{18}F-IND)

HPLC – standards at 465 nm



HPLC – product with γ detector



CONCLUSIONS

- The [^{18}F] IND and [^{11}C] VP3.36 were obtained with high radiochemical purity and an adequate yield .
- The radiochemical purity was assessed by HPLC. The HPLC methods for the assessment of radiochemical purity were optimized for both radiolabelled compounds.
- Residual solvents are below the USP defined limit.
- The physicochemical and biological evaluations are in progress.

ACKNOWLEDGEMENTS

ANII; PEDECIBA - Química; Proyecto CSIC(España)-UdelaR(Uruguay)