THE PREPARATION OF A CARBON-11 LABELLED ANALGESIC — [N-METHYL-^{11}C]MEPTAZINOL*

David R. Turton, Sajinder K. Luthra, Victor W. Pike and Malcolm J. Kensett.

Medical Research Council Cyclotron Unit, Hammersmith Hospital, Du Cane Road, London W12 OHS, U.K.

SUMMARY

A procedure for labelling the novel analgesic, meptazinol [(+)1-methyl-3-ethyl-3-(m-hydroxyphenyl)tetrahydroazepine], with the positron-emitting radionuclide, carbon-11 ($t_{\frac{1}{2}}$ = 20.4 min) has been developed in order to permit the pharmacokinetics of this analgesic to be studied in man. The procedure involves the reaction of [^{11}C]iodomethane, itself produced from cyclotron-produced [^{11}C]carbon dioxide, with normeptazinol in ethanol at 100 °C for 5 min, evaporation of any unreacted [^{11}C]iodomethane, purification by HPLC and removal of solvent. Subsequent solubilisation of the radioactive product in ethanol plus isotonic saline and sterilisation by filtration produces a safely-injectable solution of [N-methyl-^{11}C]meptazinol in 14% radiochemical yield (from cyclotron-produced [^{11}C]carbon dioxide, decay-corrected). The specific activity of the product is up to 7.4 GBq/μmol at the end of radiosynthesis (40 min from the end of radionuclide production). Preparations have been shown to be radiochemically pure and to be free of normeptazinol by analytical HPLC and TLC. That the radioactive product is [N-methyl-^{11}C]meptazinol has been unequivocally demonstrated by co-inclusion of ^{13}C-enriched iodomethane in the radiosynthesis and examination of the product by broad-band proton-decoupled Fourier transform ^{13}C-NMR spectroscopy.

Key words: meptazinol, [N-methyl-^{11}C]meptazinol, [N-methyl-^{13}C]meptazinol analgesic, carbon-11.

INTRODUCTION

Meptazinol (1) [(±)1-methyl-3-ethyl-3-(m-hydroxyphenyl)tetrahydroazepine] is a novel centrally-acting analgesic that expresses unusual opioid behaviour (1). Positron emission tomography (PET) (2) can be a uniquely direct technique for the study of pharmacokinetics in man, provided that the drug of interest can be labelled with a suitable

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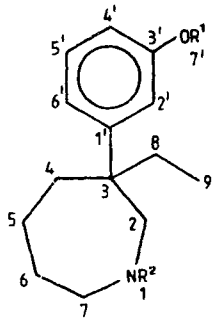
positron-emitting radionuclide. An early example is the study of the pharmacokinetics of an antibiotic, erythromycin A, using carbon-11 labelled erythromycin A (3). In order to enable the pharmacokinetics of meptazinol (**1**) to be studied by PET we sought to devise a procedure for labelling meptazinol (**1**) with the positron-emitting radionuclide, carbon-11 ($t_{\frac{1}{2}}$ = 20.4 min). Here we report a fast procedure for the production of [N-methyl-^{11}C]meptazinol (**2**) in a form acceptable for human intravenous injection, based on the N-methylation of normeptazinol (**3**) with [^{11}C]iodomethane.



(1) R^1 = H, R^2 = Me, Meptazinol

(2) R^1 = H, R^2 = $^{11}CH_3$, [N-methyl-^{11}C]Meptazinol

(3) R^1 = H, R^2 = H, Normeptazinol

(4) R^1 = H, R^2 = $^{13}CH_3$, [N-methyl-^{13}C]Meptazinol

(5) R^1 = Me, R^2 = H, O-Methyl-normeptazinol

(6) R^1 = Me, R^2 = Me, O-Methyl-meptazinol

EXPERIMENTAL AND RESULTS

Materials

Normeptazinol hydrobromide, meptazinol hydrochloride, O-methyl-normeptazinol hydrochloride and O-methylmeptazinol (**6**) were kindly donated by Wyeth Research (UK) Ltd. Diethyl ether (Analar grade; BDH Chemicals Ltd) was dried over sodium before use. All other solvents were purchased from BDH Chemicals Ltd or Fisons Ltd and were of either 'Analar' or 'HPLC grade'. Lithium aluminium hydride was purchased from

Merck Ltd. All nitrogen ('O_2-free grade'; BOC Ltd) was dried by passage through magnesium perchlorate [$Mg(ClO_4)_2+H_2O$; BDH Chemicals Ltd] immediately before use. ^{13}C-Enriched iodomethane (90 atom %) was purchased from Amersham International. Molecular sieve (4Å) was purchased from BDH Chemicals Ltd.

Preparation of free bases

Normeptazinol hydrobromide, meptazinol hydrochloride and O-methylnormeptazinol hydrobromide were each converted into the corresponding free base as follows. The salt (2 mmol) was dissolved in water (20 mL). Dichloromethane (10 mL) followed by aqueous ammonia solution (5 mL; d = 0.88) was then added. After stirring for 10 min the organic layer was removed and the aqueous layer extracted with a further portion of dichloromethane (15 mL). The organic layers were combined, dried over anhydrous magnesium sulphate and evaporated to dryness.

Analysis by HPLC (Table 1) and by TLC (Table 2) indicated each product to be pure.

Production of [^{11}C]carbon dioxide

[^{11}C]Carbon dioxide was produced on the MRC cyclotron at Hammersmith Hospital by the $^{14}N(p,\alpha)^{11}C$ reaction, essentially as described previously (4). Nitrogen at 100 KN/m^2 (15 p.s.i.) acted as bombarded substance and as sweep gas (flow rate, 140 mL/min) for the target. [^{11}C]Carbon dioxide was trapped continuously by passing the sweep gas through a column (5 cm x 0.6 cm diameter) of molecular sieve (4Å, 60-100 mesh) at room temperature. The bombardment of nitrogen with protons (7.6 MeV, 50 μA) for 1 h produced *ca* 15 GBq (400 mCi) of trapped [^{11}C]carbon dioxide. [^{11}C]Carbon dioxide was dispensed when required by passing nitrogen at 50 mL/min for 2 min through the molecular sieve heated to 230 °C. About 90% of the radioactivity was recovered under these conditions.

Compound	Capacity Factor	
	System A	System B
Meptazinol (1)	2.03 (6.5)*	3.00 (9.3)
[N-methyl-^{11}C]meptazinol (2)+	2.03 (6.5)	3.00 (9.3)
Normeptazinol (3)	6.19 (19.8)	3.68 (11.4)
O-Methylnormeptazinol (5)	2.84 (9.1)	9.35 (29.4)
O-Methylmeptazinol (6)	1.25 (4.0)	7.55 (23.4)

*Values in parentheses are retention volumes (mL).

+ Carrier added.

Table 1 - Chromatographic data for [N-methyl-^{11}C]meptazinol (2) and related compounds on two HPLC systems. System A: silica gel column ('μ-Porasil', 30 x 0.4 cm i.d.; Waters Associates) eluted at 1.0 mL/min with CH_2Cl_2/EtOH/c. NH_4OH (90: 10: 0.5 v/v). System B: silica-octadecylsilane column ('μ-Bondapak C-18', 30 x 0.4 cm i.d.; Waters Associates) eluted at 1.5 mL/min with 0.025 M Na_2HPO_4 in MeOH/H_2O (3:2 v/v) adjusted to pH 7. Stable compunds were detected by their absorbance at 280 nm.

Compound	R_f Value		
	System A	System B	System C
Meptazinol (1)	0.93	0.58	0.33
[N-methyl-^{11}C]meptazinol (2)	0.93	0.58	0.33
Normeptazinol (3)	0.64	0.28	0.22
O-Methylnormeptazinol (5)	0.85	0.50	0.17
O-Metnylmeptazinol (6)	0.96	0.89	0.24

Table 2 - R_f Values for [N-methyl-^{11}C]meptazinol (2) and related compounds in three TLC systems. System A: silica, CH_2Cl_2/EtOH/c. NH_4OH (10: 10: 1 v/v). System B: silica, CH_2Cl_2/EtOH/c. NH_4OH (75: 25: 1 v/v). System C: silica-C18 (Whatman KC-18), EtOH/1% v/v $(NH_4)_2CO_3$ soln (7: 7 v/v). Stable compounds were exposed by iodine and radioactive compounds by autoradiography.

Preparation of [^{11}C]iodomethane

[^{11}C]Iodomethane was prepared from cyclotron-produced [^{11}C]carbon dioxide as described previously (5), but with adaptation of the apparatus

to remote control. Briefly, the procedure involves the reaction of [^{11}C]carbon dioxide with lithium aluminium hydride in diethyl ether, hydrolysis of the radioactive adduct to [^{11}C]methanol and conversion of this into [^{11}C]iodomethane by treatment with hydroiodic acid. The radiochemical yield of [^{11}C]iodomethane from [^{11}C]carbon dioxide is ca 60% (decay-corrected) and the preparation time is 15 min from the end of radionuclide production.

Preparation of [N-methyl-^{11}C]meptazinol (2)

[^{11}C]Iodomethane in absolute ethanol (0.5 mL) was transferred to a small reaction vessel (volume, 2 mL) containing nor-meptazinol (3) (2 mg). The reaction pot was pressurised to 65 KN/m^2 (10 p.s.i.), sealed and then heated to 100°C with magnetic stirring for 5 min. All volatiles were then removed by opening the reaction vessel to vacuum. The radioactive residue was dissolved in dichloromethane (0.7 mL) and injected through a filter (Acro disc) onto a silica column ('μ-Porasil',

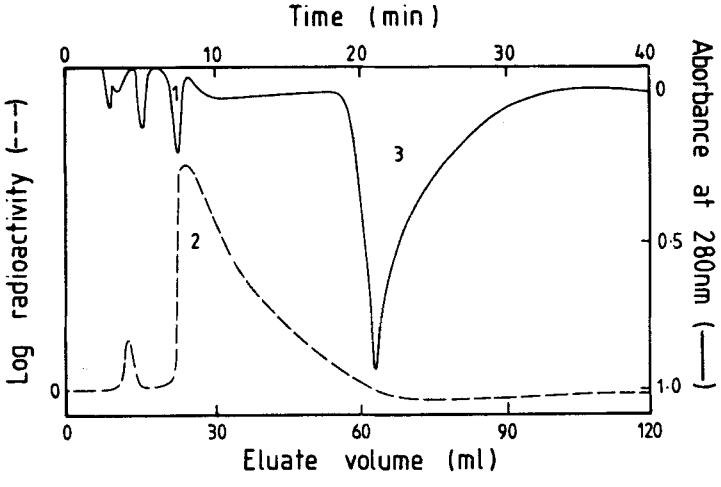


Figure 1: Chromatogram for the preparative separation of [N-methyl-^{11}C]-meptazinol (2). Peak 1: meptazinol (1), peak 2: [N-methyl-^{11}C]-meptazinol (2), peak 3: normeptazinol (3). Chromatographic conditions are described in the text.

30 cm x 0.7 cm i.d.; Waters Associates) eluted with CH_2Cl_2/EtOH/c. NH_4OH (90: 10: 0.1 v/v) at 3.0 mL/min. The eluant was monitored continuously for radioactivity and for absorbance at 280 nm. The radioactive fraction having the same retention volume (26 mL) as authentic meptazinol (**1**) was collected (Figure 1) and rotary evaporated to dryness. Unreacted normeptazinol (**3**) eluted much later (retention volume, 60 mL) and was well-separated from the collected radioactive fraction (Figure 1).

Analysis of [N-methyl-^{11}C]meptazinol (2)

The radioactive product collected from the HPLC separation was found to be radiochemically pure and to be free of normeptazinol (**3**) or related compounds (**5,6**) by HPLC on two systems (Table 1) and by TLC on three systems (Table 2).

Though normal phase HPLC analysis revealed the radioactive product to have a slightly larger retention volume than reference meptazinol (**1**), injected mixtures of the radioactive product and meptazinol (**1**) were found to co-elute (Table 1). The radioactive product and reference meptazinol (**1**) exhibited identical mobility on TLC (Table 2). No chemical impurities were detected.

Determination of the specific activity of [N-methyl-^{11}C]meptazinol (2)

HPLC System A (Table 1), equipped with radioactivity and ultraviolet absorbance detectors, was used to measure the specific activity of [N-methyl-^{11}C]meptazinol (**2**). For this purpose the response of the absorbance detector at 280 nm was pre-calibrated with respect to mass by measuring the peak areas for injections of known masses of meptazinol (**1**). Peak areas were calculated by a microcomputer (Apple II; Apple Computers) incorporting a software/hardware package (Chromatochart; Interactive Microware Inc.). The relationship between injected mass of meptazinol (**2**) and response (peak area) was linear over the measured range (0 - 0.76 μg).

For each determination, [N-methyl-^{11}C]meptazinol (**2**), eluted from

the analytical column, was measured for total radioactivity (a GBq) at a known time (t_1 min) from the end of radiosynthesis. The microcomputer was then used to calculate the corresponding mass of stable meptazinol (1) (b μmol). The specific activity of [N-methyl-^{11}C]meptazinol (2) at the end of radiosynthesis (S), was then computed according to the equation $S = \frac{a}{b} e^{t_1} \times 0.034$ GBq/ μmol. The average value of S was 3.7 GBq/μmol (100 mCi/μmol; n = 10).

Formulation of [N-methyl-^{11}C]meptazinol (2) for human intravenous injection.

The [N-methyl-^{11}C]meptazinol (2) was dissolved in absolute ethanol (0.1 mL; BP), diluted with 'normal saline for injection' (10 mL; 0.9% w/v sodium chloride, Boots Ltd), sterilised by filtration (using a 'Millex GS' filter, 0.22 μm pore size, Millipore Corporation). The resultant solution was neutral and safely injectable. [All selected preparations passed independent (Safepharm Ltd) tests for apyrogenicity and sterility].

Reverse phase HPLC and TLC demonstrated that the process of formulation had no effect on the radiochemical purity of the [N-methyl-^{11}C]meptazinol (2).

From the end of radiounclide production the preparation takes 45 min and produces an injectable solution of [N-methyl-^{11}C]meptazinol (2) in 14% radiochemical yield from [^{11}C]carbon dioxide (decay-corrected).

Further validation of the radiosynthesis

The radiosynthesis of [N-methyl-^{11}C]meptazinol (2) was carried out as described above but with the co-inclusion of ^{13}C-enriched (90 atom %) iodomethane (2 μl). The isolated mixture of ^{11}C-and ^{13}C-labelled product was then examined by broad-band proton decoupled Fourier transform ^{13}C-NMR spectroscopy (22.5 MHz). This revealed a single peak at 48.3 ppm (Table 3). When meptazinol free base (with natural abundance ^{13}C) was added to the sample this peak merged with that assigned to the N-methyl carbon of

meptazinol (base) to give an intense peak at 49.2 ppm, with the remainder of the spectrum of meptazinol at lower intensity (Table 3).

DISCUSSION

For the investigation of drug pharmacokinetics in man by PET it is necessary to label the drug with a suitable positron-emitting radionuclide by a method that ensures radiochemical and chemical purity and an adequate yield of radioactivity. Furthermore, if it is desirable to investigate the interaction of the drug with receptors, it is essential to produce the labelled drug in acceptably high specific activity.

The procedure described above has routinely provided radiochemically and chemically pure [N-methyl-^{11}C]meptazinol (2) in adequate radioactivity (370 MBq; 10 mCi) for the study of the pharmacokinetics of maptazinol in man (6). The achieved specific activity (>3.7 GBq/μmol; > 100 mCi/μmol at the end of radiosynthesis) has also enabled the possible interaction of meptazinol (1) with central opiate receptors to be investigated by PET. In this work we were restricted to the use of a 50 μA beam of 7.6 MeV protons for the production of carbon-11 by the $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen gas. Under these conditions the maximal production yield is about 14.8 GBq (400 mCi). By using a moderate beam current (ca 30 μA) of high energy protons (10-20 MeV), as is available from many modern medical cyclotrons (7), a much higher activity of [N-methyl-^{11}C]meptazinol (2) could be prepared in correspondingly higher specific activity.

Evidence from HPLC and TLC analysis, and from ^{13}C-NMR spectroscopy of the product obtained by using [^{13}C]iodomethane in the radiosynthesis, unequivocally demonstrates the identity, chemical purity and radiochemical purity of the product from our procedure.

The normal phase HPLC method of analysis (Table 1; system A) is rapid (< 6 min) and can be performed during product formulation. It therefore provides a convenient routine technique for assessing radiochemical and

chemical purity and for measuring specific activity before intravenous injection. The retention volume of [N-methyl-^{11}C]meptazinol (2), in this method of analysis, increases slightly with increasing specific activity. Such an effect has been noted by other workers in the analysis of high specific activity radiopharmaceuticals (8). Thus for unambiguous product identification it is necessary to confirm that a mixture of the radioactive product and meptazinol co-elute.

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