Radiosynthesis of $^{[11]}C$Ibrutinib via Pd-Mediated $^{[11]}C$CO Carbonylation: Preliminary PET Imaging in Experimental Autoimmune Encephalomyelitis Mice

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Ibrutinib is a first-generation Bruton’s tyrosine kinase (BTK) inhibitor that has shown efficacy in autoimmune diseases and has consequently been developed as a positron emission tomography (PET) radiotracer. Herein, we report the automated radiosynthesis of $^{[11]}C$Ibrutinib through $^{11}$C-carbonylation of the acrylamide functional group, by reaction of the secondary amine precursor with $^{[11]}C$CO, iodoethylene, and palladium–NiXantphos. $^{[11]}C$Ibrutinib was reliably formulated in radiochemical yields of 5.4% ± 2.5% (non-decay corrected; $n = 9$, relative to starting $^{[11]}C$CO$_2$), radiochemical purity >99%, and molar activity of 58.8 ± 30.8 GBq/µmol (1.55 ± 0.83 Ci/µmol). Preliminary PET/magnetic resonance imaging with $^{[11]}C$Ibrutinib in experimental autoimmune encephalomyelitis (EAE) mice showed a 49% higher radioactivity accumulation in the spinal cord of mice with EAE scores of 2.5 vs. sham mice.

Keywords: ibrutinib, multiple sclerosis, positron emission tomography, carbon-11, carbon monoxide

INTRODUCTION

Bruton’s tyrosine kinase (BTK) is critical for the maturation of B cells and in regulating hematopoietic cell circulation (1, 2). BTK inhibitors have been developed to treat B-cell malignancies and autoimmune diseases including multiple sclerosis (MS) (3, 4), where it is postulated that inhibiting BTK blocks the maturation of B cells (5–9). Ibrutinib is a first-generation BTK inhibitor used as a chemotherapeutic agent to treat B-cell cancers (10). Although ibrutinib has not been studied clinically as a BTK inhibitor in MS, it has shown efficacy in other autoimmune diseases, such as arthritis, lupus, and recently in treatment of COVID-19 (SARS-CoV-2) (11–14).

The versatility of BTK inhibitors, such as ibrutinib, in treating various B-cell-related diseases has led to their development as radiotracers for positron emission tomography (PET) (15, 16). Ibrutinib (2) has been labeled at the acrylamide functionality with carbon-11 ($^{[11]}C$) using $^{[11]}C$CO$_2$ (Figure 1) (16). However, the method resulted in low radiochemical yields (RCYs) (<5%,...
non-decay corrected) due to side reactions caused by the Grignard reagents, which also affected the chemical purity and radiochemical purity (RCP). Although the chemical purity and RCP were improved by using carrier-added CO₂, this resulted in low molar activity ($A_m$), 2.0 GBq/µmol ($\sim$55 mCi/µmol). The above-mentioned limitations (low RCP, chemical purity, $A_m$, and RCY) have restricted the widespread use of $^{11}$Cibrutinib for PET imaging. Hence, a reliable radiosynthesis of $^{11}$Cibrutinib is needed with improved RCY, RCP, and $A_m$, ideally by avoiding Grignard reagents and carrier-added CO₂.

Radiolabeling of carbonyls such as acrylamides has been extensively reported using $^{11}$C CO₂ (17, 18). Recent developments in converting cyclotron-produced $^{11}$C CO₂ to $^{11}$C CO have provided access to new radiosynthesis pathways for $^{11}$C-carbonylation (19–23). We recently reported the labeling of the acrylamide moiety of $^{11}$C tolebrutinib by $^{11}$C-carbonylation via $^{11}$C CO, iodoethylene, and the secondary amine precursor using a NiXanthos-mediated protocol under atmospheric pressure using no-carrier-added $^{11}$C CO (Figure 1) (15). In the present study, we have adapted this radiosynthesis to label $^{11}$Cibrutinib via $^{11}$C-carbonylation of 3-(4-phenoxyphenyl)-1-(piperidin-3-yl)-2,3-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1), and we report a preliminary PET/magnetic resonance imaging (MRI) study of the spinal cord in experimental autoimmune encephalomyelitis (EAE) mouse models.
**RESULTS AND DISCUSSION**

The efficacy of ibrutinib in autoimmune diseases, such as arthritis, lupus, and COVID-19 (SARS-CoV-2), makes [11C]ibrutinib an ideal BTK PET radiotracer. The N-acrylamide moiety of ibrutinib also lends this structure well for [11C]CO carbonylation utilizing a new Pd-mediated methodology that we recently reported for the radiolabeling of another BTK inhibitor, [11C]tolebrutinib (15). Our [11C]CO carbonylation of [11C]tolebrutinib (15) has advantages over the recently reported [11C]CO2 carbonylation of [11C]ibrutinib including higher RCYs and does not require carrier-added CO2, thereby also improving the $A_m$ of the radiotracer (Table 1) (15, 16). As ibrutinib is well tolerated as a pharmaceutical, and as the authentic standard and amine precursor for [11C]CO carbonylation are commercially available, [11C]ibrutinib could be rapidly validated on an automated synthesis unit for human use.

**Radiosynthesis of [11C]Ibrutinib**

The radiosynthesis of [11C]ibrutinib was fully automated using a commercial TracerMaker™ [11C] synthesis platform. The method and setup of the automated synthesis platform were adapted with only minor changes from our previously published method for [11C]tolebrutinib (15). Commercially available anhydrous tetrahydrofuran (THF) solvent was used, eliminating the need for freshly distilled THF, and the semi-preparative high-performance liquid chromatography (HPLC) employed a flow rate of 6.0 ml/min. [11C]Ibrutinib was produced in 45–50 min overall synthesis time from end of bombardment (EOB) with a retention time of 12–13 min (Figure 2A). [11C]Ibrutinib was synthesized in 5.4 ± 2.5% ($n = 9$) RCY (non-decay corrected) relative to [11C]CO2, with RCP >99%, as confirmed by co-injection of authentic standard (Figure 2B), and $A_m$ of 58.8 ± 30.8 GBq/μmol (1.55 ± 0.83 Ci/μmol) as measured by HPLC. The chromatogram from the semi-preparative HPLC purification shows only a minor radioactive by-product eluting at 3 min (Figure 2A). No radioactive by-products were seen in the analytical HPLC chromatograms (Figure 2B), indicating that the [11C]CO carbonylation reaction under these conditions is highly selective for the secondary N-piperidinyl moiety over the primary pyrimidin-4-amine.

**Preliminary PET/MR Imaging and Biodistribution in an Experimental Autoimmune Encephalomyelitis Mouse Model**

We evaluated the utility of [11C]ibrutinib in EAE mouse models of MS using female C57BL/6 mice (EAE score = 2.5) as compared with sham-treated animals (EAE score = 0.0). Regions of interest (ROIs) of the spinal cord were selected from the MRI (Figure 3A). Figures 3B,C show representative static PET/MR images with [11C]ibrutinib (0–60 min average image) in the EAE mice compared with the sham group, respectively. As demonstrated in the time activity curves (TACs) shown in Figure 4A, a 49% higher accumulation of radioactivity was measured in the sacral section of the spinal cord of the EAE mice (EAE score = 2.5) as compared with the sham at 5.5 min post-injection (p.i.), 1.27 ± 0.49 and 0.85 ± 0.04, respectively ($p = 0.0409$). Radioactivity accumulation decreased gradually but remained higher in EAE mice compared with sham mice until 40.5 min p.i., 0.48 ± 0.03 and 0.32 ± 0.05, respectively ($p = 0.0336$). Figure 4B shows that a 32% higher accumulation of radioactivity was measured in the lumbar region of EAE mice (EAE score = 2.5) as compared with the sham mice at 5.5 min p.i., 1.13 ± 0.37 and 0.80 ± 0.12, respectively ($p = 0.0359$). Radioactivity accumulation in the lumbar region of EAE mice cleared faster than in the sacral region of EAE mice, remaining higher than sham mice until 13.5 min p.i., 0.81 ± 0.09 and 0.62 ± 0.002, respectively ($p = 0.03$). Figures 4C,D show the thoracic and cervical sections of the spinal cord, respectively, for which there was no statistical difference from 0 to 60 min p.i. between the EAE mice and the sham mice. In accordance with previously published PET imaging results (16), whole-brain uptake was low, with SUV <0.5 from 5 to 60 min (Figure 4E). Biodistribution of the spinal cord (lumbar and thoracic regions combined) in mice sacrificed 60 min p.i. with [11C]ibrutinib was 0.17% ID/g and 0.09% ID/g, in EAE mice and sham, respectively. The sacral region of the spinal is too small in this mouse model to be quickly and reliably extracted. Despite this time point being past the peak of radioactivity accumulation of 5 min, as well as excluding the sacral region, the results indicate a trend toward higher radioactivity accumulation in the EAE spinal cord compared with sham spinal cord. A limitation of our PET imaging studies is the low power of our animal studies ($n = 2–3$), and future work would involve increasing the sample size.

**CONCLUSIONS**

A fully automated and reliable radiosynthesis of [11C]ibrutinib using [11C]CO has been developed. [11C]Ibrutinib was synthesized in RCYs (5.4 ± 2.5%, non-decay corrected), RCP (>99%), and $A_m$ (58.8 ± 32.8 GBq/μmol). Preliminary PET imaging studies in EAE mouse models show a trend toward increased radioactivity accumulation in the spinal cord of mice with higher EAE scores. Further studies that demonstrate the specificity of [11C]ibrutinib for imaging BTK in vivo are warranted, and the investigation of [11C]ibrutinib as a PET radiotracer in other B-cell disease models is possible.

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**TABLE 1 | Radiochemical syntheses of [11C]ibrutinib.**

| Radiochemical method                  | RCY (non-decay corrected) | RCP   | $A_m$  |
|---------------------------------------|----------------------------|-------|--------|
| [11C]CO2 carbonylation (carrier added) | 5%                         | 99%   | 2.0 GBq/μmol |
| [11C]CO2 carbonylation (no carrier added) | 2–5%                       | 90%   | 22–44 GBq/μmol |
| [11C]CO carbonylation                 | >6%                        | 99%   | 59 GBq/μmol |

RCY, radiochemical yield; RCP, radiochemical purity.
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FIGURE 2 | (A) Semi-preparative high-performance liquid chromatography (HPLC) chromatogram from production of $^{11}$Cibrutinib and (B) analytical HPLC chromatograms of $^{11}$Cibrutinib co-injected with ibrutinib standard.

MATERIALS AND METHODS

Chemistry

Reference standards of ibrutinib were acquired from Toronto Research Chemicals (Toronto, ON, Canada). 3-(4-Phenoxyphenyl)-1-(piperidin-3-yl)-2,3-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (1) was provided by Bristol Myer-Squibb Pharmaceuticals (Princeton, NJ, USA). Iodoethylene was acquired from Oakwood Chemicals (Estill, SC, USA). All other chemicals were acquired from Sigma-Aldrich (USA or Canada). No-carrier-added $^{11}$C$^2$O$_2$ was produced on a MC-17 Cyclotron (Scanditronix, Vislanda, Sweden) using the $^{14}$N(p,$\alpha$)$^{11}$C nuclear reaction in a pressurized gas target containing a nitrogen:oxygen mixture (99.5:0.5). The radiosynthesis was performed using a TracerMaker$^\text{TM}$ carbon-11 synthesis platform (Scansys Laboratorieteknik, Værløse, Denmark). Analytical HPLC was performed on a 1260 infinity II HPLC system (Agilent, Santa Clara, CA, USA) with a M177 $\gamma$-radiation detector (Ludlum Instruments, Sweetwater, TX, USA) connected in series after the UV detector.

Radiosynthesis of $[^{11}\text{C}]$ibrutinib

The reaction mixture was prepared in advance by weighing bis(dibenzylideneacetone)palladium(0) (2 mg, 3.5 $\mu$mol) and NiXantphos (2 mg, 3.5 $\mu$mol) in a capped vial under argon atmosphere, 20 min before EOB iodoethylene in THF was added (0.25% v/v, 600 $\mu$L) and vortexed for 2 min. Five minute before EOB, the iodoethylene/palladium mixture was added to a reaction vessel pre-charged with 3-(4-phenoxyphenyl)-1-(piperidin-3-yl)-2,3-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (3 mg, 7.5 $\mu$mol) under an argon atmosphere using a balloon.

Cyclotron produced non-carrier-added $^{11}$C$^2$O$_2$ was first trapped on a Hayesep D column (700 mg) cooled to $-180^\circ$C.
FIGURE 4 | Time activity curves (TACs) from 0 to 60 min associated with PET images $^{[11]C}$ibrutinib in mice with experimental autoimmune encephalomyelitis (EAE) scores 0.0 and 2.5. TACs were extracted from regions of interest (ROIs) in the (A) sacral, (B) lumbar, (C) thoracic, and (D) cervical regions of the spinal cord and in the (E) whole brain.

using liquid nitrogen on a TracerMaker™ synthesis platform. The Hayesep D column was first slowly heated to ambient temperature and further reduced online to $^{[11]C}$CO over heated (850°C) molybdenum powder (350 µm, GoodFellow, Huntingdon, UK) and concentrated on silica gel trap immersed in liquid nitrogen. After completed entrapment, the trap was heated to ambient temperature to release $^{[11]C}$CO and bubble through a closed reaction vessel (4 ml) containing the prepared reaction mixture. The reaction was then heated to 100°C, 3 min before being cooled to 50°C, and vented under helium flow (2 ml/min) for 1 min to evaporate excess THF. The mixture was redissolved in MeCN:H₂O (30:70, 3 ml) before being injected onto a semi-preparative HPLC column (Gemini C18 10 µm, 250 x 10 mm, Phenomenex, Torrance, CA, USA). $^{[11]C}$Ibrutinib was eluted with MeCN:ammonium acetate (45:55 v/v, 30 mM) at 6 ml/min ($t_R = 11–13$ min). Selected fraction was diluted with H₂O (15 ml) and pushed through a solid-phase extraction (SPE) cartridge (SepPak tC18, Waters, Milford, MA, USA). The SPE cartridge was washed with water (10 ml) before $^{[11]C}$Ibrutinib was eluted with ethanol (1 ml) and formulated with saline (0.9%, 9 ml). The overall synthesis time from EOB was 45–49 min.

RCP and molar activity were determined by analytical HPLC (Alltima C18 5 µm, 250 x 4.5 mm, HiChrom, Leicestershire, UK). $^{[11]C}$Ibrutinib was eluted with MeCN:ammonium formate (50:50 v/v, 50 mM) at 2 ml/min ($t_R = 7$ min). Identity was confirmed by co-injection with verified reference standard. $A_m$ was calculated by comparison with a reference
mice were anesthetized by isoflurane in O

For small animal PET combined with MR imaging studies, weeks of saline injection.

score of 2.5 (3T scanner (Mediso, Budapest, Hungary). Mice with an EAE TM catheterization and then transferred to a nanoScan −

the tail-vein catheter with \[ ^{11} \text{C} \]ibritinib (5.61–9.27 MBq, 151–250 \( \mu \text{Ci} \), 5.20–11.81 nmol/kg, 24–74 GBq/\( \mu \text{mol} \), 648–2,000 mCi/\( \mu \text{mol} \)). Anesthesia was maintained throughout PET/MR scanning, while body temperature and respiration parameters were monitored. A short gradient echo (GRE) scout MR was acquired for positioning the mouse in the PET field of view followed by a T1-weighted material map MR acquisition (GRE 3D, repetition time (TR) 25 ms, echo time (TE) 4.76 ms) for PET and MR co-registration and PET scatter and attenuation corrections. PET scans were initiated at the time of radioligand injection, and the list mode data were acquired for 60 min with an energy window of 400–600 keV. Following PET scanning, the animals were sacrificed by cervical dislocation, and the spinal cord was collected, weighed, and transferred to \( \gamma \)-counting tubes for biodistribution determination. Tissue radioactivity was measured with a \( \gamma \)-counter and expressed as %ID/g.

PET Data Analyses

List mode data were sorted into 33, 3D (3 × 5, 3 × 15, 3 × 20, 7 × 60, and 17 × 180 s) true sinograms (ring difference 84). 3D sinograms were converted in 2D sinograms using Fourier rebinning (24) with corrections for detector geometry, efficiencies, attenuation, and scatter, prior to image reconstruction using a 2D-filtered backprojection (FBKP) with a Hann filter at a cutoff of 0.50 cm\(^{-1}\). A static image of the complete emission acquisition was reconstructed with the manufacturer’s iterative 3D algorithm (six subsets, four iterations). The static iterative image was used for PET and MR co-registration and for presentation in figures. All data were corrected for dead-time and decay corrected to the start of acquisition. Dynamic FBKP images were used to extract TACs. Image analysis of the spinal cord (sacral, lumbar, thoracic, and cervical sections drawn manually) and brain (using the MR atlas of C57Bl/6J mice) (25) was performed using VivoQuant (4.0 patch1, Invicro LLC, Boston, MA, USA) and expressed as standardized uptake value (SUV), assuming tissue density of 1 g/ml.

Statistical Analysis

Data are represented as the mean ± SD. Statistical comparisons were performed by an unpaired \( t \)-test (\( p < 0.05 \)) with GraphPad Prism Version 8.3.1.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by CAMH Animal Care Committee.

AUTHOR CONTRIBUTIONS

AL, AB, DD, and NV designed the research. AL, AB, JT, MH, AG, TT, and DZ performed the research. AL, AB, JT, FL, DD, and NV analyzed the data. AL, AB, and NV wrote the paper. All authors reviewed the manuscript.

DEDICATION

This work is dedicated to the memory of TT, who passed away suddenly during the preparation of this manuscript. TT was a talented chemist who worked passionately on many PET ligand discovery projects. His enthusiasm for science will be missed within the field.

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Conflict of Interest: NV is a co-founder of MedChem Imaging, Inc. DD and TT are employed by Bristol-Myers Squibb Research and Development.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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