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Compartmental modeling of [^{11}C]MENET binding to the norepinephrine transporter in the healthy human brain

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ABSTRACT

Introduction: Dysregulation of the noradrenergic system has been implicated in a number of neurological conditions such as Parkinson's and Alzheimer's. [^{11}C]MENET is a novel PET radiotracer with high affinity and selectivity for the norepinephrine transporter. The applicability of different kinetic models on [^{11}C]MENET PET image quantification in healthy population is evaluated.

Methods: Six healthy volunteers (mean age: 54 years) were recruited for the study, five of whom underwent arterial sampling for measurement of the input function. Ninety minute dynamic PET scans were obtained on a high resolution research tomograph with 15 mCi of [^{11}C]MENET injected at the scan start time. Regions of interest were delineated on the PET scan aided by the corresponding MRI image for anatomical guidance. Distribution volumes and their ratios (DVRs) with respect to the occipital reference tissue were calculated using the full arterial model (FAM), the simplified reference tissue model (SRTM) and the multilinear reference tissue model (MRTM2).

Results: Among the FAMs, the single-tissue model was found to be statistically superior to the two-tissue model. [^{11}C]MENET focal uptake was observed in the NET-rich regions of the brainstem and subcortical regions including the thalamus, locus cereleus and the raphe nuclei. Highest DVRs were observed in the locus cereleus (mean $\pm$ standard deviation: 1.39 $\pm$ 0.25) and red nucleus (1.35 $\pm$ 0.25). DVRs of the thalamus were in good agreement between FAM (1.26 $\pm$ 0.13), SRTM (1.23 $\pm$ 0.15) and MRTM2 (1.21 $\pm$ 0.14). Comparing the FAM to the SRTM and MRTM2, DVRs were underestimated in the thalamus by 3 and 4% on average, respectively.

Conclusion: The single-tissue compartmental model was sufficient in describing the [^{11}C]MENET kinetics in the healthy human brain. SRTM and MRTM2 present themselves as attractive options for estimating NET DVR using an occipital reference region.

1. Introduction

Monoamine neurotransmitters such as norepinephrine (NE), dopamine (DA) and serotonin (5-HT) are critical for our mental wellbeing and have been the targets of extensive research over the past few decades [1,2]. Among these, norepinephrine is responsible for attention, stress management, as well as working and emotional memory [3,4]. The reuptake of NE is mediated by the norepinephrine transporter (NET). Dysregulation of NET is implicated in a variety of neurological conditions such as ADHD [5], depression [6], anxiety [7], Parkinson's [8], Alzheimer's [9], epilepsy [10], drug abuse [11] and post-traumatic stress disorder [4]. Thus, reliable quantitation of NET may help monitor patient disease progress and response to therapy. Such quantification is non-invasively possible using positron emission tomography (PET) imaging with a suitable radiotracer.

A number of radiotracers have been developed to estimate the concentration of NET [12,13]. However, the number of tracers relevant to the human brain has been limited. The primary hurdle remains the non-specific binding of these radioligands *in vivo*. A promising radioligand is ^{11}C labeled (*S*,*S*) 2-[(2-methoxyphenoxy)phenylmethyl] morpholine (MeNER), also called methylreboxetine (MRB), having an *in vitro* IC_{50} of 2.5 nM [14]. It has been used to investigate NET density in normal controls, cocaine addicts [11] and obese population [15]. A fluorine analog of MRB, the [^{18}F]FMeNER-D2 has been used to study NET binding density in patients with major depressive disorders [16] and ADHD [17]. [^{18}F]FMeNER-D2 has also been used in an autoradiographic study of the human brain [18]. The locus cereleus, cerebellum, cortex and the thalamus were found to exhibit NET localization. Typically, dissimilar concentrations of NET in the thalamus and the locus cereleus have been used to identify healthy volunteers from patients.

Given the success of reboxetine analogs as NET ligands, Zeng and colleagues developed (2*S*,3*S*)-2-[α-(2-methylphenoxy)phenylmethyl] morpholine (MENET) and radiolabeled it with ^{11}C to synthesize a radioligand with high affinity to NET while being selective against DAT and SERT [19,20]. [^{11}C]MENET was used *in vitro* and *in vivo* in non-human primates. Compared to [^{11}C]MeNER, [^{11}C]MENET was found to

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demonstrate more attractive imaging kinetics reaching quasi-equilibrium in less than an hour post-injection in non-human primates. [^{11}C]MeNER uptake in comparison did not reach peak equilibrium during a 90 min PET measurement and demonstrated a relatively noisy signal during the last quarter of the scan [21].

In the current work, we report the kinetics of [^{11}C]MENET in six healthy human volunteers and evaluate if the density of NET can be measured reliably in different regions of the brain using [^{11}C]MENET. The simplified reference tissue model (SRTM) and the multilinear reference tissue model (MRTM2) are compared against the compartmental model with arterial input to investigate simpler quantification methods.

2. Materials and methods

2.1. Volunteer details

Six male volunteers ranging in age from 43 and 66 years (mean age: 54 ± 8 years) participated in the study after providing written informed consent. All volunteers were considered physically and mentally healthy as judged by the absence of any history of neurological or psychiatric disorders and absence of any active medical conditions. All volunteers underwent a physical examination, baseline electrocardiogram and MINI structured diagnostic interview. Laboratory testing of urine and blood was performed 1 day prior to imaging. None of the volunteers were taking medications that acted on the central nervous system. Finally, volunteers were instructed to fast at least 4 hours prior to the PET study. This study was approved by the Emory University Institutional Review Board and was conducted under the auspices of the FDA as part of an investigational new drug (IND #112,806).

2.2. Magnetic resonance imaging

Structural MRI data were collected on all volunteers to aid in delineation of regions of interest (ROI) on PET images. A 3D T1-weighted MPRAGE of the brain was acquired with a Siemens Magnetom Trio 3-T (Siemens Medical Solutions USA, Malvern, PA). The scan parameters were 1 mm thick images with a transverse plane isotropic pixel size of 0.5 mm (repetition time/echo time 2500/4.38 ms, inversion time 900 ms, flip angle 10°, acquisition field-of-view 320×192 pixels, matrix size 320×320 pixels).

2.3. PET imaging procedure

All volunteers were scanned on a Siemens High Resolution Research Tomograph (HRRT) (Seimens Medical Solutions, Knoxville, TN) in the supine position. The HRRT has a 24/31 cm axial/transaxial field of view (FOV) and reconstructed image resolution of 2.4 mm in all directions. The volunteer's head was restrained using straps placed over the forehead and real-time motion monitoring was performed with a Polaris Vicra infrared stereo camera (NDI Medical, Waterloo, Ontario, Canada). Arterial blood samples were acquired for five (volunteers 1–5) of the six volunteers. A catheter was placed in the radial vein for administration of [^{11}C]MENET. A second catheter was placed in the radial artery on the opposite arm under local anesthesia for arterial blood sampling. [^{11}C]-MENET was obtained by methylation of (2*S*,3*S*)-*N*-*tert*-butoxycarbonyl-2-[r-(2-trimethylstannylphenoxy)phenylmethyl] morpholine with ^{11}C-CH_3I as previously described [20]. A 90 min emission list mode scan was started simultaneously with a 5 min constant bolus infusion of [^{11}C]MENET using a syringe pump. Emission list mode data were binned into the following framing sequence: 6×30 s, 4×180 s, 5×300 s, 5×600 s. Attenuation correction was measured post-emission by acquiring transmission data with a Cs-137 point source [22]. Data were reconstructed with an ordinary-poisson ordered subset expectation maximization (OP-OSEM) using 6 iterations and 16 subsets. Image data were post-filtered with 4 mm Gaussian kernel giving an in-place resolution of 4.7 mm.

Arterial samples were collected by hand in 1.5 mL aliquots every 10 s for the first 2 min. This was followed by every 30 s up to 5 min, every 60 s up to 10 min, and then at 15, 20, 30 and 60 min. Samples were chilled and centrifuged to separate the plasma fraction from the red blood cells and proteins. A total of 100 μL aliquots of the plasma rich supernatant were counted in duplicate on a NaI Packard Cobra well-counter (Perkin-Elmer, Waltham, MA.) and decay corrected to the injection time. Six additional larger samples (5 mL) were collected at 4.5, 6, 10, 20, 30, 60 min post [^{11}C]MENET injection in EDTA to determine the ratio of parent compound to its metabolic derivatives. These samples were deproteinized by the addition of 0.7 mL of acetonitrile, vortexed for 1 min and centrifuged as described above to recover the protein-free plasma supernatant. The protein free plasma was then filtered and injected onto a reverse phase Waters Xterra RP 18 HPLC column coupled to a radiometric detector to determine the percent parent. Parent blood activity concentrations for each subject were converted to standardized uptake values (SUV) and fitted to a piecewise sum of two Gaussians with three exponentials to serve as the individual arterial input to the compartment model.

2.4. Data analysis

Head motion was corrected in volunteers exhibiting greater than 4 mm head motion using a multiple-acquisition-frame approach described by Herzog and colleagues [23]. Briefly, the attenuation map was aligned to the non-attenuation corrected emission reconstruction using rigid-body transformations and optimization of the mutual information metric. Emission data were then reconstructed with attenuation and scatter correction performed using the aligned transmission maps. Inter-frame alignment was performed on the final reconstructed data with the same optimization metric.

2.4.1. Arterial input compartment model

Two compartmental models were implemented: a single-tissue model (1TC) with two rate constants (K_1 and k_2) and a two-tissue model (2TC) with four rate constants (K_1, k_2, k_3, and k_4). K_1 [mL/g/min] and k_2 [1/min] represent the unidirectional fractional rate constants, corresponding to the influx and efflux of radioligand diffusion across the blood brain barrier, respectively. k_3 [1/min] and k_4 [1/min] represent the radioligand association and dissociation rate to the specific binding sites, respectively. The blood volume was fixed to 3%. The above parameters were determined for each region by an iterative nonlinear least squares fit using the Powell method implemented in IDL (Interactive Data Language, ITT Visual Solutions Inc., Boulder, CO) as previously described [24].

The dynamic PET imaging data were summed over all frames and the resultant image for each volunteer was co-registered with their corresponding MRI for localization of regions of interest (ROI). The regions implicated for NET by Schou et al. [18] along with other regions of the brain exhibiting relatively high uptake were delineated. Neuroanatomical atlas by Haines [25] and regions of focal uptake on the PET image were used to guide ROI delineation. The complete list of segmented ROIs includes caudate, cerebellum, frontal cortex, locus cereleus, midbrain raphe (nucleus raphe dorsalis), occipital lobe, putamen, red nucleus and the thalamus. Bilateral regions were pooled, and ROIs transferred to each PET frame were then corrected for radioactive decay to generate the time–activity curves with the activity converted to SUV. A representation of these regions can be visualized in the sagittal cross-section through the average SUV PET (30–90 min) and MRI images of volunteer 2 (Fig. 1).

The distribution volumes characterizing the receptor densities in different ROIs were obtained using the following three techniques:

(a) Compartmental modeling using arterial input.
(b) Three parameter simplified reference tissue model (SRTM) with a single-tissue compartment for both reference tissue and tissue of interest.
(c) The multilinear reference tissue model (MRTM2) [26].