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Preclinical evaluation of [^{11}C]ROCK201 for imaging rho-associated protein kinase 2 in brain

Chao Zheng[1], Baosheng Chen[1], Takuya Toyonaga[1], LaShae Nicholson[2], Daniel Holden[1], Li Zhang[1], Hong Gao[1], Mingqiang Zheng[1], Krista Fowles[1], Jim Ropchan[1], Stephen M. Strittmatter[2], Richard E. Carson[1], Yiyun Huang[1], Zhengxin Cai[1]

1. PET Center, Yale School of Medicine, New Haven, CT 06520, USA
2. Program in Cellular Neuroscience, Neurodegeneration, and Repair, Departments of Cell Biology, Neurology and Neuroscience, Yale University School of Medicine, New Haven, CT, USA

Introduction: Rho-associated protein kinase (ROCK) has two isotypes, ROCK1 and ROCK2. Expression in brain is widespread for ROCK2, and limited for ROCK1[1]. ROCK2 and activation of the rho/rho-kinase pathway have been targeted for drug development for various central nervous system (CNS) disorders, including Alzheimer's disease (AD) [2]. Development of a PET imaging agent for ROCK2 will facilitate ROCK2 targeted drug development. Herein, we report the first ROCK2 specific PET radioigand, [^{11}C]ROCK201, and its evaluation in rodents and nonhuman primates (NHPs).

Methods: ROCK201 is a selective ROCK2 inhibitor with IC_{50} values of 2 nM, 17 nM and 240 nM for ROCK2, ROCK1 and protein kinase A, respectively, and no appreciable affinity for more than 40 other CNS targets [3]. [^{11}C]ROCK201 was synthesized from its phenol precursor with [^{11}C]MeI (**Fig. 1a**). PET imaging studies in rodents and NHPs were conducted on the Focus-220 scanner. Arterial blood was drawn for metabolite analysis and construction of plasma input function during the NHP scans. Self-blocking scans were performed at 10 min after the injection of 3.3 mg/kg (for rodents) or 0.05 mg/kg (for NHP) of unlabeled ROCK201. For PET scans in rats and mice, regions of interest (ROIs) were extracted from a rodent brain atlas and regional time-activity curves (TACs) were obtained by applying template ROIs to the PET images. For NHP PET, regional brain TACs were fitted with one-tissue compartment (1TC) model to obtain volume of distribution (V_T). Binding potential (BP_{ND}) was calculated using the nondisplaceable volume of distribution (V_{ND}) obtained from the blocking study, where $BP_{ND} = (V_T/V_{ND})-1$. ROCK2 protein levels in different brain regions were measured by capillary western blotting (WES).

Results: [^{11}C]ROCK201was prepared in >99.9% radiochemical purity and molar activity of 256 ± 103 GBq/μmol at the end of synthesis (n=8). LogP value was measured at 2.9. Uptake of [^{11}C]ROCK201 was observed throughout the rodent brain, with peak SUV of 2.1 in rats and 2.0 in mice (**Fig. 1b-1c**). Reduced uptake was observed in the self-blocking studies, indicating specific binding of [^{11}C]ROCK201 in the rodent brain (**Fig. 1b-1c**). In NHPs, the tracer showed a relatively fast metabolism, with parent fraction in plasma of 36% at 30 min post injection (p.i.). High quality PET images were generated with [^{11}C]ROCK201 in the monkey brain (**Fig. 1d**). The tracer entered the brain quickly and reached peak level within 10 min after injection. Higher tracer uptake levels were in the amygdala, putamen, cerebellum, frontal cortex, with peak SUV ≥ 5, and lower in centrum semiovale (peak SUV ≈ 2) (**Fig. 1d**). The 1TC V_T values ranged from 5.7 mL/cm^3 (brain stem) to 12.4 mL/cm^3 (occipital cortex). Based on the Lassen plot (**Fig. 1e**), self-blocking with 0.05 mg/kg ROCK201 occupied 43% of the binding sites, with V_{ND} of 2.62 mL/cm^3. Regional BP_{ND} values calculated using V_{ND} ranged from 1.5 (brain stem) to 3.8 (occipital cortex), indicating high specific binding signals in NHP brain regions. The specific volume of distribution ($V_s = V_T - V_{ND}$) correlated well with ROCK2 protein expression levels measured by WES in selected monkey brain regions (caudate, hippocampus, thalamus, cerebellum, occipital cortex, thalamus, and brain stem, $R^2 = 0.84$, $p = 0.004$).

Conclusions: We have successfully synthesized the first brain permeable ROCK2 radiotracer [^{11}C]ROCK201 and evaluated its imaging characteristics in rodent and NHP brains. [^{11}C]ROCK201 showed fast brain kinetics and good specific binding signals in rodents and NHPs. Further validation studies are ongoing to investigate [^{11}C]ROCK201 binding in neurological disease models.

Figure

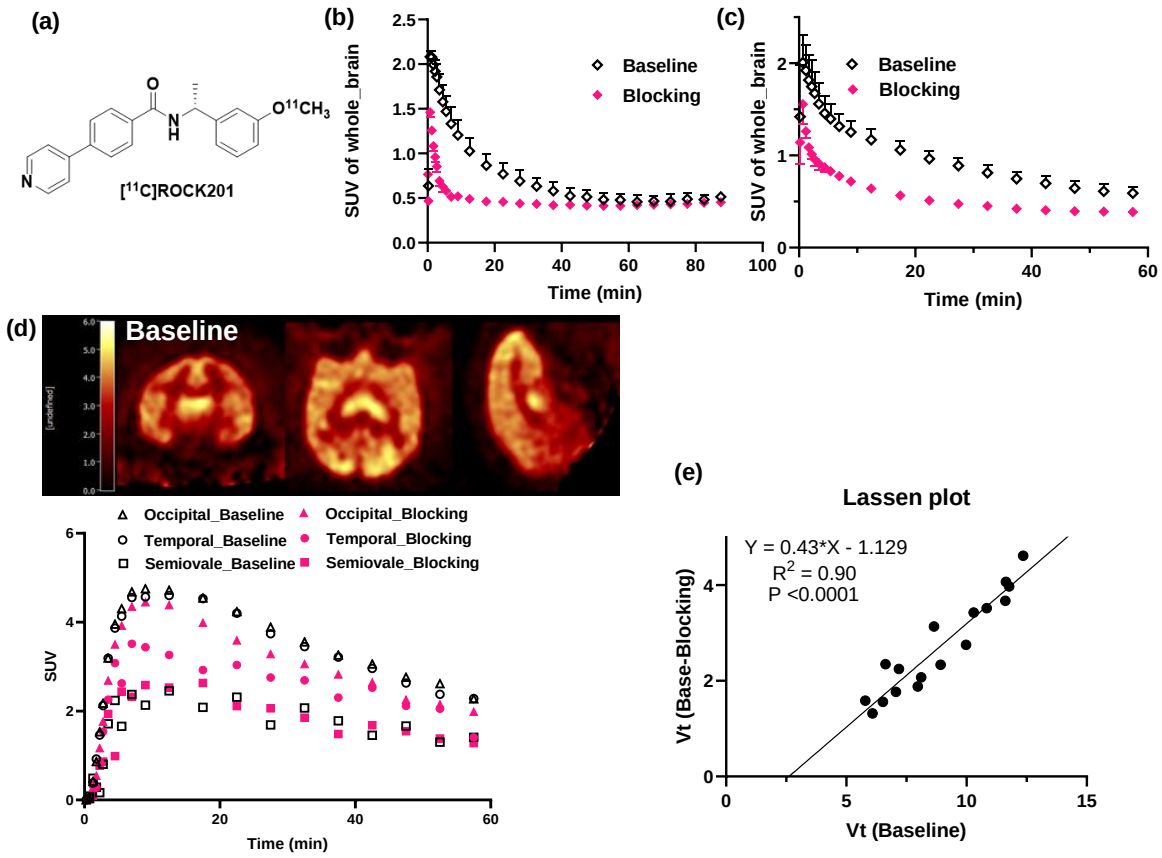

Figure 1: (**a**) Structure of [^{11}C]ROCK201; (**b**-**c**) Whole brain TACs of [^{11}C]ROCK201 in (**b**) Sprague-Dawley rats (n = 2) and (**c**) C57BL/6J mice under baseline (n = 4) and self-blocking (ROCK201, 3.33 mg/kg, i.v. n = 3) conditions; (**d**) Summed brain SUV images (15 to 30 min) from a [^{11}C]ROCK201 baseline scan in rhesus monkey, and TACs for selected brain regions under baseline and self-blocking (0.05 mg/kg, i.v.) condition; (**e**) Occupancy plot using the V_T values from baseline scan of [^{11}C]ROCK201 and the self-blocking scan with ROCK201.

References

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2. Koch JC, et al. *Pharmacol. Ther.*, **2018**, 189, 1.
3. Hobson AD, et al. *J. Med. Chem.*, **2018**, 61, 11074.

Data for reviewer:

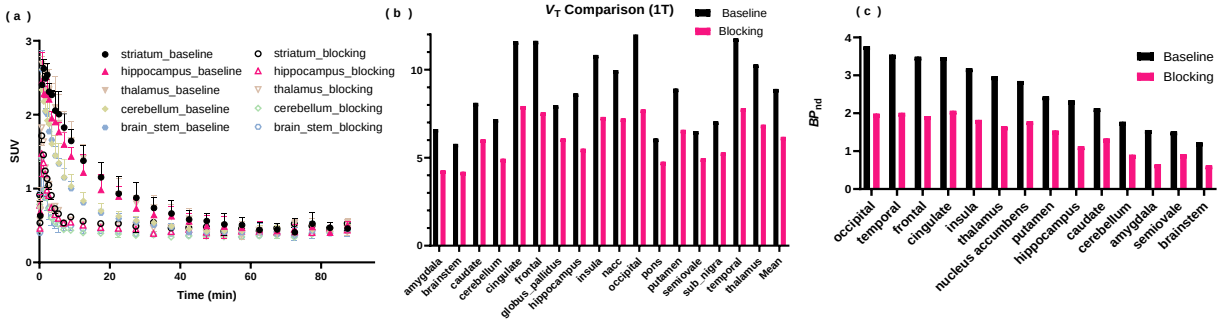

Figure S1: (**a**) Brain regional TACs of [^{11}C]ROCK201 from baseline (n = 2) and self-blocking (3.33 mg/kg, i.v.) scans in Sprague-Dawley rats; (**b**) 1TC regional V_T values from the baseline and self-blocking (0.05 mg/kg, i.v.) studies of [^{11}C]ROCK201 in monkey; (**c**) 1TC BP_{ND} values from the baseline and self-blocking studies of [^{11}C]ROCK201 in monkey.