NeuroImage xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

NeuroImage

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

Evaluation of the agonist PET radioligand [^{11}C]GR103545 to image kappa opioid receptor in humans: Kinetic model selection, test–retest reproducibility and receptor occupancy by the antagonist PF-04455242

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ARTICLE INFO

Article history:
Accepted 13 May 2014
Available online xxxx

Keywords:
^{11}C-GR103545
PF-04455242
Kappa opioid receptor
Positron emission tomography (PET)
Test–retest
Occupancy plot

ABSTRACT

Introduction: Kappa opioid receptors (KOR) are implicated in several brain disorders. In this report, a first-in-human positron emission tomography (PET) study was conducted with the potent and selective KOR agonist tracer, [^{11}C]GR103545, to determine an appropriate kinetic model for analysis of PET imaging data and assess the test–retest reproducibility of model-derived binding parameters. The non-displaceable distribution volume (V_{ND}) was estimated from a blocking study with naltrexone. In addition, KOR occupancy of PF-04455242, a selective KOR antagonist that is active in preclinical models of depression, was also investigated.

Methods: For determination of a kinetic model and evaluation of test–retest reproducibility, 11 subjects were scanned twice with [^{11}C]GR103545. Seven subjects were scanned before and 75 min after oral administration of naltrexone (150 mg). For the KOR occupancy study, six subjects were scanned at baseline and 1.5 h and 8 h after an oral dose of PF-04455242 (15 mg, $n = 1$ and 30 mg, $n = 5$). Metabolite-corrected arterial input functions were measured and all scans were 150 min in duration. Regional time-activity curves (TACs) were analyzed with 1- and 2-tissue compartment models (1TC and 2TC) and the multilinear analysis (MA1) method to derive regional volume of distribution (V_T). Relative test–retest variability (TRV), absolute test–retest variability (aTRV) and intra-class coefficient (ICC) were calculated to assess test–retest reproducibility of regional V_T. Occupancy plots were computed for blocking studies to estimate occupancy and V_{ND}. The half maximal inhibitory concentration (IC_{50}) of PF-04455242 was determined from occupancies and drug concentrations in plasma. [^{11}C]GR103545 in vivo K_D was also estimated.

Results: Regional TACs were well described by the 2TC model and MA1. However, 2TC V_T was sometimes estimated with high standard error. Thus MA1 was the model of choice. Test–retest variability was ~15%, depending on the outcome measure. The blocking studies with naltrexone and PF-04455242 showed that V_T was reduced in all regions; thus no suitable reference region is available for the radiotracer. V_{ND} was estimated reliably from the occupancy plot of naltrexone blocking ($V_{ND} = 3.4 \pm 0.9$ mL/cm^3). The IC_{50} of PF-04455242 was calculated as 55 ng/mL. [^{11}C]GR103545 in vivo K_D value was estimated as 0.069 nmol/L.

Conclusions: [^{11}C]GR103545 PET can be used to image and quantify KOR in humans, although it has slow kinetics and variability of model-derived kinetic parameters is higher than desirable. This tracer should be suitable for use in receptor occupancy studies, particularly those that target high occupancy.

Introduction

Opioid receptors belong to the superfamily of G-protein coupled receptors and are classified into at least four subtypes: μ (MOR), δ (DOR), κ (KOR), and nociceptin (Corbett et al., 2006). KOR exist abundantly in the human brain and have been implicated in a number of disorders, including substance abuse (Mash and Staley, 1999; Mello and Negus, 2000), epilepsy (de Lanerolle et al., 1997; Loacker et al.,

Abbreviations: KOR, kappa opioid receptor; MOR, mu opioid receptor; DOR, delta opioid receptor; TRV, relative test–retest variability; aTRV, absolute test–retest variability; ICC, intra-class correlation coefficient.

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http://dx.doi.org/10.1016/j.neuroimage.2014.05.033
1053-8119/

2007), Alzheimer's disease (Mathieu-Kia et al., 2001), and major depression (Barber and Gottschlich, 1997; Gerra et al., 2006; Tenore, 2008). For example, multiple lines of evidence point to the involvement of KOR in depression and related mood disorders (Beardsley et al., 2005; Carlezon et al., 2006; Mague et al., 2003; McLaughlin et al., 2003; Newton et al., 2002; Reindl et al., 2008; Shirayama et al., 2004). In turn, these findings raise the possibility that KOR antagonists might be fast-acting and efficacious antidepressants. As such, KOR is a target for development of newer antidepressants. There have been no prior reports of validated PET radiotracers for use in humans to image the KOR, although several tracers have been proposed and validated for MOR (Dannals et al., 1985), for MOR and KOR (Pert et al., 1984), and for DOR (Kinter and Lever, 1995).

GR89696 ((±)-4-[(3,4-dichlorophenyl)acetyl]-3-[(1-pyrrolidinyl) methyl]-1-piperazine carboxylate) is a potent KOR agonist (Naylor et al., 1993). Its ^{11}C-labeled form was evaluated as a PET radiotracer in mice and baboons (Ravert et al., 1999; Talbot et al., 2005). GR103545, which is the active (−)-enantiomer of GR89696, is more potent. In in vitro radioligand competition assays using recombinant cells expressing KOR, MOR or DOR, GR103545 was shown to bind to KOR with high affinity (K_i of 0.02 ± 0.01 nmol/L) and excellent selectivity over MOR (K_i of 16 ± 5 nmol/L) and DOR (K_i of 536 ± 234 nmol/L) (Schoultz et al., 2010). In initial in vivo evaluations in non-human primates (Schoultz et al., 2010; Talbot et al., 2005) [^{11}C]GR103545 was shown to have favorable characteristics: excellent brain penetration, significant washout, moderate metabolic rate in the plasma, and good specific binding signals. The uptake pattern of [^{11}C]GR103545 was in good agreement with the known distribution of KOR in the non-human primate brain. The in vivo K_D of [^{11}C]GR103545 was estimated from a study in rhesus monkeys and an appropriate tracer mass dose limit was selected for human study (Tomasi et al., 2013). However, its translation to humans was hampered by the absence of an efficient radiosynthetic method. Recently a one-pot method for the automated radiosynthesis of [^{11}C]GR103545 was developed with reliably high specific activity and radiochemical yield (Nabulsi et al., 2011).

Building upon these encouraging preliminary data in non-human primates and the development of an efficient radiosynthesis, we carried out experiments to fully validate [^{11}C]GR103545 as a radiotracer to image and quantify KOR in the human brain. First, a test–retest study was conducted to assess the suitability of kinetic models and the reproducibility of model-derived kinetic parameters. Second, the non-displaceable distribution volume (V_{ND}) was determined from a blocking study with the non-selective opioid antagonist naltrexone. Third, a receptor occupancy study was performed in humans to determine the half maximal inhibitory concentration (IC_{50}) of PF-04455242 (2-methyl-*N*-((2′-(pyrrolidin-1-ylsufonyl)biphenyl-4-yl)methyl)propan-1-amin), a potent and selective KOR antagonist (K_i of 3 nmol/L for KOR) in development as a novel therapeutic agent for depression (Grimwood et al., 2011).

Materials and methods

Human subjects

Eleven healthy subjects (25–52 years of age; 9 men and 2 women) completed the test–retest part of the study. Seven healthy subjects (26–55 years of age; 4 men and 3 women) were involved in the baseline-blocking study with naltrexone as the blocking drug, while six healthy male subjects (26–51 years of age) were enrolled in the receptor occupancy study with PF-04455242. A total of 24 subjects were enrolled in the study. There were no overlapping subjects among the three parts of the study. The maximum number of scans for any subject was 3. The receptor occupancy study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with all International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. In addition, all local regulatory requirements were followed, in particular, those affording greater protection to the safety of trial participants. These studies were performed under protocols approved by the Yale University Human Investigation Committee and the Yale-New Haven Hospital Radiation Safety Committee. Written informed consent was obtained from all subjects. As part of the evaluation procedure, magnetic resonance (MR) images were acquired on all subjects to eliminate those with structural brain abnormalities and for image registration. MR imaging was performed on a 3T whole-body scanner (Trio, Siemens Medical Systems, Erlangen, Germany) with a circularly polarized head coil. The dimension and pixel size of MR images were 256 × 256 × 176 and 0.98 × 0.98 × 1.0 mm^3, respectively.

Radiotracer synthesis

[^{11}C]GR103545 was synthesized as previously described (Nabulsi et al., 2011). Radiochemical purity of the [^{11}C]GR103545 final product solution was >95%.

Test–retest study

Among the 11 subjects who underwent two 150-min PET scans with [^{11}C]GR103545; 9 of these scan pairs were performed on the same day. The test and retest scans were 6 days apart for one subject and 2 months apart for the other subject. PET scans were performed on the High Resolution Research Tomograph (HRRT) (Siemens Medical Solutions, Knoxville, TN, USA), which acquires 207 slices (1.2 mm slice separation) with a reconstructed image resolution of ~3 mm. Prior to tracer administration, a 6-min transmission scan was conducted for attenuation correction. Each scan was acquired in list mode after intravenous administration of [^{11}C]GR103545 over 1 min by an automatic pump (Harvard PHD 22/2000, Harvard Apparatus, Holliston, MA, USA). The injected mass limit was 0.02 μg/kg body weight (Tomasi et al., 2013). Dynamic scan data were reconstructed in 36 frames (6 × 0.5 min, 3 × 1 min, 2 × 2 min, 22 × 5 min, 3 × 10 min) with corrections for attenuation, normalization, scatter, randoms, and deadtime using the MOLAR algorithm (Carson et al., 2003). Motion correction was included in the reconstruction program based on measurements with the Polaris Vicra sensor (NDI Systems, Waterloo, Canada) with reflectors mounted on a swim cap worn by the subject.

Blocking study with naltrexone

Baseline-blocking experiments were conducted with the non-selective opioid receptor antagonist naltrexone. Subjects underwent two PET scans on the same day: a baseline PET scan followed by a second scan at 75 min after an oral administration of 150 mg naltrexone. For one subject, the blocking scan with naltrexone was conducted 1 month after the baseline scan due to chemistry equipment failure.

Receptor occupancy study with PF-04455242

Subjects received three PET scans with [^{11}C]GR103545 over two days. A baseline scan was obtained on Day 1. On Day 2, the subjects first received a single oral dose of PF-04455242 and then underwent two [^{11}C]GR103545 PET scans, at 1.5 h (post-dose scan #1) and 8 h (post-dose scan #2), respectively, after drug administration. This timing was chosen based on previously acquired human pharmacokinetic data (Sawant Basak et al., 2013). The doses of PF-04455242 were 15 mg ($n = 1$) and 30 mg ($n = 5$). Eight venous blood samples were drawn from each subject at 1.5, 2.0, 2.5, 3.0, 4.0, 8.0, 9.0, and 10.5 h following PF-04455242 administration and analyzed to determine the plasma concentration of PF-04455242 over time. The plasma samples were analyzed by LC/MS/MS.

Please cite this article as: Naganawa, M., et al., Evaluation of the agonist PET radioligand [^{11}C]GR103545 to image kappa opioid receptor in humans: Kinetic model selection, test–re..., NeuroImage (2014), http://dx.doi.org/10.1016/j.neuroimage.2014.05.033