NeuroImage 87 (2014) 89–95

Contents lists available at ScienceDirect

NeuroImage

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

Retest imaging of [^{11}C]NOP-1A binding to nociceptin/orphanin FQ peptide (NOP) receptors in the brain of healthy humans ☆

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ARTICLE INFO

Article history:
Accepted 31 October 2013
Available online 10 November 2013

Keywords:
NOP receptors
Nociceptin
Test–retest imaging
PET
retest variability
intraclass correlation coefficient

ABSTRACT

[^{11}C]NOP-1A is a novel high-affinity PET ligand for imaging nociceptin/orphanin FQ peptide (NOP) receptors. Here, we report reproducibility and reliability measures of binding parameter estimates for [^{11}C]NOP-1A binding in the brain of healthy humans.

After intravenous injection of [^{11}C]NOP-1A, PET scans were conducted twice on eleven healthy volunteers on the same (10/11 subjects) or different (1/11 subjects) days. Subjects underwent serial sampling of radial arterial blood to measure parent radioligand concentrations. Distribution volume (V_T; a measure of receptor density) was determined by compartmental (one- and two-tissue) modeling in large regions and by simpler regression methods (graphical Logan and bilinear MA1) in both large regions and voxel data. Retest variability and intraclass correlation coefficient (ICC) of V_T were determined as measures of reproducibility and reliability respectively.

Regional [^{11}C]NOP-1A uptake in the brain was high, with a peak radioactivity concentration of 4–7 SUV (standardized uptake value) and a rank order of putamen > cingulate cortex > cerebellum. Brain time–activity curves fitted well in 10 of 11 subjects by unconstrained two-tissue compartmental model. The retest variability of V_T was moderately good across brain regions except cerebellum, and was similar across different modeling methods, averaging 12% for large regions and 14% for voxel-based methods. The retest reliability of V_T was also moderately good in most brain regions, except thalamus and cerebellum, and was similar across different modeling methods averaging 0.46 for large regions and 0.48 for voxels having gray matter probability >20%. The lowest retest variability and highest retest reliability of V_T were achieved by compartmental modeling for large regions, and by the parametric Logan method for voxel-based methods.

Moderately good reproducibility and reliability measures of V_T for [^{11}C]NOP-1A make it a useful PET ligand for comparing NOP receptor binding between different subject groups or under different conditions in the same subject.

Introduction

Positron emission tomography (PET) is used to measure binding site occupancy of medications and differences in the receptor density between groups by comparing the measurements within or between subjects. The sensitivity and specificity of the PET studies are influenced by variation in quantification. In this regard, the test–retest imaging, wherein the same subject undergoes two identical scans, is useful to assess both within subject variations as reproducibility or between subject variations as reliability of the outcome measures (Laruelle, 1999).

Our laboratory recently developed carbon-11-labeled NOP-1A ([^{11}C]NOP-1A) as a promising PET radioligand for in vivo imaging of nociceptin/orphanin FQ peptide (NOP) receptors (Pike et al., 2011). [^{11}C]NOP-1A has high affinity, binds selectively to the NOP receptor as an antagonist, and has appropriate lipophilicity (log $D = 3.41$) for blood–brain barrier permeability. After [^{11}C]NOP-1A injection in monkeys, about 60% of brain radioactivity reflects specific (i.e., displaceable) binding to NOP receptors (Kimura et al., 2011). We used [^{11}C]NOP-1A to visualize NOP receptors in human brain for the first time, and quantified them as total distribution volume (V_T), which is proportional to receptor density (Lohith et al., 2012). V_T values were measured both in large brain regions by compartmental modeling and in individual voxels by simpler regression analyses. In addition, V_T values were well identified

Abbreviations: AUC, area-under-the-curve; BSMSS, between-subject mean sum of squares; f_p, plasma free fraction; ICC, intraclass correlation coefficient; NOP, nociceptin/orphanin FQ peptide; PET, positron emission tomography; WSMSS, within-subject mean sum of squares; V_T, total distribution volume.

☆ Disclosures: This study was supported by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (IRP-NIMH-NIH) and by a Cooperative Research and Development Agreement with Eli Lilly & Co.

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[1] This work was performed during Dr. Lohith's postdoctoral fellowship at NIH.

1053-8119/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.neuroimage.2013.10.068

across brain regions and stable over time, which is consistent with radiometabolites not entering the brain.

However, by doing a single scan in each subject (Lohith et al., 2012), the precision of measuring binding can only be estimated mathematically based on standard errors (i.e. identifiability) to measure V_T. In the current study, we sought to determine the reproducibility and reliability of V_T by scanning each subject twice, i.e. test and retest scans. Reproducibility was measured as retest variability, and reliability was measured as intraclass correlation coefficient (ICC) (Laruelle, 1999). Retest variability and reliability were studied not only in large brain regions but also at the voxel level, because such parametric images are useful for localizing brain regions with altered binding in patient and control groups. Because voxel-wise analyses are prone to underestimate V_T, we compared two parametric methods (i.e., graphical Logan and MA1) with different sensitivities to underestimation. Furthermore, based on the results obtained we sought to determine the necessary sample size for alterations in a prospective between-subject receptor density studies.

Materials and methods

Radioligand preparation

[^{11}C]NOP-1A was labeled by [^{11}C] methylation of an *N*-desmethyl precursor, as previously described (Pike et al., 2011). The radioligand was prepared according to our Investigational New Drug Application (114,313), which was submitted to the U.S. Food and Drug Administration; a copy is available at http://pdsp.med.unc.edu/snidd/IND/nop1a.html. The radioligand was obtained with high radiochemical purity (>99%) and a specific activity of 128 ± 34 GBq/μmol at the time of injection ($n = 22$ batches).

Subjects

Eleven healthy volunteers (8 males, 3 females) participated in the brain PET scans (mean age = 29 years (range: 22–42 years); mean weight = 74 kg (range: 59–99 kg)). All subjects were free of current medical or psychiatric illnesses, as determined by medical history, physical examination, electrocardiogram, urinalysis including drug screening, and laboratory blood tests (complete blood count, serum chemistries, and thyroid function test). Subjects' vital signs were recorded before [^{11}C]NOP-1A injection and at 15, 30, 90, and 120 min after injection. Repeat urinalysis and blood tests were conducted within 2 h of PET scan completion. The protocol was approved by the Institutional Review Board of the National Institutes of Health. All subjects signed a written informed consent form.

PET scans and measurement of [^{11}C]NOP-1A in arterial plasma

All PET scans were performed on an Advance tomograph (GE Medical Systems, Waukesha, WI). Each subject underwent test and retest scans after bolus injection of [^{11}C]NOP-1A along with arterial blood sampling for metabolite corrected input function. Test and retest scans were performed on the same day separated by 3 h between radiotracer injections except for one subject whose scans occurred 10 days apart. After an 8-minute brain transmission scan using ^{68}Ge rod source, dynamic three-dimensional emission scans were acquired for 120 min as previously described (Lohith et al., 2012). Arterial blood samples were drawn manually after radioligand injection with 1.5 mL samples at 15 s intervals until 150 s, followed by 3 mL samples at 3, 4, 6, 8, 10, 15, 20, 30, 40, and 50 min, and 5 mL samples at 60, 75, 90, and 120 min. The concentration of parent radioligand and the metabolite-corrected plasma input function were obtained as previously described (Lohith et al., 2012; Pike et al., 2011). The plasma free fraction (f_P) was measured for each scan by ultrafiltration, as previously described (Gandelman et al., 1994). Assay-to-assay variation in f_P measurement was corrected based on f_P measured from a standard plasma sample along with the subject's sample (Abi-Dargham et al., 1999). Radiochemical purity was measured by incubating [^{11}C]NOP-1A from all 22 syntheses in whole blood and plasma for 30 min at room temperature.

PET images were analyzed by applying a template of 10 pre-set volumes-of-interest in Montreal Neurologic Institute space after coregistration to the same subject's magnetic resonance (MR) image using Statistical Parametric Mapping, SPM (Version 8 for Windows, Wellcome Department of Cognitive Neurology, UK) as previously described (Lohith et al., 2012). As a measure of receptor binding, V_T was calculated using brain and arterial input function from each of the test and retest scans by compartmental modeling and graphical ($Logan_{VOI}$ and bilinear $MA1_{VOI}$) analyses on large regions as well as by parametric methods ($Logan_{voxel}$ and bilinear $MA1_{voxel}$) on voxel-wise data as described before (Lohith et al., 2012). V_T/f_P was also calculated as another measure of receptor binding, because only free ligand enters the brain. Kinetic analyses and generation of parametric images were performed using pixelwise modeling software (PMOD 3.16, PMOD Technologies Ltd., http://www.pmod.com/).

Measurement of retest variability and reliability of radioligand binding

The optimal compartment model (i.e., one- vs two-tissue compartments) to determine V_T was chosen based on Akaike information criterion, model selection criterion (proposed by Micromath, Saint Louis, Missouri USA, http://www.micromath.com/products.php?p=scientist&m=statistical_analysis), and *F*-tests (Hawkins et al., 1986). V_T values measured between models or between test and retest scans in the same subject were compared using factorial repeated measures analysis of variance (rmANOVA) with Bonferroni adjustment. The retest variability of V_T was calculated as the absolute difference between test and retest V_T divided by the average between the two, expressed as a percentage. Retest variability under 10% was considered excellent; and over 10% but under 20% as moderate. The retest reliability of V_T was the intraclass correlation coefficient (ICC) calculated as follows:

$$\mathrm{ICC} = \frac{\mathrm{BSMSS} - \mathrm{WSMSS}}{\mathrm{BSMSS} + (n-1)\mathrm{WSMSS}}$$

where BSMSS and WSMSS are between- and within-subject mean sum of squares, respectively, and n is the number of within-subject observations (in this case, $n = 2$). ICC values between 0 and 1 indicated higher variability between subjects than within subjects; values close to 1 suggested good reliability. Values between −1 and 0 indicated that variability was higher within subjects than between subjects and suggested poor reliability (Landis and Koch, 1977; Shrout and Fleiss, 1979). Retest variability and ICC values were also calculated at the voxel level by SPM8 using voxelwise parametric images of V_T, yielding 3-dimensional spatial maps of reproducibility and reliability.

Sample size estimation

Because of variability in PET imaging results, it is necessary to estimate the sample size required to detect a significant effect for between-subject studies (e.g., differences in NOP density between patients and controls). The test scan results from the 11 healthy subjects in this study were pooled with single scans previously obtained by our group in 7 healthy subjects (Lohith et al., 2012). The sample size was calculated for a 10, 15 and 20% change in V_T assuming that similar variance exists between healthy subjects and patients. A 10, 15 or 20% change for the between-subject studies was chosen based on the premise that a change of at least 10–20% in outcome parameter is necessary and sufficient for establishing group differences by PET studies (Deschwanden et al., 2011; Hirvonen et al., 2012).