Institut für Interdisziplinäre Isotopenforschung Leipzig

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Optimised Radiosynthesis and Metabolite Analysis of [^{18}F]NS10743, a Radioligand for Neuroimaging of α7 Nicotinic Acetylcholine Receptor (α7 nAChR)

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Introduction

α7 nAChR, a homomeric nAChR-subtype, consists of 5 subunits (Fig.1). The outstanding diversity of cellular properties, mediated by neuronal and non-neuronal α7 nAChR, points to the diagnostic potential of this target by means of molecular imaging. α7 nAChRs are both widespread in brain regions involved in learning, memory, drug addiction and information processing and overexpressed in numerous tumor cells. It is assumed that this receptor type represents an important link between neurodegeneration, inflammation and presumably cell proliferation. The central α7 nAChR is significantly reduced in neurodegenerative diseases and schizophrenia. Apart from the recently reported PET tracers [^{11}C]CHIBA-1001 [1] (Fig.2) and [^{76}Br]SSR180711, an appropriate ^{18}F-labelled selective radioligand is currently not available for clinical application of α7 nAChR neuroimaging.

We have developed the first ^{18}F-labelled radioligand [^{18}F]NS10743 (Fig.3) for PET imaging, which demonstrated promising biological properties in mice [2]. **Here we present in detail the optimised radiosynthesis and the analysis of metabolites.**

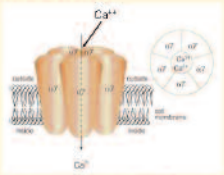


Fig.1. Structure of the α7 nAChR. The ligand-controlled ion channel is specific for Ca^{2+}.
→www.niaaa.nih.gov/Resources/GraphicsGallery/Neuroscience/

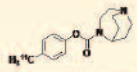


Fig.2. [^{11}C]CHIBA-1001 [1]

Fig.3 (4-[6-[4-[^{18}F]Fluorophenyl](1,3,4]oxadiazol-2yl)-1,4-diaza-bicyclo[3.2.2]nonane
→ Peters D. et al. WO 2007/038037, PCT Int. Appl. (2007)

Experimental – Radiochemistry

Radiosynthesis of [^{18}F]NS10743

A. Bromo precursor

Results

- low labelling yields (≤ 10%)
- difficult HPLC separation
  - insufficient purity
- insufficient specific activity

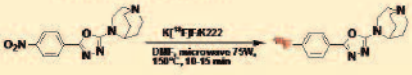
→ bromo precursor inapplicable

B. Nitro precursor

Nitro precursor does not react under thermal conditions

Partial steps

- microwave-assisted labelling
- crude purification (SPE, RP18)
  Elution: 1% HCOOH/MeOH
- reduction of excess precursor
- semipreparative HPLC separation
- final purification (SPE, RP18)
  Elution: EtOH (MeOH)



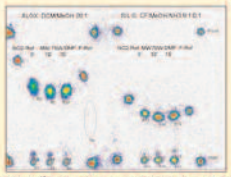


Fig.4. ^{18}F-labelling: radio-TLC of the crude product - left: ALOX (CH_2Cl_2/MeOH 20:1) right: SIL G ($CHCl_3$/MeOH/NH_3 9:1:0.1)

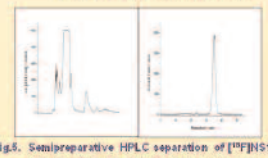
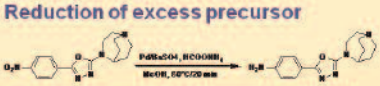


Fig.5. Semipreparative HPLC separation of [^{18}F]NS10743 left: UV- (264 nm), right: Radio-chromatogram

Reduction of excess precursor



- essential due to chromatographic similarity of the precursor and [^{18}F]NS10743
- proceeds quantitatively with $Pd/BaSO_4$
- only minor ^{18}F adsorption on Pd
- attempts for HPLC separation of the nitro compound and [^{18}F]NS10743 are in progress

Results – radiochemical parameters

- labelling yield: 55-65%
- radiochemical yield: 30-40% (n=12)
- radiochemical purity: > 99%
- specific activity: > 150 GBq/µmol
- total synthesis time: ~ 2,5 h

→ nitro precursor: excellent results

Experimental – Metabolite Analysis

Sample preparation

- homogenisation of tissue material
- removal of biological matrices:
  centrifugation, precipitation (MeCN ~0°C), purification by zeolite or RP18 phases

→ recovery rate of total activity: ≥ 90%

Analytical tools

- Radio-HPLC
- Radio-TLC
- LC-(ESI)MS
- γ-Counter
- Reference substances

In vivo metabolism in mice
(30 and 60 min p.i.)

- Brain, liver, bile: no metabolites
- Plasma, urine: one significant metabolite

Fig.6a. Radio-HPLC

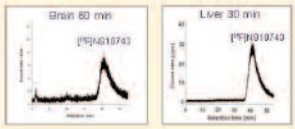
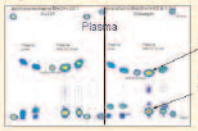
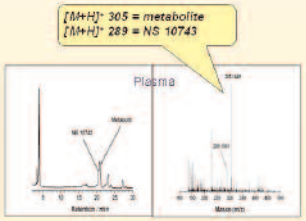


Fig. 6b. Radio-TLC



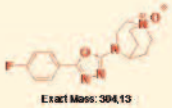
Characterisation of the major metabolite:
90 min p.i. of 75 µg NS10743

- LC-(ESI)MS in plasma samples
- ESI-MS (offline) in urine samples over time:
  Increase of $[M+H]^+$ 305
  Decrease of $[M+H]^+$ 289



Main metabolite

→ N-oxide of NS10743
probably formed via enzymatic oxidation
→ metabolism of similar structures [3]



Conclusions

- The preparation of [^{18}F]NS10743 succeeded with a microwave assisted synthesis of the corresponding nitro compound.
- The optimised radiosynthesis (RCY 30-40%) provided high purity [^{18}F]NS10743 solutions (radiochemical purity > 99%, specific activity > 150 GBq/µmol) for animal experiments.
- Metabolite studies in mice revealed metabolic stability in brain and liver (30, 60 min p.i.). In plasma and urine a single metabolite was found, which does not cross the blood-brain barrier.
- The metabolite was identified as the N-oxide of NS10743 by means of HPLC, TLC and LC-(ESI)MS.
- Based on radiochemical and biological data, [^{18}F]NS10743 is the first ^{18}F-labelled radioligand, suitable for neuroimaging of α7 nAChR with PET.

References

[1] Hashimoto, K. et al.: [^{11}C]CHIBA-1001 as a Novel PET Ligand for α7 Nicotinic Receptors in the Brain: A PET Study in conscious Monkeys. - *PLoS One* 3 (2008), e3231.

[2] Deuther-Conrad, W. et al.: Molecular imaging of α7 nAChR: design and evaluation of the promising radioligand [^{18}F]NS10743. - *Eur. J. Nucl. Med. Mol. Imaging* 36 (2009), 791-800.

[3] Shaffer, C.L. et al.: Metabolism and Disposition of a Selective α7 Nicotinic Acetylcholine Receptor Agonist in Humans. – *Drug Metab. Dispos.* 35 (2007), 1188-1195.

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