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Preclinical PET Neuroimaging of [^{11}C]Bexarotene

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Abstract

Activation of retinoid X receptors (RXRs) has been proposed as a therapeutic mechanism for the treatment of neurodegeneration, including Alzheimer's and Parkinson's diseases. We previously reported radiolabeling of a Food and Drug Administration-approved RXR agonist, bexarotene, by copper-mediated [^{11}C]CO_2 fixation and preliminary positron emission tomography (PET) neuroimaging that demonstrated brain permeability in nonhuman primate with regional binding distribution consistent with RXRs. In this study, the brain uptake and saturability of [^{11}C]bexarotene were studied in rats and nonhuman primates by PET imaging under baseline and greater target occupancy conditions. [^{11}C]Bexarotene displays a high proportion of nonsaturable uptake in the brain and is unsuitable for RXR occupancy measurements in the central nervous system.

Keywords

bexarotene, carbon-11, positron emission tomography, retinoid X receptor, neuroimaging

Introduction

Bexarotene is a potent retinoid X receptor (RXR) agonist that has shown potential for treating Parkinson's disease (PD), possibly through its ability to activate RXR-Nurr1 heterodimers,[1] and been demonstrated to induce β-amyloid clearance from a murine model of Alzheimer's disease (AD).[2] However, as bexarotene was developed to treat dermatological diseases and certain cancers such as cutaneous T-cell lymphoma (CTCL),[3] there is scant pharmacokinetic and pharmacodynamic data describing bexarotene in the central nervous system (CNS). Recently, bexarotene was shown to reduce brain amyloid in ApoE4 noncarriers diagnosed with AD, providing further evidence that bexarotene is indeed CNS active.[4] However, in that study, only a relatively high dose of bexarotene was tested, and significant elevations in triglycerides were observed in all patients treated. Furthermore, the Food and Drug Administration-approved bexarotene dosage to treat CTCL causes side effects and may presents tolerability problems in patients with PD and AD.[5,6]

Bexarotene may have neuroprotective effects at doses that are substantially lower than effective doses for treating cancer,[1] and it has been shown that "low-dose" bexarotene is well tolerated clinically and likely has CNS activity.[7,8] Low-dose treatment rescued dopamine (DA) neurons and restored behavioral function in 6-hydroxydopamine (OHDA)–lesioned rats.[1] The brain-to-plasma ratio of bexarotene in rats was approximately 1:1. The minimum effective steady-state brain concentration for the protection of DA neurons in 6-OHDA-lesioned rats was approximately 40 nmol/L, whereas the peak brain concentration of bexarotene at the minimum effective oral dose of 1 mg/kg/d was approximately 400 nmol/L. Thus, it remains unclear what dose of bexarotene would provide the optimal ratio of CNS activity–related side effects arising from peripheral exposure.

To resolve uncertainties about dose selection of bexarotene and other Nurr1-RXR agonists for patients with PD, we sought

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to evaluate the potential for measuring occupancy of midbrain RXRs by positron emission tomography (PET) with [^{11}C]bexarotene. Our preliminary work established both the feasibility of chemical synthesis of [^{11}C]bexarotene and its utility for imaging the midbrain in a nonhuman primate (NHP) in vivo using PET.[9] In addition to [^{11}C]bexarotene, retinoid A receptor ligands have been radiolabeled with carbon-11,[10,11] and more recently, a fluorine-18-labeled bexarotene analog has been reported.[12] However, to date, there are no reports exploring the specificity of these ligands for molecular imaging. Bexarotene displays comparable affinities toward RXRα, β, and γ (14, 21, and 29 nmol/L, respectively)[3] and may have greater affinity toward RXR heterodimers, such as Nurr1-RXR.[1] Retinoid X receptor subtypes are expressed differentially in mammalian organs[13] and brain regions,[14] with each exhibiting specific patterns of localization. Estimates of B_{max} in the brain have not been made, although values ranging from 0.346 to 0.567 pmol/mg in rat liver nuclear proteins have recently been reported.[15] An in vivo probe for CNS RXRs would serve as a powerful tool in drug development in the context of neurodegenerative diseases. In this brief report, we evaluate the potential of [^{11}C]bexarotene for neuroimaging of RXRs in rats and NHPs by in vivo PET imaging.

Materials and Methods

All treatment and imaging experiments were conducted according to procedures approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital. [^{11}C]Bexarotene was synthesized from [^{11}C]CO_2, as previously described[9] with minor modifications. A total of 9 radiosyntheses were completed for this study with radiochemical purity ≥98% and specific activities of 55 ± 5 mCi/μmol measured at end-of-synthesis. The range of isolated yields was 5.6 to 36.9 mCi.

Plasma Protein Binding

An aliquot of [^{11}C]bexarotene formulated in 8% ethanolic saline with 4% Tween 80 (~100 μL, 100 μCi) was added to a sample of baboon plasma (500 μL, freshly prepared by centrifugation of freshly drawn blood), diluted with water (100 μL). The mixture was gently combined by repeated inversion and incubated for 10 minutes at room temperature. Aliquots (3, 20 μL each) were withdrawn into test tubes to determine the total radioactivity in the plasma (A_T). An additional 300 μL of the incubated plasma sample was withdrawn and placed into the upper compartment of a Centrifree ultrafiltration device (Amicon, Inc, Beverly, Massachusetts). The device was centrifuged for 10 minutes, after which the upper compartment was removed and discarded. Aliquots (3, 20 μL each) from the collection cup were withdrawn into test tubes to determine the fraction of radioactivity that passed through the membrane ($A_{unbound}$). All A_T and $A_{unbound}$ test tubes were counted sequentially in a well counter with decay correction. Empty test tubes served as background samples. The above procedure was performed in duplicate. Plasma protein binding is reported as the bound fraction of radioactivity ($A_{bound}/A_T = 1 - A_{unbound}/A_T$).

Positron Emission Tomography Imaging

Rodents

Male Sprague Dawley rats ($n = 4$, 440-500 g) were anesthetized by inhalation of 3% isoflurane in a carrier of 1.5 L/min medical oxygen and maintained at 2% isoflurane for the duration of the scan. The rats were arranged in quadrants of a Siemens P4 small animal PET scanner (Siemens Preclinical Solutions, Knoxville, TN). Nonradioactive bexarotene (1 mg/kg, $n = 2$) or vehicle (10% dimethyl sulfoxide, 4% Tween 80, saline, 440-500 μL, $n = 2$) was injected via a lateral tail vein catheter 5 minutes prior to radiotracer administration ([^{11}C]bexarotene, 220-240 μCi, 310-350 μL) by the same route. Dynamic PET acquisition lasted for 60 minutes from the time of radiotracer injection, followed by a transmission scan with a ^{57}Co line source to generate an attenuation map, which was applied during image reconstruction. The dynamic PET data were binned into 32 time frames (8 × 15 seconds, 8 × 60 seconds, 10 × 120 seconds, and 6 × 300 seconds) and reconstruction of each frame via an iterative maximum likelihood expectation maximization algorithm, consisting of 16 iterations, afforded images with a resolution of approximately 2-mm full width at half-maximum. Images were analyzed using AMIDE 1.0.4 software (http://amide.sourceforge.net). Whole-brain volumes of interest (VOIs) were drawn over each image to derive time–activity curves.

Nonhuman Primates

Positron emission tomography/magnetic resonance imaging (MRI) acquisition was performed on a 3-T Siemens TIM-Trio with a BrainPET insert (Siemens, Erlangen, Germany). A PET/MRI-compatible 8-channel array coil customized for NHP brain imaging to increase signal and quality was used. Two baboons (15.1-16.4 kg) were used for baseline and nonradioactive bexarotene coadministration (0.35-0.42 mg/kg, intravenously [IV]; high occupancy) imaging conditions. During administration of [^{11}C]bexarotene (3.8-5.0 mCi), dynamic PET image acquisition was initiated and data were collected and stored in list mode for 90 minutes. Positron emission tomography data were binned into 26 time frames (6 × 10 seconds, 6 × 20 seconds, 2 × 30 seconds, 1 × 60 seconds, 5 × 300 seconds, and 6 × 600 seconds). Image reconstruction was performed using the 3-dimensional ordinary Poisson expectation maximization algorithm with detector efficiency, decay, dead time, attenuation, and scatter corrections. Image volumes were eventually reconstructed into 76 slices with 128 × 128 pixels and a 2.5-mm isotropic voxel size. Thirty minutes after scanner start, a high-resolution anatomical scan using multiecho MPRAGE sequence (repetition time [TR] = 2530 milliseconds, echo time [TE]1/TE2/TE3/TE4 = 1.64/3.5/5.36/7.22 milliseconds, T1 = 1200 milliseconds, flip angle = 7°, and 1 mm isotropic) was acquired. Image analysis was conducted

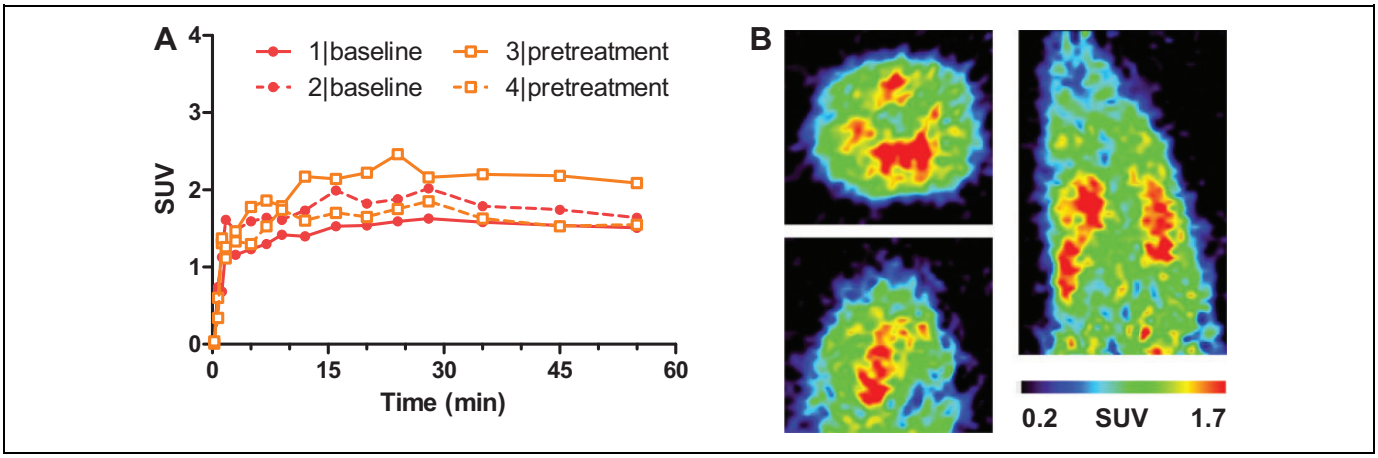

Figure 1. A, Whole-brain time-activity curves of [^{11}C]bexarotene in rat at baseline ($n = 2$) and following pretreatment (bexarotene 1.0 mg/kg, IV, 5 minutes prior to TOI, $n = 2$). B, Summed PET images at baseline 2 to 60 minutes; clockwise from top left: transverse, sagittal, and coronal slices. IV indicates intravenously; PET, positron emission tomography; TOI, time of injection.

using the PMOD 3.3 (PMOD Technologies, Zurich, Switzerland) software package. Six VOIs were drawn including whole brain, cerebellum, frontal cortex, prefrontal cortex, thalamus, and caudate to derive time–activity curves.

Results

[^{11}C]Bexarotene was prepared by copper-mediated [^{11}C]CO_2 fixation from a pinacol ester boronate precursor,[16,17] as described previously.[9] The average injected dose of [^{11}C]bexarotene was 232 $\pm$ 8 μCi (3.0 $\pm$ 1.0 μg/kg) for rats and 4.27 $\pm$ 0.51 mCi (1.8 $\pm$ 0.2 μg/kg) for primates.

Whole-brain radioactivity uptake was determined in rats at baseline and with homologous pretreatment to evaluate binding saturability. Whole-brain activity peaked within the first 2 minutes after injection of [^{11}C]bexarotene to rats at baseline and then reached ~2 standardized uptake value (SUV) with little further clearance over the duration of the 60-minute scan (Figure 1). Pretreatment with nonradioactive bexarotene (1 mg/kg, IV, 5 minutes prior to the time of injection [TOI]) effected no measurable change in whole-brain radioactivity uptake.

To assess regional uptake specificity in various brain structures and in order to mitigate the possible confound of species differences, PET neuroimaging with [^{11}C]bexarotene was further evaluated in NHPs. Our preliminary study demonstrated moderate brain uptake of [^{11}C]bexarotene at baseline with regional heterogeneity.[9] In the current study, we confirmed [^{11}C]bexarotene brain uptake and distribution at baseline ($n = 2$) and also conducted imaging with coadministration of nonradioactive drug ($n = 2$) to observe changes that would indicate saturable binding. As in our preliminary study, whole-brain uptake peaked at ~0.8 SUV, 8 to 12 minutes after TOI (Figure 2). A similar pattern of distribution throughout the brain was observed, with relatively high uptake notably in the cerebellum, frontal cortex, prefrontal cortex, thalamus, and caudate. In vitro plasma protein binding was determined from a freshly drawn blood sample, and [^{11}C]bexarotene was found to be highly protein bound (>99%), in line with previous reports in other species.[18] Drug coadministration studies showed no measurable change in whole-brain or regional uptake of [^{11}C]bexarotene over the duration of the 90-minute scans. No differences in regional heterogeneity were observed between baseline and coadministration scan images.

Discussion

Neuroreceptor imaging by PET requires radiotracers that engage in specific and saturable binding to the target of interest. In vivo target saturability can be detected by pretreatment or coadministration with nonradioactive homologous compound,[19] in this case bexarotene. The lack of observable saturation of [^{11}C]bexarotene uptake by PET imaging does not indicate an absence of specific interactions with targets such as RXR, but rather a high proportion of nonsaturable uptake, possibly including interactions with lipid membranes in the CNS.[20] Evidence also suggests that bexarotene may engage in specific interactions with multiple high-expression targets in addition to RXR hetero- and homodimers,[21-23] which could contribute to the observed sustained uptake under drug coadministration conditions.

The high lipophilicity of RXR ligands presents a challenge for the development of molecular imaging agents that demonstrate highly specific uptake in vivo. The measured log$D$ of [^{11}C]bexarotene (3.68) falls somewhat outside the typical range for successful PET neuroimaging agents that cross the blood–brain barrier (0.9-2.5).[24,25] Despite a higher than ideal log$D$ value and nearly total plasma protein binding, [^{11}C]bexarotene shows reasonable brain uptake in both rat and NHP. Due to the highly lipophilic binding site, and apparent promiscuity of known RXR ligands, development of a suitable radiotracer for neuroimaging may not be a practical goal at this time. Future efforts to develop molecular imaging probes for RXR will need to prioritize less lipophilic compounds to improve on brain extraction fraction and binding specificity in addition to the target selectivity. As interests in RXR-mediated mechanisms for

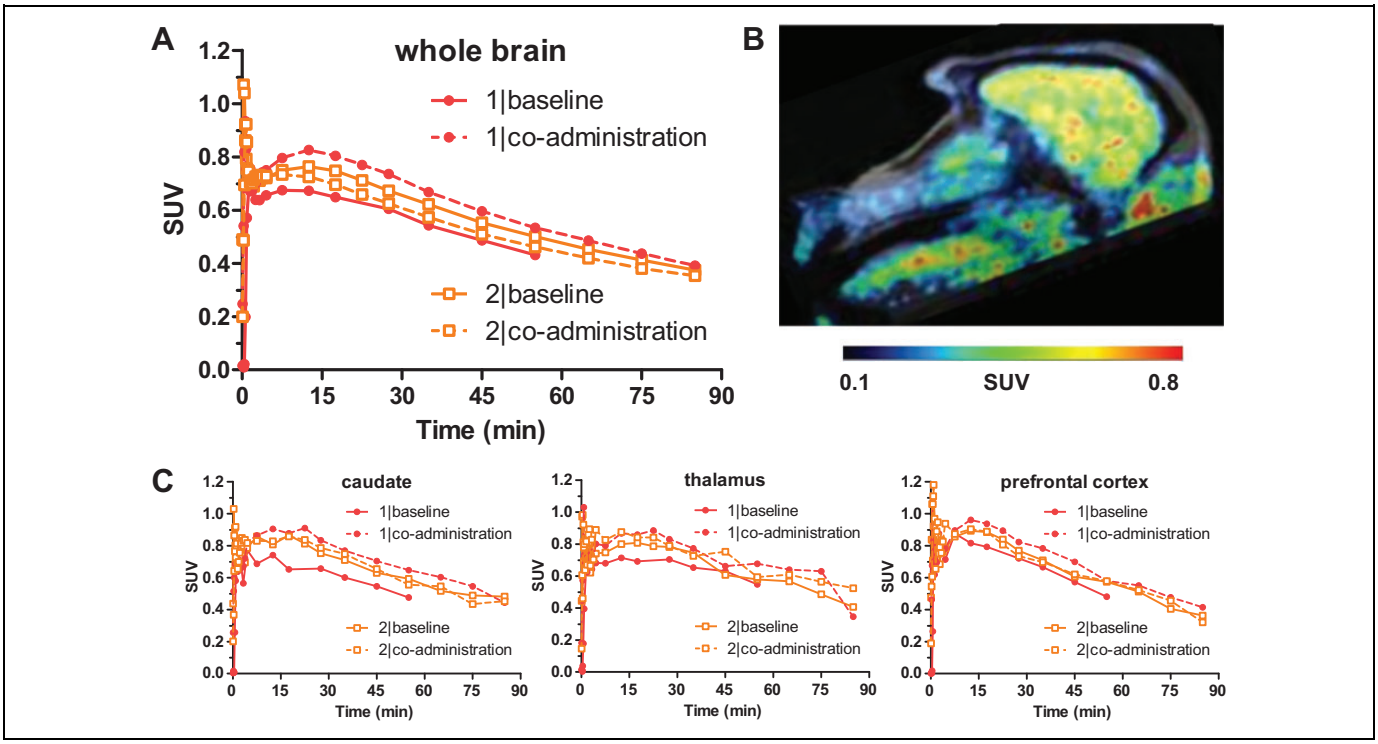

Figure 2. A, Whole-brain PET time-activity curves of [^{11}C]bexarotene in nonhuman primate at baseline and with coadministration of bexarotene (0.35-0.42 mg/kg, IV, $n = 2$). B, Summed PET-MR sagittal image at baseline 30 to 60 minutes. C, Regional time-activity curves for select brain VOIs. IV indicates intravenously; MR, magnetic resonance; PET, positron emission tomography; VOIs, volumes of interest.

the treatment of AD and PD are actively pursued, it is anticipated that new compounds under development will possess superior properties for CNS targeting of RXRs and provide an improved platform for radiotracer development for this important target.

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Declaration of Conflicting Interests

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