Synthesis of [^{76}Br]4-Bromodexetimide and [^{76}Br]4-Bromolevetimide: Radiotracers for Studying Muscarinic Cholinergic Receptors Using PET

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Summary

The muscarinic cholinergic receptor antagonist dexetimide and its pharmacologically inactive enantiomer levetimide were labelled with the positron emitter bromine-76. [^{76}Br]4-Bromodexetimide, [^{76}Br]BrDEX, and [^{76}Br]4-bromolevetimide, [^{76}Br]BrLEV, were prepared via electrophilic bromodesilylation of 4-(trimethylsilyl)dexetimide and 4-(trimethyylsilyl)levetimide with [^{76}Br]NH_4. The use of chloramine-T in acid media resulted in radiochemical yields of 80%. The radiotracers were purified by semi-preparative reverse-phase HPLC. Radiochemical and chemical purities were assessed by radio-TLC and HPLC and found to be 98%. The average time of synthesis including formulation was 60 minutes resulting in average specific activities of 300 mCi/μmol.

Key Words: dexetimide, levetimide, bromine-76, radiotracers, PET, muscarinic cholinergic receptors.

Introduction

There are five distinct receptor genes (m1-m5) which have been described for muscarinic cholinergic receptors (m-AChRs) (1). The m-AChR genes encode the M1, M2, M3 and perhaps the M4 receptors which are pharmacologically defined by the use of selective antagonists. The M1 and M3 receptors have been shown to be coupled preferentially to the stimulation of phosphoinositide metabolism, and the M2 and M4 receptors have been shown to be coupled preferentially to the inhibition of adenylate cyclase (2). The m-AChR exist throughout the mammalian brain and mediate the effects of acetylcholine and cholinergic drugs. The putative role of m-AChR in

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neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and dementias associated with Parkinson's disease (3-7) has generated considerable interest for the non-invasive mapping of m-AChR.

Introduction of non-invasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) has made possible the study of neuroreceptors in the living human brain. Such studies have proven useful in the localisation and quantification of neuroreceptors and offer insight into the relationship of these receptors in normal and pathological states (8). Successful imaging of m-AChR using [^{123}I]IQNB (9) has been achieved using SPECT and alterations in m-AChR levels have been reported for patients with dementia compared to controls (10). Radiotracers which have been developed for use with PET include [^{11}C]scopolamine (11), [^{11}C]QNB (12), [^{11}C]benztropine (13) and [^{11}C]benzetimide derivatives.

The long acting m-AChR antagonist dexetimide and levetimide, its pharmacologically inactive enantiomer, have both been labelled with carbon-11 and evaluated as PET radiotracers (14). This involved the synthesis of [α-^{11}C]benzyl iodide and its use in an alkylation reaction of the appropriate nor-benzyl amine substrate. This two-ligand approach offers advantages over previous *in vivo* strategies since the use of [^{11}C]levetimide should permit the measurement of non specific binding of [^{11}C]dexetimide *in vivo*. Recently a series of halogenated derivatives of racemic dexetimide were reported (15) which led to the development of several halogenated dexetimide radiotracers including 2-fluoro- and 4-fluorodexetimides for studying m-AChR with PET. Two radiosynthetic methods have been utilised for the preparation of the fluorodexetimides, the first involved reductive amination of [^{18}F]fluorobenzaldehydes with nor-benzyldexetimide (16) and the latter alkylation of the nor-benzyldexetimide with [^{18}F]fluorobenzyl halides (17). Both methods involved multi-step syntheses in which fluorine-18 was introduced in the first step. [^{18}F]2-Fluorodexetimide was also shown to undergo defluorination *in vivo* (17,18).

The demand for SPECT analogs has seen the successful development of [^{123}I]4-iododexetimide and [^{123}I]4-iodolevetimide (15). The preparation involved the iodination of 4-(trimethylsilyl)dexetimide and 4-(trimethylsilyl)levetimide via electrophilic iododesilylation in the final step of the synthesis. The physical half-life of iodine-123 is relatively long (13.2 h) which permits extensive tracer clearance from non-specific compartments and prolonged data acquisition. Successful imaging of m-AChR in human brain using [^{123}I]4-iododexetimide has been reported (19,20). Based on these results and the need for a facile synthesis of longer lived positron emitting dexetimide derivatives for studying m-AChR we chose to label dexetimide and levetimide with bromine-76 (16.2 h). This is a suitable isotope for PET studies due to its convenient half-life and its use in the quantification of the corresponding SPECT radioiodinated ligand. In this paper we report the radiosynthesis, purification, and quality control of [^{76}Br]4-bromodexetimide, [^{76}Br]BrDEX, and [^{76}Br]4-bromolevetimide, [^{76}Br]BrLEV.

Materials and Methods

The 4-(trimethylsilyl)dexetimide and 4-(trimethylsilyl)levetimide precursors and bromo standards were synthesised from commercially available dexetimide and levetimide (Research Biochemicals Inc) as previously described. This involved removal of the benzyl group by catalytic hydrogenolysis with formic acid as the hydrogen donor (17). Alkylation of the nor-benzyl intermediates was carried out with either 4-bromobenzylbromide or 4-trimethylsilylbenzylbromide which were both synthetically prepared as previously described (15,21). Bromine-76 was produced by irradiation of natural arsenic with a beam of 30MeV [^{3}He] ions (22). Briefly, after 15 h cooling the target was dissolved in sulphuric acid followed by oxidation with chromic acid. The radioactive bromine was carried over with a nitrogen stream and trapped in ammonia which was evaporated to dryness and reconstituted in water. Radioactivity measurements were made using a Capintec CRC-12 radioisotope calibrator.

Synthesis and Purification of [^{76}Br]4-bromodexetimide

4-(Trimethylsilyl)dexetimide.HCl (0.5 mg, 1.06 μmol) was dissolved in methanol (20 μL) followed by addition of HCl (0.1 M, 100 μL). To the reaction mixture was added [^{76}Br]NH_4 (5 mCi) and a solution (20 μL) containing chloramine-T (3.9 mg, 13.08 mmol) in HCl (0.1M ,4.0 mL). The reaction mixture was allowed to stand at room temperature for 15 minutes followed by addition of a solution of sodium metabisulfite (10 mg/mL, 30 μL). The mixture was injected onto a μ-Bondapak C-18 (10 μm, 300 mm x 7.8 mm) semi-preparative column and eluted at a flow rate of 2.5 mL/min. The mobile phase consisted of 45:55 acetonitrile:water (0.1 M ammonium acetate). The effluent from the column was monitored with a UV detector (239 nm, Waters module 440) and an in-line Geiger-Muller radioactivity detector. The radioactivity peak corresponding to [^{76}Br]BrDEX (t_R=26 minutes, k'=8) was collected and rotary evaporated to dryness. The residue was dissolved in sterile normal saline and filtered through a sterile, 0.22 mm filter (Millex GS, Millipore) into a sterile, pyrogen-free evacuated vial, and the radioactivity measured. The total synthesis time was 60 minutes with radiochemical yields of 80% calculated EOS. Radiochemical and chemical purities assessed by radio-TLC, silica gel plates (90:9.9:0.1, CH_3Cl:MeOH:TEA, R_f=0.65) and HPLC were 98%.

Synthesis and Purification of [^{76}Br]4-bromolevetimide

4-(Trimethylsilyl)levetimide.HCl (0.5 mg, 1.06 μmol) was treated as described above. Purification was carried out using the same HPLC conditions, [^{76}Br]BrLEV (t_R=26 minutes, k'=8).

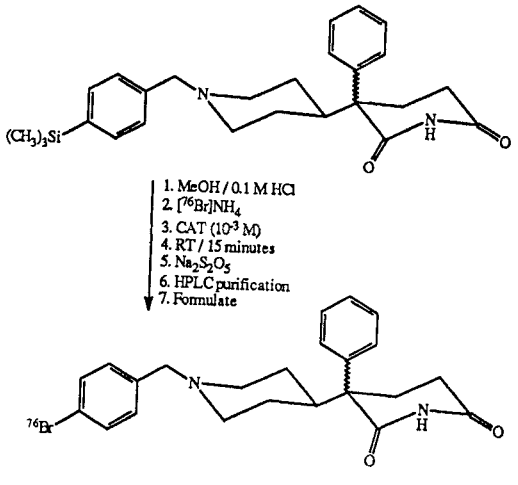
Radiochemical yields of 80% were achieved with radiochemical and chemical purities which assessed by radio-TLC and HPLC, were 98%.

Determination of Specific Activity

An aliquot of the final solution of known volume and radioactivity was applied to an analytical reverse-phase HPLC column (Waters μ-Bondapak C18 300mm x 3.9 mm). A mobile phase of 45:55 acetonitrile:water (0.1 M ammonium acetate) with a flow rate of 1 mL/min was used to elute the radioligand (t_R =16 minutes, k'=5.4). The area of the UV absorbance peak measured at 239 nm corresponding to carrier product was measured and compared to a standard curve relating mass to UV absorbance.

Results and Discussion

[^{76}Br]BrDEX and [^{76}Br]BrLEV were prepared by electrophilic bromodesilylation with no carrier added [^{76}Br]NH_4. During the preparation of these radiotracers a number of reaction conditions and reagents were examined. Chloramine-T was the optimum oxidising agent but was concentration and solvent dependent. Choramine-T in a final reaction concentration of 10^{-3}M in 0.1N HCl gave the highest radiochemical yields (Scheme 1).



(S)-[^{76}Br]BrDEX

(R)-[^{76}Br]BrLEV

Scheme 1.

The reaction mixture was allowed to stand at room temperature for 15 minutes followed by the addition of a sodium metabisulfite solution. Reverse-phase semi-preparative HPLC was used to purify the two radiotracers (figure 1). This is consistent with other reports on the use of chloramine-T as the oxidising agent for no-carrier-added labelling of aromatic biomolecules with bromine-75 and bromine-77; highest radiochemical yields were obtained at pH 1 within a few seconds at a chloramine-T concentration of 10^{-3} M in the final solution (23,24).

The average time of synthesis of [^{76}Br]BrDEX and [^{76}Br]BrLEV was 60 minutes with radiochemical yields of 80%. Comparison of the carrier peak associated with the radioactivity to that of a standard sample of BrDEX and BrLEV enabled calculation of the specific activity. The resulting chromatograms showed [^{76}Br]BrDEX and [^{76}Br]BrLEV to be of high radiochemical and chemical purities (98%), which was also checked by radio-TLC. The radioactivity products co-eluted with authentic samples while the specific activities calculated at the end of synthesis using the above reaction conditions were 300 mCi/μmol.

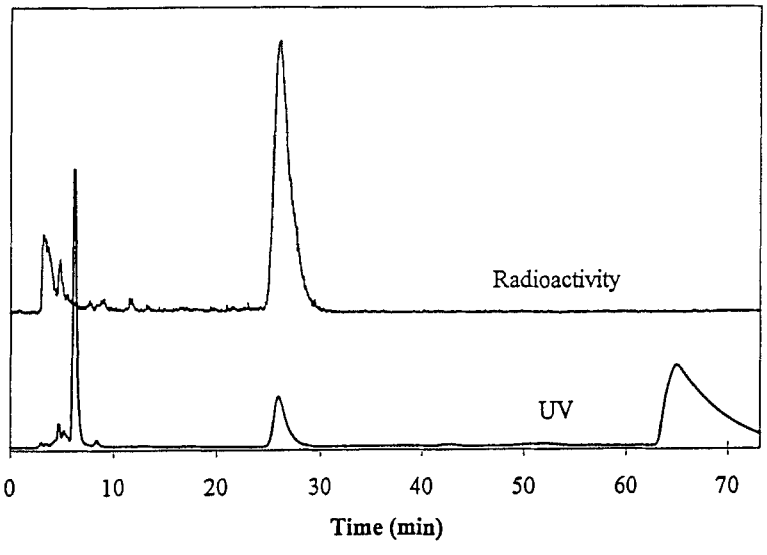


Figure 1. Preparative HPLC chromatogram of [^{76}Br]BrDEX

The initial radiolabelling conditions, for [^{76}Br]BrDEX, involved the use of chloramine-T, in a two mass equivalent to substrate followed by dissolution in trifluoroacetic acid as solvent. The rate of reaction was monitored by removal of aliquots at 1, 2, 5, 10 and 15 minutes and the radiochemical yield determined by radio-TLC. The maximum radiochemical yield was 15-16% at 5 minutes and

remained constant over the period of 15 minutes. A possible side reaction is the competing chlorodesilylation reaction which was minimised by reducing the amount of chloramine-T to one-tenth mass equivalent of the substrate. The rate of reaction was monitored with radiochemical yields of 20% achieved by 15 minutes. Increased radiochemical yields, 30% in 5 minutes, was obtained when the order of addition was reversed and chloramine-T was added last to the reaction vessel. The optimum radiochemical yields were obtained with the addition of chloramine-T last in a final reaction concentration of 10^{-3} M in 0.1N HCl (figure 2). The radiosynthesis of [^{76}Br]BrDEX and [^{76}Br]BrLEV was carried out using the optimum reaction conditions (see Materials and Methods). Under these reaction conditions no chlorinated DEX or LEV side products were observed by HPLC (figure 1). The use of other oxidising agents included peracetic acid in acetic acid as solvent and dichloramine-T in TFA solvent. These resulted in radiochemical yields of 5 and 20% respectively at 15 minutes reaction time and where both inferior to chloramine-T.

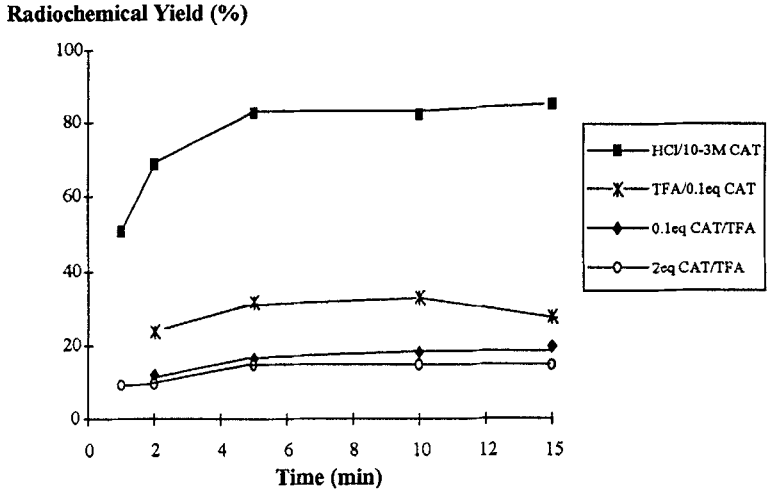


Figure 2. Rate of [^{76}Br]4-Bromodexetimide Formation.

Conclusions

[^{76}Br]BrDEX and [^{76}Br]BrLEV were prepared by bromination via electrophilic bromodesilylation of 4-(trimethylsilyl)dexetimide and 4-(trimethylsilyl)levetimide with no carrier added [^{76}Br]NH_4. The synthesis produced radiochemical and chemical pure products in quantities sufficient for *in vivo* studies of m-AChR in the brain using PET.

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