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Synthesis of 1-Benzyl-4-[(5,[^{11}C]6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine: a Promising Ligand for Visualisation of Acetylcholine Esterase by PET

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Summary

Donepezil (1-benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl-]piperidine), a potent acetylcholine esterase inhibitor, was labelled with [^{11}C]iodomethane by *O*-methylation of the *O*-6'-desmethyl precursor. Synthesis, purification and formulation were performed in approximately 30 min with an average specific activity of 39.5 GBq/μmol at EOS. The total activity available for applications was 7.5 – 11.1 GBq.

Introduction

Alzheimer's Disease (AD) is a neurodegenerative brain disorder, leading to dementia. AD afflicts between 17 and 20 million people worldwide (1). Most patients die within 10 to 15 years after the onset of the disease (2).

Multiple central neurotransmitter alterations have been identified in AD but the most consistent defects are found in the cholinergic system (3). A decrease of choline acetyltransferase (ChAT) has been demonstrated in the cortex and the hippocampus (4) and parallels the severity of the cognitive deficit. Moreover, there

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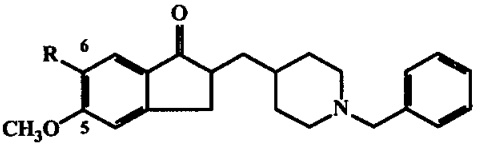
are also alterations in cholinergic receptors and decreased levels of acetyl cholinesterase (AChE) (5,6).

Symptomatic therapy of AD exists in the administration of AChE inhibitors that inhibit the metabolic breakdown of acetylcholine. Physostigmine (7) and 1,2,3,4-tetrahydro-9-aminoacridine (tacrine) (8) are two examples of AChE inhibitors. The compounds suffer from hepatotoxic side effects (9) (tacrine) or short biological half-life and non-specificity (10) (physostigmine). The recently developed N-benzylpiperidines seem to overcome the problems with the above mentioned two compounds (11). In addition they show high selectivity for AChE over butyrylcholine esterase and are selective for the brain (12) in comparison with tacrine and physostigmine. Donepezil (1-benzyl-4-[(5,6-dimethoxy-1-oxoindan-2-yl)methyl]piperidine, 1) (figure 1) is one of the most potent AChE inhibitors in this class (IC_{50} 5.7 nM) with an excellent selectivity for AChE (1250 times greater than for butyrylcholine esterase) (13). Both the S and R enantiomers have similar affinity (14).



R= OCH_3	1
R= $O^{11}CH_3$	^{11}C-1
R= OH	2

Figure 1: structure of donepezil 1, [^{11}C]donepezil ^{11}C-1 and the 6'-*O*-des-methylprecursor 2.

Several ^{11}C-labelled tracers have already been synthesized and evaluated for the visualisation of AChE *in vivo* by PET. They can be divided in two categories, the labelled substrates and the labelled enzyme inhibitors. *N*-[^{11}C]methyl-4-piperidyl-acetate (MP4A) and -propionate (MP4P) are two labelled substrate analogues which demonstrated affinity for AChE *in vivo* as observed by PET (15,16). AD patients show multiple cortical regions with reduced estimated AChE activity on MP4A PET images (17). The regional distribution in the brain of [^{11}C]physostigmine (18), a labelled enzyme inhibitor, is consistent with the present AChE activity and can be displaced by co-injection of cold physostigmine. On the other hand, the distribution

of [^{11}C]methyl-tacrine (19), a tacrine derivative, is not parallel with the AChE activity.

This report describes the radiosynthesis, purification and quality control analysis of [^{11}C]donepezil, ^{11}C-1, labelled at the methoxygroup in position 6, as a radiotracer for studying AChE *in vivo* by PET.

Results and Discussion

^{11}C-1 was synthesized by *O*-methylation of 2 in the presence of tetrabutylammonium hydroxide (TBAH) in a molar ratio of 1:1 (figure 2). An average radiochemical yield of 85% (decay-corrected, from [^{11}C]iodomethane) was obtained. At EOS 7.5 - 11.1 GBq ^{11}C-1 were produced.

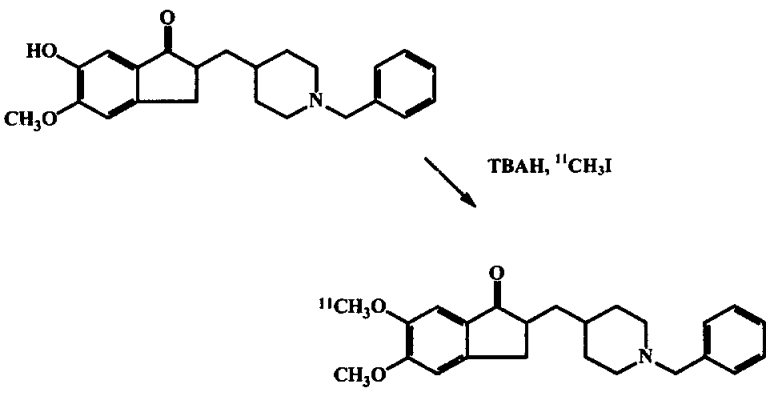


Figure 2: Radiosynthesis of ^{11}C-1.

For purification of the radioactive product, several HPLC systems were tested to obtain a good separation between the compounds in the reaction mixture. With RP HPLC using conventional columns (Alltech Econosil or Spherisorb RP-C18, 250 mm x 10 mm, 5 or 10 µm particle size) resolution was poor due to tailing of the peaks and high retention times. This could only be eliminated by the use of acidic buffer solutions (pH < 3.5) or addition of triethylamine. As triethylamine is a toxic compound, this HPLC method can only be applied for quality control of the tracer preparation. Finally, a RP-select column (Merck, 250 mm x 10 mm, 10 µm particle size) gave the best separation of ^{11}C-1 (t_R = 10.2 min) and 2 (t_R = 6.4 min) using acetate buffer (0.1 M acetic acid adjusted to pH 4 with NaOH): ethanol (67.5:32.5)

as mobile phase. With this system the collected fraction had only to be diluted 1 to 3 with phosphate buffer pH 8 (0.1 M) in order to obtain a final injectable solution with an ethanol concentration of approximately 10% and a pH of 6.0 - 7.0 (total volume after dilution: 12 mL). A typical chromatogram obtained from a production is shown in figure 3.

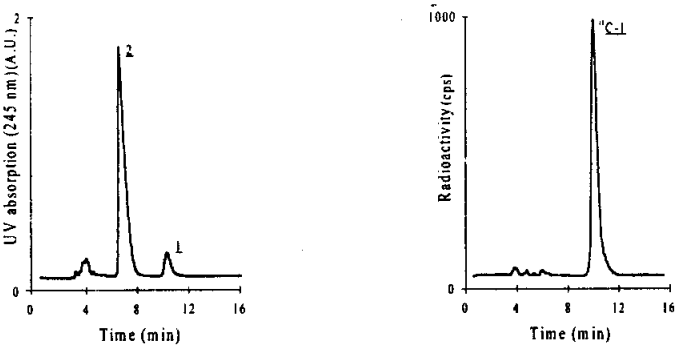


Figure 3: Typical chromatograms of product purification (left: UV chromatogram, right: radiochromatogram).

Radiochemical purity was better than 99% and a specific activity 39.5 GBq/μmol (s.d. 11.5 GBq/μmol, n=3) was obtained at EOS.

Chemical purity is performed by HPLC. UV detection at 230 nm is used for the detection and quantification of 2. With respect to the pharmacodynamic effects of receptor ligands and their derivatives and their possible interference with PET measurements, control of the mass level of 2 itself (IC_{50} value of 6.4 nM for AChE) in the final solution is mandatory. If the separation of 2 from ^{11}C-1 in the purification of the reaction mixture is not complete, the final tracer solution might contain 2 in amounts sufficient to interfere with the binding of [^{11}C]donepezil at AChE. Amounts of 2 corresponded to 3.8 nmol which means that chemical purity was higher than 98.0% and interference at AChE is negligible.

The acceptable DMF level of injectable solutions is based on the maximum acceptable daily intake (ADI). For DMF, the reaction solvent, the ADI is 6.7 mg/day for a 70 kg body mass (20). In our final purified tracer solution the concentration of DMF was below the detection limit for DMF (50 μg/ml). Considering the fact that, under normal conditions, for PET investigations only 370 MBq ^{11}C-1 are administered to humans, the injected volume ranges between 0.4 and 0.6 mL so that the intake of DMF (amounts of DMF ranging between 20 and 30 μg) is far below the ADI.

Experimental

Materials and Methods

1 and 2 were gifts from the Research Laboratories of Eisai Inc. Tetrabutylammonium hydroxide (TBAH, 40% w/v solution in water) and dimethylformamide (DMF) were purchased from Acros chimica. All other chemicals were either of 'HPLC' or 'pro analyse' grade and obtained from Aldrich, UCB or Labscan. DMF was dried over molecular sieves (3Å). HPLC was performed with a Waters 510 pump, a Pye-Unicam 4110 UV-detector and a NaI(Tl) detector or a GM-tube. Chromatograms were recorded on a dual channel integrator (Shimadzu CR5-A). For the determination of DMF a Pye-Unicam 4500 gas chromatograph, equipped with a FID detector was used.

Radiosynthesis and purification of ^{11}C-1.

Protons of 18 MeV with a beam intensity of 15 µA are used in a ^{14}N (p,α) nuclear reaction for the production of [^{11}C]CO_2. [^{11}C]CO_2 was subsequently reduced to [^{11}C]methanol and converted to [^{11}C]iodomethane semi-automatically according to the recommendations desribed by Crouzel et al. (21). [^{11}C]Iodomethane was swept by a stream of argon gas into a cooled solution of 2 (1 mg, 3 µmol) in 150 µl of DMF, containing 3 µmol of TBAH. After trapping [^{11}C]iodomethane, the reaction mixture was heated at 120° for 4 min. Water (100 µL) was added to the solution and this mixture was applied to the HPLC system. The column (Merck, RP-select, 250 mm x 10 mm, 10 µm particle size) was eluted with a mixture of acetate buffer (0.1 M acetic acid adjusted to pH 4 with NaOH): ethanol (67.5:32.5) at a flow rate of 4 mL/min. The eluent was simultaneously monitored with a UV-detector set at 254 nm and a GM-tube. ^{11}C-1 was isolated and filtered through a 0.22 µm sterile Acrodisc filter into a sterile and pyrogen free vial. Sterile pyrogen free distilled water (8 mL) was added so that the final concentration of ethanol was below 10%.

Chemical and radiochemical purity

Chemical and radiochemical quality control was performed by HPLC. The system consisted of a Nucleosil RP-C18 column (Alltech, 250 mm x 4 mm, 5 µm

particle size) eluted with phosphate buffer (50 mM phosphoric acid adjusted to pH 3.0 with NaOH): acetonitrile mixture (50:50) containing 5 mM dodecylsulphonic acid, sodium salt. The flow rate was set at 1 mL/min. Detection was achieved with a UV-detector set at 254 nm (specific activity determination) or at 230 nm (determination of 2) and a NaI(Tl) detector.

Quantification of DMF in final solution

Residual DMF in the injectable solution was determined by gas chromatography. The analysis was carried out on a chromatograph equipped with a 2 m x 2.5 mm I.D. stainless steel column packed with Porapack Q (80 - 100 mesh). The temperatures of the injector and FID detector were 250 and 200 °C, respectively. Column temperature was set at 190°C. Nitrogen gas was used as carrier at a flow rate of 30 mL/min. 1.0 µL of the final solution or DMF standard in water was injected into the GC system. The detection limit for the system was approximately 50 µg/mL.

Conclusion

The synthesis of ^{11}C-1, a potential PET imaging agent for AChE is described. 7.5 - 11.1 GBq could be produced with a synthesis time of 30 min corresponding to a radiochemical yield of 85%. Chemical and radiochemical purity were higher than 98 and 99%, respectively and a specific activity of 39.5 GBq/µmol was obtained.

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