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Research Article

Synthesis and preliminary PET study of the 5-HT$_7$ receptor antagonist [^{11}C]DR4446

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Summary

DR4446 (1-methyl-2a-[4-(4,5,6,7-tetrahydrothieno[3,2-*c*]pyridin-5-yl)butyl]-2*a*,3,4,5-tetrahydro-1H-benz[*cd*]indole-2-one) is a potent 5-HT$_7$ receptor antagonist ($K_i = 9.7$ nM) with a high selectivity over other 5-HT family receptors (K_i for 5-HT$_{1A}$: 770 nM; for other 5-HT receptors: > 1000 nM). As a positron emission tomography (PET) tracer for the 5-HT$_7$ receptor, [^{11}C]DR4446 was synthesized at high radiochemical purity ($> 98\%$) with specific activity of 73–120 GBq/μmol at the end of synthesis by the alkylation of the desmethyl precursor (1) with [^{11}C]CH_3I in the presence of NaH. A PET study in monkey demonstrated that [^{11}C]DR4446 had good permeability into the brain, and had a specific binding component in the brain regions including the thalamus, possibly an area in the 5-HT$_7$ receptors. Metabolite analysis showed that [^{11}C]DR4446 was relatively stable and low percentages of two radio-labeled metabolites were detected in the plasma of monkey using HPLC. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: 5-HT$_7$ receptor; DR4446; carbon-11; PET

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Introduction

The initial division of 5-HT receptors into the 5-HT_1 and 5-HT_2 classes has now expanded to seven structurally and pharmacologically distinct classes.[1,2] 5-HT_7 receptor is the most recent addition to the family of 5-HT receptors.[2,3] The 5-HT_7 receptor has been cloned from rat,[4,5] mouse,[6] guinea pig[7] and human[8] cDNA. In the periphery, this receptor stimulation causes relaxation of the blood vessels in monkey,[9] dog[10] and rabbit.[11] In the CNS, the 5-HT_7 receptor may play a role in mediating 5-HT-induced phase shifts of neuronal activity in the suprachiasmatic nucleus of the hypothalamus.[12] These data suggest that the 5-HT_7 receptor might be linked to the control of circadian rhythms.[13] Moreover, this receptor may be involved in the pathophysiology of sleep disorders,[7] depression[8] and schizophrenia,[14,15] although the evidence is very preliminary. In particular, clozapine, an atypical antipsychotic, exhibits a high affinity for the 5-HT_7 receptor ($K_i = 5.1$ nM), suggesting that therapeutic activity may be exerted partially via this receptor.[14] The greatest abundance of 5-HT_7 mRNA is found in the brain, where it is discretely located within the thalamus, hypothalamus, and various limbic and cortical regions.[5,8,13,16] Autoradiography has also shown that the distribution of the 5-HT_7 receptor in rat and guinea pig brain matches the brain mRNA distribution to a large extent.[4,17] However, it is clear that better pharmacological tools including the utilization of a potent and selective ligand for this receptor are necessary to establish the exact location of the 5-HT_7 receptor in brain.

The visualization of receptors *in vivo* using external imaging with positron emission tomography (PET) technique forms an extremely powerful method for the study of receptors. Therefore, the *in vivo* imaging of the presence and functioning of the 5-HT_7 receptor would be of benefit to determine the distribution of this receptor and to understand its changing role in the pathophysiology of mental disorders. Until now, several potent and selective ligands[10,18–21] for this receptor have been developed. Of these compounds, DR4446 (1-methyl-2a-[4-(4, 5, 6, 7-tetrahydrothieno[3, 2-*c*]pyridin-5-yl)butyl]-2*a*,3,4,5-tetrahydro-1H-benz[*cd*]indole-2-one, Figure 1) displayed a relatively high affinity for the 5-HT_7 receptor and high selectivity over the other 5-HT receptors.[21] DR4446 was shown to bind with high affinity ($K_i = 9.7$ nM) to human recombinant 5-HT_7 receptors expressed in mammalian cells, whereas its affinity at 5-HT_{1A} receptor was 770 nM.

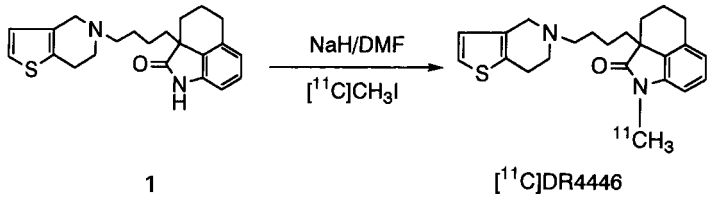

Figure 1. Radiosynthesis of [^{11}C]DR4446

Moreover, the compound displayed negligible affinity ($K_I > 1\ \mu M$) for the other 5-HT subtypes (5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_2$, 5-HT$_{2c}$, 5-HT$_3$, 5-HT$_4$, 5-HT$_6$). It also displayed weak or negligible affinity for other neurotransmitter receptors including dopaminergic and adrenergic receptors. Therefore, we selected DR4446 as a candidate for PET imaging of the 5-HT$_7$ site, with the hypothesis that it might serve as a suitable *in vivo* radioligand for the 5-HT$_7$ receptor. In this study, we synthesized [^{11}C]DR4446 by *N*-methylation of 1 (desmethyl precursor) with [^{11}C]methyl iodide ([^{11}C]CH$_3$I). Using PET with monkey brain, we determined the regional distribution of [^{11}C]DR4446 and the blocking effect on the binding of [^{11}C]DR4446 with non-radioactive DA4446. Finally, we measured the unchanged [^{11}C]DR4446 as well as metabolites in monkey plasma by high-performance liquid chromatography (HPLC).

Results and discussion

Methylation of *N*-, *O*-, or *S*-nucleophile with [^{11}C]CH$_3$I is the most widely used tool for introducing a short-lived ^{11}C positron emitter into organic molecules. This strategy was successfully employed in the radiosynthesis of [^{11}C]DR4446 by the reaction of the corresponding desmethyl precursor 1 with [^{11}C]CH$_3$I (Figure 1). The [^{11}C]CH$_3$I for radiosynthesis was prepared by reduction of [^{11}C]CO$_2$, which was generated by proton bombardment of ^{14}N$_2$ gas, with LiAlH$_4$ and subsequent treatment with HI. The [^{11}C]CH$_3$I obtained was trapped into a mixture containing the secondary amide 1 and NaH at -15°C to -20°C. The 5-min methylation reaction at 30°C afforded [^{11}C]DR4446 at a high radiochemical yield of 15–28% (corrected for decay) from the total [^{11}C]CO$_2$ at the end of synthesis (EOS).

It was of paramount importance in the synthesis of [^{11}C]DR4446 to remove all traces of unreacted precursor (1), because 1 has been shown to potently inhibit the 5-HT$_7$ receptor *in vitro* ($K_i = 6.5$ nM)[21] and may compete with [^{11}C]DR4446 in the PET study. Complete separation of the product ($t_R = 7.9$ min) and precursor ($t_R = 4.6$ min) could be accomplished using the reverse-phase column system. The HPLC purification procedure gave radiochemically and chemically pure [^{11}C]DR4446 (>98%). Under these conditions, [^{11}C]DR4446 was obtained at up to 1.9–2.2 GBq of an intravenous injectable solution with a specific activity of 73–120 GBq/μmol at the EOS. In the final product solution (7 ml), no significant 1 peak was determined (<0.1 μg/7 ml). The total synthesis time was 21–23 min from the end of bombardment (EOB).

The *in vivo* distribution of [^{11}C]DR4446 in monkey brain was studied using PET. One dose of 98 MBq of [^{11}C]DR4446 (specific activity: 110 GBq/μmol) was administered to a monkey, and PET images were required during a 90 min period. Figure 2a shows the time–activity curves (TACs) in the monkey brain for 90 min scan after intravenous injection of [^{11}C]DR4446. Immediately following administration of [^{11}C]DR4446, radioactivity began to accumulate in the all regions

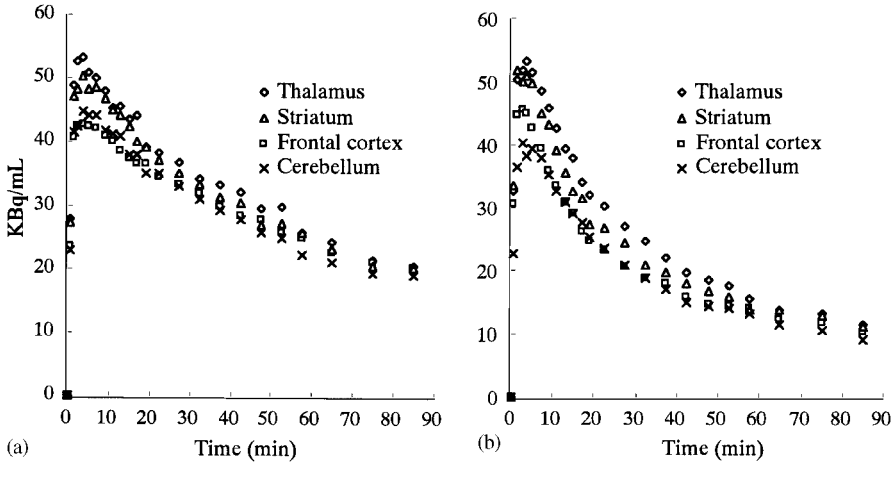

Figure 2. Time—activity curves of [^{11}C]DR4446 in the thalamus, striatum, frontal cortex and cerebellum of the monkey brain. Each point was normalized to the injected activity of 98 MBq: (a) [^{11}C]DR4446 (98 MBq); (b) [^{11}C]DR4446 (98 MBq) + DR4446 (1 mg/kg)

examined. The initial uptake of [^{11}C]DR4446 in the brain was high, reflecting a high penetration of the tracer across the blood–brain barrier (BBB) consistent with its partition coefficient of 3.7 (log *P*). As can be seen, the radioactivity in all regions reached a peak by 3–5 min post injection and then began to clear from all structures and was at 50% of the peak uptake by 55–60 min. The radioactivity in the thalamus, a possibly rich area in 5-HT$_7$ sites,[16,17] was slightly higher than in the other regions. In the blocking study, there was a consistent change in the uptake or kinetics of [^{11}C]DR4446 after a simultaneous injection of [^{11}C]DR4446 with non-radioactive DR4446 (1 mg/kg) as compared to the control study which was obtained on the same conditions (Figure 2b). The radioactivity in the thalamus, striatum, frontal cortex and cerebellum was reduced to 71–79% of control at 15 min, to 55–60% of control at 45 min, and to 47–52% of control by the end of PET scan. This observation confirms that [^{11}C]DR4446 may have some specific binding in the monkey brain.

Blood samples were processed and plasma was isolated and extracted. The recovery of total radioactivity from the plasma was higher than 80%. The percentages of total radioactivity in plasma representing the unchanged compound and two metabolites obtained after the injection of [^{11}C]DR4446 (98 MBq) are shown in Figure 3. The fraction of the total radioactivity representing unchanged [^{11}C]DR4446 in monkey plasma as determined using HPLC was 91% at 2 min, 70% at 30 min, and 46% at 90 min after the injection. Only tiny radio-labeled metabolites that were less lipophilic than the parent compound [^{11}C]DR4446 were observed on the HPLC chromatogram.

There were some reports about the established regional distribution of the 5-HT$_7$ receptors.[5,8,13,16] Although there may be differences in relative abundance of 5-HT$_7$ protein and its mRNA between brain regions and across species, the thalamus was generally considered to have a more abundant presence than the other brain regions. In our PET study, the *in vivo* distribution of [^{11}C]DR4446 demonstrated a somewhat higher uptake in the thalamus than in the other regions. However, whether this distribution pattern was due to blood flow differences or due to a specific binding component of [^{11}C]DR4446 in the brain was not clear. Therefore, the blocking effect on the binding sites of [^{11}C]DR4446 in the brain was determined with non-radioactive DR4446. As can be seen in Figure 2b, the simultaneous injection of non-radioactive DR4446 with [^{11}C]DR4446 reduced the uptake of

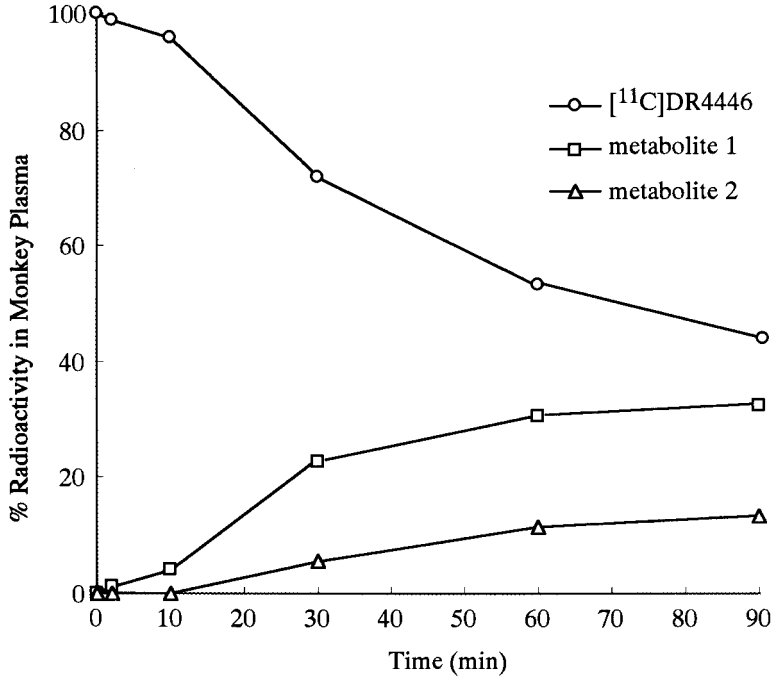

Figure 3. The percentage of unchanged [^{11}C]DR4446 and metabolites versus time in monkey plasma

radioactivity in the all brain regions including the thalamus. This observation suggested that this PET tracer had some specific binding sites in the brain. The relative stability of [^{11}C]DR4446 in the plasma may also support this finding. The next step of our study is to determine whether the specific binding of [^{11}C]DR4446 is due to the 5-HT$_7$ receptors in the primate brain.

Experimental

General

DR4446 and the corresponding desmethyl precursor 1 (2a-[4-(4,5,6,7-tetrahydrothieno[3, 2-*c*]pyridin-5-yl)butyl]-2*a*,3, 4,5-tetrahydro-1H-benz [*cd*]indole-2-one) were gifts from Meiji Seika Kaisha Ltd. (Yokohama, Japan). ^{11}C was produced by ^{14}N(p, α)^{11}C nuclear reaction using a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industry Co. Ltd., Tokyo, Japan). Radioactivity was determined using a dose calibrator

(IGC-3R Curiemeter, Aloka, Tokyo). HPLC was performed using a JASCO HPLC system (JASCO, Tokyo, Japan): effluent radioactivity was determined using a NaI (Tl) scintillation detector system. All chemical reagents were purchased from Aldrich Chem. Co. (Milwaukee, WI) and Wako Pure Chem. Ind., Ltd. (Osaka, Japan). The radiosynthesis, PET study and metabolite assay were performed at the National Institute of Radiological Sciences. Experimental animals used in the present study were treated and/or handled according to the 'Recommendations for Handling of Laboratory Animals for Biomedical Research' compiled by the Committee on the safety and handling regulations for laboratory animal experiments, National Institute of Radiological Sciences.

Preparation of [^{11}C]DR4446

Radioactive ^{11}C was generated by the ^{14}N (p, α)^{11}C nuclear reaction using the cyclotron. Preparation of [^{11}C]CH_3I, ^{11}C-methylation of 1 to [^{11}C]DR4446, HPLC purification and formulation were achieved automatically using specially designed equipment.[22] The [^{11}C]CH_3I obtained was trapped in 300 µl of anhydrous DMF containing the amide precursor 1 (1.0 mg) and NaH (10 µl, 0.5 g/20 ml DMF) at -15°C to -20°C, and then the reaction mixture was heated to 30°C and kept for 5 min. The radioactive mixture containing [^{11}C]DR4446 was diluted with 0.5 ml of HPLC mobile phase, and transferred onto a column (10 mm ID $\times$ 250 mm, CAPCELL PAK C_{18}, SHISEIDO, Tokyo, Japan) attached to the JACSO HPLC system. Elution with $CH_3CN/H_2O/Et_3N$ (70/30/0.05) at a flow rate of 6 min/ml gave a radioactive fraction corresponding to pure [^{11}C]DR4446 ($t_R = 7.9$ min). The fraction was collected in a rotary evaporator and evaporated to dryness at about 90°C under reduced pressure. The residue was dissolved in 7 ml sterile isotonic saline, and passed through a 0.22-µm Millipore filter. At EOS, 1.9–2.2 GBq of [^{11}C]DR4446 was obtained with a radiochemical yield of as an injection solution after 10–15 min of proton bombardment at a beam current of 10–15 µA.

Radiochemical purity and specific activity determinations

Radiochemical purity was assayed by analytical HPLC (column: CAPCELL PAK C_{18}, SHISEIDO, 4.6 mm ID $\times$ 250 mm, UV at

254 nm, mobile phase: $CH_3CN/H_2O/Et_3N = 70/30/0.05$) and was always greater than 98%. The retention time was 6.6 min for [^{11}C]DR4446 at a flow rate of 1.5 ml/min. Confirmation of the identity of [^{11}C]DR4446 was achieved by co-injection with the non-radioactive DR4446. For the determination of specific activity, the mass (μmol) of [^{11}C]DR4446 with a known radioactivity (GBq) was determined by HPLC comparison of UV absorbance at 254 nm of this radioligand with those of known concentrations of the corresponding non-radioactive DR4446. The specific activities of [^{11}C]DR4446 were 73–120 GBq/μmol at EOS.

PET of [^{11}C]DR4446 in Monkey

All PET scans were performed using a high-resolution SHR–7700 PET camera (Hamamatsu Photonics, Hamamatsu, Japan) designed for laboratory animals, which provides 31 transaxial slices 3.6 mm (center-to-center) apart and 11.4 cm field of view. The spatial resolution for the reconstructed images was 2.6 mm FWHM. A male rhesus monkey (*Macaca mulatta*) weighing about 5.0 kg was fixed with a fixation apparatus. A magnetic resonance image (MRI) of the monkey brain was obtained with a Philips Gyroscan S15/ACS II (1.5 T) with a three-dimensional T_1-weighted axial MRI sequence. After transmission scans for attenuation correction were performed for 1 h using a 78 MBq ^{68}Ge-^{68}Ga source, dynamic emission scan in 3D acquisition mode was performed for 90 min (2 min × 5 scans, 4 min × 10 scans, 10 min × 4 scans).

All emission scan images were reconstructed with a Colsher filter of 4 mm, and circular regions of interest (ROIs) with a 5-mm diameter were placed over the thalamus, striatum, frontal cortex and cerebellum using an image analysis software. The software included: (1) anatomical definition on MRI using atlas (manual); (2) ROI setting on MRI; (3) co-registration of PET and MRI; (4) applying ROIs to PET; (5) determination of radioactivity. ROIs were defined over ten slices (Nos. 11–20) of total 31 slices. A solution of [^{11}C]DR4446 (98 MBq) with a specific activity of 110 GBq/μmol was injected intravenously into the monkey, and time-sequential tomographic scanning was performed on a transverse section of the brain for 90 min. In the blocking study, non-radioactive DR4446 (1 mg/kg) was mixed with the solution of [^{11}C]DR4446 (98 MBq, specific activity: 90 GBq/μmol) and then was injected intravenously into the monkey. The TACs in the ROIs were obtained for each scan of the brain.

Metabolite assay in plasma

Blood samples (1 ml) were obtained from the monkey at 2, 10, 30, 60, and 90 min after intravenous injection of 98 MBq [^{11}C]DR4446. All samples were centrifuged at 10 000 rpm for 2 min at 4°C to separate plasma, which (250 µl) was collected in a test tube containing 0.5 ml of CH_3CN and 10 µl of authentic non-radioactive DR4446 (1.1 mg/5 ml). The tube was vortexed for 15 s and centrifuged at 10 000 rpm for 1 min for deproteinization. The extraction efficiency of radioactive metabolites into the CH_3CN ranged from 81 to 93% of total radioactivity in the plasma. An aliquot of the supernatant (100–500 µl) was injected into an HPLC column with a high-sensitive detector[23] for radioactivity and analyzed for metabolites by the same HPLC conditions as used for the radiochemical purity determination. The radioactivity in a certain fraction was divided by the total radioactivity and expressed as a percentage of the total radioactivity.

Conclusion

The novel 5-HT$_7$ receptor PET tracer [^{11}C]DR4446 was successfully synthesized by the reaction of the desmethy precursor 1 with [^{11}C]CH_3I. The radiochemical yield was 15–28% (corrected for decay, based on the total [^{11}C]CO_2) and the specific activity was 73–120 GBq/µmol at EOS. The PET study in monkey demonstrated that [^{11}C]DR4446 had a specific binding component in the brain regions including the thalamus, a possibly area in the 5-HT$_7$ receptor sites. A detailed PET characterization of [^{11}C]DR4446 binding in primate brain is currently underway and the results will be presented elsewhere.

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References

1. Eglen RM, Jasper JR, Chang DJ, Martin GR. *Pharmacol Sci* 1997; **18**: 104–107.
2. Vanhoenacker P, Haegeman G, Leysen JE. *Trends Pharmacol Sci* 2000; **21**: 70–77.
3. Johns BJ, Blackburn TP. *Pharmacol Biochem Behav* 2002; **71**: 555–568.
4. Shen Y, Monsma FJ, Metcalf MA, Jose PA, Hamblin MW, Sibley DR. *J Biol Chem* 1993; **268**: 18 200–18 204.
5. Kinsery AM, Wainwright A, Heaven R, Sirnathsinghji DJ, Oliver KR. *Brain Res Mol Brain Res* 2001; **88**: 194–198.
6. Plassat JL, Amlaiky N, Hen R. *Mol Pharm* 1993; **44**: 229–236.
7. Tsou AP, Kosaka A, Bach C, *et al. J Neurochem* 1994; **63**: 456–464.
8. Bard JA, Zgombick J, Adham N, Vaysse P, Branchek TA, Weinshank RL. *J Biol Chem* 1994; **268**: 23 422–23 426.
9. Leung E, Walsh LKM, Pulido-Rios MT, Eglen RM. *Br J Pharmacol* 1996; **117**: 926–930.
10. Cushing DJ, Zgombick JM, Nelson DL, Cohen ML. *J Pharmacol Exp Ther* 1996; **277**: 1560–1566.
11. Martin GR, Wilson RJ. *Br J Pharmacol* 1995; **114**: 383.
12. Sleight AJ, Carolo C, Petit N, Zwingelstein C, Bourson A. *Mol Pharmol* 1995; **47**: 99–103.
13. Ruat M, Trafiffort E, Leurs R, *et al. Proc Natl Acad Sci USA* 1993; **90**: 8547–8551.
14. Roth BL, Craigo SC, Choudhary MS, *et al. J Pharmacol* 1996; **117**: 926–930.
15. Meltzer HY. *Neuropsychopharmacology* 1999; **21** (Suppl 2): 106S–115S.
16. To ZP, Bonhaus DW, Eglen RM, Jakeman LB. *Br J Pharmacol* 1995; **115**: 107–116.
17. Gustafan EL, Durkin MM, Bard JA, Zgombick J, Branchek TA. *Br J Pharmacol* 1996; **117**: 657–666.
18. Forbes IT, Dabbs S, Duckworth DM, *et al. J Med Chem* 1998; **41**: 655–657.
19. Kikuchi C, Nagaso H, Hirayama T, Koyama M. *J Med Chem* 1999; **42**: 533–535.
20. Kikuchi C, Ando Y, Watanabe Y, Nagaso H, Okuno M, Koyama M. *J Med Chem* 2002; **45**: 2197–2206.
21. Kikuchi C, Nagaso H, Hirayama T, Koyama M. *Bioorganic & Med Chem Lett*, submitted.
22. Suzuki K, Inoue O, Hashimoto K, Yamasaki T, Kuchiki M, Tamate K. *Appl Radiat Isot* 1985; **36**: 971–976.
23. Takei M, Kida T, Suzuki K. *Appl Radiat Isot* 2001; **55**: 229–234.

