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A new technique for labeling of [^{11}C]-choline, a positron-emitting tracer for tumor imaging

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[^{11}C]-choline has been reported as a potential tracer for imaging a variety of human tumors with positron emission tomography (PET). A new labeling technique for [^{11}C]-choline was established depending on parameters optimized, such as reaction time, volume, temperature, and the quantity of DMAE. The synthesis yield was improved from 82.0% to 96.5% (EOB), while the consumption of DMAE precursor decreased from 60 to 2 mg. Absolute yield of [^{11}C]-choline was 2500 MBq for a 10-minute irradiation at 15 μA, and a total synthesis time of less than 8 minutes from [^{11}C]-CH_3I to [^{11}C]-choline.

Introduction

Like 2-[^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]-FDG), [^{11}C]-choline has been reported of potential in PET imaging of a variety of tumors.[1–4] It was suggested that the uptake of [^{11}C]-choline in tumors correlates with the increased synthesis of membrane phosphatidyl-choline of the malignant tissue. Due to its negligible distribution in normal cerebral cortex, myocardium and urinary system, [^{11}C]-choline was found particularly helpful in imaging tumors in brain, lung, esophagus, rectum, prostate and bladder, resulting from a higher target to background signal ratio as compared with [^{18}F]-FDG.

So far, three methods have been proposed in the literature for the radio-synthesis of [^{11}C]-choline. In the first method, [^{11}C]-methyl iodide was reacted with 100–500 μl of 2-dimethylamino-ethanol (DMAE) at temperatures above 100 °C for 5 minutes, followed by a 25-minute synthesis and HPLC purification to achieve a radiochemical yield of over 95%.[5,6] The synthesis yield was reportedly further improved by using high specific activity [^{11}C]-methyltriflate instead of [^{11}C]-methyl iodide.[7,8] In the second method, 80 μl of DMAE was pre-loaded on a C-18 cartridge at room temperature. The final product was purified with a cation exchange resin instead of HPLC with a radiochemical yield of 87% 12 minutes after EOB.[9,10] The third method employed a loop instead of C-18 cartridge.[11–13] [^{11}C]-methyl iodide was directly delivered to the loop containing 60 μl DMAE, then the loop was eluted with ethanol. [^{11}C]-choline was trapped in the loop and purified on a cation exchange cartridge, resulting in yields of over 80% using 60 μl of DMAE in 20 minutes.

Although DMAE is harmless and used sometimes as a nutrition additive, excess amounts of the chemical may compete with [^{11}C]-choline in the uptake of living cells.[14] We developed a new technique for the labeling of [^{11}C]-choline, in which very small amount of DMAE in acetone was allowed to react with [^{11}C]-methyl iodide, in order to increase of the synthesis yield and to decrease the quantity of precursor at the same time.

Experiment

Reagents

DMAE, acetone and choline chloride were purchased from Aldrich (USA); 1 mol/l lithium aluminum hydride in THF from ABX; acetonitrile from Lab-Scan (HPLC, Ireland); Sep-Pak CM cartridges from Waters (USA).

Production of [^{11}C]-CH_3I[15]

[^{11}C]-CO_2 was produced via the ^{14}C(p,α)^{11}C nuclear reaction in the RDS111 cyclotron (CTI, USA) with 10-minute irradiation at 15 μA. [^{11}C]-CH_3I of 5550MBq was obtained in an automatic synthesis module (PET-II, Beijing PET Co., China) with a single vessel in the liquid phase. Then, [^{11}C]-CO_2 was delivered too and trapped in the loop cooled with liquid nitrogen. The loop trap was warmed up in air to 50 °C, then [^{11}C]-CO_2 was transferred with nitrogen gas with a flow rate of 30 ml/min into the reaction tube inside the module containing 0.2 ml 1 mol/l lithium aluminum hydride in THF. Then the tube was heated to 190 °C to remove THF by nitrogen gas flow. 0.2 ml 57% HI was added to the tube after THF had been removed completely. The tube was heated once again to 190 °C. [^{11}C]-CH_3I was carried out by nitrogen gas with a flow rate of 30 ml/min. The synthesis yield of [^{11}C]-CH_3I was 85.3±1.7% (EOB, $n = 60$), within 10 minutes from [^{11}C]-CO_2 to [^{11}C]-CH_3I.

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Optimization of the labeling reaction

A stock solution of [^{11}C]-CH_3I was prepared by bubbling the [^{11}C]-CH_3I into a flask filled with 1 ml of acetone at 0 °C. To determine their effects on the final labeling yield, a stock solution of DMAE (in acetone, RT) was prepared and reacted with freshly prepared [^{11}C]-CH_3I under varied labeling parameters, such as reaction volume, reaction temperature, pH, reaction time, and quantity of DMAE. The radiochemical yield was assessed by HPLC using a Nova-Pak C-18 HPLC column (3.9×150 mm^2) and CH_3CN/0.1 mol/l ammonium formiate of pH 4.0 (79/21 V/V) as mobile phase at a flow rate of 1 ml/min.

Radiochemical yield

The radiochemical yield was analyzed by HPLC with the results shown in Fig. 1. It can be seen that the reference retention time of [^{11}C]-choline was 4.1 minutes because of its solubility in water, while the retention time of [^{11}C]-CH_3I, a lipophilic compound, was 9.3 minutes. Thereby, it was easy to calculate the percentage of [^{11}C]-CH_3I being transformed to [^{11}C]-choline.

Automated radiosynthesis

The [^{11}C]-CH_3I was bubbled into the reactor of an automated remote controlled synthesizer (PET-I Methylation Module, Beijing PET Co., China). The reactor, containing 2 mg DMAE was dissolved in 50 µl acetone at 0 °C, warmed up to room temperature for 2 minutes when [^{11}C]-CH_3I has been developed. The reaction mixture was then diluted with 10 ml of ethanol and delivered to the cation exchange cartridge (CM). [^{11}C]-choline was captured by the CM column, washed with 10 ml water, and then the purified [^{11}C]-choline was eluted by 5 ml 0.9% NaCl and measured in a dose calibrator.

Production of [^{11}C]-choline on the C-18 cartridge[9,16]

Sixty µl DMAE was loaded on to the C-18 cartridge and joined with the CM column. [^{11}C]-methyl iodide was distilled across the C-18 and the CM cartridge at a nitrogen flow rate of 30 ml/min. Ethanol (10 ml) and water (10 ml) were then passed through the C-18 and the CM cartridge to wash out any unreacted precursor and [^{11}C]-methyl iodide. The product was desorbed from the CM column by elution with 5 ml saline.

Results and discussion

Effect of the reaction time

A stock of [^{11}C]-CH_3I was added into a flask filled with DMAE (the quantity was intentionally reduced to 0.33 mg) dissolved in acetone with a total reaction volume of 50 µl at RT. The radiolabeling yield was assessed by taking off 3 µl each of the reaction solution at different time. It was obvious that the labeling yield increased with reaction time, reaching over 95% at 20 minutes (Fig. 2). Due to the short half-life of ^{11}C, the labeling process must be completed as rapidly as possible. It was possible to get high labeling yield in less time by increasing the amount of the DMAE precursor.

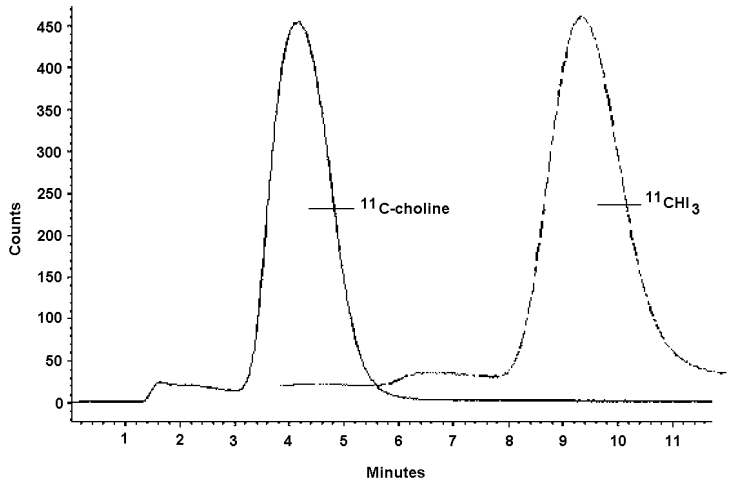

Fig. 1. Radiochromatogram of [^{11}C]-CH_3I and [^{11}C]-choline

Effect of the quantity of precursor DMAE

The labeling process was repeated with a stock of [^{11}C]-CH_3I added into a flask filled with DMAE then incubated at RT for 3 minutes. The quantity of DMAE dissolved in acetone varied from 0.33 to 2.0 mg. The influence of the quantity of DMAE on the radiolabeling yield is shown in Fig. 3. The radiolabeling yield increased with the amount of DMAE, reaching 95–100% after 1–2 mg DMAE was added. Thereafter, 2 mg DMAE was used in the automated radiosynthesis. The results were the same as those from the loop method,[11] but a plateau was reached with 80 µl (about 70 mg) DMAE at 83.5%.

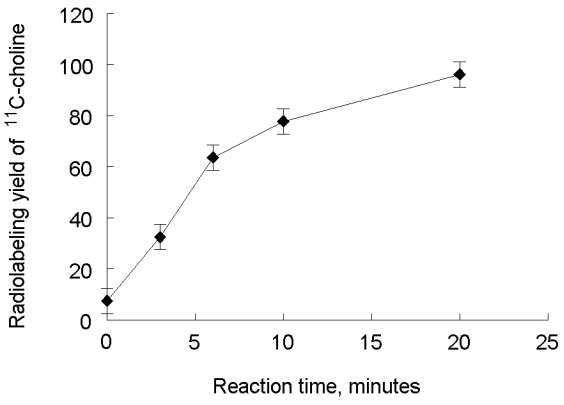

Fig. 2. Effect of the reaction time on labeling yield of [^{11}C]-choline ($n = 3$)

Effect of the reaction volume

The effect of the reaction volume was studied while 1 mg DMAE was incubated with [^{11}C]-CH_3I at RT for 3 minutes, in various reaction volumes (Fig. 4). The radiolabeling yield decreased as the reaction volume increased. Higher concentrations of DMAE result in better labeling efficiency. The best reaction volume was suggested as 50 µl.

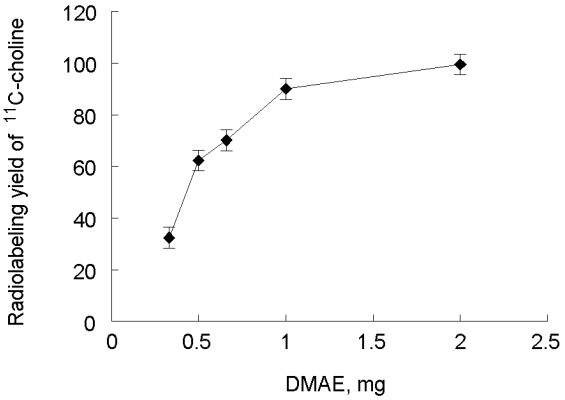

Fig. 3. Radiochemistry yield and the amount of precursor DMAE ($n = 3$)

Effect of the reaction temperature

[^{11}C]-CH_3I was bubbled into the reactor cooled to 0 °C, containing 1 mg DMAE in 50 µl acetone. One part of the mixture was analyzed directly by HPLC, other parts at RT or at 40 °C for 3 minutes before the HPLC analysis. The labeling yield was nearly 0 at 0 °C, but quickly rose as the temprature increased, being over 95% at RT and at 40 °C. There was no difference between RT and 40°C in the labeling yields. After [^{11}C]-CH_3I has been bubbled into the reactor at 0 °C, the reactor had to be taken off the ice-bath to increase the labeling efficency.

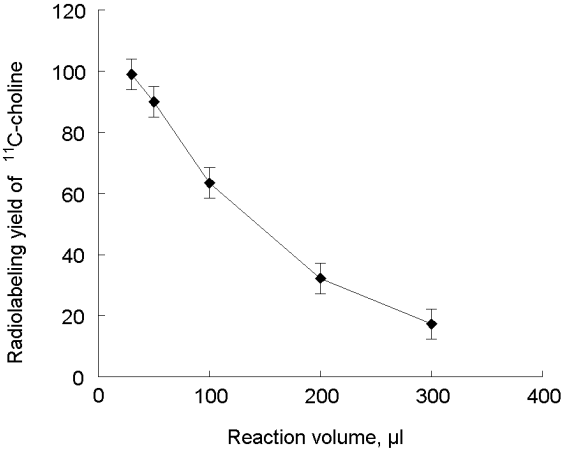

Fig. 4. Effect of reaction volume on the labeling yield of [^{11}C]-choline ($n = 3$)

Effect of pH

With most of the fixed labeling parameters, i.e., 1 mg DMAE incubated with [^{11}C]-CH_3I at RT for 3 minutes in a final reaction volume of 50 µl, the labeling efficiency was quite stable without the addition of acid or base. The labeling yield decreased from 95% to 85% when 10 µl 5N NaOH was added into the mixture. This was different from other methylation reactions.[17,18]

Automated radiosynthesis

To facilitate the reliable and efficient production of [^{11}C]-choline for clinical uses, the results obtained in the bench experiments were employed in the design of a remotely controlled synthesizer module. As described above, [^{11}C]-CH_3I was bubbled into the reaction vessel of the synthesizer filled with 2 mg DMAE in 50 µl acetone at 0 °C, and subsequently the labeling reaction was carried out for 2 minutes at room temperature. The mixture was transferred to a Sep-Pak CM column with 10 ml diluted ethanol, washed with 10 ml water, then eluted with 5 ml saline and sterile-filtered to render the product ready for clinical use.

In ordinary production runs, the average yields of the radiosynthesis was 2500±300 MBq of [^{11}C]-choline ($n = 3$). The procedure took about 8 minutes from [^{11}C]-CH_3I to [^{11}C]-choline. The radiochemical yield based on [^{11}C]–CH_3I was 96.5±2.3% (EOB). On the other hand, the method of carbon-11 methylation on a C-18 Sep-Pak cartridge, taking about 6 minutes, was able to produce 2000±215 MBq of [^{11}C]-choline ($n = 55$), with a yield of 82.0±8.5% (EOB).

Conclusions

A relatively efficient synthesis route for the production of [^{11}C]-choline in a remote controlled synthesizer was developed. The synthesis yield was improved from 82.0% to 96.5%, and the quantity of DMEA decreased significantly from 60 to 2 mg. The absolute production yield of [^{11}C]-choline was 2500 MBq after a 15 µA irradiation for 10 minutes, and the total synthesis time from [^{11}C]-CO_2 to [^{11}C]-choline was less than 20 minutes.

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