technical note

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Synthesis of a [^{11}C]Methoxy Derivative of α-Dihydrotetrabenazine: a Radioligand for Studying the Vesicular Monoamine Transporter

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The synthesis of [^{11}C]TBZOMe, a [^{11}C]methoxy derivative at the 2-hydroxy position of α-dihydrotetrabenazine, was carried out by an *O*-[^{11}C]methylation reaction. The product [^{11}C]TBZOMe (100–200 mCi) was obtained in 15–40% radiochemical yield (corrected for decay) within 37 min, and in high specific activity (2000–2500 Ci/mmol) and radiochemical purity (>97%). [^{11}C]TBZOMe is a potential new radioligand for studying the vesicular monoamine transporter using positron emission tomography.

Introduction

Tetrabenazine (TBZ) and dihydrotetrabenazine (TBZOH, α and β isomers), the major metabolites of TBZ (Schwartz *et al.*, 1966; Mehvar and Jamali, 1987), are specific inhibitors of the vesicular storage of monoamines, and bind with high affinity to the synaptic vesicle amine transporter (TBZ and α-TBZOH, $IC_{50} = 3$ nM; β-TBZOH $IC_{50} = 20$ nM) (Scherman *et al.*, 1988). We have previously reported the synthesis and *in vivo* biological evaluation of TBZ labeled with carbon-11, a short-lived ($t_{1/2} = 20.4$ min) radionuclide, for non-invasive *in vivo* imaging of monoaminergic terminals by positron emission tomography (PET) (DaSilva and Kilbourn, 1992; DaSilva *et al.*, 1993a,b). Quantification of monoaminergic terminal losses would be of immense value for studying the development and progression of neurodegenerative disorders such as Parkinson's disease. [^{11}C]Tetrabenazine showed good brain penetration and significant specific binding in brain regions with high levels of monoaminergic innervation (DaSilva and Kilbourn, 1992). However, [^{11}C]TBZ is rapidly and extensively metabolized *in vivo* to give mainly α- and β[^{11}C]TBZOH, and the presence of potential radiolabeled metabolites may complicate quantitative pharmacokinetic modeling. As large substituents can be attached at the hydroxyl function of α-TBZOH and such derivatives retain high binding affinity for the monoamine vesicular transporter (Scherman *et al.*, 1988; Aranda *et al.*, 1990), we have investigated derivatives of TBZOH as possible improved, less metabolized *in vivo* imaging agents. We report here the synthesis of the [^{11}C]methyl ether derivative of the 2-hydroxyl function of α-TBZOH in order to explore the effect of an *O*-[^{11}C]methoxyl group on the specific brain uptake and retention, and metabolism, of such TBZ derivatives.

Experimental

Synthesis of 5-O-methyl dihydrotetrabenazine (TBZOMe)

The Na-alkoxide salt of **1** (50 mg, 0.18 mmol) was prepared by stirring with dry NaH (~30 equiv.) in DMF at 80°C for 15 min. After cooling the reaction vessel at 0°C, CH_3I (1 equiv.) was added and the mixture was stirred for 5 min. The mixture was then poured in cold water (~5 mL, 0°C), passed through a C18 Sep-pak (Waters Assoc., preactivated with 10 mL MeOH and 20 mL water), rinsed with water (20 mL) to remove NaOH and DMF, then eluted with CH_2Cl_2 (30 mL) and MeOH (20 mL). The CH_2Cl_2 fraction was evaporated to dryness and the residue purified (3×) by silica gel chromatography (gradient CH_2Cl_2–CH_2Cl_2:MeOH 19:1) to mainly give **2** (TLC $R_f = 0.35$, eluent $CHCl_3$:MeOH 24:1). TBZOMe was further purified by silica gel semi-preparative HPLC (CH_2Cl_2:hexane:(isopropanol:diethylamine 24:1) 17:82:1; 5 mL/min; T_R = 9 min) to provide **2** as an oil (2 mg, ~3%). ^{1}H-NMR ($CDCl_3$, 360 MHz) δ 0.945 (m, 6H, CH—$(CH_3)_2$), 2.45 (dt, 1H, $C(OCH_3)H$), 3.44 (s, 3H, $CHOCH_3$), 3.84 (s, 3H, $ArOCH_3$), 3.86 (s, 3H, $ArOCH_3$), 6.58 (s, 1H, ArH) and 6.68 (s, 1H, ArH); MS (C.I., NH_3) *m/z* 334 ($[M+1]^+$, 100%); Anal. (high resolution-exact mass) Calcd for $[C_{20}H_{31}NO_3—H]^+$: 334.2382. Found: 334.2368.

Synthesis of [^{11}C]TBZOMe

The sodium-alkoxide salt of **1** was formed *in situ* just prior to the *O*-[^{11}C]methylation, by heating (80°C) the reaction vessel containing **1** (1.0 mg) and dry NaH (2–5 mg, ~18–44 equiv.) in dry DMF (200 mL) under N_2 for 5–10 min. This reaction vessel was then placed in a custom-made apparatus, and all subsequent steps in the synthesis performed by remote control from outside of the closed hot cell. [^{11}C]Carbon dioxide was produced via the $^{14}N(p,\alpha)^{11}C$ reaction using proton irradiation of a nitrogen target, and converted to [^{11}C]methyl iodide by $LiAlH_4$ reduction followed by treatment with HI. The vial containing the Na-alkoxide salt of **1** was cooled to −30 to −40°C, [^{11}C]CH_3I (carried by a stream of N_2) was bubbled into the reaction mixture, and the vial sealed and warmed to 0°C for 5 min to afford [^{11}C]**3**. Water (0.5 mL) was added at 0°C to stop the reaction and decompose excess NaH, then the solution was transferred onto a short reversed phase column in-line with the HPLC injector, and which was filled with C_{18} Sep-pak packing that had been pre-washed with MeOH (50 mL) and water (100 mL). The extraction column was rinsed with water (1.0 mL) and blown dry with N_2 (tank pressure 80 psi) for 2 min. [^{11}C]TBZOMe was purified using a silica gel semi-preparative HPLC column, by passing the HPLC solvent (CH_2Cl_2:hexane:(isopropanol:diethylamine 24:1) 37:62:1; 6 mL/min) through the extraction column and onto the HPLC column. The radioactive peak corresponding to [^{11}C]**2** ($T_R = 6.0$ min; **1** $T_R > 15.0$ min) was collected into a sterile vial placed in a warm bath (30–40°C) and the solvent removed by N_2 flow. The residue was formulated in a sterile solution of isotonic phosphate buffer (pH 6.0) and then filtered through a 0.2 μm alumina filter (Anotop) into a sterile 10 mL multidose vial (radiochemical yield 15–40% at end of synthesis, decay corrected, based on [^{11}C]CO_2). [^{11}C]TBZOMe was prepared for i.v. injection within 37 min from end of bombardment in high chemical

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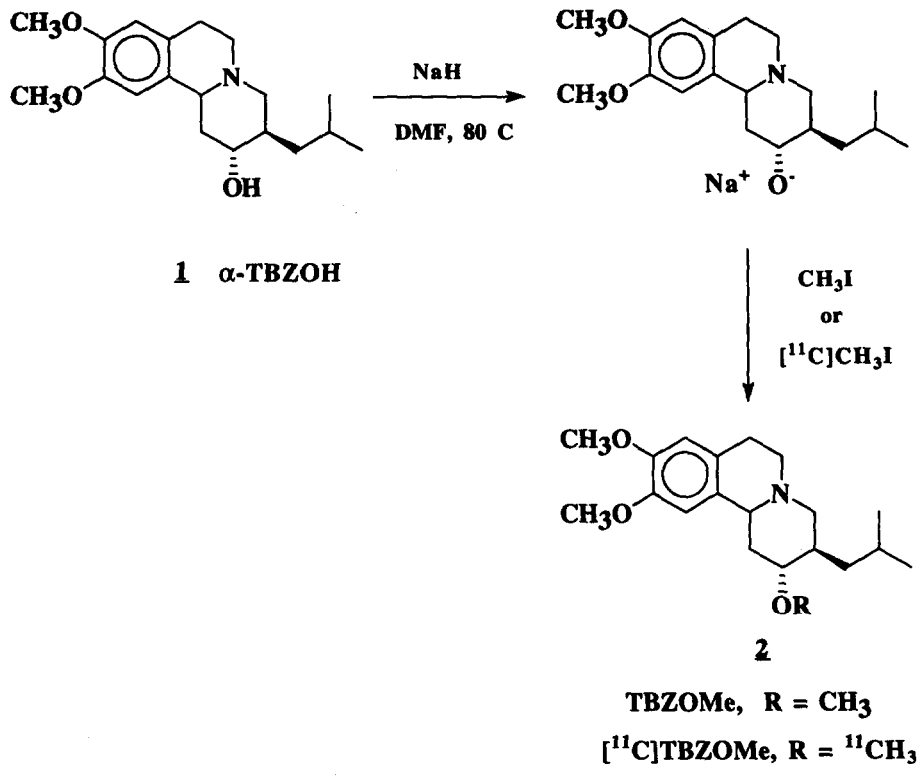

Scheme 1

and radiochemical purities (>97%), and specific activities varied between 2000–2500 Ci/mmol at end of synthesis.

Analysis of an aliquot of the final formulation of [^{11}C]2 was performed on a C_{18} 5 micron HPLC column (acetonitrile: KH_2PO_4 (10 mM) 3:1; 2.0 mL/min; T_R = 6.4 min), in series with u.v. (286 nm) and γ-radioactivity detectors. HPLC analyses showed that [^{11}C]TBZOMe was identical to authentic TBZOMe (2).

Discussion

The synthesis of [^{11}C]TBZOMe first required synthesis of α-TBZOH, which was prepared by modification of reported methods (Shwartz *et al.*, 1966; Aranda *et al.*, 1990; Scherman *et al.*, 1981). Sodium borohydride reduction (3.5 equiv.) of TBZ in dry EtOH for 90 min afforded two diastereomers of dihydrotetrabenzine [α-TBZOH (1) and β-TBZOH] in >95% yield, which were separated by silica gel chromatography (CH_2Cl_2:MeOH 49:1) in a ratio of about 4:1 with the desired α-isomer predominating.

A sample of authentic, unlabeled 2 was prepared by *O*-alkylation with one equivalent of methyl iodide (Scheme 1). Extensive purification (column chromatography and HPLC) was needed to separate the desired product from a minor impurity (which was not seen in alkylations with no-carrier-added [^{11}C]CH_3I), resulting in a low overall yield of the synthesis. No attempts were made for improvement, since TBZOMe was required only for confirmation of chemical structure, and as an analytical standard. Finally, the carbon-11 form of TBZOMe was prepared by *O*-[^{11}C]methylation of α-TBZOH, followed by HPLC purification. The final product was obtained in high radiochemical purity (>95%) and high specific activity (>2000 Ci/mmol at end-of-synthesis). Chromatograpic conditions were chosen such that the product, [^{11}C]TBZOMe, eluted before the starting material TBZOH, allowing for complete separation. Beginning with 2.5 Ci of [^{11}C]CO_2, this reaction sequence has been used to prepare 100–200 mCi batches of [^{11}C]TBZOMe suitable for *in vivo* animal studies. The evaluation of the pharmacological specificity, metabolism, primate imaging, and regional brain pharmacokinetics of this new radioligand will be reported elsewhere.

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