MICROPET IMAGING STUDIES OF THE INITIATION AND PROGRESSION OF A MURINE MODEL OF RHEUMATOID ARTHRITIS

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Keywords: microPET, copper-64, rheumatoid arthritis, antibody, glucose-6-phosphate isomerase

Introduction: The use of a dedicated small animal positron emission tomography (microPET) scanner has provided new insights into the mechanism and disease progression of a murine model of rheumatoid arthritis. The K/BxN murine model of rheumatoid arthritis (RA) shares many of the pathologic characteristics of human RA. K/BxN mice spontaneously develop joint inflammation by 4-5 weeks of age, and this disease can be transferred into naïve mice by injection of K/BxN serum or purified autoantibodies specific for the glycolytic enzyme glucose-6-phosphate isomerase (GPI). These features have provided a mechanism for studying the initiation and progression of the disease though *in vivo* PET imaging (1).

Previously published result: The ubiquitous expression of GPI and the systemic circulation of GPI-specific antibody seem incongruous with the tissue specificity of this disease. The use of *in vivo* imaging with the microPET enables the tracking and quantitation of Cu-64 labeled GPI-specific polyclonal antibody during the initiation of joint inflammation in naïve mice. Through the use of both dynamic and static imaging, we have shown that polyclonal GPI-specific antibody specifically localized to distal joints in the front and rear limbs within minutes of intravenous injection, reached saturation by 20 minutes, and remained localized for at least 24 hours; in addition to causing inflammation of these same joints (2). In contrast, control antibodies did not localize to the joints or cause inflammation in naïve mice.

Result and discussion: In order to elucidate the triggering events of the observed localization, we have used PET imaging and classical biodistributions to examine the roles of Fc RI, Fc RIII, C5, mast cells and neutrophils. Dynamic imaging experiments were utilized in these studies; radiolabeled antibody is first injected via catheter and the animals scanned for 15 min, at which point K/BxN serum from diseased animals was injected and the scan continued for an additional thirty minutes. This study protocol has been found to be extremely reproducible, and the results of ROI analysis compare favorably with those from classical biodistribution. The crucial components found in these studies were Fc RIII , neutrophils, and mast cells. The results of these studies have led to a proposed mechanism wherein the formation of antibody – antigen immune complexes trigger the initial antibody localization both anti-GPI and control, followed by subsequent inflammation in the distal joints.

Conclusions: We have found that the antibody localization can be triggered through the mediation of irrelevant immune complexes, modification of our dynamic imaging protocol by replacement of the K/BxN serum with a preformed immune complex (mouse peroxidase – antiperoxidase, mPAP) led to identical results. These studies have shown the benefits of *in vivo* PET imaging in addressing immunological questions. Our imaging protocols have been found to be extremely reproducible with the results of ROI analysis comparing favorably to those from classical biodistribution while requiring far fewer animals which is beneficial when dealing with transgenic or gene-disrupted animals.

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EVALUATION OF CU-64-DOTA-CTT, A MMP-9 INHIBITOR, AS A POTENTIAL PET LUNG INFLAMMATION IMAGING AGENT

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Keywords: matrix metalloproteinases (MMPs), copper-64, inflammation imaging, PET

Introduction: MMP-9 (gelatinase B, 92 kD type IV collagenase) is one of a family of matrix metalloproteinases (MMP) enzymes that can degrade many extracellular matrix proteins, including basement membrane components such as type IV collagen. Overexpression of MMPs have been implicated in the tissue destruction associated with various inflammatory lung diseases such as chronic obstructive pulmonary disease. Likewise, the inhibition of MMP-9 has been reported to reduce lung injury in animal models. Thus, a method that could be used to monitor lung inflammation non-invasively by quantifying MMP-9 tissue concentrations could be useful both experimentally and clinically. **Methods:** A cyclic peptide, CTTHWGFTLC (CTT), which has been reported to be a selective inhibitor for MMP-2 and MMP-9 (1), was synthesized, and the N-terminus of CTT was conjugated with the chelator DOTA (1,4,7,10-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid) for labeling with Cu-64 [$T_{1/2}$ = 12.7 h; 39% beta minus; 17.4% positron]. A murine model of pulmonary infection and inflammation was established by embedding *Pseudomonas aeruginosa* organisms in agarose beads and instilling $6x10^4$ cfu/mouse into the right lung of C57BL/6 mice. Three days post-administration of the beads, ^{64}Cu-DOTA-CTT was administered i.v. An hour later, the lungs were retrieved, and radioactivity in each lung was separately determined. In addition, the lungs were analysed by gelatin zymography to quantify MMP-9 activity. **Results:** The uptake of ^{64}Cu-DOTA-CTT in the right lung in infected mice (5.97 1.00 %ID/g) was significantly higher than in the left uninfected lung (4.3 0.78 %ID/g) or in the lungs of normal mice (right: 4.01 0.70 %ID/g, left: 3.99 0.47 %ID/g). MMP-9 activity by gelatin zymography (in both proMMP-9 and active MMP-9 forms) was 3-4 times higher in the right lung of infected mice than in right lung of normal mice, demonstrating a correlation between higher lung uptake of ^{64}Cu-DOTA-CTT and increased MMP-9. **Conclusions:** MMP-9 concentrations are increased in a murine model of pulmonary infection and inflammation, assayed by both the uptake of ^{64}Cu-DOTA-CTT and zymography. PET imaging using ^{64}Cu-DOTA-CTT might be a viable option for imaging lung inflammation.

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EVALUATION OF A RADIOLABELLED PBR LIGAND IN AN ANIMAL MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS: A PROBE FOR IMAGING "MULTIPLE SCLEROSIS"

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Key words: Multiple Sclerosis, PBR, Imidazopyridines, Experimental autoimmune encephalomyelitis, SPECT

The Peripheral Benzodiazepine Receptor (PBR), is over-expressed in neoplastic tissues, in the central nervous system in a number of neurodegenerative disorders and in patients with multiple sclerosis (MS). Recently it has also been shown that the PBR density increased in a model of experimental autoimmune encephalomyelitis (EAE) in rats. EAE is an inflammatory, demyelinating disease of the central nervous system that is used extensively as a model of the human demyelinating disease MS. A specific radiopharmaceutical incorporating either PET or SPECT radionuclides may therefore have clinical potential as an imaging agent for MS.

The aim of this study was to evaluate the iodine-123-labelled imidazopyridine: N,N-diethyl-6-chloro-(4'-iodophenyl)imidazo(1,2-a)pyridine-3-acetamide ([^{123}I]-CLINDE), as a potent radioligand for the PBRs in EAE. CLINDE is a high affinity (IC_{50} = 1.7 nM) ligand for the PBR whilst it exhibits significantly lower binding to the central benzodiazepine receptors (IC_{50} = 450 nM). [^{123}I]-CLINDE has been prepared by classical iododestannylation reactions with peracetic acid in > 80% radiochemical yield and >98% radiochemical purity.

Groups of male Lewis rats were injected in the footpad of both hind feet with an emulsion of myelin basic protein (50 g) and complete Freund's adjuvant (100 l per foot). Eleven days after the induction the animals were divided into groups according to the severity of the disease and a score from 0 (no clinical sighns) to 5 (hindlimb paralysis) was assigned. The animals were injected with [^{123}I]-CLINDE (0.6 MBq in 100 l) and the time course distribution of the tracer in the spinal cord, brain areas and other tissues was evaluated. Histopathological assessment of disease was also performed.

Preliminary results indicate an enhanced uptake of [^{123}I]-CLINDE activity in all animals induced with EAE compared to controls. The enhanced uptake reflected the ascending nature of the inflammatory lesions ie. uptake in the lumbar spinal cord > thoracic cord > cervical cord > medulla > cerebellum. In addition, the 3 h uptake of [^{123}I]-CLINDE in the lumbar and thoracic spinal cord correlated with disease severity. Typically a 2 fold enhancement in PBR expression was observed in a clinical score of 0 (no clinical signs but with lesions in the cord) up to a 7 fold enhancement in a clinical score of 5. A similar 2 to 5 fold enhancement in PBR expression was observed in the medulla and cerebellum of rats that scored 3 and above in disease progress. After 24 h, the uptake of [^{123}I]-CLINDE in the spinal cord, medulla and cerebellum increased by 10 and 6 times, respectively, in the EAE rats compared to the control animals. Moreover, a high spinal cord to blood ratio in all the EAE animals was observed.

The above results clearly demonstrate the ability of [^{123}I]-CLINDE to measure *in vivo* changes of PBR density according to area of involvement and the severity of disease. This suggests that [^{123}I]-CLINDE warrants further investigation as a potential SPECT marker for the study of inflammation and multiple sclerosis.

[^{11}C]LOPERAMIDE AS HIGHLY SENSITIVE PET PROBE FOR MEASURING CHANGES IN P-GLYCOPROTEIN FUNCTIONALITY

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Keywords: loperamide, PET, P-glycoprotein, brain

Introduction: P-glycoprotein, an ATP-driven transmembrane efflux pump, is known to play a major role in multidrug resistance leading to therapy failure in *e.g.* oncology and antiretroviral treatment. Loperamide, an opiod receptor agonist, has been shown to be a good substrate for P-glycoprotein (P-gp) with 13.5-fold higher concentration in the brain of mdr1(-/-) knock-out versus wild type mice. The aim of the work described below was to assess the utility of [^{11}C]loperamide as a PET probe for measuring changes in P-gp functionality.

Methods: [^{11}C]loperamide was prepared by reacting 1mg desmethyl-loperamide with [^{11}C]MeI in 300µL DMSO using 2 mg KOH as a base for 5 min at 80°C (1.04±0.9GBq, 32±19GBq/µmol, n=8). Ketamine induced isoflurane aneasthetised pigs (n=2, yorkshire landrace, 37.5kg) received an arterial cannula in one of the femoral arteries and a venous cannula in the contralateral femoral vein before being placed in the PET camera (Siemens ECAT HR). Both animals received 4 consecutive doses of [^{11}C]loperamide, 1 scan at baseline conditions and 3 additional scans following iv. administration of increasing doses of the known competative P-gp substrate cyclosporin A (CsA), 20-30 min prior to administration of [^{11}C]loperamide; pig1: 1, 10, 30 mg/kg CsA, pig2: 5, 15, 30mg/kg CsA. A [^{15}O]CO and a [^{15}O]H_2O scan were performed prior to each [^{11}C]loperamide scan to correct for changes in blood volume and to measure changes in blood flow.

Results: Under baseline conditions, little uptake (<1% ID/L) was observed in CNS. Pretreatment with CsA led to a dose dependent increase in [^{11}C] derived radioactivity signal in the CNS as depicted in Figure 1. Whole brain CNS levels increased by a maximum of 7-fold compared to baseline conditions, with regional increases as high as 9-fold. Rate of metabolism was slightly decreased at doses of CsA>10mg/kg (from 60% parent to ~80% parent at 60 min post admin). Clearance of [^{11}C]loperamide from plasma was also slightly reduced at doses of CsA>10mg/kg, leading to increased plasma concentrations at later timepoints.

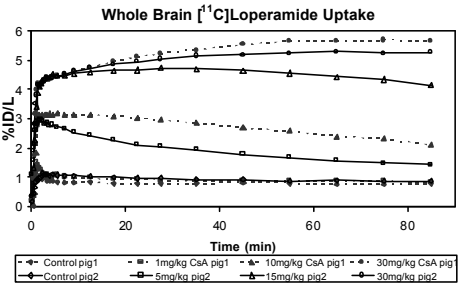

Figure 1: Whole brain time-activity curves, corrected for cerebral blood volume

Conclusion/Discussion: [^{11}C]Loperamide appears to be a very promising PET probe for measuring changes in P-gp functionality *in vivo*. The large difference between baseline and ~100% P-gp inhibition indicates that [^{11}C]loperamide could be used as a research tool to investigate the effect of small (15%) changes in P-gp functionality on pharmacology (*e.g.* drug-drug interactions, disease therapy). Further studies are planned in healthy volunteers and in specific patient populations to assess the utility and safety of this novel PET probe in man.

SYNTHESIS AND EVALUATION OF [^{18}F]-DESBROMO-DUP-697 AS PET TRACER FOR CYCLOOXYGENASE-2 EXPRESSION

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Keywords: Cyclooxygenase-2, Positron emission tomography, Inflammation, Brain, Fluorine-18.

Cyclooxygenase (COX) is an important enzyme in the biosynthesis of prostaglandins and thromboxanes. Expression of COX-2, the inducible isoform of this enzyme, is increased in response to inflammatory stimuli. Upregulation of COX-2 expression has also been observed in, for example, cerebral and cardiac ischemia, Alzheimer's disease and in a variety of tumors, where it was associated with disease progression and poor prognosis. To elucidate the role of COX-2 and for diagnostic purposes, a non-invasive imaging technique to assay COX-2 expression can be of great help. Here we report the synthesis and preliminary evaluation of [^{18}F]desbromo-DuP-697 ([^{18}F]-**2**) as a PET tracer for COX-2 expression.

As depicted in Figure 1, the selective COX-2 inhibitor [^{18}F]-**2** was prepared via a nucleophilic aromatic substitution reaction on nitro precursor **1** with the K[^{18}F]F/kryptofix complex (DMF, 160 ^{o}C, 15 min). After purification by normal phase HPLC, radiochemically pure (>99%, HPLC) [^{18}F]-**2** with a specific activity of 21-47 GBq/μmol was obtained in 2-6% decay corrected yield.

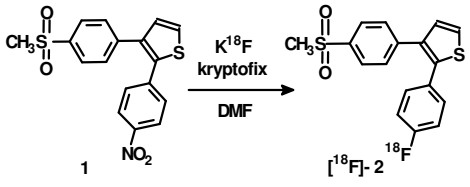

Figure 1: *Labeling of COX2 inhibitor [^{18}F]-**2**.*

Biodistribution studies of [^{18}F]-**2** were carried out in Sprague-Dawley rats, in which a sterile inflammation was induced by injection of carrageenan in a hind paw. To study the distribution of specific binding, either the COX-2 selective inhibitor NS-398 (1.5 mg/kg) or the non-selective COX inhibitor indomethacin (1.5 mg/kg) was injected in the tail vein 5 min prior to the tracer. The animals were sacrificed after 120 min of tracer distribution.

Tracer uptake was significantly reduced by both NS-398 and indomethacin in heart (SUV: 0.34±0.15, block: 60% and 61%), kidney (SUV: 0.51±0.11, block: 46% and 52%), brain (SUV: 0.18±0.03, block: 40% and 40%) and blood cells (SUV: 0.077±0.003, block: 16% and 57%). These tissues were all reported to express high levels of COX-2 (1,2). Largest decrease in tracer uptake after pretreatment with NS-398 or indomethacin was observed in lung (SUV: 0.66±0.47, block: 71% and 75%), but this decrease was not statistically significant due to the wide variation in the tracer uptake in lungs of control animals. This is in agreement with literature data showing wide inter-individual variation in COX-2 expression in rat lung (2). High non-specific tracer uptake was found in fat and intestine. Surprisingly, uptake of [^{18}F]-**2** in the inflamed paw did not significantly differ from tracer uptake in the control paw. This was ascribed to insufficient induction of COX-2 expression in the inflamed paw, which was confirmed by measurement of COX peroxidase enzyme activity in the inflamed and in the control paw. Autoradiography studies showed regional differences in tracer distribution in the brain, with high uptake in various cortical regions.

Taken together, these results indicate that [^{18}F]-**2** could be suitable as a PET tracer especially for imaging of COX-2 expression in the brain.

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SYNTHESIS OF [^{18}F]FLUOROQUINOLONE ANTIBIOTICS FOR HUMAN PET STUDIES

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Keywords: PET, [^{18}F]Fluoroquinolone, Ciprofloxacin, Norfloxacin, Pefloxacin

Fluoroquinolones are a widely prescribed class of antibiotic agents with a broad spectrum of antibacterial activity. Labeling of fluoroquinolones with fluorine-18 (^{18}F) is of interest for the performance of pharmacokinetic measurements and the visualization of bacterial infections in humans with positron emission tomography. Previously synthesized ^{18}F-labeled fluoroquinolones (lomefloxacin, trovafloxacin) were obtained by a direct isotopic ^{18}F for ^{19}F exchange reaction on the unprotected target molecules themselves (1,2). In our hands the ^{18}F-labeled fluoroquinolones [^{18}F]ciprofloxacin (1-cyclopropyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid, [^{18}F]-**1**), [^{18}F]norfloxacin (1-ethyl-6-[^{18}F]fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic acid, [^{18}F]-**2**) and [^{18}F]pefloxacin (1-ethyl-6-[^{18}F]fluoro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-quinoline-3-carboxylic acid, [^{18}F]-**3**) could not be synthesized by such a direct exchange reaction.

In order to circumvent the lack of chemical activation of compounds **1, 2** and **3** for a direct exchange reaction a novel two-step, one-pot radiosynthetic approach towards [^{18}F]-**1,** [^{18}F]-**2** and [^{18}F]-**3** was developed. A 6-fluoro-7-chloro-substituted precursor molecule (i.e. 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**4**) for [^{18}F]-**1** and 7-chloro-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5**) for [^{18}F]-**2** and [^{18}F]-**3**) was first reacted with K[^{18}F]F-K_{222} (40 min, 180°C) to obtain the radiolabeled intermediates [^{18}F]-**4** and [^{18}F]-**5** in yields of 20-30%. In the second step these intermediates were coupled in 7-position with the amines piperazine (for [^{18}F]-**1** and [^{18}F]-**2**) or 1-methylpiperazine (for [^{18}F]-**3**). In order to enhance the reactivity of [^{18}F]-**4** and [^{18}F]-**5** for the coupling reaction they were in situ converted into their respective boron complexes by addition of trimethylborate prior to addition of the amine (3). After 40 min heating at 180°C the [^{18}F]fluoroquinolones [^{18}F]-**1**, [^{18}F]-**2** and [^{18}F]-**3** were obtained in yields of 50-70% based on [^{18}F]-**4** and [^{18}F]-**5**. Crude reaction mixtures were purified by semipreparative reversed-phase HPLC followed by on-line solid-phase extraction with a strong cation exchange cartridge for removal of HPLC solvent. By this approach [^{18}F]-**1**, [^{18}F]-**2** and [^{18}F]-**3**, readily formulated for intravenous injection, could be obtained in overall radiochemical yields of 3-4% in a total synthesis time of 130 min. Co-injection on analytical HPLC with the unlabeled reference molecules confirmed the identity of the radiolabeled compounds. The chemical purity was better than 96% and the radiochemical purity exceeded 99%. Since pharmacokinetic measurements involve co-administration of the unlabeled molecules the obtained specific radioactivity (400-900 MBq/µmol at the end of synthesis) was sufficiently high for the prospective applications of these compounds.

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SYNTHESIS, MICROPET IMAGING, BIODISTRIBUTION AND DOSIMETRY DATA OF D- AND L-3[C-11]LACTIC ACID

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Keywords: lactic acid, PET, [C-11]methyl iodide, microPET, biodistribution.

Introduction: In order to assess the metabolic fate of lactic acid in the human heart and also, to correlate lactate transport activity during functional brain activation in humans, we synthesized both D- and L-lactic acid, labeled with carbon-11 in the carbon-3 position. We determined the biodistribution of both tracers in rats by microPET and calculated the dosimetry for human use.

Radiochemical Synthesis: The tracers were synthesized in a modified two-step enzymatic synthesis previously described (1). Our simplified procedure used 3 mg of N-(diphenylmethylene)glycine t-butyl ester dissolved in 200 µl of acetonitrile:dimethylformamide (3:1 v/v) and 4 µl of 5N KOH to trap the [C-11]methyl iodide (2). After 4 minutes heating at 85 ºC, the reaction was mixed with 350 µl of 6N HCl diluted with acetonitrile and transferred to a conical flask to be hydrolized. The dry contents were redissolved with Tris buffer and after pre-mixing the two enzymes systems and cofactors were added: α-ketoglutarate, pyridoxal-5-phosphate, flavin-adenine-dinucleotide, catalase, glutamic-pyruvic-transaminase, D-amino-acid-oxidase and either D- or L- lactic dehydrogenase with β-nicotinamide-adenine-dinucleotide reduced form. The mixture was incubated for 5-6 minutes at 50 ºC. The enzymes were heat-denatured and removed by passing the mixture through a Oasis® HLB (Hydrophilic-Lipophilic Balance) solid phase purification system (Waters Inc). The final product was filtered through a 0.22 µ filter to render a sterile and pyrogen-free, clear and colorless solution ready for injection. The total radiochemical yield at EOS was 30-45% We have consistently obtained 555-1,480 MBq (15-40 mCi) product with a radiochemical purity of 95% or better, in a total synthesis time of 50 minutes that includes 12 minutes preparation of [C-11]methyl iodide. The product was found to be enzyme free and contained less than 5 pmm total of non-radioactive lactic acid, in a 5-mL injectate. The enantiomeric purity was determined on a specialty column Chirex D-penicillamine (Phenomenex) and found to be higher than 98%. We have developed an efficient remote system for the routine production of D- and L-3[C-11]lactic acid.

Biodistribution and Radiation Dosimetry Studies: Dosimetry calculations were performed for the D and L isomers using biodistribution in Sprague Dawley rats. Rats receiving either D or L-3[C-11]lactic acid were imaged on a microPET R4 scanner, (Concorde Microsystems Inc) prior to dissection. It was observed that the distribution of both isomers was fairly uniform throughout the muscle of the body. The primary and secondary critical organs were the urinary bladder and the kidneys, having absorbed doses of 36 and 33 mrem/mCi respectively. The heart wall absorbed dose was 29 mrem/mCi.

Conclusion: D and L-3[C-11]lactic acid have been synthesized with an acceptable yield and radiopharmaceutical quality appropriate for human use. Washington University Radioactive Drug Research Committee (RDRC) approval has recently been obtained.

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[^{11}C]PJ34: A PET RADIOTRACER FOR IMAGING THE ROLE OF PARP-1 IN NECROSIS

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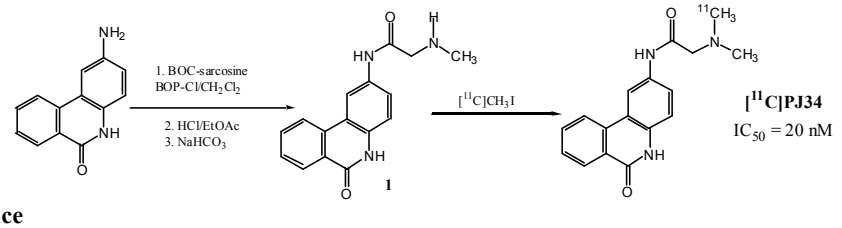
Keywords: PET, necrosis, PARP

Poly(ADP-ribose) polymerase-1 (PARP-1) is an abundant nuclear enzyme of eukaryotic cells that has been implicated in response to DNA injury. PARP-1 detects single strand DNA breaks induced by a variety of genotoxic insults. Upon binding to DNA strand breaks, PARP-1 catalyzes the transfer of ADP ribose units from NAD^+ to a variety of nuclear proteins, including DNA polymerase and histones. The physiological function of PARP-1 is not completely understood, but it is currently believed that PARP-1 plays a key role in DNA repair, cell differentiation, DNA replication, and control of the cell cycle. However, the role of increased PARP-1 activation in a variety of pathological conditions has been well established. In conditions of severe DNA injury, overactivation of PARP-1 leads to the rapid depletion of intracellular NAD^+ and ATP pools, resulting in cellular dysfunction and death. The overactivation of PARP-1 levels and depletion of NAD^+ pools is currently believed to be the principal pathway responsible for cellular death via necrosis. Therefore, PARP-1 is thought to play a major role in a variety of pathological conditions, including ischemia-reperfusion injury (i.e., myocardial infarction and stroke), septic shock, diabetic cardiomyopathy, and neurodegeneration. The measurement of PARP-1 levels is currently limited to the use of immunohistochemistry techniques in tissue slices. The development of a radiotracer that could image PARP-1 levels with PET would provide a useful tool in studying the role of this enzyme in a variety of pathological conditions.

Previous studies have shown that the phenanthridinone derivative, PJ34, has a high affinity for PARP-1 (1). The presence of the dimethylaminoacetamido group of PJ34 indicates that it is possible to prepare the ^{11}C-labeled compound via N-alkylation of the des-methyl precursor, **1**, with [^{11}C]methyl iodide. The synthesis of **1** was achieved via condensation of 2-amino-6(5H)-phenanthridone (1) with Boc-sarcosine under standard coupling conditions (Scheme 1). Deprotection under acidic conditions followed by neutralization afforded **1** in high yield. Treatment of **1** with [^{11}C]methyliodide afforded [^{11}C]PJ34 in 60% yield and a specific activity of ~2,000 mCi/ mol (decay corrected to E.O.B.). In vivo biodistribution studies in male Sprague-Dawley rats revealed a rapid clearance of activity from tissue (e.g., heart and lung), which is consistent with a low expression of PARP-1 in normal tissue. However, a microPET imaging study in a rat model of myocardial ischemia-reperfusion injury showed a higher uptake of radioactivity in heart relative to that in the lungs. These data suggest that [^{11}C]PJ34 may be useful for imaging elevated PARP-1 levels in necrosis.

Scheme 1



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