PK 11195 as radiotracer for peripheral benzodiazepine binding sites (PK binding sites)

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The isoquinoline carboxamide PK 11195 is a compound that binds specifically to the so-called "peripheral benzodiazepine binding sites" (PK binding sites). Although they are found at low levels in whole brain homogenates they are not neurotransmitter receptors. They are widespread and found in the kidney, adrenal gland, testicles, ovaries, lung and heart, where they are localized in the outer mitochondrial membrane; yet, their exact function is unclear.
It was found that the levels of PK binding sites in rat brains significantly increase in case of neuronal damage; for example after intrastriatal or systemic injections of neuronal excitotoxins. This is associated with a macrophage invasion that occurs after the brain insult. Macrophages are known to be rich in PK binding sites. Therefore, [N-methyl-^{11}C]PK 11195 has a potential to be used as a tracer to study stroke, tumours, and diseases involving cell loss, e.g. Parkinson's disease.

This project was focused on developing HPLC conditions for the semi-prep and the analytical column, and then doing a practice run. These have been our first steps towards a PET study with [N-methyl-^{11}C]-PK11195.

The precursor is N-desmethyl-PK 11195, obtained from ABX. It is labelled at the nitrogen position with a ^{11}C-methyl group derived from ^{11}C-methyliodide. The reaction vessel wass preloaded with 1 mg N-desmethyl-PK 11195, which was dissolved in 0.4 mL DMSO and 10 mg finely powdered KOH. The whole reaction mixture was heated for 1.5 min at 90°C and then purified by semi-prep HPLC (Water µ-Bondapak-C18, 300 x 7.8 mm, 10 µm particle size; flow: 6 mL/min; solvent: 60% 0.01 M H_3PO_4, 40% acetonitrile). The retention time of the product was 10.8 min and that of the precursor was about 16 min. The solvent was removed by rotary evaporation, and the product was taken up in about 3 mL sterile saline.

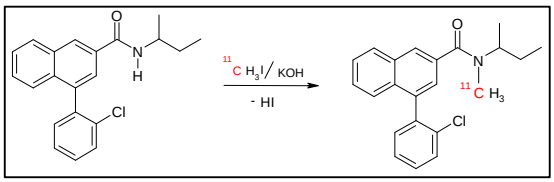

Fig.1: Labelling of PK 11195

For quality control, an aliquot of the product was analyzed by analytical HPLC and by radio-TLC. The analytical column for this purpose was Water µ-Bondapak-C18, 300 x 7.8 mm, 10 µm particle size, the flow was 1 mL/min and the solvent was the same as that used for the semi-prep (60 % 0.01 M H_3PO_4, 40% acetonitrile). The product had a retention time of 11.6 min. For the radio-TLC 50% cyclohexane and 50% ethyl acetate was used as a solvent. The R_f of PK 11195 was 0.2 (see fig.2).

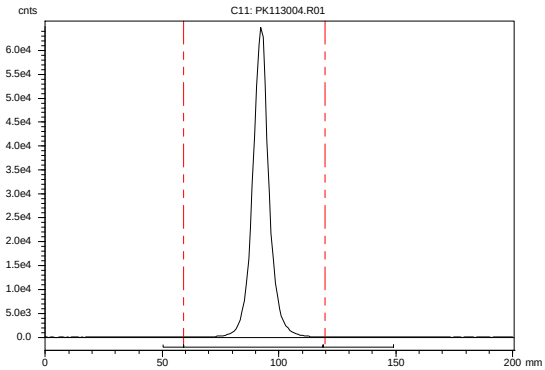

Fig.2: Radio-TLC, 99.85 % radiochemical purity

The practice run showed that N-desmethyl-PK 11195 can be labelled very easily with carbon-11. The radiochemical yield for this reaction was very high (about 78%). Unfortunatly, much of the activity was trapped in the millipore filter; therefore, a smaller millipore filter will be used for the next run to avoid this problem. Also, the pH of the saline-product-solution was too low (~ 1.0), so 0.1 mL sodium bicarbonate solution had to be added to obtain a pH of about 5.5. The radiochemical purity of the product was 99.85%. The PPB was low, suggesting that most of the tracer doesn't bind to the protein and can therefore reach the brain (not sure about this).

More studies with this tracer are being planned.

References

V.W. Pike et al., Radioligand for PET Studies of Central Benzodiazepine Receptors and PK Binding Sites, Nucl. Med. Biol., Vol. 20, No. 4, 503-525, 1993

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