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Synthesis of 3,4-dihydro-5-[^{11}C]methoxy-1(2H)-isoquinolinone as a Potential Tracer for Poly(ADP-ribose) Synthetase

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SUMMARY

Synthesis of 3,4-dihydro-5-[^{11}C]methoxy-1(2H)-isoquinolinone ([^{11}C]MIQO), a potent poly (ADP-ribose) synthetase inhibitor, was devised in order to evaluate whether it is possible to image excessive activation of poly(ADP-ribose) synthetase (PARS) by positron emission tomography. [^{11}C]MIQO was prepared by O-[^{11}C]methylation of 3,4-dihydro-5-hydroxy-1(2H)-isoquinolinone, obtained by a Schmidt reaction with 4-hydroxy-1-indanone, sodium azide and trichloroacetic acid, with [^{11}C]methyl triflate. Total synthesis time from EOB was 35 minutes. The radiochemical yield based on [^{11}C]carbon dioxide was 31±8% (n=8; decay corrected). The final product had a specific activity of 76 GBq/μmol at EOS, and the radiochemical purity of [^{11}C]MIQO was over 99%.

KEY WORDS: poly(ADP-ribose) synthetase, 3,4-dihydro-5-[^{11}C]methoxy-1(2H)-isoquinolinone, [^{11}C]methyl triflate, ischemic injury, PET

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INTRODUCTION

Poly(ADP-ribose) synthetase (PARS) is a nuclear enzyme which is activated by DNA strand breaks and participates in DNA repair. It catalyzes the transfer and polymerization of ADP-ribose units provided by NAD onto both itself and other nucleoproteins. Excessive PARS activation, however, can deplete tissue stores of NAD, and the depletion of NAD, an important co-enzyme in energy metabolism, leads to cell death due to resultant depletion of ATP. PARS inhibitors dramatically protect tissue from ischemic damage in focal cerebral ischemia (1,2), and myocardial infarction (3). These findings suggest that a positron-labeled PARS inhibitor may be useful for imaging regions of ischemic injury in tissue by positron emission tomography (PET). With similar intention, Andersson *et al.* (4) prepared several labeled PARS inhibitors and evaluated their biodistribution in normal monkeys, however they failed to obtain sufficient PET images with them in monkeys.

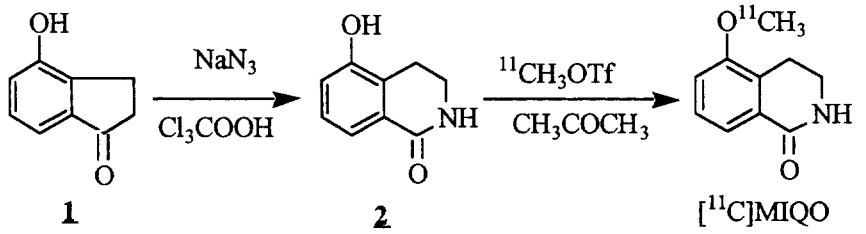
PARS inhibitors are divided into 3 groups on the basis of their structure: 3-substituted benzamides (5), 5-substituted isoquinolinones (6), and 6-amino-2-benzpyrones (7). We chose 3,4-dihydro-5-[^{11}C]methoxy-1(2H)-isoquinolinone ([^{11}C]MIQO), one of the 5-substituted isoquinolinones, as the candidate for a labeled PARS inhibitor, because its PARS inhibitory activity has been reported to be 24 fold as high as that of 3-amino-benzamide (6). Details of the synthesis are described below.

RESULTS AND DISCUSSION

The synthetic pathway from 2,3-dihydro-4-hydroxy-1-indanone (**1**) to [^{11}C]MIQO via 3,4-dihydro-5-hydroxy-1(2H)-isoquinolinone (**2**) is shown in Scheme 1. The precursor (**2**) was obtained in a yield of 15.8% by heating with **1**, sodium azide, and trichloroacetic acid.. The elemental analysis data for the product obtained agreed with the calculations for the ring expansion product **2**. The EI/MS spectrum revealed fragment peaks of 163; M^+, 134; $(M\text{-}CH_3N)^+$, 106; $(134\text{-}CO)^+$, and 28; CO^+, which also supported the 3,4-dihydro-1(2H)-isoquinolinone structure for **2** (not the quinolinone type). Finally, compound **2** was identified by comparing its ^{1}H-and ^{13}C-NMR spectral data with those of an authentic sample of **2** provided by Dr. Nakagawa.

Radiolabeling of [^{11}C]MIQO was accomplished by O-[^{11}C]methylation of the demethyl precursor **2** with [^{11}C]methyl triflate, obtained from [^{11}C]carbon dioxide via [^{11}C]methyl iodide according to the procedure reported by Jewett (8).

Scheme 1 Pathway for the synthesis of [^{11}C]MIQO



1 **2** [^{11}C]MIQO

$^{11}CH_3OTf$: [^{11}C]methyl trifluoromethanesulfonate

[^{11}C]MIQO was separated from unreacted **2** and radioactive impurities by a semi-preparative HPLC. Total synthesis time from EOB was 35 minutes, and the radiochemical yield (EOB) from [^{11}C]carbon dioxide was 31±8% (n=8). The final product had a specific activity of 76 GBq/μmol at EOS, and the radiochemical purity of [^{11}C]MIQO was over 99%.

Andersson *et al.* (4) pointed out that a PET radiotracer for PARS should possess high affinity for PARS. We also thought that it should be rapidly washed out from the normal tissue to facilitate detection of PARS activity *in vivo* by PET. Preliminary biodistribution studies in normal rats and monkeys have revealed a rapid clearance of [^{11}C]MIQO from the target tissues. Details of these results will be published elsewhere (9). [^{11}C]MIQO may enable detection of PARS activity by PET in tissue with ischemic injury.

EXPERIMENTAL

General

Reagent grade chemicals were purchased from commercial suppliers and used without further purification. [^{11}C]Carbon dioxide was prepared by the $^{14}N(p,\alpha)^{11}C$

nuclear reaction using a nitrogen gas target and 18 MeV protons produced by a Sumitomo Heavy Industry CYPRIS HM-18-type cyclotron.

^{1}H-NMR spectra were measured with a JNM-A500 spectrometer (JEOL, Japan). Electron impact mass spectra (EI/MS) were measured with a JMS-HX 110 spectrometer (JEOL, Japan). Elemental analyses were measured with MT-6 (YANACO, Japan). Column chromatography was carried out on a Merck Kieselgel 60.

3,4-Dihydro-5-hydroxy-1(2H)-isoquinolinone (2)

A mixture of 4-hydroxyindanone (3.79 g, 25.6 mmol) and trichloroacetic acid (46.8 g, 286.2 mmol) was heated at 60-65°C until the mixture became homogeneous. Sodium azide (3.33 g, 51.6 mmol) was then added to the mixture in one portion, and the mixture heated continuously at 70°C for 18h. The mixture was cooled, diluted with ice-water (2000 ml), extracted with ether (500 ml), and washed with a saturated $NaHCO_3$ solution. The ether layer was dried (Na_2SO_4) and evaporated under reduced pressure to give a dark oil (5.29 g). The oil was chromatographed on silica gel to give 2 (426 mg,15.8 %) as colorless needles, which were then recrystallized from AcOEt. ^{1}H-NMR ($C_5D_5N_1$): δ 3.16 (2H, t, 4-CH_2, J_{3-4}=7 Hz), 3.52 (2H, td, 3-CH_2, J_{3-4}=7Hz, J_{2-3}=3 Hz), 7.26 (1H, dd, 6-H, J_{6-7}=8 Hz J_{6-8}=2 Hz), 7.29 (1H, AMX-q, 7-H, J_{6-7}=8 Hz J_{7-8}=7 Hz), 8.11 (1H, dd, 8-H, J_{7-8}=7 Hz J_{6-8}=2 Hz), 8.51 (1H, br-s, 2-NH), 11.75 (1H, s, phenolic OH). ^{13}C-NMR ($C_5D_5N_1$): δ 22.46 (C-4), 39.95 (C-3), 118.56 (C-6), 119.05 (C-7), 127.12 (C-8), 127.3 5 (C-10), 131.88 (C-9), 155.13 (C-5), 166.24 (C-1). EI/MS m/z: 163 (M^+), 134 (M^+-CH_3N), 106 (134-CO). 13C-NMR (Anal Calcd for $C_9H_9NO_2$: C, 66.25; H, 5.56; N, 8.58. Found: C,66.20; H, 5.53; N, 8.60.

The authentic reference 2. ^{1}H-nmr (C_5D_5N): δ 3.16 (2H, t, 4-CH_2, J_{3-4}=7 Hz), 3.52 (2H, td, 3-CH_2, J_{3-4}=7Hz, J_{2-3}=3 Hz), 7.26 (1H, dd, 6-H, J_{6-7}=8 Hz J_{6-8}=2 Hz), 7.29 (1H, AMX-q, 7-H, J_{6-7}=8 Hz J_{7-8}=7 Hz), 8.11 (1H, dd, 8-H, J_{7-8}=7 Hz J_{6-8}=2 Hz), 8.51 (1H, br-s, 2-NH), 11.76 (1H, s, phenolic OH). ^{13}C-NMR ($C_5D_5N_1$): δ 22.47 (C-4), 39.94 (C-3), 118.56 (C-6), 119.05 (C-7), 127.12 (C-8), 127.3 4 (C-10), 131.89 (C-9), 155.13 (C-5), 166.24 (C-1).

3,4-Dihydro-5-[^{11}C]methoxy-1(2H)-isoquinolinone ([^{11}C]MIQO)

The [^{11}C]carbon dioxide was converted to [^{11}C]methyl iodide using a Sumitomo Heavy Industry [^{11}C]methyl iodide production system, and [^{11}C]methyl triflate was then prepared by passing the carrier gas (N_2) containing [^{11}C]methyl iodide vapor through a column packed with graphite-impregnated silver triflate according to the procedure of Jewett (8). The [^{11}C]methyl triflate produced was continuously bubbled with the carrier gas in a sealed reaction vessel containing **2** (0.4 mg, 2 μmol) and 0.5 M sodium hydroxide solution (5 μl) in acetone (800 μl). The reaction mixture was allowed to stand for 5 min at 30°C, and the HPLC mobile phase (600μl) was then added. The mixture was chromatographed by HPLC on a silica gel column (YMC-SIL A022, 12 mm x 150 mm, 5μm), eluted with dichloromethane-diethyl ether (1:1 v/v). [^{11}C]MIQO had a retention time of 13 min at a flow rate of 6.0 ml/min. After removing the solvent, the radioactive residue was dissolved in a solution of dimethyl sulfoxide-water (2:1 v/v). Total synthesis time from EOB was 35 minutes. The radiochemical yield (EOB) based on [^{11}C]carbon dioxide was 31±8% (n=8). The final product had a specific activity of 76 GBq/μmol at EOS. The radiochemical purity of [^{11}C]MIQO was over 99%.

3,4-Dihydro-5-methoxy-1(2H)-isoquinolinone (MIQO)

Unlabeled 3,4-dihydro-5-methoxy-1(2H)-isoquinolinone (MIQO) was synthesized as follows: Methyl trifluoromethansulfonate (98 mg, 1.2 mmol) was added to a mixture of **2** (82 mg, 0.5 mmol) and 0.5N sodium hydroxide solution (1 ml, 0.5 mmol) in acetone (25 ml), and the reaction mixture was stirred at 20°C for 3 h. The solvent was then evaporated under reduced pressure, and the residual solid was triturated with ice-water and acidified with 0.5N hydrochloric acid (HCl). The precipitate was separated from the solution by filtration, washed with water, and dried under reduced pressure to give MIQO (79 mg, 90%) as colorless needles, which were then recrystallized from AcOEt. Unlabeled MIQO was used to investigate the HPLC conditions for separation of [^{11}C]MIQO from the unreacted **2**. ^{1}H-NMR (C_5D_5N): δ 2.92 (2H, t, 4-CH_2, $J_{3\text{-}4}$=7 Hz), 3.46 (2H, td, 3-CH_2, $J_{3\text{-}4}$=7Hz, $J_{2\text{-}3}$=3 Hz), 3.73 (3H, s, 5-OCH_3), 6.98 (1H, dd, 6-H, $J_{6\text{-}7}$=8 Hz

J_{6-8}=2 Hz), 7.32 (1H, A_2X-t, 7-H, J_{6-7}=8 Hz J_{7-8}=8 Hz), 8.13 (1H, dd, 8-H, J_{7-8}=8 Hz J_{6-8}=2 Hz), 8.58(1H, br-s, 2-NH). EI/MS m/z 177 (M^+), 148 (M^+-CH_3N), 120 (148-CO). Anal Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.76; H, 6.28; N, 7.90.

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