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Short Research Article

SPECT imaging of the $\alpha 4\beta 2$ nicotinic receptor using (5-^{123}I)A85380†

SALLY L. PIMLOTT[1,*], MARGARET PIGGOTT[2], JAMES PATTERSON[3], JONATHAN CAVANAGH[4] and DAVID J. WYPER[3]

[1]Department of Clinical Physics, University of Glasgow, Glasgow, UK
[2]Institute for Ageing and Health, Newcastle General Hospital, Newcastle, UK
[3]Institute of Neurological Sciences, Southern General Hospital, Glasgow, UK
[4]Sackler Institute of Psychobiological Research, University of Glasgow, UK

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Abstract: In order to image nAChR density *in vivo* in humans, [5-^{123}I]-iodo-3-[2(S)-2-azetidinylmethoxy]pyridine ([5-^{123}I]A85380) has been developed as a SPECT imaging tracer. This article outlines the radiochemical synthesis, *in vitro* validation in human post-mortem tissue and some initial *in vivo* clinical studies using the ligand [5-$^{123/125}$I]A85380. Limitations of the usefulness of the tracer for routine clinical use are also discussed. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: nicotinic; receptors; 5-IA85380; SPECT; imaging

Introduction

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that are widely distributed in the human brain where the majority of high-affinity nAChRs in the brain are of the $\alpha 4\beta 2$ subtype.[1,2] Reductions in nAChR density have been shown in a number of neurodegenerative disorders that include Alzheimer's disease (AD), dementia with Lewy bodies (DLB) and Parkinson's disease (PD).[3–5] The ability to measure nAChRs levels in living patients, using either positron emission tomography (PET) or single photon emission tomography (SPECT), is therefore a useful tool in increasing our understanding of the mechanisms underlying disease processes, in the diagnosis and staging of diseases and in the development of therapies to treat a disease.

There have been a number of different radioligands developed for use in positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging of nAChRs in humans. Initially nicotine itself was radiolabelled, followed by a number of different analogs of various nicotine related compounds (some of which are shown in Scheme 1).

[^{11}C]Nicotine **1** has been used in a number of PET imaging in humans,[6–10] however [^{11}C]nicotine demonstrates rapid dissociation from the receptor–ligand complex, high levels of non-specific binding, and strong dependency of accumulation on cerebral blood flow.[11,12]

Epibatidine **4** is an azabicycloheptane alkaloid isolated from the skin of the Ecuadorian poison frog (*Epipedobates tricolor*) that demonstrates a high affinity for nAChRs *in vitro*[13–15] and a regional distribution *in vivo* in mice consistent with nAChRs.[16] Epibatidine however has 300 to 1000 times more potent activity than (-)nicotine[17] and as a result its high toxicity limits human imaging studies with epibatidine and many of its analogs.

The search for compounds with lower toxicity than epibatidine for human imaging studies, led to the development of a series of halogenated analogs **13–17**,[18] based on a 3-pyridyl ether group of compounds.[19] 5-iodo-3-[2(S)-2-azetidinylmethoxy]pyridine (5-IA85380 **17**), is a highly selective $\alpha 4\beta 2$ SPECT ligand,[20] that has been shown to be suitable for human imaging studies, with adequate safety and dosimetry.[21] This article outlines the radiosynthesis of [5-^{123}I]A85380, *in vitro* human post-mortem studies, clinical studies and limitations of clinical studies.

*Correspondence to: Sally L. Pimlott, Department of Clinical Physics, University of Glasgow, Glasgow, UK. E-mail: s.pimlott@clinmed.gla.ac.uk
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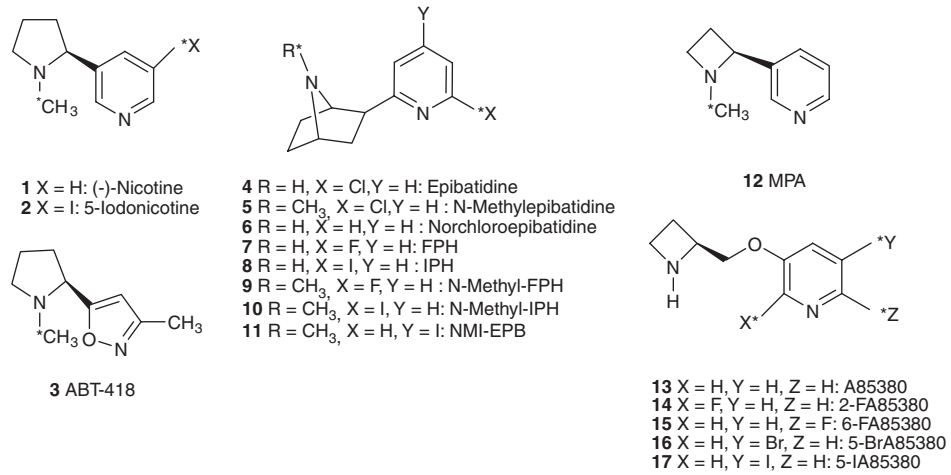


1 X = H: (-)-Nicotine
2 X = I: 5-Iodonicotine

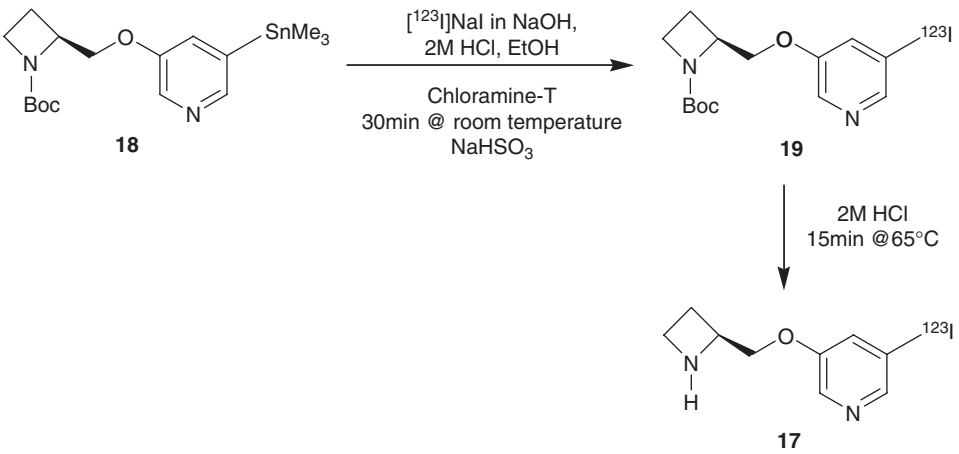
3 ABT-418

4 R = H, X = Cl,Y = H: Epibatidine
5 R = CH_3, X = Cl,Y = H : N-Methylepibatidine
6 R = H, X = H,Y = H : Norchloroepibatidine
7 R = H, X = F, Y = H: FPH
8 R = H, X = I, Y = H : IPH
9 R = CH_3, X = F, Y = H : N-Methyl-FPH
10 R = CH_3, X = I, Y = H: N-Methyl-IPH
11 R = CH_3, X = H, Y = I: NMI-EPB

12 MPA

13 X = H, Y = H, Z = H: A85380
14 X = F, Y = H, Z = H: 2-FA85380
15 X = H, Y = H, Z = F: 6-FA85380
16 X = H, Y = Br, Z = H: 5-BrA85380
17 X = H, Y = I, Z = H: 5-IA85380

Scheme 1



Scheme 2

Results and discussion

The synthesis of radiolabelled [5-^{123}I]A85380 has been previously published via eletrophilic iododestannylation of the corresponding a *t*-butoxycarbonyl (BOC) protected trimethlystannyl precursor **18**.[22,23] The method reported here (Scheme 2), via a simple and rapid one-pot synthesis, is a modification of these previously published methods. Electrophilic iododestannylation of **18** was achieved using chloramine-T as an oxidizing agent, where the reaction was left for 30 min at room temperature. The reaction was then quenched using sodium bisulphite. Deprotection of the intermediate compound **19** was achieved using 2 M hydrochloric acid and incubation for 15 min at 65°C to obtain the final product. The reaction mixture was purified using preparative HPLC, evaporated and formulated into a patient dose through a 0.22 μm filter. This method consistently produced 150 MBq patient doses with an isolated radiochemical yield of 61% ± 1.3 ($n = 25$), a radiochemical purity of >98% and a specific activity of 149 Ci/μmol ± 29.4. The stability of [5-^{123}I]A85380 was found to be adequate for clinical use, where the radiochemical purity is greater than 95% after 24 h.

Pre-clinical studies carried out in human post-mortem tissue can be used to provide reference data for clinical research SPECT studies. In a study investigating [5-^{125}I]A85380 binding in post-mortem brain tissue from normal elderly individuals, the binding distribution of [5-^{125}I]A85380 was confirmed to be consistent with the reported distribution of other high affinity nicotinic ligands (Figure 1).[24] In addition to high thalamic and moderate striatal and temporal cortex density, moderate [5-^{125}I]A85380 binding was also seen in white matter tracts in cingulate, occipital

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and temporal areas, indicating the presence of nAChRs along nerve fibre tracts, which has not been reported in other high affinity nicotinic agonist distribution studies.[24] Further study of [5-^{125}I]A85380 binding in disease tissue found a loss of striatal [5-^{125}I]A85380 in PD, DLB and AD, but not in vascular dementia (VaD).[24] These important post-mortem tissue studies demonstrated [5-$^{123/125}$I]A85380 to be a potentially useful ligand for both *in vitro* and *in vivo* human studies investigating disease symptoms and progression, response to acetylcholinesterase-inhibiting drugs and in differentiating primary degenerative dementia from VaD.

To date 5-[^{123}I]-A-85380 is the only nAChR SPECT ligand to be used clinically in humans. [5-^{123}I]A85380 imaging in normal human volunteers demonstrated high brain uptake, consistent with the known distribution of $\alpha4\beta2$ receptors and with acceptable dosimetry.[25–27] There have only been a few pilot clinical studies reported to date, investigating [5-^{123}I]A85380 binding in different neurological disorders such as AD and PD.

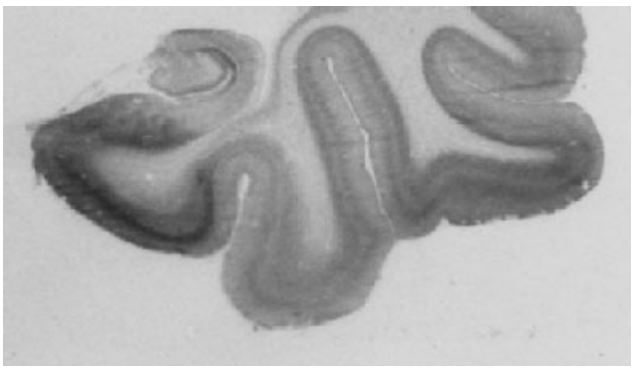

Figure 1 [5-^{125}I]A85380 binding distribution in the temporal cortex.

Preliminary results in AD, demonstrated reductions in [5-^{123}I]A85380 binding in cholinergic rich brain regions.[28,29] In PD a widespread significant decrease in [5-^{123}I]A85380 binding has been reported in both cortical and sub-cortical regions.[30] All *in vivo* data to date is consistent with the reported deficits seen in human post-mortem tissue.

A limitation in the usefulness of nicotinic receptor imaging, is the direct displacement of nicotinic tracers, such as [5-^{123}I]A85380, by nicotine itself. This is demonstrated in Figure 2, where we have shown decreased [5-^{123}I]A85380 binding in a smoker after recently smoking a cigarette, indicating occupancy of the nicotinic receptor by nicotine. This is consistent with a recent study investigating [2-^{18}F]A85380 binding in smokers where nearly complete occupancy of nAChR was shown.[31] Imaging studies investigating disease aspects such as progress and response to therapy are thereby limited to non-smoking patients, hampering the usefulness of [5-^{123}I]A85380 in routine clinical use. This characteristic however does lend [5-^{123}I]A85380 to be useful in imaging occupancy of nicotinic receptors by nicotine in studies investigating smoking and smoking cessation. An initial study investigating [5-^{123}I]A85380 binding during smoking cessation has shown increased receptor availability in abstinent smokers compared to non-smokers, where these levels normalise to control levels over time.[32]

Conclusion

[5-^{123}I]A85380 can be synthesised, via a rapid and simple one-pot synthesis, reproducibly with a high isolated chemical yield and adequate radiochemical

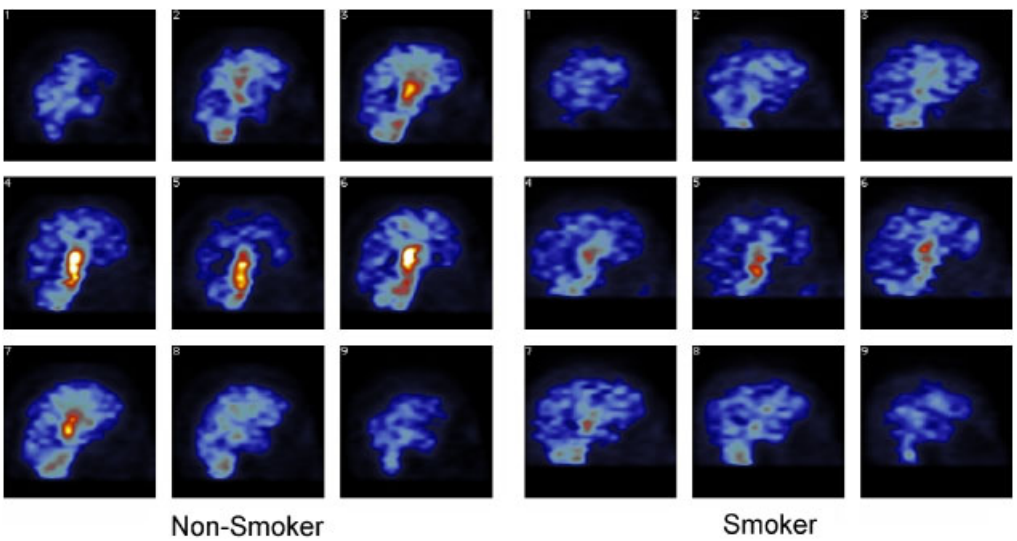

Figure 2 [5-^{123}I]A85380 sagittal scans through the midline. Figure available in colour online at www.interscience.wiley.com

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purity for human imaging studies. *In vitro* human post-mortem studies provide useful reference data for clinical imaging studies, where clinical studies investigating AD, PD and other neurological disorders are ongoing. !The effects of drugs on $\alpha 4\beta 2$ nicotinic neuro-receptors can be investigated with this tracer, but in clinical studies it is important to constrain protocols to avoid confounding effects—in this case the effect of smoking.

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