Radiolabelling and evaluation of a gavestinel-based ligand for imaging the NMDA-receptor status using PET

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Introduction: The NMDA-receptor is involved in a multiplicity of neurological functions in the central nervous system. Due to its role in neurodegenerative disorders such as cerebral ischemia, epileptical seizures, Parkinson's and Alzheimer's disease it is of major importance [1]. But also in terms of neuroexcitatory effects such as neuronal development, learning and neuronal plasticity, the NMDA-receptor is of high interest [2]. The aim of this work is to design a ligand for imaging the NMDA-receptor status via PET.

Experimental: From biological studies of a series of fluoroethoxy substituted gavestinel derivatives the most promising candidate with low nanomolar affinity was chosen for ^{18}F-labelling. Two corresponding precursor molecules (precursor A, ethyl ester and precursor B, carboxylic acid) were synthesized in a seven or eight step reaction, respectively (Figure 1).

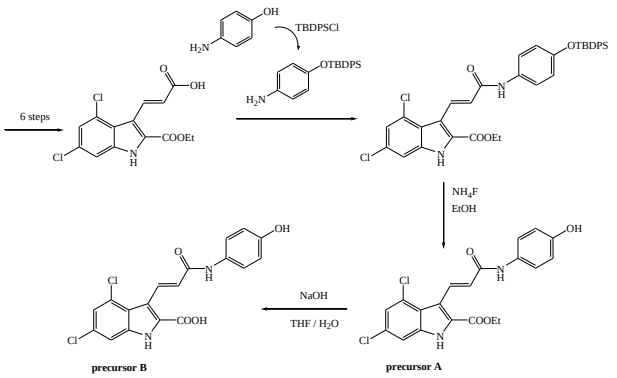


Figure 1. Reaction scheme of the precursors

The ^{18}F-labelling was achieved by the use of 2-[^{18}F]fluoroethyltosylate via the reaction of [^{18}F]F$^-$ and ethylene ditosylate (Figure 2).

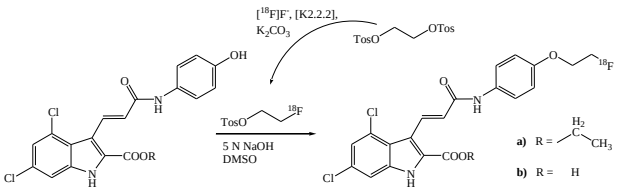


Figure 2. Reaction scheme of the radiosyntheses a) [^{18}F]fluoroethylation of the ester, using 0.95 eq. 5 N NaOH b) [^{18}F]fluoroethylation of the carboxylic acid, using 1.9 eq. 5 N NaOH

Radiochemistry: According to the optimized labelling process (Figure 3), precursor B (2 mg, 5,11 µmol) was dissolved in DMSO (300 µL), mixed with 5 N sodium hydroxide solution (1.94 µL, 1.9 eq.) and heated to 100 °C. 2-[^{18}F]fluoroethyltosylate in DMSO (700 µL) was added and the mixture was stirred at 100 °C for 20 min. To quench the reaction, the mixture was diluted with water (1 mL). The isolation of the [^{18}F]-labelled product was performed on a semipreparative Dionex HPLC system equipped with a LiChrosphere column (250 mm × 10 mm) by means of an eluent system of a mixture of NaOAc buffer (0.25 M + 5 mL/L CH_3COOH, pH 5.01) and acetonitrile at a flow of 4 mL/min. The HPLC product fraction (10 mL) was diluted with water (40 mL), passed through a strata-X cartridge and washed with water (10 mL). The product was eluted with EtOH (2 mL). The solvent was evaporated *in vacuo* and the product was formulated in isotonic NaCl-solution. Quality control was performed on a Sykam HPLC system, using the above mentioned solvent system and a LiChrosphere column (250 mm × 4 mm) at a flow of 1 mL/min. Additionally, a quality control by radio TLC was performed (R_f = 0.48, petrol ether / ethyl acetate 1 / 2 + 3 % formic acid). The new radiotracer was applied to a small animal µ-PET study using a healthy rat.

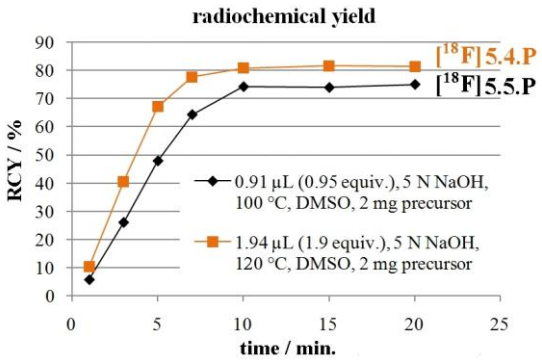
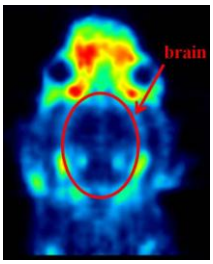


Figure 3. Results of the optimization of the ^{18}F-labelling of both precursor molecules



Results: Two new precursors were successfully synthesized. Corresponding radiolabelling via 2-[^{18}F]fluoroethyltosylate resulted in radiochemical yields of 70 – 80 %. In a small animal µ-PET study the tracer, containing the carboxylic acid, showed no brain uptake. It is assumed that the new radiotracer is not able to sufficiently penetrate the blood-brain-barrier. In further studies, autoradiographic experiments and an alternative pro-drug design of the tracer are being planned.

References

[1] Cooke SF, Bliss TVP, Brain, 2006; 129: 1659 - 1673
[2] Gozlan H, Ben-Ari Y, Trends in TiPS, 1995; 11: 368 - 374

Acknowledgement

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