Development of a ^{18}F labelled acetylcholinesterase tracer for diagnosis of Alzheimers disease

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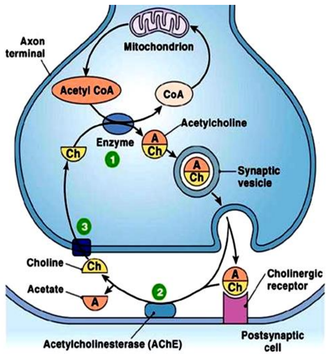
Background

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? → Results in brain damage



Treatment strategies:

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

Utility of PET for imaging cholinergic function → Determine 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.

Objective

The aim of this work is to develop a potential ^{18}F labelled radiopharmaceutical as marker of the activity of acetylcholinesterase (AChE) in Alzheimer`s disease .

A new AChE inhibitor [3-(benzyloxy)-1-(5-bromopentyl)-5-nitro-1*H*-indazole, IND], was evaluated *in vitro* for its anti-cholinesterase activity ($IC_{50,AChE}$ = 6.0 ± 0.8 μM) and was selected for labeling.

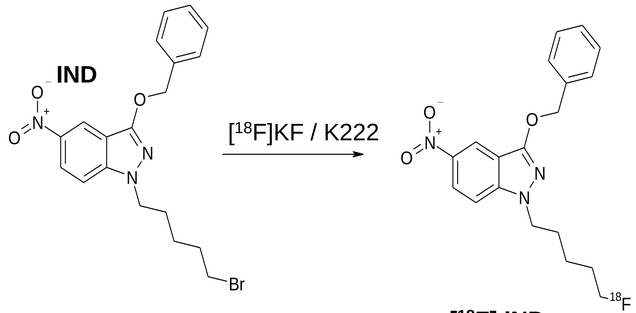
Materials & Methods

LABELING

A GEMS PETtrace cyclotron was used to generate $[^{18}F]$Fluoride by the $[^{18}O(p,n)^{18}F]$ nuclear reaction. After bombardment, the $[^{18}F]$Fluoride was transferred to a GEMS TRACERlab FX_{FN} synthesizer.

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 (IND), dissolved in 1 mL DMSO was treated with $K^{18}F$ in presence of Kryptofix-222, conditions were optimized by varying the mass precursor, the temperature and time of incubation.

The crude $[^{18}F]$IND solution was transferred to the HPLC and purified by semipreparative using a Mancherey-Nagel, Nucleodur C18, 5 um, VP 125/10 and NH_4OAc 0,1 M : CH_3CN (30:70) movil phase with a flow rate 4,0 mL/min.



$[^{18}F]$ IND

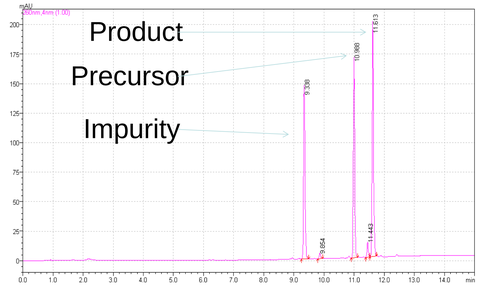
AChE (IC_{50}, μM) = 6.0 ± 0.8

QUALITY CONTROL

Radiochemical purity:

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



The radionuclide purity was assessed by gamma spectrum, and residual solvents by GC using the general conditions described in the USP.

BIODISTRIBUTION

Biodistribution was performed using normal female mice, Swiss, 2 months old.

The administration was performed in the tail vein and the animals were sacrificed by cervical dislocation.

Results

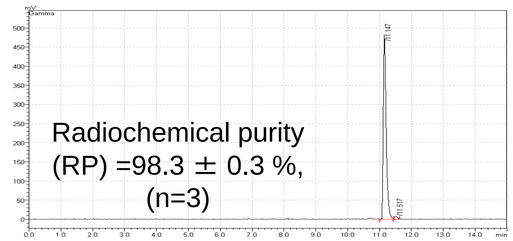
The labeling was optimized by varying the precursor mass, the temperature and time of incubation. One of the best results was obtained in condition 2 (seeTable 1). This condition was selected because it represents the best balance between yield, precursor mass and reaction time. The labelled compound was obtained with a yield of 25.0 ± 5.5 % (n = 5) (not decay corrected (NCD)) and radiochemical purity of 98.3 ±0.3 %, (n=5).

Residual solvents are below the USP defined limit.

Table 1 - Optimization of synthesis parameters

Conditions	Mass (mg)	Temperature (ºC)	Time (min)	Yield % (NDC)
1	10	160	10	27.3
2	5	160	10	25.0
3	2.5	160	10	17.8
4	2.0	160	10	6.4
5	1.0	160	10	1.0
6	5	180	10	24.9
7	5	120	10	18.7
8	5	160	5	15.2
9	5	160	20	25.8

HPLC analysis of labeled IND (γ detection)



The distribution in normal animals showed the characteristic profile of a highly lipophilic compound, high hepatobiliar elimination (22.6 ± 0.3 % dose after 30 minutes, n = 3) and low urinary excretion (12.4 ± 3.5 % dose after 30 minutes, n = 3).
The compound crossed the blood brain barrier (BBB) and an uptake in the brain of 3.5 ± 0.3 % dose/g (n = 3) was observed at 30 minutes post-injection.

Conclusions

$[^{18}F]$IND crosses the BBB and might be an interesting radioligand for *in vivo* comparative studies of the AChE brain activity in health animals and animals with AD.
The ligand selected showed interesting properties to continue the study as a potential diagnostic agent in AD.

References

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