Applied Radiation and Isotopes 129 (2017) 57–61

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Automated synthesis of 1-[^{11}C]acetoacetate on a TRASIS AIO module

Kiran Kumar Solingapuram Sai[a,*], H. Donald Gage[a], Frankis Almaguel[a], Bryan Neth[b], Timothy M. Hughes[b], Sebastien Tremblay[c], Christian-Alexandre Castellano[c], Stephen C. Cunnane[c], Matthew J. Jorgensen[d], Suzanne Craft[b], Akiva Mintz[a]

[a] Department of Radiology, Wake Forest School of Medicine, Winston Salem, NC, USA
[b] Department of Internal Medicine, Wake Forest School of Medicine, Winston Salem, NC, USA
[c] Research Center on Aging, Sherbrooke Molecular Imaging Center, Université de Sherbrooke, Sherbrooke, Quebec, Canada
[d] Department of Pathology-Comparative Medicine, Wake Forest School of Medicine, USA

HIGHLIGHTS

- Automated and simplified production of 1-[^{11}C]acetoacetate using TRASIS AIO module.
- 1-[^{11}C]acetoacetate radiochemistry can be directly translated and easily adapted to any automated modules for human injections.
- 1-[^{11}C]acetoacetate production was validated through monkey PET imaging studies for the first time.

ARTICLE INFO

Keywords:
Automation
Ketone metabolism
PET imaging
Ion-exchange column
Formulations
Non-Human primates

ABSTRACT

We automated radiochemical synthesis of 1-[^{11}C]acetoacetate in a commercially available radiochemistry module, TRASIS AllInOne by [^{11}C]carboxylation of the corresponding enolate anion generated *in situ* from isopropenylacetate and MeLi, and purified by ion-exchange column resins.1-[^{11}C]acetoacetate was synthesized with high radiochemical purity (95%) and specific activity (~ 66.6 GBq/μmol, n = 30) with 35% radiochemical yield, decay corrected to end of synthesis. The total synthesis required ~ 16 min. PET imaging studies were conducted with 1-[^{11}C]acetoacetate in vervet monkeys to validate the radiochemical synthesis. Tissue uptake distribution was similar to that reported in humans.

1. Introduction

Under normal conditions, glucose is the brain's primary fuel; however during prolonged fasting or at lower plasma glucose concentrations, fat-derived ketones constitute the brain's main fuel (Cunnane et al., 2016; Willis et al., 2002). Ketone bodies consist of acetoacetate, *β*-hydroxybutyrate and acetone. These play a vital role in brain lipid synthesis in the fetus and in infant brain development (Roy et al., 2012; Yudkoff et al., 2004). During long-term starvation in adults, plasma ketones reach a concentration of 2–6 mM and can supply up to ~ 70% of the brain's fuel needs (Bianchi and Davis, 1996; Owen et al., 1967).

Alzheimer's disease (AD) is the most common neurodegenerative disease and the leading cause of severe dementia, especially among the older adults. The occurrence of AD exponentially increases with age once individuals reach 65 years of age or older. There is no specific treatment regimen to preclude the symptoms of AD nor are the presently available ones are effective. Low uptake of 2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) in AD-susceptible regions has been proposed as a key markers in AD pathophysiology (Mosconi, 2005). Considerable scientific evidence suggests that brain glucose metabolism is inhibited during AD (Bianchi and Davis, 1996; Castellano et al., 2015). As the main fuel alternate to glucose, ketones can account for up to 60% of brain metabolic demands in order to help mitigate states of decreased glucose metabolism in AD. One of the ketone-based strategies studies aimed at slowing the progression of AD in the high Fat-ketogenic diet. One of the several research pathways studied to improve the conditions of AD is 'ketogenic diet"(Cunnane et al., 2010; Elwood et al., 1960; Loessner et al., 1995; Owen et al., 1967; Pifferi et al., 2008). Cognitive function can be significantly improved in memory-impaired adults by a ketogenic diet intervention (Courchesne-Loyer et al., 2012; Krikorian et al., 2010; Nugent et al., 2014; Pifferi et al., 2008; Yudkoff et al., 2004).

Current strategies to assess brain metabolism using [^{18}F]FDG provide no information regarding the brain's innate ability to use ketone

Abbreviations: [^{11}C]AcAc, 1-[^{11}C]acetoacetate; FDG, 2-[^{18}F]fluoro-D-glucose; PET, Positron Emission Tomography; NHP, Non-Human Primate; EOS, End Of Synthesis

* Corresponding author.
E-mail address: ksolinga@wakehealth.edu (K.K. Solingapuram Sai).

http://dx.doi.org/10.1016/j.apradiso.2017.07.066
Received 17 December 2016; Received in revised form 24 May 2017; Accepted 31 July 2017
Available online 02 August 2017
0969-8043/

bodies. PET imaging using [^{11}C]acetoacetate ([^{11}C]**AcAc**) can provide quantitative analysis of brain ketone utilization that can help uncover the role of ketone metabolism in early AD; such information could potentially lead to new preventive and therapeutic strategies (Authier et al., 2008; Courchesne-Loyer et al., 2016; Nugent et al., 2013). These promising translational imaging applications of [^{11}C]**AcAc** stimulated efforts for the development of a more robust and simple automated radiochemistry procedure. While previous investigators have automated [^{11}C]**AcAc** synthesis in a custom-built radiochemistry module (Tremblay et al., 2007), simplifying this production in a more commercially available automated unit will pave the path for widespread use of the radiopharmaceutical in clinical settings. TRASIS AIO is an automated, commercially available radiosynthesis module that produces GMP grade PET radiopharmaceuticals for human injections and is being widely used in several PET centers across the world. Here, we report for the automated synthesis [^{11}C]**AcAc** in TRASIS AllInOne module and report its utility in non-human primates (NHP).

2. Materials and methods

The following chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO) and were used without any additional purification: methyllithium (MeLi, 1.6 M diethylether), isopropenylacetate (IPA), anhydrous tetrahydrofuran (THF), sodium hydroxide solution (NaOH, 1.0 M), citric acid monohydrate (CAM), trisodium citric acid (TCA), sodium chloride (NaCl) and lithium acetoacetate (LAA). All reactions were carried out using anhydrous solvents unless otherwise stated. Both the resin materials *i.e.,* Dowex × 8–100 (cation resin material) and AG 1X-8 (anion exchange resin material), chromatography flex columns and the analytical HPLC column heater were purchased from Fisher Scientific (Hampton, NH). Aqueous 5 mM sulfuric acid (H_2SO_4) solution was purchased from VWR scientific (Radnor, PA). Analytical HPLC was performed using Varain ProStar system, which includes quaternary gradient pump, manual injector, a variable wavelength detector and a standard Bioscan radioactivity-HPLC-flow detector. Aminex HPX-87 H analytical column (300 × 70 mm) was purchased from BIO-RAD (Hercules, CA). Sterile pyrogen-free filters were purchased from Millipore Corp (Billerica, MA).

2.1. TRASIS AIO preparation

TRASIS AllInOne (AIO) is a commonly used radiochemistry module for clinical GMP-grade radiopharmaceutical production (AIO, 2014). [^{11}C]**AcAc** radiosynthesis was carried out with TRASIS AIO module using the ports as shown in Fig. 1.

2.2. Cartridge conditioning and citrate buffer setup

Citrate buffer solution, pH 4.0 for eluting the final radioactive product [^{11}C]**AcAc** was made according to the previously published procedures (Tremblay et al., 2007). Briefly, for a stock solution (25 mL), CAM (0.3 g, 1.42 mM), TCA (0.18 g, 0.7 mM) and NaCl (0.12 g, 1.95 mM) were added in a sterile laminar fume hood. The solution was stored at 4 °C and was filtered through a 0.22 μm pyrogen-free Millipore filter for every batch synthesis. Both the cation and anion exchange resin cartridges were pre-conditioned following published procedures. Briefly, Dowex 50WX8-100 was placed in a small chromatography flex column and washed with sterile water (10 mL). AG 1X-8 was placed in a separate flex column and washed with aqueous NaOH solution (5 mL, 1.0 M) followed by sterile water (25 mL). Both the ion exchange resin materials had a final pH between 7.0 and 7.5

2.3. Radiochemical synthesis of [^{11}C]*AcAc*

2.3.1. Production of [^{11}C]CO_2

[^{11}C]CO_2 was produced in the Wake Forest PET Center cyclotron facility on a GE PETtrace- 800 cyclotron. [^{11}C]CO_2 was produced from the nuclear reaction $^{14}N(p,\alpha)^{11}C$ by proton bombardment of a niobium target to a pressure of 17.2 bar (250 psi). A nitrogen target containing 0.2% oxygen was irradiated for 15–20 min with a 45 μA beam of 16 MeV protons, to produce up to 40–42 GBq of [^{11}C]CO_2 (Solingapuram et al., 2014). [^{11}C]CO_2 released from the GE PET-trace cyclotron was directly bubbled and trapped into the reaction vial assembled in the TRASIS AIO module using anhydrous potassium perchlorate ($KClO_4$, 8 g) trap.

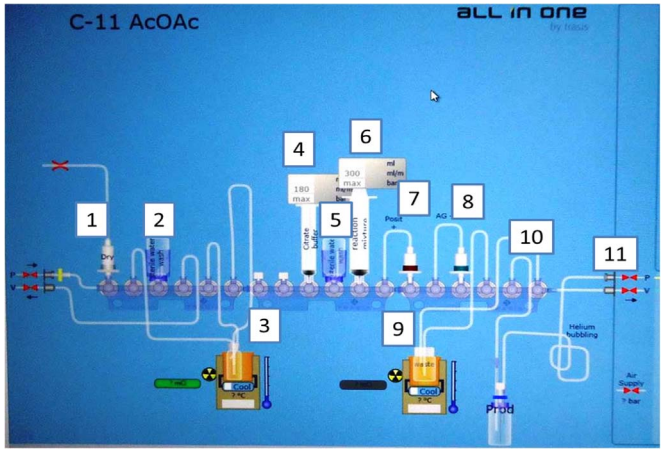


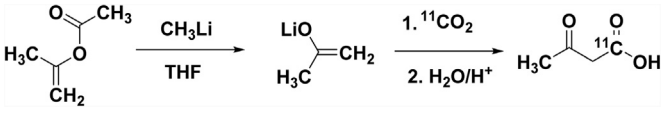
Fig. 1. TRASIS AIO reaction program screenshot for [^{11}C]**AcAc** radiosynthesis. Port#1 [^{11}C]CO_2; Port #2 sterile water setup-hydrolysis; Port #3 reaction vial holder 1; Port# 4 citrate buffer solution (10 mL syringe) 5 mL, pH 4.0; Port#5 sterile water setup-washing AG 1X-8 cartridge; Port#6 reaction mixture syringe (20 mL syringe); Port #7 cartridge holder for Dowex × 8–100 (cation exchange resin material); Port#8 cartridge holder for AG 1X-8 (anion exchange resin material); Port#9 reaction vial holder 2 (waste vial); Port#10 product line; Port #11 helium degassing line.

2.3.2. Precursor reaction mixture setup

The enolate anion of acetone was prepared following a previously published method with slight modification (Tremblay et al., 2008, 2007). Briefly, IPA (1.0 eq) was slowly added drop-wise to the glass vial loaded with MeLi (1.6 M in diethyl ether, 1.85 eq) at − 75 °C under inert conditions. Anhydrous THF (0.75 mL) was slowly added to the reaction mixture and allowed to stir for 1 h at − 40 °C to − 70 °C under an inert atmosphere (Scheme 1). MeLi afforded better yields of final radioactive product when used as a base, compared to *n*BuLi. The crude enolate solution formed *in situ* was used for radiolabeling as is without any additional purification.

2.3.3. [^{11}C]*AcAc* production

Optimized radiosynthesis of [^{11}C]**AcAc** was completed *via* carboxylation, hydrolysis, resin cartridge purification and citrate buffer formulation (Tremblay et al., 2007). The TRASIS AIO setup for radiosynthesis is shown in Fig. 2. Firstly, the crude enolate solution (0.8–1.3 mL) synthesized from MeLi-base catalyzed reaction of isopropenylacetate was placed in the reaction vial holder#1of the TRASIS AIO module, once the module was ready to receive [^{11}C]CO_2 gas from the cyclotron. The vial was then cooled to − 10 °C to − 40 °C using liquid nitrogen cooling setup from the module. [^{11}C]CO_2 released from the cyclotron was bubbled and trapped into the reaction vial with the enolate solution at the same temperature. After complete transfer of radioactivity (3–4 min), the radioactive reaction mixture was allowed



Scheme 1. Radiosynthesis of [^{11}C]**AcAc**.