Technical Note

Journal of Labelled Compounds and Radiopharmaceuticals

Received 20 July 2012, Revised 17 September 2012, Accepted 2 October 2012 Published online 25 October 2012 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.2979

Facile and improved synthesis of [^{11}C]Me-QNB

Vanessa Gómez-Vallejo, Mikel González-Esparza and Jordi Llop*

[^{11}C]Me-QNB is a muscarinic acetylcholinergic receptor antagonist that has been used for the assessment of myocardial muscarinic receptors density in different cardiovascular pathologies. In the current technical note, we report a facile, highly efficient and fully automated method for the preparation of this radiotracer. The radiosynthesis was performed by reaction of [^{11}C]CH_3I with the desmethylated precursor (QNB) at room temperature using the captive solvent method. Excellent radiochemical yield (91.1 ± 2.4%, decay-corrected) and radiochemical purity (>99.5%), and good specific activity (137 ± 5 GBq/μmol) were obtained when the purification was performed by reverse phase HPLC in overall synthesis time <31 min. Purification using solid-phase extraction offered lower radiochemical yield (27.6 ± 3.1%, decay-corrected) and radiochemical purity (>95%) but higher specific activity (244 ± 18 GBq/μmol) in shorter reaction times (<21 min). These results, especially concerning radiochemical yield, significantly improve those previously reported in which the reaction was performed in a vial and the purification step was based on ionic chromatography.

Keywords: Carbon-11; Me-QNB; positron emission tomography; muscarinic receptor

Introduction

Me-QNB (**1**, *N*-methyl-quinuclidin-3-yl benzilate, Scheme 1) is a hydrophobic, non-metabolized and highly specific muscarinic acetylcholinergic antagonist,[1] which has been previously labeled with Carbon-11[2,3] and characterized as a radiotracer for the *in vivo* imaging of myocardial muscarinic receptors using positron emission tomography.[2,4,5] This radiotracer has been used for the assessment of myocardial muscarinic receptor density and affinity constants in heart transplant patients,[6] in chronic idiopathic dilated cardiomyopathy patients,[7] and in familiar amyloid neuropathy patients.[8]

The radiosynthetic procedure for the preparation of [^{11}C]Me-QNB, on the basis of the reaction of the desmethyl precursor (QNB) with [^{11}C]methyl iodide ([^{11}C]CH_3I), was first developed by Mazière *et al.*[2] After a purification step using HPLC, average decay-corrected radiochemical yields of 23.3 ± 10% with respect to [^{11}C]CH_3I were obtained. A more efficient strategy based on the use of [^{11}C]methyl triflate ([^{11}C]MeOTf) as labeling agent was developed later.[9] Decay-corrected radiochemical yields of 48.5 ± 10% (based on [^{11}C]MeOTf) could be obtained with associated decay-corrected specific radioactivities of 98 ± 36 GBq/μmol.

Recently, we have developed methodologies for the preparation of different ^{11}C-labeled radiotracers[10,11] using the captive solvent method first developed by Wilson and co-workers.[12] This method has been proven to improve radiochemical yields; in addition, reactions that take place only at high temperature using the conventional *in vial* reaction can be performed at room temperature with equivalent results.[11]

In this study, we describe the fully automated radiosynthesis of [^{11}C]Me-QNB using the captive solvent method and [^{11}C]CH_3I as a methylating agent. The reaction was completed in 1 min at room temperature. When the purification step was performed by HPLC, significantly higher radiochemical yields than those previously reported[2,9] were obtained. Implementation of a purification step on the basis of solid-phase extraction lowered decay-corrected radiochemical yield and radiochemical purity values; however, the whole preparation could be completed in shorter times, and consequently, higher specific activities were obtained.

Materials and methods

General

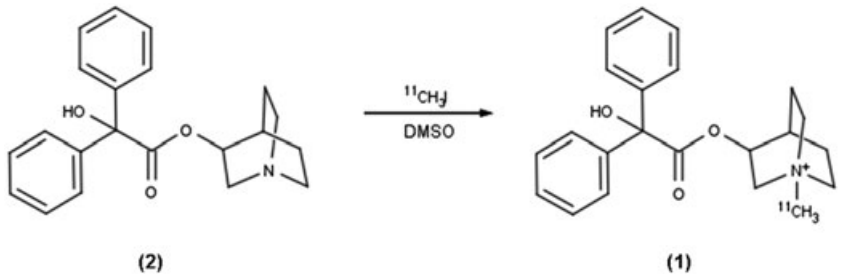
All chemicals and solvents (unless otherwise specified) were of analytic or HPLC grade from Panreac Química (Madrid, Spain) or Sigma-Aldrich (Spain). C-18 Light Sep-Pak cartridges were obtained from Waters Cromatografía, S.A. (Spain) and were preconditioned sequentially with ethanol (5 mL) and water (5 mL). Accell Plus CM Sep-Pak cartridges were obtained from Waters Cromatografía, S.A. (Spain) and used without preconditioning. QNB and Me-QNB were purchased from ABX (Avanced Biomedical Compounds, Radeberg, Germany). Purified water (ultrapure, Type I water, ISO 3696) was obtained from a Milli-Q® Integral system (Millipore Iberica S.A.U., Madrid, Spain).

Production of [^{11}C]CH_3I

The synthesis of [^{11}C]CH_3I was carried out using a TRACERlab FX_C Pro synthesis module (GE Healthcare, Buckinghamshire, UK) starting from cyclotron produced [^{11}C]CH_4, which was generated in an IBA Cyclone 18/9 cyclotron by bombardment (target current = 22 μA, integrated current = 1 μAh) of a gas N_2/H_2 mixture (99/1, starting pressure = 16 bar) with high energy (18 MeV) protons.

Radiochemistry Department, CIC biomaGUNE, Paseo Miramón 182, San Sebastián 20009, Spain

**Correspondence to: Jordi Llop, Radiochemistry Department, CIC biomaGUNE, Paseo Miramón 182, San Sebastián 20009, Spain.*
E-mail: jllop@cicbiomagune.es



(2) (1)

Scheme 1. Radiosynthesis of [^{11}C]Me-QNB.

Synthesis of [^{11}C]Me-QNB: purification with HPLC

[^{11}C]CH_3I synthesized as described previously was trapped in a 2-mL stainless steel HPLC loop (AutoLoop™ system) pre-charged with a solution of QNB (1 mg) in dimethyl sulfoxide (80 μL). After complete trapping of [^{11}C]CH_3I, the reaction was allowed to occur for 1 min at room temperature (Scheme 1). The reaction mixture was purified by means of HPLC using a Mediterranean Sea18 column (250 × 10 mm, 5 μm; Teknokroma, Barcelona, Spain) as stationary phase and sodium acetate aqueous solution (2 g/L) adjusted to pH = 10.0 with 1M NaOH/CH_3CN 50/50 as a mobile phase at a flow rate of 5 mL/min. The purified fraction (retention time ~8 min, radiometric and UV detection) was collected and reformulated in the TRACERlab FX_C Pro synthesis module by diluting with water (20 mL), trapping in a C-18 cartridge, rinsing with water, elution with ethanol (1 mL), further elution with physiologic solution (9 mL) and final filtration through a 0.22-μm filter (Millex®-GS, Millipore).

Synthesis of [^{11}C]Me-QNB: purification with solid-phase extraction

The production of [^{11}C]CH_3I and the methylation reaction were performed as described in this section. The reaction mixture was pushed from the loop with purified water (10 mL), and the resulting eluate was passed through an Accell Plus CM cationic exchange cartridge. The cartridge was rinsed with ethanol (2 mL) and purified water (3 mL). Finally, the retained radioactive product was eluted with 10% ethanol in physiologic saline solution (total volume = 3 mL) and filtered through a 0.22 μm filter (Millex®-GS, Millipore).

Irrespective of the purification method, the amount of radioactivity of the final radiotracer was measured in a dose calibrator (PETDOSE HC, Comecer; Ravenna, Italy), and a sample was submitted to quality control. Chemical and radiochemical purity as well as specific radioactivity were determined by HPLC, using an Agilent 1200 Series HPLC system (Madrid, Spain) with a multiple wavelength UV detector (λ = 207 nm) and a radiometric detector (Gabi, Raytest; Straubenhardt, Germany). An RP-C18 column (Agilent XDB-C18, 50 × 4.6 mm, 1.8 μm) was used as stationary phase, and ammonium formate aqueous solution (3.15 g/L) adjusted to pH = 6.0 with HCOOH/CH_3CN 75/25 was used as a mobile phase. The retention times for QNB and Me-QNB were 3.67 and 3.08 min, respectively.

Radiochemical yields were calculated with respect to [^{11}C]CH_3I. For that purpose, one synthesis of this labeling agent was performed each working day between [^{11}C]Me-QNB runs. [^{11}C]CH_3I was collected in AutoLoop™ system, where the loop had been pre-charged with dimethyl sulfoxide (80 μL). After complete trapping, the solution was pushed with CH_3CN (5 mL) into a vial and the activity was measured in the dose calibrator. This value was used for the calculation of radiochemical yields in all syntheses performed within the same day.

Results and discussion

The synthetic strategy reported here is based on the captive solvent method first described by Wilson and co-workers.[12] Historically, this methodology has offered better results than the traditional *in vial* reaction because of three main factors: First, direct introduction of the reaction crude into the purification system (usually HPLC) prevents loss of radioactivity derived from liquid transfers; second, less solvent can be used in the preparation of the precursor solution, resulting in a higher concentration, which favors the methylation reaction; third, the precursor solution is distributed on the loop surface creating a thin film, which results in improved interaction of the precursor with the labeling agent with subsequent increase in radiochemical conversion values. This improved reactivity allows to complete reactions at room temperature in shorter or equivalent reaction times (as compared with *in vial* reactions) minimizing thus the formation of undesired by-products.

Our first syntheses were performed without purification to optimize experimental conditions. Dimethyl sulfoxide was used as a solvent, and reaction time was fixed at 5 min. Although the reaction was carried out at room temperature, radiochemical conversion values (determined by analytical HPLC) close to 100% were obtained. Thus, the reaction time was progressively shortened, and surprisingly, equivalent results were obtained when the reaction time was fixed at 3 and 1 min. Because of these excellent results, the experimental conditions were not further optimized.

The purification step was performed at first instance by HPLC. The methodology described by Dolle *et al.*[9] was based on ionic chromatography; thus, a mixture of 0.9% aq. NaCl/EtOH (65/35) was used as a mobile phase, and a semi-preparative Spherisorb® SCX Column was used as stationary phase. Such mobile phase has the advantage that no reformulation step is required, because dilution of the collected fraction with the appropriate volume of physiologic saline solution results directly in an injectable solution. However, as mentioned in the paper, under these chromatographic conditions, the retention times for the precursor and the desired radiotracer were 5.5–6.5 min and 8.0–9.5 min, respectively; because of this fact, the use of higher amount of precursor was restricted because the separation of the radiotracer and the precursor was not successful in all batches. In our case, taking into account that we were using

1 mg of precursor and that most of the tracers, which are routinely produced in our laboratory, are purified using RP-C18 columns, the development of a new method on the basis of reverse phase chromatography in which the retention time of the precursor was longer than the retention time of the radiotracer was anticipated to be more convenient. Although the radiotracer is a cation at any pH < 10.5, pK_a calculations showed that the neutral form of the precursor is the major specie present in solution at 8.8 < pH < 11.0. By adjusting the pH at 10.0 and using a reverse phase C-18 column, we found that the elution order could thus be easily reversed. As can be seen in Figure 1, under optimized chromatographic conditions, the retention time of the radiotracer was ~8 min, whereas the retention time of the precursor was 9–12 min. Despite the broadness of the latter peak and the severe tailing effect, the purified fraction could be collected and reformulated, yielding a radiotracer with a radiochemical yield of 91.1 ± 2.4% ($n = 5$) with respect to [^{11}C]CH_3I (decay-corrected) and specific radioactivity of 137 ± 5 GBq/μmol in overall synthesis time of 30–31 min (Table 1, Entry 3). The presence of the precursor in the final solution could not be detected by analytical HPLC (limit of detection: 0.05 μg/mL), and no other peaks were detected in the UV chromatogram. The radiochemical purity was above 99.5% in all cases (Table 1). These results are significantly better than those previously reported by Dollé *et al.* [9] especially in terms of radiochemical yield. The slight improvement in specific radioactivity values (137 ± 5 vs. 98.4 ± 36) might be due to the fact that Dollé and co-workers used cyclotron produced [^{11}C]CO_2, whereas in our case, the primary labeling agent was [^{11}C]CH_4, which is known to provide radiotracers with higher specific radioactivities.[13]

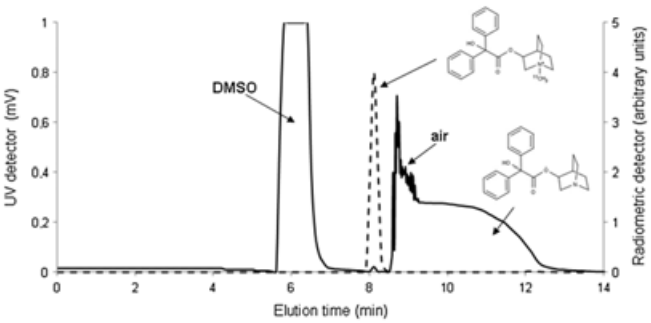


Figure 1. Chromatographic profiles corresponding to the purification of [^{11}C]Me-QNB. Radiometric detector (dotted line) and ultraviolet (UV) detector (solid line) profiles are shown.

The cationic nature of the radiotracer led us to consider the implementation of a purification step using solid-phase extraction cartridges. After reaction, the crude was eluted from the loop with purified water (10 mL), and the resulting solution was eluted through an Accell Plus CM cartridge. The cartridge was rinsed with ethanol (2 mL) to remove the unreacted precursor, with purified water (3 mL) to remove the residual ethanol, and the retained radiotracer was eluted into a vial using 10% ethanol in physiologic saline solution (total volume = 3 mL). As can be seen in Table 1 (Entry 4), the synthesis could be completed in 20–21 min. Decay-corrected radiochemical yield was considerably lower than the one obtained using HPLC (27.6 ± 3.1% vs. 91.1 ± 2.4%), probably due to partial elution of the radiotracer from the cartridge while removing the unreacted precursor. However, higher specific radioactivity (244 ± 8 GBq/μmol vs. 137 ± 5 GBq/μmol, decay uncorrected) values were obtained, and the amount of radioactivity should be sufficient to approach preclinical and clinical studies. The latter strategy could be particularly useful when high specific activities are required or no HPLC system is available. Moreover, shorter preparation times should increase the throughput in the synthetic systems.

Table 1. Experimental conditions, radiochemical yield, specific activity, and chemical and radiochemical purity for the preparation of [^{11}C]Me-QNB

Entry	QNB	Rt (min)	RT (°C)	[^{11}C]Me-QNB (GBq)	RCY (%)	A_S (GBq/μmol)	ST (min)	RCP (%)	CP
1[a]	0.64 mg	2	200	4.8 ± 1.2[b]	34.4 ± 10[c]	3.7–74[d]	<30	–	–
2[e]	0.64 mg	1	100	6.8 ± 1.4[b]	48.5 ± 10[f]	98.4 ± 36[g]	27–28	>99	>95%[h]
3	1.0 mg	1	30	0.81 ± 0.12[i]	91.1 ± 2.4[c]	137 ± 5[j]	30–31	>99.5	ND[k]
4	1.0 mg	1	30	0.39 ± 0.05[i]	27.6 ± 3.1[c]	243 ± 18[j]	20–21	>95	ND[k]

[a]Obtained from Syrota *et al.* [1]
[b]Starting from 44.4 GBq of [^{11}C]CO_2 (integrated current in target not specified).
[c]Based on [^{11}C]CH_3I, decay-corrected.
[d]In the original publication, the authors did not specify whether values were decay-corrected or not.
[e]Obtained from Dollé *et al.* [9]
[f]Based on [^{11}C]CH_3OTf, decay-corrected.
[g]Decay corrected.
[h]Obtained from chromatographic profile.
[i]Amount of [^{11}C]CH_4 not measured, irradiation conditions as detailed in materials and methods.
[j]Decay-uncorrected.
[k]no other peaks apart from Me-QNB detected in the UV chromatogram.
Rt: Reaction time; RT: Reaction temperature; RCY: Radiochemical yield; A_S: Specific radioactivity; ST: total synthesis time (including purification); RCP: Radiochemical purity; CP: Chemical purity. Mean ± standard deviation values are reported for [^{11}C]Me-QNB amount of radioactivity, radiochemical yield and specific radioactivity. For entries 3–5, $n = 5$.

Conclusions

In this study, an improved method for the preparation of the muscarinic receptor antagonist [^{11}C]Me-QNB has been reported. The methodology based on the reaction of QNB with [^{11}C]CH_3I at room temperature using the captive solvent method considerably improves the previously reported results in terms of decay-corrected radiochemical yield (91.1 ± 2.4% vs. 48.5 ± 10%). The implementation of a purification step using reverse phase semi-preparative HPLC reverses the elution order of the precursor and the radiotracer; thus, higher amounts of precursor can be used without affecting the chemical purity of the final radiotracer. Alternatively, a purification method using solid-phase extraction can be applied. As a result, lower radiochemical yield and radiochemical purity values are obtained, but overall preparation time could be shortened from 30–31 min to 20–21 min.

Acknowledgements

The authors would like to thank the Departamento de Industria, Comercio y Turismo of the Basque Government for financial support.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] A. Syrota, P. Merlet, J. Delforge. The Heart, chapter 37, Section 2. In *Cardiac Neurotransmission in Principles of Nuclear Medicine*, 2nd Ed. (Ed.: H. N. Wagner), W. B. Saunders Company: Philadelphia – London – Toronto – Montreal – Sydney – Tokyo, **1995**, pp. 759–773.
[2] M. Mazière, D. Comar, J. M. Godot, P. H. Collard, C. Cepedat, R. Naquet, *Life Sci.* **1981**, *29*, 2391.
[3] M. Mazière, G. Berger, J. M. Godot, C. Prenant, J. Sastre, D. J. Comar, *Radioanal. Chem.* **1983**, *76*, 305.
[4] A. Syrota, G. Paillotin, J. M. Davy, M. C. Aumont, *Life Sci.* **1984**, *35*(9), 937.
[5] A. Syrota, D. Comar, G. Paillotin, J. M. Davy, M. C. Aumont, O. Stulzaft, B. Mazière, *Proc. Natl. Acad. Sci. US.* **1985**, *82*, 584.
[6] D. Le Guludec, J. Delforge, A. Syrota, M. Desruennes, H. Valette, I. Gandjbakhnch, P. Merle, *Circulation* **1994**, *90*, 172.
[7] D. Le Guludec, A. Cohen-Solal, J. Delforge, N. Delahaye, A. Syrota, P. Merlet, *Circulation* **1997**, *96*, 3416.
[8] N. Delahaye, S. Dinanian, M. Slama, H. Mzabi, D. Samuel, D. Adams, P. Merlet, D. Le Guludec, *Eur. J. Nucl. Med.* **1999**, *26*, 416.
[9] F. Dollé, F. Hinnen, F. Vaufrey, S. Demphel, Y. Bramoulle, D. Fournier, M. Ponchant, H. Valette, C. Crouzel, *J. Labelled Compd. Radiopharm.* **2001**, *44*, 337.
[10] V. Gómez, J. D. Gispert, V. Amador, J. Llop, *J. Labelled Compd. Radiopharm.* **2008**, *51*(1), 83.
[11] V. Gómez-Vallejo, J. Llop, *Nucl. Med. Commun.* **2011**, *32*(11), 1011.
[12] A. A. Wilson, A. Garcia, L. Jin, S. Houle, *Nucl. Med. Biol.* **2000**, *27*, 529.
[13] V. Gómez-Vallejo, V. Gaja, J. Koziorowski, J. Llop. *Positron Emission Tomography – Current Clinical and Research Aspects*. Chapter 7. Intech Ed: Croatia, Europe, **2012**, pp. 183–210.